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Genome analysis and performance traits in cattle (Genomanalyse und Leistungsmerkmale beim Rind). T. Leeb - Tierärztliche Hochschule Hannover, Institut für Tierzucht und Vererbungsforschung, Bünteweg 17p, D-30559 Hannover

Introduction

Our knowledge of mammalian genomes has greatly increased with the initial completion of the human genome sequence [1] and the genome sequences of other model organisms such as mouse [2] and rat [3]. These genome sequencing projects are extremely valuable for livestock researchers as ~80 % of the mammalian genes have unambiguously conserved orthologs across mammalian species and ~99 % of the genes have at least a related paralogous gene in other mammalian species [2]. It has become evident that the number of protein coding genes in the mammalian genome lies between 25,000 and 30,000 - much lower than most estimates before the realization of the genome sequencing programs. Whereas the presumed number of genes steadily decreased in the last few years, it also became clear that the complexity of a mammalian organism is maintained by a very complex network of gene regulation and that a great amount of protein diversity is caused by alternative splicing and posttranslational modification. Additionally, more and more RNA coding genes are being discovered whose functions are mostly unclear at the moment. Although the protein coding genes have been intensively studied for many years, only about half of the 25,000 - 30,000 genes have been experimentally characterized and functionally annotated. In the past, functional characterization was mostly performed on single genes and in many cases the isolated analysis of a single gene and its protein product was greatly hampered by missing knowledge on the context of the analyzed gene product in complex regulatory networks.

The knowledge of complete genome sequences has facilitated novel approaches towards the functional characterization of genes. One of the most important technical advances has been the development of methods for the simultaneous monitoring of the expression levels of all mRNAs (transcriptomics) or even all proteins (proteomics) of a given cell type or tissue. These novel expression profiling methods are usually based on microarray hybridizations for mRNA quantifications or on high-throughput mass spectroscopy analyses of proteins. Taken together transcriptomics and proteomics are sometimes also termed "functional genomics". The term functional genomics illustrates that expression profiles can be used to extract physiological information on genes. A typical experiment compares the expression profiles of a tissue under two different physiological states. It is thus possible to identify genes that are up- or down-regulated between the two different physiological states. Analysis of such genes can reveal the interaction of different genes in regulatory circuits and consequently accelerate the elucidation of metabolic pathways or signal transduction cascades.

Status of the bovine genome project

Efforts are now well under way to determine the complete sequence of the cattle genome. In 2002 cattle joined the list of organisms that are considered high scientific priority for genome sequencing by the NHGRI [4]. Important prerequisites for the sequence assembly have been established such as large-insert clone libraries [5], RH maps [6] and high-resolution clone-based physical maps [7]. It is expected that whole genome shotgun (WGS) sequencing of about 6x coverage of the bovine genome will take place at the Baylor College of Medicine Human Genome Sequencing Center in Houston, Texas in close collaboration with Texas A&M University. The gene annotation of the bovine genome sequence will be facilitated by the large amount of EST data that are available for cattle [8]. In addition of the pure sequencing of the cattle genome, the international bovine genome consortium

aims also for the generation of a single nucleotide polymorphism (SNP) map that will complement the existing microsatellite maps for genetic mapping experiments.

One of the general aims of genome analysis in cattle is the discovery of DNA variations that underlie heritable traits. Heritable traits can be classified in single-gene traits that are controlled by a single gene in contrast to polygenic traits that are influenced by many genes. Quantitative traits such as most performance traits are poly- or at least oligogenic traits, where variations at one genetics locus are responsible for only a fraction of the total variance of the trait. Single gene traits as well as polygenic traits may further be modulated by environmental factors. Historically, there have been two major routes towards the identification of genes and functionally important mutations within these genes: the candidate gene approach and the positional cloning approach.

Candidate gene approach

In the so-called candidate gene approach, a candidate gene is selected based on knowledge of its physiological relevance for a specific trait and this gene is then intensively analyzed. A successful example for this approach was the discovery of the causative mutation for the deficiency of uridine monophosphate synthase (DUMPS) in cattle, an embryonic lethal autosomal recessive disease [9] (Table 1). The responsible gene coding for uridine monophosphate synthase (UMPS) was identified as it was noticed that the levels of orotic acid (the substrate for UMPS) were elevated in the milk of heterozygous carriers of the disease. Unfortunately, the candidate gene approach is not always feasible as it is often difficult to deduce rational candidate genes purely based on phenotypic descriptions.

Positional cloning gene approach

For this approach a linkage analysis of the trait of interest with polymorphic markers across the genome has to be performed ("genome scan"). Following the initial genome scan fine mapping is performed with additional markers in those chromosomal regions that show linkage to a trait of interest. Once a sufficiently small genomic interval has been identified, a mutation analysis of all genes in this interval should reveal the causative mutation for the trait of interest. Pure positional cloning approaches were rarely used in livestock genomics, as it often requires a heroic effort to do a complete mutation analysis of regions that may easily span several Mb. A more economical modification of the pure positional cloning is the so-called positional candidate cloning. This strategy also starts with a linkage analysis to narrow down the chromosomal position of the gene of interest. However, once a linked genomic interval is identified, the genes in this interval are first inspected with regard to their putative functions and only those genes that seem reasonably well connected to the phenotype are considered as positional and functional candidates. This combination of positional and functional information often reduces the number of purely positional candidates to more manageable numbers. A good example for the positional candidate approach has been the recent finding that a mutation in the gene *DGAT1* encoding diacylglycerol O-acyltransferase (EC2.3.1.20) is responsible for differences in milk fat content [10-12] (Figure 1).
Before the *DGAT1* gene was identified, several linkage studies were performed that detected a quantitative trait locus (QTL) for milk fat content on bovine chromosome 14 (BTA 14) [13]. The QTL interval was narrowed down to about 3 cM on BTA 14 by further fine mapping [14]. Through comparative analyses of this region on BTA 14 with the syntenic regions in the human and mouse genome it was established that the best functional candidate among the ~50 genes in this region was the *DGAT1* gene. The evidence for a possible effect of the *DGAT1* gene on milk composition came from the biochemical knowledge that the enzyme DGAT1 is involved in triglyceride synthesis and from the fact that *Dgat1* knock out mice are deficient in lactation [15]. Following the discovery of the relevance of the *DGAT1* polymorphism for milk composition, evidence is now increasing that this polymorphism in cattle additionally affects the intramuscular fat deposition also called marbling, which is another economically relevant trait [16].

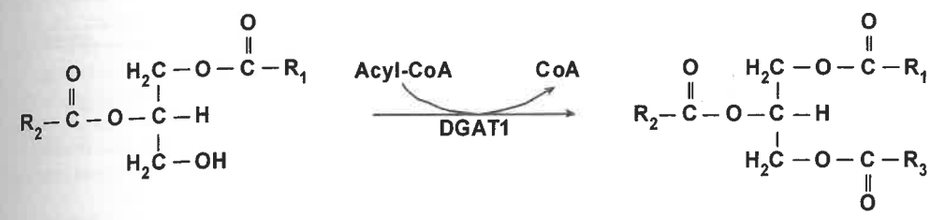

Figure 1. Enzymatic activity of DGAT1.
The enzyme diacylglycerol O-acyltransferase (DGAT1) catalyzes the addition of the third acyl group to a diacylglyceride. This reaction represents the last and rate-limiting step of triglyceride synthesis. A point mutation in a conserved domain of the DGAT1 enzyme (K232A) is found in several cattle breeds. The lysine variant is responsible for increased milk fat content and other characteristic milk composition traits [12].

Another recent example for the successful application of a positional candidate strategy has been the molecular dissection of a QTL for milk yield on BTA 20 [17]. Similar to the chain of events in the case of *DGAT1*, this QTL was mapped relatively early to BTA 20, but the causal mutation could not be resolved [18]. Only after extensive fine mapping had narrowed down the QTL region considerably, it became possible to define a rational set of positional candidate genes for this QTL that included the genes for the prolactin receptor (*PRLR*) and the growth hormone receptor (*GHR*). Finally, it turned out that a phenylalanine-to-tyrosine exchange (F279Y) in the bovine growth hormone receptor is responsible for an increase in milk yield. However, this increase in milk yield is associated with a decrease in protein and fat percentage so that the total protein and fat yield remains almost constant.

Comparative mapping strategies

Positional cloning efforts in livestock species are very often supplemented with a comparative mapping strategy. Comparative mapping exploits the fact that mammalian chromosomes were rarely rearranged during evolution. Therefore, mammalian species share large chromosome fragments with conserved gene order, the so-called syntenic blocks. For example between cattle and human there might be as few as 105 syntenic blocks [6, 7]. As the sequence and gene annotation of the human genome is known, the gene content of a bovine genomic fragment can be extrapolated from the gene content of the homologous human genome region if the borders of conserved synteny are known. Comparative mapping is now routinely used in livestock genetics to define the gene content of regions that have been identified by linkage mapping. One example for this approach has been the discovery that mutations in the bovine *ED1* gene cause developmental defects of ectodermal structures such as hair, teeth, and certain eccrine glands [19, 20]. The chromosomal region responsible for this phenotype was originally identified by linkage mapping on the long arm of the X chromosome. Comparative mapping of this cattle genome region revealed that in the syntenic region of the human genome mutations in the human *ED1* gene cause anhidrotic ectodermal dysplasia, a disease characterized by similar symptoms as the bovine counterpart. Thus the comparative mapping approach greatly facilitated the identification of the causative gene defect in cattle.

Table 1: Known causative gene mutations for performance and other traits in cattle (modified from [21])

trait	gene	chromosome	mutation	reference
milk fat content	diacylglycerol O-acyltransferase *(DGAT1)*	BTA 14	K232A	[10-12]
milk yield	growth hormone receptor *(GHR)*	BTA 20	F279Y	[17]
muscular hypertrophy (mh)	myostatin *(GDF8)*	BTA 2	various deletions	[22, 23]
anhidrotic ectodermal dysplasia (ED1)	ectodysplasin *(ED1)*	BTA X	various mutations	[19, 20]
bovine leukocyte adhesion deficiency (BLAD)	ß2-integrin *(CD18)*		D128G	[24]
black or red coat color	melanocortin 1 receptor *(MC1R)*	BTA 18	various mutations	[25]
Chediagk-Higashi Syndrome (CHS)	Chediagk-Higashi syndrome 1 *(CHS1)*	BTA 28	H2015R	[26]
chondrodysplasia (BCD, Japanese Brown cattle)	limbin *(LBN)*	BTA 6	splice site mutation affecting exon 11	[27]
citrullinaemia	argininosuccinate synthetase *(ASS)*	BTA 11	R86X	[28]
deficiency of uridine monophosphate synthase (DUMPS)	uridine monophosphate synthase *(UMPS)*	BTA 1	G405X	[9]
Ehlers-Danlos Syndrome (Dematosparaxis)	procollagen I N-propeptidase *(ADAMTS2)*	BTA 7	deletion	[29]
glycogen storage disease II (Pompe's disease)	acidic α -glucosidase *(GAA)*	BTA 19	various mutations	[30]
glycogen storage disease V (McArdle's disease)	myophosphorylase *(PYGM)*	BTA 29	R489W	[31]
mannosidosis, alpha	α-mannosidase *(MAN2B1)*	BTA 7	various mutations	[32]
mannosidosis, beta	ß-mannosidase *(MANBA)*	BTA 6	W858X	[33]
maple syrup urine disease (MSUD)	branched chain alpha-ketoacid dehydrogenase, E1α subunit *(BCKDHA)*	BTA 18	various mutations	[34]
myoclonus (ICM)	glycine receptor α1 *(GLRA1)*	BTA 7	K24X	[35]
protoporphyria (PP)	ferrochelatase *(FECH)*	BTA 24	X417L	[36]
renal dysplasia	claudin 16 *(CLDN16)* (formerly paracellin 1)	BTA 1	deletion of exons 1-4	[37]
spherocytosis	solute carrier family 4, anion exchanger *(SLC4A1)*	BTA 19	R646X	[38]
trimethylaminuria	flavin-containing mono-oxygenase 3 *(FMO3)*	BTA 16	R238X	[39]

Functional genomics in livestock species - Transcriptomics

Whereas hundreds of QTLs have been mapped in cattle during the last decade, only two of the responsible molecular DNA alterations for these QTLs have been elucidated (Table 1). Scientists are therefore eager to develop new experimental strategies that facilitate the molecular analysis of QTLs. One of the most challenging tasks in this process is the definition of candidate genes for a given trait. As described above the two main approaches towards the identification of suitable candidate genes were based either on functional or on positional information, respectively. The functional approach was greatly hampered by our insufficient knowledge of the cattle genome. As only a small fraction of the genes were known, many genes were inadvertently omitted from the candidate lists. Furthermore,

our understanding of the cellular processes that underlie a complex phenotype is also often incomplete. These complex processes often result from the interaction of whole networks of genes that are poorly understood. One aspect that might allow insight into these complex gene networks is their expression pattern. A reasonable assumption requires that a candidate gene should be expressed in those tissues that are phenotypically affected. Further clues may be derived from the fact that co-regulated genes may encode proteins that are part of the same metabolic pathway. The observation of gene expression patterns on the mRNA level has the tremendous advantage that all genes of an organism can be monitored simultaneously. Therefore measurements of transcript levels in specific tissues or even in individual cells have great potential for our understanding of gene functions.

Technically, mRNA expression profiles can be obtained by several methods, of which microarray techniques are becoming increasingly popular [40-42]. For the purpose of transcript quantification the microarrays consist of glass slides ("chips") upon which single stranded DNA is bound. Glass chips are non-porous, which ensures faster hybridization rates as opposed to porous substrates such as nylon membranes [43]. The DNAs on the microarrays can either be oligonucleotides or long cDNA molecules that are prepared by PCR and spotted onto the chip surface [44]. Alternatively, oligonucleotides can also be synthesized directly on the glass surface either using sophisticated photolithographic techniques such as photochemical deprotection for the growing oligonucleotide chains or by delivering the compounds of the oligo synthesis by inkjet technology [45]. With *in situ* synthesis several hundred thousand targets can be synthesized on one chip whereas spotted chips can accommodate a few tens of thousands of targets. On both types of chips it is possible to spot at least one hybridization target for every gene in the mammalian genome, if gene sequences are available. While the production of such global microarrays has become routine for human and mouse, it is currently not possible to produce bovine chips of the same quality because the bovine genome sequence is not yet available. In the past, some efforts were undertaken to perform heterologous hybridizations with animal RNA on human microarrays [46]. This approach has severe limitations as only a subset of highly conserved transcripts can be monitored. However, the ongoing efforts of the cattle genome community have already provided a great amount of EST data, so that cDNA sequences of a large fraction of the bovine genes have become available and the first bovine cDNA chips are now being produced [47-49].

To acquire an expression profile high-quality RNA is isolated from tissue and labeled fluorescently. The labeled RNA is hybridized to the microarray and the fluorescence at each microarray spot is recorded using a microarray scanner. The fluorescence intensity at each spot reflects the abundance of the corresponding transcript. Typically, microarrays are only used for one single hybridization and for accurate quantification it is necessary that each spot within a microarray but also across microarrays contains the same amount of target DNA. These stringent quality criteria are mainly fulfilled by *in situ* synthesized oligo chips but not by spotted chips. To overcome this technical problem microarray experiments are often performed as competitive hybridizations with two differently (i.e. red and green) labeled RNAs. Expression levels are then measured as relative ratio between the red and green signals and variations in the amount of target DNA on the chips are compensated. In competitive hybridizations the two RNAs can be derived from different tissues or from one tissue in two different physiological states and the results allow conclusions about up- and down-regulated genes between the two samples.

An extremely important aspect of microarray experiments is the design of the experiment and subsequent data analysis. Great care must be used to ensure that differences in gene expression levels are actually due to the physiological process under investigation and not by other differences between the samples or random variation. For example if the gene expression profile of a distinct cell type with respect to two different diets is compared, the samples should be obtained from animals of the same sex, breed, age, lactation status etc. Obviously it is of utmost importance to use defined populations of cells for expression profiling. Expression data from complex mixtures of cell types and tissues are almost impossible to interpret. Finally, enough replicates must be performed to ensure

reproducible results [50] and appropriate statistical tests must be employed to discriminate significant changes in gene expression from random fluctuation [51]. As the measurement of an expression profile is basically a descriptive observation, epidemiological methods can be used to avoid biases and control confounding [52].

Functional genomics in livestock species - Proteomics

The monitoring of expression levels on the protein level has many advantages over mRNA based expression profiling [53]. In some cases changes in the mRNA level are not directly related to changes in protein concentration. In other cases it is not the total protein concentration that is physiologically relevant but only a certain modified fraction of the protein, e.g. only the phosphorylated form of a protein has enzymatic activity while the non-phosphorylated form represents an inactive intermediate. However, although proteomics promises to be an ideal tool for the investigation of complex physiological processes, it still faces many technical problems that are not easily overcome. Due to posttranslational modifications the number of different proteins in a cell is typically larger than the number of mRNAs. Furthermore, proteins may have dramatically different properties, which makes their simultaneous monitoring more demanding than mRNA expression profiling. One approach to obtain a protein expression profile is 2D-gel electrophoresis followed by quantification of the individual protein spots. While this procedure is now routinely used in microorganisms, its application in livestock species is still very limited [54]. One difficulty is the enormous protein complexity of mammalian cells that cannot completely be resolved on 2D-gels. Another current problem are the lacking mass data of most bovine proteins and peptide fragments. The identification of 2D-gel spots is normally achieved by mass spectroscopy, however in contrast to human or mouse the protein mass databases in cattle and other livestock organisms are still very rudimentary, so that protein identification by mass spectroscopy is currently not possible in cattle.

Summary and conclusion

Cattle genome analysis is progressing rapidly. The techniques to determine a complete mammalian genome sequence are now well-established and sequencing capacities at the major sequencing centers allow the WGS sequencing of several mammalian species per year. Cattle is scheduled to be among the first livestock species to be completely sequenced within the very near future. Researchers put great hope in the determination of the bovine genome sequence as knowledge of the genome sequence might help to accelerate the dissection of genetic variation that is responsible for differences in performance as well as other heritable traits. With traditional candidate gene and positional gene approaches the first QTL mutations have now been discovered. Knowledge of the genome sequence will allow the realization of functional genomics experiments that might complement the traditional gene identification strategies. The hallmark of these experiments is the parallel analysis of complex gene networks rather than the classical analysis of single genes. This global view of functional genomics experiments may help us to better understand the complex genetics of performance traits. During the breeding history of livestock animals a number of performance traits were subject to intensive selection. Therefore, the analysis of modern high-performing animals has great potential to reveal some of the genetic mechanisms that underlie basic physiological processes. Insights in this field could have implications not only for animal production but also for human physiology and medicine.

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1. **Effects of different phosphorus sources in the diet on phosphorus content in blood and on bone stability (breaking strength) in broiler chicks** (Der Einfluss verschiedener Phosphorquellen im Futter auf den Phosphorgehalt im Blut sowie die Knochenstabilität [Bruchfestigkeit] bei Broilern). A. Hemme*, M. Spark, H. Paschertz and J. Kamphues – Hannover

Knowledge of the availability of phosphorus from the specific source is crucial to achieve an adequate supplementation without an excess. To elucidate this question, the influence of three different phosphorus sources [monocalcium phosphate (MCP), dicalcium phosphate (DCP) and defluorinated phosphate (DFP)] in the diet (all with a P concentration of 6 g P/kg dm) for broiler chicks was tested on Ca and P content in serum and on bone stability.

Methods: Two experimental periods, with 100 one-day-old broiler chicks each, were conducted (4 groups of 25 chicks). During the first ten days all chicks received a conventional starter diet (12.6 MJ ME; per kg dm: 213 g XP, 9 g Ca, 6 g P). After ten days the feeding was different in the 4 groups: while the control group was fed with an usual fattening diet (13.0 MJ ME; per kg dm: 207 g XP, 9 g Ca, 6 g P) with Ca-Na-phosphate and phytase (0.15 g/kg dm), the three other groups received the same basic mix with different sources of P, supplemented to the same total of the phosphorus concentration. The feed conversion and the daily gains were estimated continually. Out of each group, five animals were sacrificed on the tenth day to determine basic values. On the 23rd day further five animals out of each group were examined, whereas the remaining broiler chicks were slaughtered at the end of the experiment (after 36 days). Serum samples were examined on Ca and P contents. Further to determine the breaking strength in Newton (N), tibia and humerus of every broiler were fractured by a material-test-machine type „Zwicki-Z2,5/TNIS" (Zwick-Roell, Ulm, Germany).

Results: With same basic values (Ca, P, breaking strength) at day 10 there were significant differences on day 23, these differences increased at the end of the trials. The use of DFP as P source led to significantly adverse values of P in serum and breaking strength of long bones.

Table 1: Experimental design and results of both experiments

group		Control	DCP	MCP	DFP
diet (day 1 – 10)[1]		9.0 / 6.0			
diet (day 11 – 36)[1]		9.0 / 6.0	9.8 / 5.7	8.9 / 5.3	10.3 / 5.5
daily gains, day 11 – 36 (g)		71.5	69.9	66.3	64.7
(g) feed/ (g) gain, day 11 – 36		1.533	1.606	1.615	1.622
serum P	day 10	1.55 ± 0.22	1.65 ± 0.24	1.69 ± 0.22	1.68 ± 0.26
[mmol/ l]	day 23	2.10 ± 0.25	1.53 ± 0.20	1.62 ± 0.37	1.14 ± 0.21
(n = 2 x 5)	day 36	1.85 ± 0.24	1.64 ± 0.18	1.96 ± 0.19	1.42 ± 0.32
bone strength [N]	humerus (r)[2]	339 ± 72.8	364 ± 81.2	333 ± 60.7	275 ± 81.3
(n = 2 x 15)	tibia (r)[2]	266 ± 86.5	283 ± 69.5	257 ± 67.8	209 ± 63.3

[1] g Ca/ g P per kg dm of the diet [2] r = right

Although in both experiments identical genetic lines and absolutely comparable diets were used, differences in the breaking strength were observed (experiment 2: very high outside temperatures → in all groups reduced values of breaking strength were detected, indicating that eventually climatic effects exist on this parameter).

Conclusions: The significantly reduced content of serum P and the decreased breaking strength are indicating the low phosphorus availability of the rather cheap phosphorus source DFP. If there is suspicion for nutrition caused skeletal diseases in broilers, it is not sufficient to measure the total P content in the diet, but it makes sense to focus the thoughts on availability of the P sources that are used (estimation of P content in the serum may be informative).

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2. Alteration of regulative P-excretions from urine to faeces by growing Saanen goat kids, fed either with a solid or a liquid diet (Verlagerung der regulativen P-Ausscheidungen vom Harn in den Kot bei wachsenden Ziegenlämmern, gefüttert entweder mit fester oder flüssiger Nahrung).

May Looff*, Korinna Huber, Julia Hattendorf, G. Breves, E. Pfeffer – Bonn/ Hannover

While adult and ruminating goats excrete excess phosphorus (P) with the faeces, goat kids regulate P-excess via the kidney - even at that time of complete weaning (Boeser et al. 2003). The aim of this study was to ascertain the time when this change in P-regulation takes place and to determine the influence of the solid diet on this process.

Methods: Twelve Saanen kids, divided into two groups, were given a diet containing 0,5 g P/MJ ME, which means a level of 150% compared to original goat milk. The first group was offered solid feed (hay and pelleted concentrates in a fixed ratio of 1:4) ad libitum and was weaned at a bodyweight of 11 kg. The second group was continuously fed with goat milk, supplemented with di-sodium hydrogen phosphate, and given straw as roughage. To achieve an equivalent growth in both groups, milk for the second group was allocated in "pair-fed"-manner, that means that the received energy was equal to the energy-intake of the first group. Keeping the kids in metabolic cages enabled the individual registration of feeds, faeces and urine to draw up P-balances.

Results: Resulting from the nearly equal energy-intake, average daily bodyweight gain was the same (158 g) in both groups till week 16, when the experiment was finished for the milk-fed kids (group II). P-intake, dependent on the feed consumption of the solid-fed kids (group I), raised continuously during the experiment. In the beginning there was no difference between the two groups in the pathway of P-excretion (see table 1), nearly all excess P was excreted via the kidney. In the course of the experiment, the excretory way for excess P changed gradual from the urine to the faeces in group I, whereas the kidney continued to be the main excretory way for P in group II (see table 1). P-retention was comparable for both groups. Three to four months after weaning the P-excretion via the kidney, seen as % of intake, was as low as known for adult goats in group I.

Table 1

		week 2		week 16	
		group I	group II	group I	group II
ME-intake	(MJ/d)	2,67 ± 0,21	2,68 ± 0,01	7,35 ± 1,2	7,87 ± 0,45
P-intake	(g/d)	1,38 ± 0,11	1,38 ± 0,00	3,87 ± 0,63	3,85 ± 0,14
P-retention	(% of intake)	57,8 ± 3,7	59,2 ± 3,0	28,1 ± 3,3	30,2 ± 5,8
P via urine	(% of intake)	41,1 ±3,4	40,0 ± 2,7	21,0 ± 5,6	57,5 ± 9,4
P via faeces	(% of intake)	1,2 ± 1,2	0,8 ± 0,3	50,9 ± 6,2	12,3 ± 4,8

Conclusions:
From this experiment it can be concluded, that the change from an excretion of surplus P via the kidney to an excretion via the faeces is a gradual process, which is finished not before several months after weaning. Without feeding hay or concentrates, P-excretion with the urine was not reduced. From another experiment, though, there are indications for a faster progress of this alteration in P-excretion if kids were brought up in the beginning with original goat milk.

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3. Effects of different phosphorus supply on the PTH and vitamin D_3 receptor expression in small ruminants (Auswirkungen einer unterschiedlichen Phosphor-Versorgung auf die PTH- und Vitamin D_3-Rezeptor-Expression bei kleinen Wiederkäuern).

Alexandra Muscher*, Julia Hattendorf, Korinna Huber, E. Pfeffer, G. Breves – Hannover/Bonn

In phosphorus (P) homeostasis PTH stimulates the renal phosphate (P_i) excretion, whereas vitamin D_3 increases the intestinal P_i absorption. In goats different P supply does not cause adaptive changes in PTH and/or vitamin D_3 hormone levels as it is the case in monogastric animals (1,2). Possibly, the extent of PTH and vitamin D_3 receptor expression could be affected. Another receptor which should be analysed by molecular biological methods is the calcium sensing receptor (CaR). Together with the sodium dependent P_i transporter type IIa (NaPi IIa) it is coexpressed in the kidney.

Animals and Methods:
White Saanen goats aged five to six months were used. They were allocated to one of two dietary P concentrations: aP (adequate P supply) 0,3 g/MJ ME or dP (double P supply) 0,6 g/MJ ME.
After slaughtering jejunal mucosa, kidney cortex and parotid glands were immediately removed and frozen in liquid nitrogen. DNA probes which were needed for the Northern Blot Analyses, were created by RT-PCR. The specifity of the probes was verified by sequencing. Isolated poly $(A)^+$RNAs from kidney and jejunum were transferred by capillary blotting onto nitrocellulose membranes. Radioactive labeled CaR, PTHR, VDR and ß-actin probes were hybridised to the mRNAs. Specific bands were detected by a phosphorus imager. Using ß-actin as an internal standard relative amounts of specific mRNA were found.
Salivary and plasma P_i concentrations were determined colorimetrically by the molybdate-vanadate-method described by Kruse-Jarres (3).

Results:
Neither in jejunum nor in kidney VDR mRNA expression was affected by different P supply. The renal CaR mRNA could also be detected. Likewise the expression was not modulated by dP whereas PTHR mRNA was strongly decreased in the kidney. This receptor mRNA could also be found in jejunum, but it did not seem to be influened by dP.

Conclusion:
Unaffected mRNA expression must not be reflected on protein level or on functional properties of receptors as posttranscriptional or postranslational processes could modulate the receptor expression beyond mRNA level.
Under dP supply it was expected that the expression of PTHR mRNA should increase due to the higher renal P_i excretion NaPi IIa should be decreased. PTH was known to modulate the P_i transporter removal from the apical membranes of proximal tubular cells. The goats were kept on dP from their first days of life and therefore the renal sodium dependent P_i transport was decreased. This lack of the PTH target could lead to the PTHR mRNA downregulation. The ability to regulate renal P_i reabsorption could be retarded and this assumption is supported by *in vivo* studies which were performed in the same animals.

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4. Changes in bone markers and intact parathyroid hormone in Hanoverian Warmblood foals (Veränderungen von Knochenmarkern und intaktem Parathormon bei hannoverschen Warmblutfohlen).

Ingrid Vervuert*, M. Coenen, Sarah Winkelsett, L. Christmann, O. Distl, E. Bruns and B. Hertsch - Hannover/Göttingen/Verden/Berlin

As bone markers are known to reflect bone metabolism and intact parathyroid plays an important role in calcium metabolism, the present study was designed to monitor changes in bone markers and intact parathyroid hormone in growing foals with special emphasis on age, sex, growth rates, and osteochondrosis.

Material and methods: 629 Hanoverian Warmblood foals born between December 26, 2000 and July 01, 2001 (308 males, 321 females) from 83 farms in Germany were included in this study. Over a period of six month (from birth until six month of age) foals were monitored monthly (body weight, withers height, feeding practice, and housing conditions). Additionally, blood samples were collected and osteocalcin (ON, marker of bone formation), PICP (carboxyterminal propeptide of type I collagen, marker of bone formation), ICTP (carboxyterminal telopeptide of type I collagen, marker of bone resorption), and intact parathyroid hormone (intact PTH) were analysed in a total of 284 foals. Information on osteochondrosis was obtained between the fifth and tenth month after birth using x-ray (fetlock joints, hocks, and stifles). All results are presented as means.

Results: Bone markers in plasma decreased with age ($P<0.05$), whereas intact PTH remained nearly constant during the first months of life. Sex did not influence these results. Furthermore, there were no differences in bone markers and intact PTH between healthy foals (N = 165) and those foals that developed ostechondrotic lesions (N = 119). However, different developments in plasma bone markers were noticed between foals, born early in the year (before 31 March, N = 154) compared to those foals who were born after the 31 March (N = 130, Table 1) without any distinctions between healthy foals and foals with OC. Additionally, body weight to withers height ratio was higher in early born foals. Intact PTH did not reveal these differences.

Table 1 Plasma bone markers in early and late born foals during the first 200 days of life

age (d)	ICTP [µg/L]		PICP [µg/L]		ON [µg/L]	
	early born	late born	early born	late born	early born	late born
1 - 50	38.2^{a}	39.0^{a}	883^{a}	940^{a}	173^{a}	172^{a}
51 - 100	33.7^{b}	34.3^{b}	869^{a}	883^{b}	142^{b*}	127^{b*}
101 - 150	31.0^{c*}	29.2^{c*}	836^{a*}	801^{c*}	116^{c*}	89.5^{c*}
151 - 200	30.6^{c*}	25.7^{d*}	835^{a*}	766^{c*}	98.8^{d*}	80.3^{d*}

means in the same column with different superscripts are different ($P<0.05$), *differences between early and late born foals ($P<0.05$)

Discussion: As expected, an age-related decrease was noticed in bone markers, whereas intact PTH concentrations remained constant during the first 200 days of life. The development in bone markers and intact PTH was similar for OC affected and healthy foals. Amazingly, the fall in bone markers was different regarding the date of birth. The varying trend in bone markers was related to the housing conditions. Early born foals were kept in stables in the first months of life, whereas the late born foals had free access to pasture immediately after birth. Housing foals predominantly in stalls during wintertime may impair normal bone development, compared to foals maintained on pasture immediately after birth. Although there was no clear link between the development of osteochondrosis and bone markers, other orthopaedic diseases are implicated in growth. As bone development is affected by housing conditions, the measurement of bone markers might be a useful tool to monitor bone metabolism in growing foals.

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5. The effect of inorganic and organic copper and zinc supplementation on growth performance and copper and zinc status of weanling pigs (Wirkung von anorganischen und organischen Kupfer- und Zinkzulagen auf Wachstum und Kupfer- und Zinkstatus von Absetzferkeln).

C. Rapp*, D. Büttner, U. Geier, A. Oster and H. Schenkel – Eden Prairie/ Forchheim/Stuttgart-Hohenheim

Zinc and copper are essential components in numerous enzyme systems that are involved in protein synthesis, epithelial tissue integrity, iron metabolism and immune response. Antagonists such as phytic acid are binding to trace minerals and thus inhibit trace mineral absorption and reduce bioavailability. The bioavailability of trace minerals varies with source and has been shown to be improved in organic sources such as zinc methionine compared to inorganic zinc forms. The objective of the present studies was to evaluate whether replacing inorganic copper and zinc by organic sources has an effect on growth performance and copper and zinc status of weanling pigs. Two hundred ninety-four cross bred female and male castrated pigs with an average initial weight of 8.9 kg were allotted to three treatments with five replicates (Experiment 1 and 2) and four replicates (Experiment 3) with seven to six pigs per replicate pen each. Pigs were fed a barley-wheat-soybean meal based mash feed diet. Treatments consisted of a diet without added copper and zinc (Control), a diet supplemented with 20 mg/kg copper and 65 mg/kg zinc from sulphate forms (Sulfates) and a diet with 20 mg/kg copper and 40 mg/kg zinc from organic sources and 25 mg/kg zinc in sulfate form (Organics). Experimental diets were fed from weaning until 40 days after weaning. Organic copper and zinc used were complexes of single amino acids and the respective trace mineral in a one to one molar ratio (Availa®Cu, Availa®Zn, Zinpro Corporation, Eden Prairie, USA). At day 40 after weaning, four pigs per treatment per experiment (Exp.) were killed; liver, one kidney and tail bones harvested and copper and zinc concentrations determined (LCu, LZn, KCu, KZn, BCu, BZn). Pigs fed the copper and zinc supplemented diets had consistently increased average daily gain (ADG) and higher BZn levels than Controls ($P<0.05$). In two of the three studies copper and zinc supplementation improved feed/gain (F/G) and increased LZn, KCu and KZn levels compared to Controls ($P<0.05$). Although not statistically different, in two of the three studies ADG of pigs fed Organics tended to be higher than in pigs fed Sulfates. Similarly, there was a consistent trend for increased average daily feed intake (ADFI) in pigs fed Organics compared to pigs fed Sulfates. In conclusion, copper and zinc supplementation improved growth and zinc status in pigs. It seems that replacing inorganic copper and zinc with organic forms may increase feed intake and growth in pigs.

	ADG[a]	ADFI[a]	F/G[a]	LCu	LZn	KCu	KZn	BCu	BZn
		G		mg/kg DM					
Exp. 1									
Control	263^{y}	473	1.80	65	104^{y}	13.3^{y}	85^{y}	1.5^{y}	64^{y}
Sulfates	291^{yz}	486	1.68	32	118^{yz}	24.8^{z}	102^{z}	1.3^{yz}	108^{z}
Organics	316^{z}	552	1.78	37	158^{z}	21.8^{z}	98^{z}	1.2^{z}	112^{z}
SE	12	28	0.08	11	14	1.3	4	0.1	8
Exp. 2									
Control	272^{y}	608^{y}	2.29^{y}	59	82^{y}	12.5	81	1.8	47^{y}
Sulfates	345^{z}	617^{y}	1.79^{z}	38	123^{z}	21.5	95	1.8	86^{z}
Organics	373^{z}	698^{z}	1.87^{z}	28	108^{z}	18.5	83	1.4	86^{z}
SE	16	19	0.11	10	6	2.1	5	0.2	7
Exp. 3									
Control	217^{y}	529	2.48^{y}	34^{yz}	120	11.5^{y}	80^{x}	2.3	47^{y}
Sulfates	326^{z}	543	1.64^{z}	25^{y}	113	19.3^{z}	95^{y}	3.3	79^{z}
Organics	324^{z}	568	1.78^{z}	36^{z}	121	17.5^{z}	86^{yz}	1.7	79^{z}
SE	18	17	0.13	3	24	1.8	2	1.2	6

[a] Least Squares Means

[x,y,z] Within a column and an experiment, means lacking a common superscript letter differ ($P < 0.05$)

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Cu-Werte trotz Zulage in Versuchsgruppe niedriger als in Kontrolle!

6. Selenate reduces insulin resistance in type II diabetic dbdb mice whereas selenite has no effect (Selenat reduziert die Insulinresistenz in Typ II diabetischen dbdb Mäusen, während Selenit keinen Effekt zeigt).

A. S. Müller* and J. Pallauf – Giessen

Introduction:
Oral or intraperitoneal administration of selenate (SeVI) was found to normalize glucose and fatty acid metabolism in type I diabetic rats (1, 2). The antidiabetic mechanism of selenate was attributed to an enhancement of the phosphorylation status of single components of the insulin signalling pathway downstream the insulin receptor (3). In a recent study we could show that selenate displayed insulinomimetic properties also in type II diabetic dbdb mice. A diminished insulin resistance was found to be responsible for the improved glucose metabolism (4).

Experimental design:
To examine the mechanism by which selenate diminishes the insulin resistance in type II diabetic animals in comparison to selenite and selenium deficiency, 21 diabetic adult female dbdb mice were assigned to 3 experimental groups of 7 animals each and fed a selenium deficient diet based on torula yeast. Group 0Se was fed the Se deficient diet for 10 weeks while the mice of the groups SeIV and SeVI were supplemented with sodium selenite or sodium selenate in weekly increasing doses up to 30% of their individual LD_{50} in week 10. Glucose tolerance and insulin resistance of the mice were tested before subjecting the animals to the specified dietary conditions and again after 10 weeks of special feeding. After 10 weeks the mice were killed and the activity of protein tyrosine phosphatases in liver and muscle was measured.

Results:
After 10 weeks the dbdb mice of the selenate treated group SeVI showed a significantly improved glucose tolerance and reduced insulin resistance in comparison to selenium deficient 0Se mice and selenite treated mice. The activity of protein tyrosine phosphatases which act as inhibitors of insulin signalling was significantly reduced by selenate treatment (Table).

Table: Activity of protein tyrosine phosphastases (mU/g FM) in the liver and skeletal muscle cytosol of dbdb mice

Organ	0 Se (Se deficient)	Se IV (selenite)	Se VI (selenate)
Liver	220±87.0[a]	190±46.0[a]	72±24.0[b]
Skeletal muscle	20±4.0[a]	18±2.0[a]	14±2.0[b]

Conclusion:
The results of the present study and of an additional inhibition study with tyrosine phosphatases (data not shown) indicate that the antidiabetic role of selenate in type II diabetic animals is the result of the inhibition of protein tyrosine phosphatases by intermediary selenate metabolites.

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7. Effects of dietary phytase activity on inorganic phosphorus concentration in blood plasma of Nile Tilapia - *Oreochromis niloticus* fed low phosphorus plant based diets (Einfluss der Phytaseaktivität im Futter auf die Konzentration an anorganischem Phosphor im Blutplasma von Nil Tilapien, denen pflanzliche Basaldiäten mit niedrigem Phosphorgehalt verabreicht wurden).

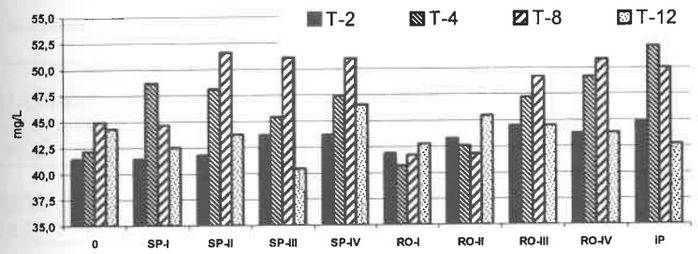
L. Portz* and F. Liebert – Göttingen

Approximately 70% of the total phosphorus in plant feedstuffs are in form of phytate and practically not available for fish species, lacking an intestinal phytase. The main objective of this study was to determine the concentration of inorganic phosphorus in the blood plasma as an additional indicator of the metabolic availability of phosphorus following a graded application of two different sources of microbial phytase in plant based diets for Nile Tilapia.

Methods: The plant based diet (soybean meal 24.5%; wheat gluten 11.5%; corn 22.5%; wheat 32.5%), containing 30.2% crude protein and 0.26% phytate P, was supplemented with 500 (I), 750 (II), 1000 (III), 1250 (IV) FTU/kg of the experimental phytase SP-1002 CT and Ronozyme® P5000 (Roche Vitamins Ltd, Basel), respectively. A control diet (0.88% total P) was supplemented with mono-sodium-phosphate (1.5 g P/kg). The experiment was conducted in 6 sedimentation systems ($0.15m^3$ /system) with Nile Tilapia (average BW 125g) after an adaptation period of 30 days. The blood sampling took place in three repetitions at 2 - 4 - 8 - 12h after feeding, corresponding to T-2, T-4, T-8 and T-12. Totally nine fishes per treatment were anaesthetized and the caudal peduncle vein was punctured. Inorganic phosphorus was determined in the individual plasma samples using the ammonium molybdate method. Data were analyzed as one-way completely randomized design, submitted to ANOVA ($P < 0.01$ and $P < 0.05$) and Tukey's test using the SAS-program.

Results: Phosphorus concentration in the blood plasma (Figure) was significantly increased ($P < 0.01$ and $P < 0.05$) after the control diet with inorganic phosphorus addition as well as after diets with supplemental microbial phytase, but depending on the level of dietary phytase activity and the source of microbial phytase.

Figure: Inorganic P concentration in blood plasma of Nile tilapia fed two different microbial phytases



Conclusions: The course of inorganic phosphorus concentration in blood plasma of Tilapia indicates significant effects of inorganic P addition and phytase supplementation on improvement of the metabolic availability of phosphorus. The results also indicate that phytase SP-1002 CT increased plasma inorganic P concentration more quickly. This observation is in general agreement with the results of P utilization from growth studies (1) and remaining phytate P concentration in the digesta of the hepatic loop (2).

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8. Effect of different sources of microbial phytase on parameters of phytate degradation in the digestive tract of Nile Tilapia - *Oreochromis niloticus* (Einfluß unterschiedlicher Phytasequellen auf Parameter des Phytatabbaus im Verdauungstrakt von Tilapien). L. Portz* and F. Liebert - Göttingen

Excessive phosphorus (P) load from intensive fish farming needs scientific based strategies to improve P-utilization mainly of omnivorous fish feeds of plant origin. The number of studies investigating the mechanisms of phytate hydrolysis in fish species is very scarce. The main objective of this study was to evaluate the level of degradation of phytate following the incorporation of two different sources of microbial phytase in plant based diets for Nile Tilapia.

Methods: The basal diet (soybean meal 24.5%; wheat gluten 11.5%; corn 22.5%; wheat 32.5%) contained 30.2% CP, 0.15% non-phytate P and 0.26% phytate P. This diet was supplemented (prior to pelleting) with graded levels (500, 750, 1000, 1250 FTU/kg) of two different sources of microbial phytase (experimental phytase SP-1002 CT or Ronozyme® P5000, Roche Vitamins Ltd, Basel). A control diet was supplemented with 1.5 g P/kg from mono-sodium-phosphate (0.88% total P). Titaniumdioxid (0.3%) was incorporated as indigestible marker for determination of protein and phosphorus digestibility with 6 sedimentation systems (0.15m^3/system) using 10 animals per system (average BW 125g). After finishing the digestibility trial, two hours after application of a single meal, the content of the hepatic loop was collected (n=3), pH was determined and samples were frozen up to the analysis of phytate P content. Experimental data were analyzed as one-way completely randomized designs, submitted to ANOVA ($P < 0.05$ and $P < 0.01$) and Tukey's test using the SAS-program.

Results: The phytate P concentration in the contents of hepatic loop (average pH=4.0) was significantly reduced ($P < 0.01$) after application of phytase supplemented diets, indicating an important capacity for phytate degradation in this proximal part of the digestive tract of Tilapia. Consequently, after these treatments the phytate P concentration in the feces was stepwise reduced (Table) and P digestibility improved significantly. Additionally, apparent digestibility of CP tended to increase but only partly with significance.

Table: Phytate P in the digesta and apparent digestibility of protein and phosphorus in Tilapia fed plant based diets with two different sources of microbial phytase

	Phytate phosphorus content (%)[1]									
	0 [2]	SP-500	SP-750	SP-1000	SP-1250	RO-500	RO-750	RO-1000	RO-1250	NaH_2PO_4
Feces	0.50^{a}	0.33^{c}	0.26^{de}	0.25^{e}	0.25^{e}	0.39^{b}	0.29^{d}	0.26^{de}	0.27^{de}	0.49^{a}
Chyme [3]	0.17^{a}	0.12^{abc}	0.10^{bc}	0.09^{c}	0.10^{bc}	0.16^{a}	0.16^{a}	0.14^{ab}	0.14^{ab}	0.16^{a}
	Apparent digestibility (%)									
CP	91.81^{e}	93.24^{d}	95.79^{a}	95.33^{ab}	95.59^{a}	93.70^{d}	94.97^{ab}	95.36^{ab}	94.68^{bc}	93.87^{cd}
P	43.13^{f}	60.61^{d}	64.56^{cd}	70.84^{ab}	75.66^{a}	50.01^{e}	65.00^{cd}	67.41^{bc}	73.01^{a}	75.55^{a}

1) As freeze dried substance
2) Basal native phytase activity 250 FTU/kg
3) Hepatic loop

Conclusions: Results indicate that both sources of microbial phytase support an efficient phytate degradation during digestion of plant based Tilapia diets. It is also indicated that the experimental phytase SP-1002 CT expressed a more pronounced effect on phytate degradation in the proximal part of the digestive tract of Tilapia. More detailed studies are ongoing for investigation of the mechanisms of phytate degradation in the gut of Nile Tilapia and other fish species.

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9. Inevitable phosphorus losses of laying hens (Unvermeidliche Phosphorverluste von Legehennen). R. Hempel*, E. Strobel and M. Rodehutscord - Halle (Saale)

In laying hens, the experimental basis for estimating factors of phosphorus (P) requirement is weak (GfE, 1999). This study was conducted to quantify the amount of P that is inevitably lost by hens both until the terminal ileum and with excreta.

Methods: Six diets were used on the basis of a wheat by-product, dried beet pulp, and maize starch with graded levels of monobasic calcium phosphate. Analysed P concentrations (g/kg DM) were 0.6, 1.7, 3.3, 3.8, 6.0, and 7.5. Ca concentration varied between 41 and 51 g/kg DM. Other nutrients were contained according to the recommendations for laying hens. Thirty-six 32-wk old hens were individually kept in balance crates, and 6 hens were allocated to each diet. Average BW was 1.65 kg. Feed allowance was 90 g/d, as reduced intake had to be expected in response to the deficit in dietary P. Consequently, average laying performance was 72%, and average egg weight was 42 g. Excreta were quantitatively collected for 8 consecutive days after 3 d of adjustment to the respective P level. After the end of the collection period hens received the diets for another 3 d ad libitum. Then hens were killed by CO_2 asphyxiation, and ileal content was immediately collected. Digesta of 3 hens was pooled into one sample in order to achieve a sufficiently large sample. Diets contained TiO_2 as the marker. P analysis was performed with ICP-OES after repeated acid hydrolysis. P flow at the terminal ileum was calculated based on concentrations of TiO_2 and P in diets and digesta, and on measured feed intake. Results were subjected to regression analysis, and inevitable losses were calculated by extrapolation to zero P intake.

Results: Hens significantly responded in P excretion to increased P supply (Fig.). The estimated P excretion at zero P intake was 82 (SE 20) mg/d, corresponding to 50 mg/kg BW d^{-1}. For the terminal ileum, no significant response in P flow depending on P intake was detected, indicating that the net absorption of supplemented P in the small intestine was complete, irrespective of the level of supply. Estimated ileal P flow at zero P intake was 67 (SE 12) mg/d.

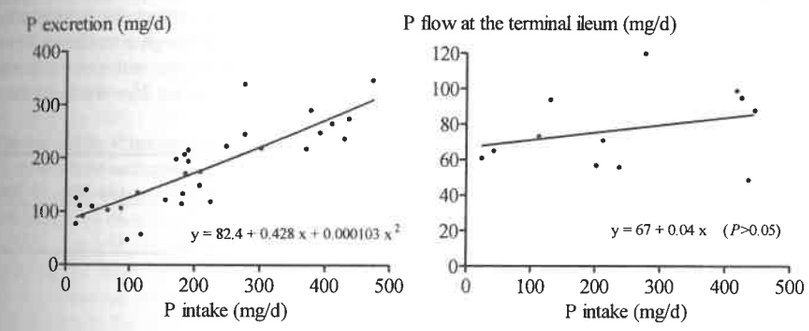

Figure: Excretion (left) and ileal flow (right) of P depending on P intake of hens

Conclusions: Current requirement estimates assume daily inevitable P losses of 83 mg/kg BW, without a solid experimental basis (GfE, 1999). The value determined herein (50 mg/kg BW) indicates that this is an overestimate. A comparison of total excretion and ileal P flow reveals that about 80% of inevitable P losses happen in the small intestine. The adaptation to increased P intake, however, seems to be relevant only post-ileal, likewise due to variation in urinary excretion.

1) GfE (1999): Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler). DLG-Verlag, Frankfurt am Main

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10. Influence of low copper and different manganese contents in milk replacers on performance of calves, concentration of these elements in the liver and Cu-retention

(Einfluss unterschiedlicher Kupfer- und Manganversorgung im Milchaustauscher von Kälbern auf die Entwicklung und die Spurenelementeinlagerung in der Leber). M. Spolders*, U. Meyer, G. Flachowsky, M. Grün – Braunschweig/Jena

In the last years there is an increasing discussion that imbalances in the supply with micro elements might promote bovine spongiform encephalopathy (BSE, BERGMANN and BERINGER 2001). In this context a lack of copper and an excess of manganese are of major concern. To contribute to this subject a feeding experiment with calves was designed to study the influence of different dietary Cu- and Mn-contents on growth performance and their accumulation in various organs and tissues. The present paper deals with trace element concentrations in the liver and Cu-retention from feed.

Methods: The feeding trial followed by slaughter investigations on day 100 and 200 was carried out in 2002 on the experimental station of the Federal Research Centre (FAL) in Braunschweig under loose housing conditions with computer based feeding and drinking units. Twenty 2 weeks old male calves of the German Holstein Breed having an average initial live weight of 50 kg were allotted to two feeding treatments. Four calves were slaughtered at the start of the experiment. The milk replacer fed to group I was formulated to have a copper content of 5.8 mg/kg DM and 55 mg Mn/kg DM. The milk replacer fed to group II had the same composition, but the copper content was extremely low with 0.95 mg/kg DM and the Mn-content extremely high amounting to 450 mg/kg DM.

Results: At start of the experiment the Cu-concentration in the liver was found to be 443 mg/kg DM. After feeding the milk replacer with the higher copper content over 100 days, the Cu-concentration increased to 1040 mg/kg DM and remained on this level until exp. day 200 (Table 1). Feeding the milk replacer with the lower Cu-content, a continuous increase of the Cu-concentration in the liver was observed as well, but the slope was not so high as compared to that of group I indicating that the storage capacity of the liver was not totally exhausted. However, a comparison of the Cu-concentrations in the livers of group I and group II demonstrates that they reflect the contents in the milk replacers. Corresponding effects were not detected for manganese, although a tendency towards a higher Mn-concentration as dependent on application time was observed, but not pronounced directly to daily intake. The mean Cu-retention in the liver was estimated to be 26 – 46 % of the Cu-intake with higher retention-rates in group II.

Table 1: Cu-and Mn-concentrations in the liver (mg/kg DM)

Day	0 (n = 4)	100 (n = 4)	200 (n = 6)
Copper			
I	443^{A} ±163	1040^{aB} ±270	1083 ±536
II	443 ±163	565^{b} ±62	675 ±225
Manganese			
I	5.9^{A} ±0.9	9.2^{B} ±1.7	8.3^{aB} ±0.2
II	5.9^{A} ±0.9	11.8^{B} ±0.8	11.3^{bB} ±1.1

a < b, A < B; p < 0.05

Table 2: Cu-retention(% of Cu-intake)

	Cu-retention in the liver		
Day	0-100	100-200	0-200
I	30.5	26.2	28.4
II	31.8	45.5	40.4

Conclusions: Although the Cu-content of milk replacers was very low, the Cu-concentration in the liver increased remarkably. The discrepancies of copper and manganese deposition in calves require further investigations to give definitive information on the transfer rate of these micro elements predominantly into the liver.

BERGMANN, W., BERINGER, H. (2001): Opinion-paper: Kupfermangel, ein möglicher BSE-auslösender Faktor? J. Plant Nutr. Soil Sci. 164, 223-235.

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11. The efficiency of different selenium compounds on the repletion of the activity of functional selenoproteins and selenium content in the liver of selenium depleted rats

(Repletionseffizienz verschiedener Selenverbindungen auf funktionelle Selenoproteine und den Selengehalt der Leber bei depletierten Ratten). Claudia Parys* and J. Pallauf - Giessen

The trace element selenium develops its biological activity as an integral part of functional selenoproteins. In human food and animal feed selenium is derived from various sources. Plants and yeasts contain selenium predominantly as selenomethionine, whereas selenocysteine is the main form of selenium in animal proteins. Inorganic forms of selenium such as selenite (SeIV) and selenate (SeVI) are used in human and animal nutrition for an efficient selenium supplementation. Due to different absorption and utilization mechanisms for selenium compounds the efficiency during selenium repletion of selenium deficient animals varies with the selenium compound used.

The study was designed to examine the efficiency of four different selenium compounds (selenate, selenite, selenomethionine and ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one)) on the restoration of the activity of the functional selenoproteins cellular glutathione peroxidase (GPx1), plasma glutathione peroxidase (GPx3) and selenium concentration in the liver in selenium depleted rats.

Methods: The repletion efficiency of selenium compounds was investigated in 180 weanling male albino rats with an initial weight of 40.8±2.8 g. The rats were selenium depleted for 10 weeks by feeding a selenium deficient diet (< 0.015 mg Se/kg) based on torula yeast. After the depletion period the animals were randomly assigned to four repletion groups of 36 animals each and received diets supplemented with 0.2 mg selenium/kg diet as sodium selenate, sodium selenite, selenomethionine or ebselen for up to 28 days. 2, 5, 8, 14, 21 and 28 days after onset of selenium repletion six animals of each dietary group were sacrificed and selenium concentration in the liver as well as the activities of GPx1 in liver and GPx3 in plasma were measured. There were also a selenium deficient negative control group (n=18) and a selenium supplemented positive control group (n=18).

Results: Starting from a GPx1 activity of 9.30±1.10 mU/mg protein in the liver of selenium depleted rats, the repletion with selenate was most effective in the restoration of GPx1 activity after 28 days (736±32.5) in comparison to selenite (686±87.0) and selenomethionine (595±79.8). On days 2, 5, 8, 14 and 21 of the resupplementation period the differences between the above mentioned selenium compounds were not yet significant. In contrast resupplementation of the rats with ebselen had no effect on liver GPx1 activity. The activity remained comparably as low as in the selenium depleted rats. After 28 days of repletion no differences between selenate (40.2±7.84 mU/mg protein), selenite (37.5±4.13) and selenomethionine (38.6±1.98) occurred with regard to the restoration of GPx3 activity (end of depletion 2.12±0.28). Comparable to GPx1 the activity of GPx3 (1.78±0.36) was also not enhanced by ebselen.

Selenium content in the liver in all resupplementation groups reached a plateau which was comparably as high as in the selenium supplemented positive control group (968±94.8 µg Se/kg liver). As observed for GPx1 and GPx3 the selenium level was not enhanced by ebselen (18.5±0.83) in comparison to the selenium deficient control animals (18.5±3.71).

Conclusions: The differences observed in the restoration of liver GPx1 can be explained by differences in the absorption mechanism and the intermediary metabolism for the selenium compounds investigated. Presumably selenium from selenate was most effective because it is absorbed unmodified and subsequently reduced predominantly in the liver to the oxidation state –II from which it can be utilized for the synthesis of functional selenoproteins. Selenite reacts with thiols prior to its absorption and is distributed to a wider variety of tissues whereas selenomethionine can be incorporated unspecifically into body proteins instead of methionine. Selenium from ebselen is apparently not available.

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12. Effects of dietary manganese concentration on growth, manganese concentration in organs and cartilage composition of piglets (Einfluss der Mangankonzentration in der Diät auf Wachstum, Mangankonzentration in Organen und Knorpelzusammensetzung von Ferkeln).

C. Dreisbach*, J. Pallauf and Erika Most - Giessen

Manganese (Mn) activates different enzymes, such as galactosyltransferase I, arginase and manganese dependent superoxide dismutase. Manganese deficiency is reflected in bone malformation, which is due to insufficient activation of cartilage galactosyltransferase I and subsequently reduced glucosaminoglycan (GAG) synthesis. The existing feeding recommendations for growing pigs differ in a wide range from 2 - 4 mg Mn/kg diet (NRC 1998) to 40 mg Mn/kg diet (INRA 1989). Commercial feeds for pigs contain frequently even noticeably more than 40 mg Mn/kg. The aim of this experiment therefore was to study the manganese requirement of fast growing piglets.

Methods: 36 weaned male 21 day old piglets (BW-hybrid x Pietrain) with about 6 kg weight were kept over a 5 day adaptation period. Subsequently they were randomized into 6 groups of 6 animals each and housed in stainless steel metabolic cages for 42 days. They were fed a semisynthetic diet containing skim milk powder, corn starch, soy bean oil, glucose, cellulose, DL-methionine, L-threonine, mineral and vitamin premix as well as manganese at concentrations of 0.3, 2, 4, 8, 16 and 32 mg/kg diet (Mn added as $MnSO_4xH_2O$). At the end of the trial, the piglets were slaughtered without scalding and blood and organ samples were collected. The manganese concentration in different tissues was measured by graphite furnace atomic absorption spectroscopy after wet ashing. Uronic acid (URA) was measuered as component of GAG in joint cartilage from the os metacarpale after papain digestion.

Results: There were no differences between the groups in zootechnical parameters. In all groups daily weight gain reached an average of 710±62 g/d at an age of 9 weeks. Feed utilisation was 1.45±0.05 kg feed/kg weight gain over the whole trial. Weight at slaughtering was 29.1 ± 0.1 kg (at 10 weeks). The haematological parameters HB, PVC, RBC, MCV, MCH, MCHC, WBC showed no significant differences between the groups. Manganese concentration in the liver was significantly reduced in groups receiving less than 8 mg Mn/kg feed. In animals receiving 8 mg Mn/kg there was still a tendency for a lower Mn concentration compared to animals receiving 16 and 32 mg Mn/kg. URA in joint cartilage was significantly reduced in groups receiving 2 and 4 mg Mn/kg.

Table 1: Concentration of manganese in diet and organs as well as uronic acid in joint cartilage

Group	Manganese [mg/kg FM]					URA [g/kg FM]
	Diet	Liver	Kidney	Lung	Heart	Joint cartilage
I	0.3	0.279 ± 0.069 [a]	0.259 ± 0.022 [a]	0.066 ± 0.014 [a]	0.060 ± 0.015 [a]	2.193 ± 0.266 [a,b]
II	2	0.769 ± 0.059 [b]	0.463 ± 0.037 [b]	0.101 ± 0.013 [a,b]	0.070 ± 0.015 [a]	1.815 ± 0.260 [a]
III	4	1.074 ± 0.158 [b]	0.748 ± 0.097 [c]	0.146 ± 0.025 [b,c]	0.114 ± 0.037 [b]	1.908 ± 0.285 [a]
IV	8	1.591 ± 0.195 [c]	0.997 ± 0.166 [d]	0.163 ± 0.044 [c]	0.165 ± 0.050 [b]	2.541 ± 0.102 [b,c]
V	16	1.937 ± 0.260 [c,d]	1.062 ± 0.069 [d]	0.190 ± 0.049 [c,d]	0.238 ± 0.020 [c]	2.881 ± 0.310 [c]
VI	32	2.028 ± 0.378 [d]	1.137 ± 0.083 [d]	0.230 ± 0.014 [d]	0.276 ± 0.045 [c]	2.600 ± 0.309 [b,c]

mean ± SD, different superscript letters indicate significant differences with P<0.05 (Tukey-HSD)

Conclusion: Under the conditions investigated growing pigs receiving less than 8 mg Mn/kg diet showed a significant decrease in manganese storage. Below 8 mg Mn/kg diet, the biochemical marker URA in joint cartilage was slightly reduced. Although the animals did not show signs of bone malformation, feeding 8 mg Mn/kg seems to be advisable to prevent bone disorders in fast growing piglets.

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13. Reduction in feed mineral content – Effects on piglet development and mineral excretion (Effekte reduzierter Mineralstoffgehalte im Ferkelfutter auf Ferkelentwicklung und Mineralstoffausscheidungen).

Mechthild Freitag*, Franca Berardi – Soest

To reduce possible soil accumulation of certain minerals by excreta from pig farming a reduction of trace element concentration in feed is discussed by EU legislation

	Accepted mineral concentration in pig feed (mg/kg feed)			
	Copper (Cu)	Zinc (Zn)	Manganese (Mn)	Iron (Fe)
to date	175/35*	250	250	1250
discussed	30/20**	100(120)***	150(100)	600(500)

* < /≥16 weeks of age, **< /≥ 2 months of age, *** () alternatives less likely

Latter concentrations include natural mineral content in feedstuffs. To achieve more information on the effects of reduced feed mineral content on piglet growth under farm conditions an experiment was carried out in a commercial piglet rearing farm.

Methods: A total of 386 piglets with an average weight of 6.9±0.7 kg were divided into 3 groups. The control group (C, n=130) received standard feed, the second group (E1, n=127) a reduced mineral content with inorganic trace minerals and the third group (E2, n=129) reduced mineral content with organic and inorganic trace elements. Amounts of added minerals were as follows

Group	Feeding	Cu (mg/kg)		Zn (mg/kg)		Mn (mg/kg)		Fe (mg/kg)	
	period	inorganic	organic	inorganic	organic	inorganic	organic	inorganic	organic
C	1	156	-	200	-	67	-	256	-
	2	168	-	200	-	100	-	105	-
	3	129	-	100	-	50	-	164	-
E1	1	15	-	50	-	60	-	150	-
	2	15	-	50	-	60	-	150	-
	3	10	-	50	-	60	-	150	-
E2	1	-	15	20	30	30	30	120	30
	2	-	15	20	30	30	30	120	30
	3	-	10	20	30	30	30	120	30

Results: Initial piglet weight did not differ between groups. During the first 4 weeks weight gain was less in group E2 (p<0.001) compared to C and E1. This difference was compensated until the end of the 8 week experiment. Serum concentration of Cu, Fe and haemoglobin did not differ at any time. However, marked differences between groups were analysed in faecal Cu and Zn excretion.

	Sample period	C (mg/kg)	E1 (mg/kg)	E2 (mg/kg)
Copper	beginning	990.5±268	1135.0±92	965.0±78
	week 5	968.8±53 [a]	128.8 ±2 [c]	184.2±19 [b]
	week 8	684.0±63 [a]	89.6 ±6 [c]	156.0±7 [b]
Zinc	beginning	1315.0±332	1455.0±134	1250.0±57
	week 5	1278.0±43 [a]	502.4 ±15 [b]	549.4±52 [b]
	week 8	1230.0±209 [a]	420.6 ±43 [b]	1382.0±60 [c]

Conclusions: Under commercial farm conditions reduction of trace element supplementation does not affect piglet growth and serum Cu, Fe and haemoglobin content. Reduction of trace elements to a level proposed by the EU does not adversely affect piglet rearing while significantly reducing mineral accumulation in soil.

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14. Influence of the Ruminal Nitrogen-Balance (RNB) on rumen fermentation, microbial protein synthesis, amount of utilisable crude protein and milk urea (Einfluss der ruminalen Stickstoffbilanz auf Pansenfermentation, mikrobielle Proteinsynthese, nXP-Menge und Milchharnstoff).

Andrea Riemeier*, P. Lebzien, G. Flachowsky - Braunschweig

The recommandation on the ruminal N-balance (RNB = [crude protein intake - utilisable crude protein] : 6.25) has to consider 2 different goals. On one hand N-shortage in the rumen has to be avoided for reasons to ensure maximal rumen fermentation. On the other hand a surplus of degradable N leads to an uneconomic protein utilisation, induce N-burdens for the animal and the environment and increase the urea content in milk. This leads to the question an the lowest quantity of N, available in the rumen, without negative effects on rumen fermentation and microbial protein synthesis.

Methods: The series of experiments involved 4 periods with 5 lactating dairy cows each. They were fitted with duodenal and ruminal cannulae. NEL and uCP supply of the animals met their requirements. The basal ration (RNB –0.6 g/MJ ME) was formulated on 50% of corn silage and 50% of concentrate mixture on dry matter basis. To establish RNB-values of –0.3, 0 and +0.3 g/MJ ME different amounts of urea were incorporated into the concentrate mixture. Samples were taken from milk, rumen fluid and duodenal digesta.

Results: The NH_3-N concentration increased 3 hours after feeding starting in the rumen fluid from 1.6 to 28.5 mg/100 ml due to higher RNB-values and differed significantly between RNB-group –0.6, 0 and +0.3. The NH_3-N concentration in group –0.6 which amounted to 1.6 mg/100 ml was found to be remarkably lower than 5.6-8.4 mg/100 ml, which were postulated to be necessary for an optimal rumen fermentation (1). However, concerning the NH_3-N content in the duodenal chyme (increased from 2.9 to 11.3 mg/100 ml) differed not significantly except betwenn group –0,3 and 0. Urea excretion with milk amounted to 13.6^a, 18.8^a, 42.0^b and 63.0^c mg/100 ml, when RNB was –0.6, -0.3, 0 or +0.3 g/MJ ME. Effects on the pH-value in the rumen due to differences in the ruminal nitrogen balance were not detected. Table 1 shows the influence of the ruminal N-balance on fermented organic matter (FOM), undegraded feed protein (UDP), the efficiency of microbial protein synthesis and the amount of utilisable crude protein (uCP).

Tabele 1. Quantity of fermented organic matter, undegraded feed protein, utilisable crude protein and efficiency of microbial protein synthesis

RNB (g/MJ ME)	**-0.6**	**-0.3**	**0**	**+0.3**
FOM (kg/d)	9.2^a	9.4^{ab}	9.9^b	9.8^{ab}
UDP (g/d)	338^a	441^{ab}	499^b	451^b
uCP (g/d)	1658^a	1792^{ab}	1953^b	1733^{ab}
microbial protein (MP) (g/d)	1320	1382	1454	1373
(g/MJ ME)	7.68	7.84	8.31	7.98
(g/kg FOM)	144	143	148	142

Different superscripts, in one line, indicate sign. differences ($p<0.05$)

Conclusion: A negative RNB of –0.6 g/MJ ME resulted in a significantly reduced amount of FOM compared to RNB = 0. Due to the reduced MP and UDP the quantity of utilisable crude protein (uCP) in the RNB-group –0.6, was almost 170 g lower as compared to the average of the other three groups. Negative RNB of –0.3 g/MJ ME shoes no negative effect on uCP. But the positive RNB of +0.3 shows a negativ trend on uCP (p-value = 0.06) compared to RNB 0.

1) PIATKOWSKI, B., GÜRTLER, H., VOIGT, J. (1990): Gustav Fischer Verlag Jena

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15. Standardization of the gut segment to be sampled in studies on praecaecal amino acid digestibility in broiler chickens (Standardisierung des Darmabschnittes zur Probennahme in Untersuchungen zur praecaecalen Verdaulichkeit von Aminosäuren bei Broilern).

H. Kluth* and M. Rodehutscord - Halle (Saale)

In studies on the praecaecal amino acid (aa) digestibility in chickens it became common practise to sample digesta from the terminal small intestine beginning at Meckel's diverticulum (Md), presuming no protein digestion behind Md. It was the aim of this study to investigate whether aa digestion is completed already up to Md. Therefore, the net disappearance rate (NDR) of aa was studied in different sections of the terminal small intestine of chickens that were fed two different dietary proteins.

Methods: Two isonitrogenous diets (27% crude protein) with protein mainly originating from solvent extracted soybean meal (SES) or a 1:1 mix of SES and peas were used. Peas were included at the expense of maize starch. The other ingredients (maize, wheat gluten, premixes) were unchanged. Diets contained TiO_2 as the indigestible marker, and they were pelleted through a 3-mm die. Male broiler chickens were raised in floor pens with a commercial starter feed until d 14 of age. Body weight was 294 g (SEM 3 g) on d 14. From d 14 to 21, the experimental diets were offered ad libitum. Eight pens of 12 birds each were allocated to each diet, and the experimental unit was the pen (n=8). On d 21, birds were killed by CO_2 asphyxiation. The body cavity was immediately opened, and the section between Md and 2 cm prior the ileo-caecal-colonic junction was isolated. This section was then separated into three parts of equal lengths (proximal, middle, terminal). The content was gently flushed with deionised water and pooled on a pen-basis. Samples were immediately frozen and subsequently freeze-dried to await analysis of crude protein, aa and TiO_2.

Results: Birds gained, on average, 43 g BW/d during the experimental period. The amount of freeze-dried digesta was not significantly different between the three sections. Two-way ANOVA showed no interaction between diet and gut section, and a significant diet effect on NDR was detected only for methionine (see table). While no significant differences were detected in aa NDR between the middle and the terminal section, NDR were significantly lower in the proximal section. Differences were 3 percent units for crude protein, and were between 3 and 6 percentage units for individual aa.

Table: Net disappearance rate of amino acids in the three sampled gut sections (%)

	Section			ANOVA			
	proximal	middle	terminal	SEM	Diet	Section	Diet×Section
Crude protein	83^a	86^b	86^b	0.8	0.773	0.002	0.671
Lysine	82^a	85^b	85^b	1.0	0.402	0.020	0.631
Methionine	80^a	86^b	85^b	1.4	0.007	<0.001	0.244
Cystine	70^a	74^{ab}	76^b	1.4	0.610	0.011	0.722
Threonine	75^a	80^b	80^b	1.1	0.901	0.001	0.493
Arginine	86^a	89^b	89^b	0.8	0.216	0.002	0.712
Phenylalanine	84^a	87^b	88^b	0.8	0.859	<0.001	0.697
Isoleucine	81^a	84^b	85^b	0.8	0.576	0.002	0.574
Leucine	82^a	86^b	86^b	0.9	0.803	<0.001	0.619
Valine	78^a	83^b	82^b	1.0	0.783	0.003	0.612

Conclusions: Absorption of aa is not yet completed at Md. When aa digestibility is to be determined to describe the capacity of a feedstuff, this effect must be considered. Therefore, as part of a standardized method, the gut section under study should comprise only the last two thirds of the part between Md and 2 cm prior the ileo-colonic-caecal junction.

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16. Marginal efficiency of methionine utilisation in broiler chicks depending on the supply of methionine and non essential amino acid nitrogen (Grenzverwertung von Methionin bei Broilern in Abhängigkeit von der Zufuhr an Methionin und nicht essentiellen Aminosäuren).

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We examined the effect of dietary crude protein (CP) concentration on the efficiency of utilisation of increasing limiting methionine intake and the magnitude to which increased CP concentration may elevate the requirement for dietary methionine (Met) in growing male broiler chicks.

Methods: Two basal diets mainly based on maize, peas, field beans, wheat gluten and free amino acids were used. They were calculated to be equal in their essential amino acids and Met contents but different in their CP content: 209 (L) and 253 (H) g/kg. This was achieved by different inclusions of non-essential amino acids. The analysed basal levels of Met (Cys) were 1.7 (2.5) (L) and 1.8 (2.5) (H) g/kg. Both basal diets were supplemented with the same incremental doses of DL-Met in 7 further isonitrogenous diets (see figure). Ross broilers were allotted in groups of 10 per pen to each dietary treatment, and 3 pens were used per treatment. Diets were offered ad libitum from d 8 to 21 posthatch. All birds were killed at the end of the experiment, and representative birds were killed on d 8. Accretions of protein and amino acids were determined in fat free body homogenates by comparative whole body analysis. A four-parameter sigmoidal function was fitted to the data. Marginal efficiency was calculated as the first derivative of the function of Met accretion depending on Met intake.

Results: Non-linear responses were detected for growth and protein accretion (see Fig.). The y_{max} in protein accretion were 87 (L) and 82 (H) g/bird, and to achieve 95% of this y_{max}, 3.3 and 3.5 g/kg Met were required. Dietary Met concentrations needed to achieve 95% of y_{max} in other growth response criteria were also higher for H compared to L. Marginal efficiency of utilisation of incremental Met achieved a maximum of 102 (L) and 95% (H). With further increase in Met intake, efficiency of utilisation was lower in H than in L (see Fig.).

Conclusions: The response to incremental Met intake is non-linear, and the efficiency of utilisation is not constant at low level of supply. Factors other than Met may further limit protein accretion with increased dietary CP content. The oxidation of Met may be elevated with increased CP intake as indicated by the differences in marginal efficiency.

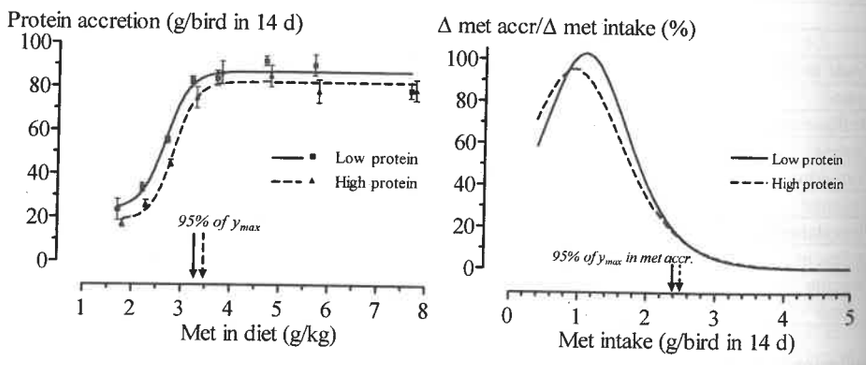

Figure: Protein accretion and marginal efficiency of methionine utilisation (average and SEM, n=3; arrows indicate the x-value to achieve 95% of y_{max} in protein and methionine accretion)

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17. Influence of the dietary tryptophan concentration on performance of lactating sows and the growth of their piglets (Zum Einfluss der Tryptophanversorgung auf die Laktationsleistung von Sauen und das Wachstum ihrer Ferkel).

F.G. Pampuch*, Brigitte R. Paulicks and Dora A. Roth-Maier – Freising-Weihenstephan

In the present study the effects of increasing tryptophan (Trp) concentrations in the feed of lactating sows on feed intake, milk yield and litter performance were determined.

Methods: The data of 72 lactations, 28 days long, of a total of 61 multiparous sows (German Landrace) were used. After a gestational feeding starting at day 60 of gravidity with 2.6 kg/d (1.2 g Trp/kg feed), the sows were continously assigned to one of the six treatments (I-VI) on day 110 of gestation. The treatments differed only in the Trp concentration of the lactation feed (g Trp/kg feed: 1.2, 1.5, 1.8, 2.4, 3.0, 4.2, resp.). The nutrient values of the feeds were 156 g CP, 10.6 g Lys and 13.2 MJ ME per kg according to the recommendations and covered the nutrient requirements of lactating sows. L-tryptophan was supplemented to the basic feed mixture (I, native content of 1.2 g Trp/kg corresponding to 0.8 g apparent ileal digestible Trp/kg) for the graded levels of Trp concentrations. Litter size was standardized to 10-12 piglets. Sows' feed intake, weight loss during lactation, milk yield (weigh-suckle-weigh method), milk composition (fat, protein, lactose, energy, dry matter), and growth performance of the piglets were determined.

Results: Dietary Trp contents of up to 2.4 g/kg increased feed intake of the sows during lactation significantly by about 40 % to 5.53 kg/d compared with 3.25 kg/d with only 1.2 g Trp/kg. Further dietary Trp supplementations did not affect feed intake. Weight loss of the sows during lactation, milk yield, and piglet weaning weight reacted correspondingly. Concentrations of milk fat (7.6 %), protein (4.7 %), lactose (5.8 %), energy (5.0), as well as dry matter (19.2 %) were not affected by dietary Trp. Due to the lower milk supply the piglets grew slower and even when they were offered an additional starter feed in the 4th lactation week they could not compensate this delayed growth performance up to weaning with 28 days.

Treatment	I	II	III	IV	V	VI
Trp concentration (g/kg feed)	1.2	1.5	1.8	2.4	3.0	4.2
Feed intake (kg/d)	3.25^{c}	4.39^{b}	4.39^{b}	5.53^{a}	4.66^{b}	4.34^{b}
Weight loss during lactation (kg)	30.8^{a}	16.9^{ab}	19.2^{ab}	9.5^{b}	14.1^{ab}	13.1^{ab}
Milk yield (g/d)	6840^{b}	8192^{ab}	8362^{ab}	9035^{a}	8618^{ab}	8140^{ab}
Piglet weaning weight (kg)	5.85	6.60	6.70	6.71	6.66	6.46

a, b, c significantly different means (Student-Newman-Keuls test; P<0.05)

Conclusion: As a result of the present investigation a Trp concentration of 2.4 g/kg feed is required for lactating sows corresponding to 2.0 g apparent ileal digestible Trp per kg feed mixture to avoid impairments of feed intake and lactation performance. Including metabolic parameters (1) and a persistant high performance a supply of 3.0 g Trp/kg (2.6 g apparent ileal digestible Trp/kg) is recommended.

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18. Bioavailability of lysine in shrimp meal determined by the slope-ratio assay in pigs

(Bioverfügbarkeit von Lysin aus Shrimpmehl ermittelt mit dem Slope-Ratio-Assay beim Schwein). A. O. Fanimo and A. Susenbeth* - Abeokuta/Kiel

The slope-ratio assay is usually considered as the ultimate standard against which other methods are judged, because it measures a response (growth, protein retention) that is of great practical importance, and it indicates the net effect of all components that can affect bioavailability i.e. digestion, absorption and utilization (1). This study was conducted to determine the bioavailabilty of lysine of shrimp meal (SM) by the slope-ratio assay in pigs, using nitrogen retention as the response criterion.

Methods: Twelve crossbred barrows with an average initial live-weight of 35 ± 3 kg were used. The experimental set-up was an incomplete block design with four diets and two periods of 14 days each. In each period, 7 days were for adaptation to diets and 7 days for faeces and urine collection. A basal diet consisted of (g kg^{-1}) 440 barley, 440 wheat, 55 soybean meal, 25 oil, and 40 mineral/vitamin premix. 1.12 kg of this diet was offered to all animals daily to provide 5.5 g lysine/d . Sun-dried SM (mainly head, exoskeleton and appendages) was ground to pass through a 3 mm screen (coarse SM) or a 0.5 mm screen (fine SM). The basal diet was supplemented with either casein (reference), coarse SM or fine SM to supply additional 4 g lysine/d, respectively. The effects of lysine supplied by adding casein and SM to the basal diet on N retention were determined. The ratio of the slope of the coarse SM or the fine SM diets to the slope of the casein diet was used as the estimate of lysine availability assuming that lysine from casein is 100 percent available.

Results: Chemical analysis showed SM to contain (g/kg DM) crude protein, 215; lysine, 8.0; methionine, 3.6; crude fibre, 200; ash, 560; ADF, 234; NDF, 284 and chitin, 155. Taking the availability of lysine in casein to be 100 percent, the availability of lysine in coarse SM and fine SM were 34 % and 38 %, respectively, lower (Table 1). The reduction in lysine availability of 4 % in the fine SM compared to the coarse SM was not significant. High fibre, ash and chitin may have caused the relatively low availability of lysine in SM since apparent digestibility of amino acid is known to be adversely affected by the presence of fibre and chitin (2). It is open to question, whether these components cause low intestinal availability of lysine or increase endogenous protein losses.

Table 1: Bioavailability of lysine in shrimp meals determined by the slope-ratio assay.

Lysine source	slope[1]	SE (slope)	Lysine availability[2] (%)	Comparison with the reference slope
Casein (reference)	1.71	0.13	100	-
Shrimp meal, coarse	1.13	0.18	66	P< 0.0017
Shrimp meal, fine	1.07	0.18	62	P< 0.0016

[1] (N retention for the test diet – N retention for the basal diet)/(Lysine intake of the test diet – Lysine intake of the basal diet); [2] Ratio of the respective slope to the reference slope.

Conclusion: Shrimp meals with low protein content show only moderate lysine availability compared to other protein sources of animal and plant origin, which is not improved by grinding.

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19. Long-term effects of a high dietary protein intake on protein metabolism in [^{15}N]-labeled rats as animal model

(Untersuchungen zum langfristigen Einfluss einer hohen Proteinzufuhr auf den Proteinstoffwechsel am Tiermodell ^{15}N-markierter Ratten). A. Vikari*, W. Windisch and F. X. Roth – Freising-Weihenstephan/Wien

Levels of protein intakes in Western developed countries are increasing and high protein intakes are often advocated for maintaining beneficial health effects according to public opinion. However, experimental studies on the influence of a high dietary protein intake on protein metabolism are mostly performed over short-term periods and therefore effects of a longer-term or chronic intake are difficult to judge (1). For this reason the objective of this study was to assess the dynamics of protein metabolism by the use of [^{15}N] as tracer in specific organs, tissue fractions and the whole-body of adult rats ingesting a long-term high protein diet.

Methods: The experiment consisted of two periods. During the first period 32 juvenile female Sprague-Dawley rats were reared from 48 to 179 g body mass with a [^{15}N]-labeled semisynthetic diet for 7 weeks. In order to trace all amino acids within the whole-body protein pool homogenously, the dietary protein source (casein) was supplemented with a mixture of ^{15}N-amino acids proportionally to the casein-specific AA ratio (in total: 1.01 atom% isotope enrichment). Subsequently, the traced rats were divided in two groups (n=14) and received a tracer free diet of 8.0 g daily (restrictive) with either 10% casein (adequate protein, AP-10 group) or 30% casein (high protein, HP-30 group) for 7 weeks. Faeces and urinary excretions were collected quantitatively. The animals were killed under anaesthesia on day 0, 2, 7, 13, 20, 27, 34, 41 and dissected quantitatively in single organs, plasma, muscle, skin, hairs and skeleton. Samples were then analyzed for total N and [^{15}N] by GC-C-IRMS.

Results: At the end of the labeling period (day 0) [^{15}N]-enrichment reached 0.91 atom% ^{15}N (AP) in the whole-body (91% of dietary isotopic enrichment) with only minor variations among organs and tissues. After day 0, absolute whole-body N mass increased slightly (0.7% per day) without being affected by the level of protein intake. Tissue N content was fairly well maintained. Over 7 weeks of treatment, [^{15}N]-elimination from the whole-body was more pronounced in rats ingesting the HP-30 meal than in those given the AP-10 meal (Table 1). This accounted for 69.2% (AP-10), respectively 59.4% (HP-30) of the initial enrichment (day 0) at the end of the study ($p < 0.05$). Among all organs and tissues, [^{15}N]-signature decreased most markedly in plasma, digestive tract and liver followed by kidney, spleen, lung, skeleton, skin, brain and finally heart and skeletal muscle.

Tab.1: Whole-body [^{15}N]-elimination in % of initial tracer enrichment (day 0)

day	0	2	7	13	20	27	34	41
AP-10 [%]	100±2.1	99.9±0.7^{a}	89.8±2.0^{a}	84.1±1.3^{a}	82.9±1.9^{a}	78.7±0.0^{a}	76.4±1.0^{a}	69.2±1.0^{a}
HP-30 [%]	100±2.1	98.0±1.0^{a}	84.5±1.9^{a}	77.9±0.4^{b}	72.8±3.8^{a}	68.7±1.9^{b}	62.7±0.4^{b}	59.4±3.0^{b}

Values are MEANS ± SD,^{a,b}: P < 0.05, HP-30 group vs. AP-10 group within experimental day.

Conclusion: Increasing the dietary protein intake from adequate to high level did not affect absolute whole-body N and tissue N content over a period of 7 weeks. However, the extent of body N exchange by dietary N was elevated in single organs, tissues and the whole-body in rats ingesting a long-term high protein diet compared to an adequate protein intake. We suppose that this phenomenon indicates a lower degree of recycling of amino acids along with the synthesis and degradation of body protein and possibly with a higher rate of whole-body protein turnover itself.

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20. Influence of variety and location on the crude protein content and amino acid composition of grain legumes (Einfluss von Sorte und Standort auf den Rohproteingehalt und die Aminosäurenzusammensetzung von Körnerleguminosen)

Annette Simon* and W. Köhn - Berlin

The aim of the analyses was to compare the crude nutrient content and amino acid composition of the different species of legume grains depending on location and variety.

Material and Methods: The analysed samples derived from four different locations of the experimental site of the Institute of Plant Science, Humboldt-University Berlin. These grain legumes were cultivated in 2001 in the course of an experiment dealing with the suitability of different cultivars of legume grains on light soils. Additional samples of blue lupins from three locations were provided by Saatgut Steinach GmbH. The content of crude nutrients and amino acids was analysed for peas (n= 14), field beans (n = 13), yellow lupins (n = 17) and for blue lupins (n = 13). The analyses of crude nutrients followed the Weende analyses and additional fibre analysis according to Van Soest.

Results: The mean crude protein content of all samples of peas, field beans, yellow lupins and blue lupins amounted to 22.9 %, 29.3 %, 44.5 % and 30.8 %, respectively. The differences in the crude protein content of all samples of peas were small and ranged between 22 and 24 %. The influence of location (cultivation sites Berge, Blumberg and Dahlem) on the crude protein content of varieties *Eiffel* and *Swing* (peas) was also marginal. The differences amounted to only 1.9 and 1.1 %, respectively. The additional irrigation at the Berge location caused an increased yield per hectare in all investigated species and varieties, which was accompanied by a remarkable decrease in crude protein content. This decrease was more pronounced in the variety *Eiffel* than in *Swing* (a decrease of crude protein of 3.1 and 1.9 %, respectively).

Among field beans the *Scirocco* variety contained more crude protein than the *Music* variety. In the mean of three locations the crude protein content of these varieties amounted to 30.2 % and 28.1 %, respectively. However, after additional irrigation the decrease in the crude protein content of the variety *Scirocco* was more pronounced then in *Music* (1.7 % and 0.4 %, respectively).

The mean crude protein content of the varieties *Borsaja* and *Juno* (yellow lupins) amounted to 45.2 % and was not different at the four investigated locations. The better quality of soil at the location Berge is considered to be the reason for the 5 % increase of crude protein content at this location. After irrigation an especially noticeable decrease in crude protein content of 4 % was observed for the variety *Juno*. In the same way as observed in field beans the decrease in crude protein content of yellow lupins was connected with an increase in crude fibre content.

Related to the amount of amino acids per 16 g N, the analysed samples showed an expected low content in sulphur-containing amino acids. The content ranged from 1.62 g/16 g N for field beans to 2.18 g/16 g N for yellow lupins. The methionine content was particularly low in yellow and blue lupins (0.6 g/16 g N) and relatively high in peas (0.84 g/16 g N).

Between the crude protein content of grain legumes and the content of essential amino acids, negative correlations were observed, which partly showed a high significance. For peas this was true for methionine and cysteine, in field beans for lysine and threonine, in yellow lupine for threonine and leucine and in blue lupins for methionine. For example, the decrease of methionine and cysteine in peas amounted to 4 and 9 % of the mean content of the amino acid, respectively.

Conclusions: Variety, as well as location have had an influence on the crude nutrient content of grain legumes. Better soil quality especially improved the crude protein content of yellow lupins. Additional irrigation decreased the protein content of grain legumes by 1 to 4 %. This was most pronounced in peas (cv. *Eiffel*) and yellow lupins (cv. *Juno*). Futhermore, it can be expected that in blue lupins and peas, an increased crude protein content is connected with a decrease in protein quality, because of diminishing amounts of sulphur-containing amino acids.

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21. Plasma Amino Acid Levels in Horses under the Influence of Digestion and Exercise (Untersuchung der Plasmaaminosäuren bei Pferden unter Berücksichtigung postabsorptiver und leistungsbedingter Einflüsse).

Sigrun Hackl*, J. Zentek, R. v.d. Hoven, J. Spona, Michaela Zickl - Wien

Only few publications can be found giving reference values for amino acid levels in blood plasma of horses. The aim of this study was to contribute to the determination of normal values for blood concentrations of 20 different amino acids in horses under standardized conditions. The study was designed in two parts, one focussing on postabsorptive changes and the other investigating the influence of exercise on the amino acid levels in blood plasma.

Methods: Postabsorptive effects were investigated in blood samples taken repeatedly of a group of 10 yearlings. Fasting levels were determined in the morning, additional samples were taken at two hour intervals up to eight hours after the meal. To evaluate influences of exercise blood samples were taken from 37 standardbred trotters immediately before and after standardized training. Blood plasma was analysed for leucine, isoleucine, valine, arginine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, glutamine, glutamic acid, asparagine, aspartic acid, citrulline, glycine, tyrosine, serine, taurine and ornithine using HPLC technique (OPA derivatization). Additional parameters (creatine kinase, alanine aminotransferase, lactate, 3-methylhistidine) were included for characterization of muscle metabolism during exercise. Statistics was performed according to the distribution of data (ANOVA or non parametric tests).

Results: Plasma amino acids increased significantly after feeding with some showing higher increases than others. Between two and four hours after feeding largest differences between amino acids were seen with some decreasing rapidly (arginine, lysine, leucine, isoleucine and threonine), others slowly and some staying at the elevated level (ornithine, aspartic acid, glutamic acid, serine, citrulline and glutamine). At six and at eight hours after the meal most amino acids showed a slow decrease back to near baseline levels. Only glycine increased again between six and eight hours after the meal. A significant increase was seen after exercise for ornithine, lysine, aspartic acid, glutamic acid, threonine, tyrosine, valine, methionine, phenylalanine, leucine, isoleucine and taurine. Glutamine, asparagine, serine, histidine and tryptophan levels decreased. Glycine, citrulline, arginine and 3-methylhistidine showed no significant changes. Lactate, CK and AST increased significantly but with a high variation between individuals.

Conclusions: The increase of amino acids after the meal was to be expected but varied considerably. For example glutamic acid showed a peak increase of 16%, whereas lysine showed the highest peak increase of all amino acid (275%). These differences might be due to faster digestion or absorption of some amino acids compared to others. Russel et al. (1986) described peaks of plasma methionine at 4 hours after the meal when horses where fed twice daily. However, our results show peak levels after two hours and decreasing levels after four hours. The changes of amino acid plasma levels after exercise can not be fully explained. Increases of lactate, CK and AST hinted at changes in muscle metabolism during exercise, but so far it is unknown how far that also influences amino acids. Studies on muscle metabolism in humans usually explain increased levels of amino acids as a result of protein breakdown in muscle (Graham et al. 1991). But in our study, 3-methyl-histidine, a marker of muscle breakdown, showed no changes due to exercise.

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22. Influence of dietary protein quality and quantity on urine composition of cats (Untersuchungen zum Einfluss der Proteinqualität und -menge im Futter auf die Harnzusammensetzung bei Katzen). J. Zentek, Annette Schulz –Hannover/Wien

Urolithiasis is still an important and worldwide seen problem in cats. In the present investigation the influence of varying dietary protein quality and quantity was investigated with regards to the urine composition and the urinary excretion of nitrogen and related metabolites, oxalate and crystalluria in cats.

Methods: Six diets with low or high concentrations of different protein sources (greaves meal, soybean protein isolate or horse meat) were sequentially fed to seven adult cats (table 1).

Table 1: Ingredients of the experimental diets (%)

Diet	Protein source		Rice	Animal Fat	Cellulose	Porcine liver	Vit./Min. supplement
G I	Greaves	85	9	-	3	-	3
G II		25	50	19	3	-	3
S I	Soy protein isolate	70	9	10	3	5	3
S II		25	45	19	3	5	3
H I	Horse meat	95.5	2.8	-	0.9	-	0.9
H II		55	30	11.3	1.9	-	1.8

Each diet was fed for at least 14 days before urine samples were collected. The urinary concentrations and excretion of nitrogen, urea, ammonia, creatinine, protein, oxalate and additionally the pH, specific gravity and urinary sediment were investigated. Statistics: Two factorial ANOVA (factors protein quality and quantity) and a paired t-test was used for the comparison of the feeding periods. Probability values of less than 0.05 were taken as significant.

Results: The urine volume was determined mainly by total water intake. The specific gravity of urine was dependent on water and protein intake and the resulting urinary concentration of urea. The renal excretion of nitrogen, urea, creatinine and protein was affected by the dietary protein concentration and the protein source (table 2). The nitrogen intake was inversely correlated to the renal excretion and the urinary concentration of oxalate. After sediment analysis, calcium oxalate dihydrate crystals were observed only as an exception in one cat, while struvite crystals were found regularly and in higher numbers with some relation to the intake of magnesium and the urinary pH

Table 2: Urinary excretion of N-metabolites and oxalic acid (mg/kg BW.d)

Diet	nitrogen	urea	ammonia	creatinine	protein	oxalic acid
GI	$1065^{a}\pm351.61$	$2870^{a}\pm916$	$29.46^{a}\pm6.71$	$50.65^{a}\pm12.0$	$4.93^{a}\pm1.36$	$2.83^{a}\pm0.89$
GII	$440^{b}\pm149.15$	$1301^{bc}\pm417$	$15.30^{b}\pm3.76$	$38.77^{b}\pm7.89$	$2.38^{b}\pm0.93$	$13.70^{b}\pm4.32$
SI	$960^{a}\pm282.62$	$2557^{a}\pm960$	$21.60^{c}\pm7.10$	$33.21^{c}\pm3.72$	$5.02^{a}\pm2.14$	$1.17^{c}\pm0.53$
SII	$553^{c}\pm120.66$	$1488^{b}\pm365$	$14.55^{b}\pm4.21$	$28.54^{d}\pm2.55$	$4.18^{ac}\pm1.12$	$4.79^{d}\pm2.73$
HI	$1426^{d}\pm209.53$	$3672^{d}\pm556$	$55.15^{d}\pm10.6$	$39.09^{b}\pm3.25$	$4.64^{a}\pm1.27$	$0.94^{c}\pm0.29$
HII	$399^{b}\pm58.48$	$1086^{c}\pm240$	$14.15^{b}\pm2.82$	$36.60^{b}\pm4.90$	$3.46^{c}\pm0.86$	$3.62^{d}\pm2.44$

Conclusion: Dietary protein intake should be considered in the diet formulation as important factor with regards to urine composition and the related risk for feline lower urinary tract disease.

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23. Effect of the ratio ammonia to short chain fatty acids in the rumen on microbial protein synthesis in lactating dairy cows (Einfluss des Verhältnisses von Ammoniak zu kurzkettigen Fettsäuren im Pansen auf die mikrobielle Proteinsynthese bei Milchkühen). T. Kaswari* and P. Lebzien – Braunschweig

Microbial protein (MP) synthesis mainly depends on carbohydrates and nitrogen availability in the rumen. It has been reported that relatively stable rumen ammonia and energy in the rumen through the day offer favourable conditions for MP synthesis. Few experiments correlated rumen ammonia and fatty acids concentration in the rumen with MP synthesis. The objective of the present experiments was to prove if variations of the ratio of ammonia (NH_3-N) and short chain fatty acids (SCFA) affect MP synthesis in the rumen.

Methods: Eight lactating dairy cows fitted with rumen and duodenal cannulas, were used in each of two experiments (exp.). Diets containing (DM basis) 7 kg corn silage, 1.3 kg grass hay, 4.2 kg wheat and 2.5 kg pea (exp.1) ; 10.3 kg grass silage, 5.2 kg corn and 1.5 kg soybean meal (exp.2) were given at four feeding times. Roughages in both exp. were offered in equal parts twice daily. Concentrates were offered in 4 hours interval according to the following design : (A) Equal amounts of protein and energy sources were offered at each feeding time; (B) 1st and 3rd feeding time were offered only the energy sources (wheat or corn), 2nd and 4th feeding time were offered only the protein sources (pea or soybean meal); (C) Separate feeding of energy and protein sources as in "B", but in different sequence. Cr_2O_3 was used as a marker for duodenal flow and MP in the duodenal fluid was estimated by NIRS (Lebzien and Paul 1997).

Results: The following table shows the effect of different feeding methods on rumen ammonia and SCFA, ratio ammonia to SCFA, MP synthesis, and efficiency of MP synthesis. Feeding sequence altered rumen ammonia and SCFA concentrations. In exp.1, the range of SCFA and NH_3-N concentrations in the rumen differed much more between sequences during 480 min. after the first feeding than in the exp 2. In both experiments, feeding energy first (sequence B) resulted in less variation of the ratio ammonia and SCFA in the rumen. In addition, MP synthesis and efficiency of MP synthesis were also higher on sequence B. However, differences in either MP synthesis or efficiency of MP synthesis for both experiments were not significant.

	exp. 1			exp. 2		
Sequence	A (n=4)	B (n=4)	C (n=4)	A (n=4)	B (n=4)	C (n=4)
Rumen Parameters						
- SCFA (mmol/l)	69 - 122	81 - 119	81 - 149	89 - 111	87 – 115	87 - 114
- NH_3-N(mmol/l)	1.2 – 15.0	1.9 – 16.8	1.0 – 16.8	3.5 – 16.9	3.3 – 13.9	**3.4 – 18.7**
Ratio NH_3-N to SCFA (mol/l : mol/l)						
Mean	0.08	0.05	0.06	0.10	0.08	0.10
min - max	0.01-0.18	0.02-0.09	0.01-0.16	0.04-0.18	0.03-0.15	0.04-0.17
Microbial Protein						
- g/ day	1515	1672	1576	1724	2036	1776
- g/ MJ ME	9.0	10.2	9.5	8.9	10.6	9.2

Conclusion: This experiments demonstrate that less variation of the ratio rumen ammonia to fatty acids leads to more effective nitrogen utilization resulting higher MP synthesis in the rumen.

LEBZIEN,P. and PAUL,C. (1997): Anim. Feed Sci. Technol., 68: 225-233.

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24. Effect of varying ruminal N-balance on feed intake and milk yield of dairy cows (Einfluss unterschiedlicher ruminaler Stickstoffbilanz bei Milchkühen auf Futteraufnahme und Milchleistung).

V. Kriete*, U. Meyer, P. Lebzien and F. Liebert - Braunschweig/Göttingen

Excessive supply of degradable N results in an inefficient N utilisation. This fact raises the question which is the lowest quantity available N in the rumen without negative effects on dry matter intake, milk yield and milk composition. A feeding experiment was designed to study the effect of two levels of ruminal N balances (RNB) on these parameters.

Methods: The experiment comprising 30 dairy cows lasted 121 days. The animals were divided into 2 groups (RNB min; RNB plus) according to the stage of lactation and milk yield. The experiment started on day 155±33 of lactation. The ration was based on corn silage and concentrates (TMR) in equal portions on dry matter base and offered ad libitum. The composition of the concentrate (main components: soybean meal, barley, wheat, corn and dried sugar beet pulp) for both groups was the same except of urea supplements for group RNB plus. The energy and nutrient supply was scheduled to meet the recommendations of GfE (2001). Feed intake and milk yield were individually registered daily and milk composition was analysed twice a week.

Results: The supply of energy and uCP (utilizable Crude Protein - nXP) was sufficient to meet the requirements during the whole experiment. The mean energy concentration was 7.5 MJ NEL/kg DM and the mean uCP concentration 153 g/kg DM and was found to be similar for both rations. The CP contents differed amounting 103 g/kg DM in the ration of group RNB minus versus 167 g/kg DM for group RNB plus resulting in a RNB of –8 and +2 g/kg DM or –0.65 and +0.18 g/MJ ME, respectively. Feed intake, milk yield and milk composition of the 2 experimental groups are summarized in Table 1. With exception of milk fat content and daily milk fat yield, all parameters showed significant differences. The daily DM intake of group RNB plus was found to be 2 kg higher and daily milk yield increased by 3 kg. Daily milk protein yield was also 105 g higher and the corresponding milk urea concentration was 2.5 times higher.

Table 1: Feed intake, milk yield and milk composition

Group		Feed intake	Milk yield	Milk fat		Milk protein		Milk urea content
	n	(kg DM/d)	(kg/d)	(%)	(g/d)	(%)	(g/d)	(mg/100ml)
RNB min	16	16.4^{A} ± 2.1	21.6^{A} ± 3.3	3.49 ± 0.7	746 ± 143	3.22^{A} ± 0.3	693^{A} ± 100	11.6^{A} ± 1.9
RNB plus	14	18.5^{B} ± 2.2	24.7^{B} ± 4.1	3.20 ± 0.6	782 ± 157	3.50^{B} ± 0.3	859^{B} ± 116	29.1^{B} ± 4.1

$^{A, B}$ indicate significant differences within a column ($p < 0.05$, t-test)

Conclusions: Feed intake and milk yield were significantly decreased when RNB was reduced from +0,18 to – 0.65 g/MJ ME. According to RIEMEIER et al. (2003) this is caused by an insufficient N-supply of rumen microbes when RNB was lower than –0.6 g/MJ ME, but not at –0.3g/MJ ME.

GFE (2001): Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlags GmbH, Frankfurt am Main.
RIEMEIER, A., LEBZIEN, P., FLACHOWSKY, G. (2003): EAAP publication No. 109 (eds SOUFFRANT, W.B., METGES, C.C.) Wageningen Academic Publishers, 645-647.

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25. Lysine requirement in adult miniature pigs assessed by an indicator amino acid oxidation method (Lysinbedarf adulter Miniaturschweine bestimmt mittels Indikator Aminosäuren Oxidation).

U. Hennig*, Gunda Backes, K. J. Petzke, Angelika Elsner, P. Junghans, M. Derno, G. Nürnberg, Cornelia C. Metges – Dummerstorf/Bergholz-Rehbrücke

Minipigs are increasingly used as model animals in nutrition research but no information is available concerning their requirements of amino acids (AA). The indicator amino acid oxidation (IAAO) technique which is based on the concept that an imbalanced AA supply caused by the deficiency of an indispensable AA results in the oxidation of any surplus of the other indispensable AA is a tracer approach to estimate the AA requirement. The aim of this investigation was to assess the lysine requirement using ^{13}C-leucine and to calculate the required dietary concentrations of Met+Cys, Thr, and Trp for adult minipigs based on the Lys requirement estimate and AA relations in maintenance.

Methods: Five pigs per group with a mean BW of 23 kg were fed 40 g DM per $kg^{0.75}$ BW over 10 days. Two diets based on wheat gluten as the only protein source were prepared. Diet 1 and 2 contained 2.7 and 6.6 g Lys per kg DM, respectively (diet 2 supplemented with 5 g L-Lysine*HCl). Infusion studies were performed with ^{13}C-leucine and ^{13}C-sodium bicarbonate. After an overnight fast and withdrawal of baseline samples of breath and blood infusions were started and continued for 10 h (4 h without and 6 h with feed). Samples were drawn during the fasted and fed state. Daily Leu oxidation rates were calculated based on plasma α-^{13}C-ketoisocaproic acid enrichment and ^{13}C in breath corrected by the $^{13}CO_2$ recovery factor. Methods are described in detail elsewhere (Backes et al. (2002)). The Lys requirement was estimated by plotting the lysine intakes against the Leu balance (= subtracting the amounts oxidized from the intake). The Lys requirement is met when the Leu balance is neutral (intersection point on the x-axis).

Results: $^{13}CO_2$ bicarbonate recoveries were significantly lower in Diet 1 vs. 2 and higher in the fed vs. fasted state, which is in general agreement with values measured in human subjects. The daily ^{13}C-leucine balance was significantly affected by the dietary lysine level (Table 1).

Table 1 Leucine balance of minipigs fed two lysine levels (LSM ± SE)

	Diet 1	Diet 2	±SE
	mg/kg/d		
Intake	162	162	
Oxidation	194.6^{a}	109.5^{b}	21.5
Balance	-29.1^{a}	$48.2^{b;A}$	21.3

$^{a, b}$ diet groups are significantly different at P<0.05
^{A} significantly different from zero

Table2 Proposed dietary AA for adult minipigs (g/kg DM)

Lys	4.6 *
Met+Cys	5.3 **
Thr	5.7 **
Trp	1.6 **

* experimentally derived in the study
** calculated based on relations of maintenance requirements

Based on the Leu balance data given in Table 1 the Lys requirement was assessed to be 84 mg per kg BW, or 2 g per day, or 4.6 g Lys/kg DM (Table 2) considering the actual BW and daily feed intake. The concentrations proposed for other indispensable AA based on relations of maintenance requirements are given in Table 2.

Conclusion: Further studies in adult minipigs are necessary to evaluate whether our proposed concentrations for Lys, Met+Cys, Thr, and Trp are adequate.

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26. Effect of the true ileal digestible threonine supply on performance of grower and finisher pigs (Einfluss der Versorgung mit wahr ileal verdaulichem Threonin auf die Mastleistung von Schweinen in der Anfangs- und Endmast).

T. Ettle*, Dora A. Roth-Maier, J. Bartelt and F.X. Roth - Freising-Weihenstephan/Cuxhaven

The wide range for threonine (Thr) requirements of growing pigs given in literature may be caused by different factors as the varying digestibility of different feedstuffs or a different body weight range of the animals used in different studies. For this reason the present study investigated the influence of the threonine supply on the performance of grower and finisher pigs and used the true ileal amino acid digestibility as a basis for diet calculation and derivation of Thr requirements.

Methods: The study used a total of 96 ad libitum fed crossbred pigs in a body weight range of 33 – 65 kg (grower stage) and 65 - 110 kg (finisher stage). Ensuring to have chosen an adequate lysine (Lys) supply, in the grower as well as in the finisher stage four different levels of true ileal digestible Thr where combined with two dietary levels of true ileal digestible Lys, resulting in 8 dietary treatments. The true ileal digestible Thr concentrations of the grower diets 1 – 8 were 4.24; 4.64; 5.14; 5.44; 5.04; 5.34; 5.94 and 6.54 g/kg at mean true ileal digestible Lys levels of 7.8 (Lys level 1, diets 1 – 4) and 9.2 g/kg (Lys level 2, diets 5 – 8), respectively. The true ileal digestible Thr concentrations of the finisher diets 1 – 8 were 3.23; 3.53; 3.83; 4.43; 3.23; 3.73; 3.93 and 4.33 g/kg, at mean ileal digestible Lys levels of 6.0 (Lys level 1, diets 1 – 4) and 6.6 g/kg (Lys level 2, diets 5 – 8). The optimum true ileal digestible Thr supply was estimated separately for grower and finisher pigs by the broken line model from means of daily true ileal digestible Thr intake of all treatments.

Results: In the grower stage, at Lys level 1 increasing the true ileal digestible Thr concentration from 4.24 to 5.44 g/kg resulted in a numerically increased daily gain ($P < 0.1$) of about 10 % and a significantly reduced feed/gain ratio. Increasing the true ileal digestible Lys concentration of the diet lead to a significantly improved growth rate and feed/gain ratio. In the finisher stage, increasing the true ileal digestible Thr concentration from 3.23 to 4.43 g/kg at Lys level 1 increased daily gain significantly from 834 to 907 g. Feed intake increased and feed/gain ratio declined at least in numerical terms. At the Lys level 2, average daily gain and feed/gain ratio were significantly improved when the dietary true ileal digestible Thr concentration increased from 3.23 to 3.73 g/kg. The optimum daily intake of true ileal digestible Thr for daily gain and feed conversion rate in the grower and the finisher stage were calculated to be 10.1 or 10.3 g and 10.7 or 10.5 g, respectively.

Lys level	1				2			
Thr level	1	2	3	4	5	6	7	8
Grower stage								
True ileal digest. Thr intake (g/d)	7.5	8.3	9.3	9.9	9.3	9.7	10.8	12.0
Weight gain (g/d)	774	805	832	848	851	848	858	867
Feed/gain (kg/kg)	2.29^{a}	2.22^{ab}	2.19^{ab}	2.16^{b}	2.16	2.15	2.12	2.13
Finisher stage								
True ileal digest. Thr intake (g/d)	8.2	9.2	10.0	11.7	8.3	9.8	10.3	11.4
Weight gain (g/d)	834^{b}	879^{ab}	893^{ab}	907^{a}	809^{b}	869^{a}	879^{a}	902^{a}
Feed/gain (kg/kg)	3.06	2.96	2.94	2.91	3.22^{a}	3.03^{b}	2.98^{b}	2.94^{b}

Conclusions: In the present study, the estimation of the Thr requirement based on daily intake was more reliable than that based on the concentration of Thr in the diet. The calculated optimum daily intake of true ileal digestible Thr for optimising growth rate and feed conversion rate in growing and finishing pigs corresponded to an optimum true ileal digestible Thr : Lys ratio of 0.73 and 0.69 g/g.

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27. Conclusions about the optimal lysine:threonine ratio for growing pigs, based on requirement studies (Schlussfolgerungen zum Lysin:Threonin Verhältnis für wachsende Schweine, basierend auf Bedarfsableitungen).

B. Wesseling* and F. Liebert – Göttingen

The dietary balance of essential amino acids is of special importance for a high level of protein utilization, mainly under conditions of high growth rates and corresponding to reduction of nitrogen excretion. Additionally, the ideal protein concept can be used for estimating amino acid requirements. Ideal amino acid ratios have been worked out mostly on total or digestible basis. The aim of this study was to estimate optimal lysine:threonine ratios based on calculated requirement data (1,2).

Methods: Growing barrows in the body weight range 40-55 kg from genotype [Piétrain x (Duroc x Landrace)] were fed isoenergetic diets (13,6 MJ ME) with lysine as the first limiting amino acid for calculation of lysine requirement (2). Threonine limiting diets were used to estimate the threonine requirement (1). N balance studies were conducted with a five day adaptation period and five day collection period (n=7). Data analysis was based on an exponential N utilization model (3), adapted for the determination of efficiency and requirement for the limiting amino acid (4), and potential for protein deposition of the actual genotype (5).

Results: Lysine and threonine requirement data, based on varying efficiency of lysine and threonine are summarized in the table. The results indicate that, corresponding to varying efficiency of lysine and threonine, the optimal lysine:threonine ratio for growing barrows (40-55kg) is in the range between 1: 0,5 and 1: 0,58.

Table: Efficiency of lysine and threonine and corresponding amino acid requirement for 145g daily protein deposition *

Lysine efficiency **	40	55	70
Lysine requirement (g/d)	24,81	18,04	14,18
Threonine efficiency **	80	100	120
Threonine requirement (g/d)	12,40	9,92	8,27
Calculated lysine:threonine ratio ***	50	55	58

* 50 kg body weight

** bc^{-1} within the exponential model (4)

*** expressed as percentage of lysine

Conclusion: The results indicate a rather wide ratio of lysine to threonine. The efficiency of lysine and threonine in the diet clearly affects the concluded amino acid requirement and consequently the calculated ratio lysine:threonine. Assuming practical ranges for lysine and threonine efficiency (6, 7), the ratio 1:0,55 is most likely. Further studies have to clarify the effect of body weight on this ratio.

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28. Selected parameters of protein and serotonin metabolism in milk and blood of lactating sows in response to the dietary tryptophan supply (Ausgewählte Parameter des Eiweiß- und Serotoninstoffwechsels in Milch und Blut von laktierenden Sauen in Abhängigkeit von der alimentären Tryptophanversorgung).

Brigitte R. Paulicks*, F.G. Pampuch and Dora A. Roth-Maier – Freising - Weihenstephan

Studying the effects of increasing tryptophan (Trp) concentrations in the feed of lactating sows (1) the contents of several components were examined in the milk and in the blood of the sows.

Methods: A total of 72 lactations of 61 multiparous sows (German Landrace) was the basis to form six treatments (I-VI) differing only in the Trp concentration of the lactation feed (g Trp/kg: 1.2, 1.5, 1.8, 2.4, 3.0, 4.2, resp.). All other influences, e.g. nutrient concentrations (156 g CP, 10.6 g Lys, 13.2 MJ ME per kg feed), gestation feeding (2.6 kg/d with 1.2 g Trp/kg), housing, or litter size (10-12 piglets/litter), were standardized. L-Trp was supplemented to the basic feed (I, native content of 1.2 g Trp/kg corresponding to 0.8 g apparent ileal digestible Trp/kg) for the graded levels of Trp concentrations. Milk and blood samples of the sows, taken after the 3 weeks of lactation, were analyzed for several parameters (1) e.g. for the concentrations of amino acids, urea, and serotonin.

Results: The concentrations of amino acids in the milk were not affected by the different Trp supplementations. The contents (g/kg) in milk were: Lys 3.4, Met 0.9, Cys 0.6, Thr 1.9, Trp 0.6, Leu 3.9, Ile 1.9, Val 2.4, Phe 1.9, Tyr 1.8, His 1.2, Ala 1.6, Arg 2.2, Asp 3.7, Glu 9.6, Gly 1.5, Ser 2.3. In blood plasma Trp concentration was correlated with alimentary Trp intake (r = 0.99). The average contents of other amino acids in blood plasma were in mg/l: Lys 43.2, Met 20.6, Cys 1.9, Thr 35.7, Leu 24.8, Ile 14.2, Val 20.8, Phe 21.5, Tyr 18.9, His 17.7, Ala 65.0, Arg 31.1, Asp 8.1, Asn 2.5, Glu 87.3, Gln 33.1, Pro 40.2, Hyp 2.8, Gly 65.9, Ser 11.0. Essential amino acids showed some tendency to lowest concentrations for group V whereas the non essentials showed tendency to lowest values for group I. Milk urea decreased from 211 mg/l in group I continuously to 173 mg/l in group V and then increased again to 188 mg/l in group VI. Values in blood plasma urea and increase of serotonin in blood serum between gravidity and lactation reacted correspondingly.

Conclusion: Although performance parameters indicated a requirement of 2.4 g Trp/kg lactation feed (1), the results of the present investigation show that less than 3.0 g Trp/kg feed will not meet the requirement of lactating sows according to metabolism parameters. Particularly for persistently high performance 3.0 g Trp/kg lactation feed (2.6 g apparent ileal digestible Trp) are necessary. Longterm studies seem to be required.

Treatment		I	II	III	IV	V	VI
Trp concentration (g/kg feed)		1.2	1.5	1.8	2.4	3.0	4.2
Milk	Trp (g/kg)	0.55	0.59	0.59	0.62	0.59	0.61
	Urea (mg/l)	211	201	185	184	173	188
Blood plasma	Trp (mg/l)	2.74^{d}	5.51^{cd}	8.67^{c}	15.54^{b}	15.73^{b}	23.91^{a}
	Urea (mg/l)	272	243	231	229	213	235
Blood serum	Serotonin increase[1] (ng/ml)	-248^{b}	-168^{b}	-27^{a}	$+38^{a}$	$+90^{a}$	$+76^{a}$

a, b, c, d significantly different means (Student-Newman-Keuls test; P<0.05)
[1] increase of serotonin in blood serum between gravidity and lactation

(1) PAMPUCH, F.G., PAULICKS, B.R., ROTH-MAIER, D.A. (2004): Proc. Soc. Nutr. Physiol. 13.

The experiment was supported by Lohmann Animal Health GmbH & Co. KG, Cuxhaven.

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29. Apparent ileal and total amino acids digestibility and nitrogen balance in young chickens fed diets with different protein level (Scheinbare praecaecale und Gesamt-Aminosäuren-Verdaulichkeit sowie Stickstoff-Bilanz bei Küken mit differenziertem Vorsorgungsniveau an Protein).

T. Wertelecki*, D. Jamroz - Wrocław

Feeding of chickens with reduced protein level diets and with balanced exogenous amino acids content may allow to obtain similar production effect in broilers as with standard diets (1). In present study the chickens fed diets containing different levels of crude protein and endogenous amino acids. The ileal and total amino acids (AA) digestibility and nitrogen balance has been determined. This information is important for correction of early chicks feeding.

Methods: 300 male Hubbard chickens at age 0-14 days of life were used in whole experiment, to the digestibility – balance trial 60 chickens were chosen (20 birds/group). Birds fed three diets based on maize (52-62%) and soyabean meal (38-29%) and containing different crude protein (CP) level: I – 220, II – 200 i III – 185 gkg^{-1}. For similar balance four exogenous AA, pure AA were added to each mixture and balanced in groups II and III to level applied in diet I (Lys 1.2%, Met+Cys 0.8%, Try 0.2%, Tre 0.8%). Mixtures were isoenergetical (12.5 MJ kg^{-1}). During 8-13 days of life the nitrogen balance and total apparent AA digestibility were estimated. Instead on day 14th of life the apparent ileal AA digestiblity was determined using indicator method (Cr_2O_3, 5 gkg^{-1}). Chickens were killed and from small intestine (from *Meckel* to end of small intestine) content was collected.

Results: The feed intake was similar in all groups - in mean 195.5 g/head. The increased nitrogen retention was in contrast proportional to feed's crude protein content. The reduced level of CP in diets significantly (P<0.05) increased: the ileal apparent AA digestibility coefficients (between group I in comparison to group II – III) for Ileu by about 24.4% - units, for Phe+Tyr by 2.4%, Lys by about 3.3% and total apparent AA digestibility, for Ileu by about 1.5% only. Simultaneously the ileal and total digestibility of some AA were significantly decreased: Asp.a. by about 7.9 and 2.0%, Tre by about 4.2 and 2.6%-units (P<0.05). Significant differences in AA digestibility are present in Table 1.

Table 1 Nitrogen balance, apparent ileal and fecal AA digestibility in young chickens

Body weight on 14 d [g]	I	II	III	N balance:	I	II	III
	353,1	355,5	335,7	N retention %	$61,3^{a}$	$63,0^{b}$	$63,4^{b}$
Apparent ileal AA digestibility [%]				Apparent fecal AA digestibility [%]			
significantly increased coefficients							
Ileu	60,9	71,3	85,3	Ileu	94,6	95,7	96,1
Phe+Tyr	78,1	79,3	80,5				
His	80,1	80,8	85,3				
Lys	83,0	84,4	86,3				
Try	82,9	85,4	85,9				
significantly decreased coefficients							
Asp. a	81,3	76,3	73,4	Asp. a	90,9	89,4	88,9
Tre	79,7	79,1	75,5	Tre	90,8	89,8	88,2
Ser	78,0	73,0	72,1	Ser	91,4	90,5	87,1
Glut. a	86,9	85,2	83,1	Glut. a	93,9	92,6	92,4
Cys	79,6	73,9	73,8	Pro	96,9	93,8	95,1
Gly	79,3	71,1	65,0	Cys	82,8	82,6	77,2
Ala	87,8	86,1	86,0	Gly	84,6	83,6	82,8

Scheinbare praecaecale und Gesamt-Aminosäuren-Verdaulichkeit sowie Stickstoff-Bilanz bei Küken mit differenziertem Vorsorgungsniveau an Protein

Conclusion: The reduction of crude protein in feed from 220 to 185 gkg^{-1} significantly increased digestibility of Ileu, Lys and Try and decreased digestibility of some endogenous amino acids: Ser, Asp.a., Glut.a., Gly.

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30. Disappearance of amino acids from yolk sac of chickens fed diets with different protein level (Verschwinden von Aminosäuren aus dem Dottersack bei Küken nach der Verfütterung unterschiedlicher Proteinniveaus). T. Wertelecki*, D. Jamroz - Wrocław

In the first 72-120 hours of life the chickens may use parallel two sources of nutrients: endogenous from the yolk sac content and from feed-mixture. The dynamic of yolk sac resorption is depend on intensity of nutrition and kind of feeds (1). The aim of present study was to determine the resorption rate of main nutrients from yolk sac content in chickens fed mixtures containing different levels of crude protein and endogenous amino acids.

Methods: 240 male Hubbard chickens were randomly assigned to three groups, and kept at age 0-7 days. Birds fed mixtures containing different crude protein content: I – 220, II – 200 and III – 185 gkg^{-1}. For similar balance of four exogenous AA, pure amino acids were added to each mixture and they were balanced in groups II and III to the level of I^{st} diet: (in %) Lys (1.2), Met+Cys (1.0), Try (0.2) and Tre (0.8). The basal compounds were barley (13.5%), wheat (37-48%), soyabean meal (36-26%). The diets were isoenergetical (12.5 $MJkg^{-1}$). The 20 birds/group were individually weighed (BW), killed and yolk sac removed and weighed (YS) in 6 hours after hatchery leave (0d), 72, 120 and 168 hours of life. In lyophilized yolk sac crude protein (CP) and amino acids (AA) concentration were determined.

Results: The better gain of BW in 0-168 h were observed in group III (79.1 g) in comparison to the other groups ($P<0.05$). The higher CP disappearance from yolk sac was observed in 72 h of life in chicks from group III (17.2% units). The lowered level of CP in feed decrease concentration of AA in yolk sac' protein during 0-168 h of life: Tre, Ser, Val, Met, Try while Lys* and Glut.a.* only in 72 h, and also increased level of Pro, His (in 0-168 h), while Lys* and Glut.a.* only in 120 – 168 h. The higher disappearance of some AA from yolks protein could prove its faster utilisation in metabolism, except feed source of that same AA: Met in 72 h (0.5% gr.I) and contrary in 120 h (0.4% gr. III); Val in 120 h (1.6% gr. III), Try 120-168 h (0.3-0.35% gr. II – III) and Lys in 72 h (25.3% gr. III).

Table 1. CP content in YS [%] and changes of AA concentration at YS CP [% CP]

		0d	72h	120h	168h
CP content in YS	I	24.6	21.6	14.0	13.3[a]
	II	24.8	22.9	17.6	16.8[a]
	III	25.2	20.8	15.1	19.8[b]
Tendency of AA increasing content					
Pro	I	1.9	2.1	3.9	2.6[a]
	II	2.4	2.0	3.2	3.9[b]
	III	2.2	1.9	3.0	3.4[b]
His	I	1.8	1.7	3.4	3.7
	II	2.1	1.8	3.2	3.4
	III	2.2	2.2	4.1	3.5
Lys*	I	6.9	8.0	8.3	9.5
	II	7.5	7.4	8.3	8.5
	III	7.5	5.9	8.3	9.1
Glut.a.*	I	15.5	13.5	14.9	15.7[b]
	II	14.4	13.2	16.2	17.2[a]
	III	14.6	12.4	15.4	15.4[b]

Tendency of AA decreasing content		0d	72h	120h	168h
Tre	I	3.5	3.7	3.7	3.3
	II	4.1	3.8	3.7	3.5
	III	4.1	3.9	3.2	3.5
Ser	I	8.5	7.1	5.7	5.5
	II	7.4	7.7	6.4	5.2
	III	7.9	7.1	4.4	7.1
Ala	I	5.0	5.0	4.7	3.6
	II	4.3	4.6	4.2	2.9
	III	4.4	5.2	4.2	4.9
Val	I	4.8	5.2[ab]	3.5[b]	2.7
	II	4.7	4.8[a]	3.5[b]	3.0
	III	4.6	6.1[b]	1.9[a]	3.9
Met	I	3.1	2.2	2.4[b]	2.2
	II	2.9	2.5	2.2[ab]	1.6
	III	2.8	2.9	1.9[a]	2.1
Try	I	1.5	1.1	1.4	1.1
	II	1.4	1.2	1.2	1.0
	III	1.4	1.2	1.0	0.7

Conclusion: The reduced level of CP in feed has lead to higher disappearance of Met and Try from yolk sac's protein during 0-168 h of life. From the other side the higher increasing of concentration in yolk's protein was observed for Pro, His and Glut.a. in comparison to group I at the same time.

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31. Estimation of the potential for protein deposition of extensive genotypes of growing chicken in different age periods (Schätzung des Proteinansatzvermögens extensiver Broilergenotypen in unterschiedlichen Altersabschnitten). Samadi* , F. Liebert and A. Sünder - Göttingen

The potential for protein deposition in growing animals is considerably affected by genotype, age and sex. Information about this potential are important for evaluation of the breeding success as well as a reference standard for conclusions about the amino acid requirement of growing animals within the exponential N utilization model (1). The objective of these studies was to estimate the potential for protein deposition in extensive genotypes of growing chicken depending on age (I 657 and Red JA) at different age by rising feeding studies. This information is essential for further calculation of estimation of maximum capacity for nitrogen deposition and also conclusion about amino acid requirements within physiology based N utilization model for growing animals.

Methods: Two extensive Hubbard ISA genotypes (I 657 and Red JA) of day old male growing chicken were raised under defined conditions. N rise experiments with graded protein supply (N1=6,6%, N2=12,9%, N3=18,6%, N4=24,9%, N5=30,6% and N6=35,1% CP in DM) from HP soybean meal with supplements of L-lysine and DL-methionine were conducted in 4 age periods (10-25d, 30-45d, 50-65d, 70-85d). N balance experiments (n=6) were divided into 5d adaptation and 5d collection period. Nitrogen maintenance requirement (NMR) was calculated by quadratic regression between N intake and N excretion. The calculated NMR values were used for further calculation of PDmaxT (N balance+NMR) as the theoretical maximum for N retention (2). The determination of PDmaxT was based on the fitting of N balance data depending on N intake by an exponential function with given NMR, corresponding to the principle of *Levenberg-Marquardt-algorithm* for threshold value estimation within the SPSS program (version 11.5).

Results: The example of data fitting for genotype I 657 corresponding to the age period 10-25d [$y = 2967\,(1-e^{-0,000436x})$; $r^2=0,98$] indicates 2967mg/$LW_{kg}^{0,67}$/d as PDmaxT. The summarised results of the other age periods as well as the data of genotype Red JA are given in the table.

Table: Results of estimation of NMR and PDmaxT for different age periods of extensive genotypes of male growing chicken

Age period (d)	Genotype			
	I 657		Red JA	
	NMR (mg/$LW_{kg}^{0,67}$/d)	PDmaxT (mg/$LW_{kg}^{0,67}$/d)	NMR (mg/$LW_{kg}^{0,67}$/d)	PDmaxT (mg/$LW_{kg}^{0,67}$/d)
10-25	258	2967	288	2742
30-45	341	2874	345	2816
50-65	195	1675	125	1564
70-85	108	1375	90	1160

Conclusions: The results indicate lower levels for PDmaxT in extensive genotypes comparing to data for intensive genotypes Cobb and Ross (3) and a strong decrease of PDmaxT in the older chicken. The algorithm used for NMR-determination has to be compared with other methods/functions and further approved for physiological relevance.

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32. First conclusions about threonine requirement of male growing chicken of extensive genotype depending on age and protein deposition (Erste Schlussfolgerungen zum Threoninbedarf männlicher Broiler extensiven Genotyps in Abhängigkeit von Alter und Proteinansatz). Samadi* and F. Liebert-Göttingen

Different methods are available for scientific based conclusions about the amino acid requirement of growing chicken. Based on the efficiency of the limiting amino acid, the amino acid requirement can be determined within the exponential N utilization model (1). Important parameters of this model related to extensive genotypes of growing chicken were investigated with threonine limiting diets of graded protein content (2). The purpose of this study is to evaluate the threonine requirement of extensive male growing chicken depending on age and genotype.

Methods: The calculations are based on N balance data of N rise experiments with totally 288 male growing chicken of two extensive genotypes from Hubbard ISA (I 657; Red JA). The diets were based on HP soybean meal with supplementation of L-lysine and DL-methionine. Threonine concentration (c=3,87g / 16gN) and the relative amino acid pattern (Lys : Met+Cys : Thr = 1 : 0.85 : 0,54) identified threonine as the limiting amino acid in all diets. The nutrient content of the experimental diets is summarized in table 1.

Table 1.: Nutrient content of experimental diet

Diet	N1	N2	N3	N4	N5	N6
CP (%DM)	6,6	12,9	18,6	24,9	30,6	35,1
ME(MJ/kg DM)	13,81	13,85	14,43	14,72	14,49	14,59

The calculation of threonine requirement data was based on the exponential N utilisation model (1,3) for a defined level of N retention (80% of PDmaxT in the age periods) and given daily protein deposition, respectively.

Results: The results of calculations for the threonine requirement are summarized in table 2.

Table 2: Daily threonine requirement of extensive male broiler at different age and genotypes

Age period (d)	10-25		30-45		50-65		70-85	
Genotype	I 657	Red JA	I 657	Red JA	I 657	Red JA	I 657	Red JA
PDmaxT [1]	2967	2742	2874	2816	1675	1564	1375	1160
bc^{-1}_{Thr} [2]	113	127	120	117	205	174	201	221
Mean BW (g)	285	281	940	992	1833	1994	2672	2936
CP-deposition(g/d) [3]	5,7	5,1	11,7	11,9	10,7	11,2	12,0	10.8
Thr-Requirement ($mg/BW_{kg}^{0,67}$) [4]	890	793	838	860	490	578	500	455

[1] Potential for daily N retention ($mg/BW_{kg}^{0,67}$) [2] Parameter of Thr-efficiency [3] Calculated from N balance data and corresponding to 80% of PDmaxT [4] for 80% of PDmaxT

Conclusion: The results give a first indication that the threonine requirement between the genotypes used could be different. This observation might be affected also by the variation of threonine efficiency, calculated based on the mathematical estimated function between N intake and N retention for each N-rise experiment of the study. The results have to be approved in growth trials with determination of nutrient deposition and under conditions of varying Thr efficiency.

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33. Effect of DL-Methionine supplementation on various fattening and slaughter traits of broilers under organic farming conditions (Einfluss einer DL-Methionin-Supplementierung auf verschiedene Mast- und Schlachtkriterien in der biologisch-organischen Broilerhaltung). A. Lemme*, K. Damme, A. Petri - Hanau/Kitzingen

In organic farming supplementation of crystalline amino acids is prohibited. In order to meet the minimum requirements of essential amino acids - particularly methionine (Met) or methionine + cysteine - dietary protein levels are relatively high. Currently up to 20% of conventional produced feed components are allowed in all mash diets for organic broiler production (EU- directive 1804/99). However, from August 2005 on raw materials exceptionally produced under organic farming conditions will be allowed resulting in a reduced number of possible ingredients which in turn will lead to even higher dietary protein levels. Therefore, an experiment was performed in order to compare the effects of three diets formulated according to current practice, future practice, and future practice if supplementation of DL-Met would be allowed.

Methods: A total of 1500 mixed-sex day-old ISA J 257 broiler chicks were equally distributed to 15 floor pens (á 10 m^2) with 100 birds each. Broilers were fed starter diets from day 1 to 28 and grower diets from day 29 to 69. Three types of diets were fed: 1) according to current guidelines 2) according to future practice (both 1 and 2 according to EU-directive 1804/99) and 3) as 2) but with supplementation of DL-Met. Diets contained 0.40% Met with corresponding crude protein contents of 25.5, 27.2 and 21.9% in the starter and 22.8, 27.8 and 21.0% in the grower diets for treatments 1, 2 and 3, respectively. While in the diets of treatment 1 potato protein and corn gluten meal with relative high Met contents were included in addition to full fat soybeans, the dietary Met in treatment 2 originated almost exclusively from soybean products (full fat beans, cake). Supplementation of small amounts (0,1%) of DL-Met in treatment 3 reduced the percentage of soybean products and allowed inclusion of peas. All ingredients were grown under organic farming conditions and feed formulations were adapted to prior amino acid analyses. Body weights of birds, feed as well as water consumption were determined in two-week intervals. At day 69 representative samples of 50 broilers per treatment were taken for slaughter traits evaluation and carcass and breast meat yield were determined.

Results: Compared to the other both treatments particular birds of treatment 2 gained less, had a higher water feed ratio (Table). This might be in direct relationship to the overload in protein intake which is indicated by the protein conversion data. Whilst best carcass yield was achieved with birds in treatment 2, broilers of treatment 3 had highest breast meat yield.

Treatment	Gain day 1-69 (g)	FCR* day 1-69 kg/kg	WFR* day 1-69 l/kg	Carcass yield %	Breast yield* %	Protein conversion g/kg
1, current guidelines	2665 [ab]	2.468	2.05 [b]	71.7 [c]	32.4 [b]	579
2, future practice (2005)	2594 [b]	2.452	2.41 [a]	73.9 [a]	32.7 [b]	684
3, future practice +DL-Met	2679 [a]	2.438	2.07 [b]	73.2 [b]	33.8 [a]	521

* FCR: Feed conversion ratio (corrected), WFR: Water-Feed ratio, Breast: in % of carcass; [abc] Means within columns with different superscripts are significantly different (P<0.05)

Conclusion: Feeding broilers according to the EU directive 1804/99 with 100% organic feed components from Aug. 2005 on will result in impaired performance, higher metabolic stress which is indicated by the high water to feed ratio, and reduced protein utilisation (an thus higher nitrogen excretion). However, these adverse effects can be solved by supplementing small amounts of DL-Met. In addition, the inclusion of local protein sources is supported by DL-Met supplementation.

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34. Variability of lysine efficiency of diets for Nile-Tilapia (*Oreochromis niloticus*) containing different batches of peas (Variabilität der Lysinwirksamkeit von Diäten für Nil-Tilapien unter Verwendung von verschiedenen Erbsensorten).

K. Benkendorff* und F. Liebert - Göttingen

Especially protein sources from plant origin are increasingly important in the nutrition of cultured fish. Based on our results about the efficiency of lysine in different feed protein mixtures for Nile-Tilapia (1, 2), the evaluation of the amino acid efficiency within an exponential N utilization model is also useful to compare protein sources for growing fish. The present study was carried out in order to investigate the variability of lysine efficiency in plant feed proteins.

Methods: A growth trial was conducted with male Nile-Tilapia (average initial BW = 11.0 g) assigned to 34 tanks in a recirculating system. Each tank contained 18 fishes. The fishes were fed with 8 semi-purified lysine limiting diets with a crude protein content ranging between 32.6 and 33.7% DM for 56 days. The diets were calculated to be limiting in lysine by combination of protein sources and supplementation of crystalline amino acids. Each treatment consisted of 4 repetitions. The diets contained 9.7% fish meal, 17.7% wheat gluten, 24.1% wheat and 27.2% of six different batches of peas (A-F). Additionally, batch A was exposed to two different heat-treatments (T_1: 1 min at 85°C and T_2: 10 min at 100°C, respectively). The amount of dietary lysine originating from the peas varied between 31.6 and 35.1%. At the end of the experimental period, the body composition was determined in representative samples of each tank and used for calculation of nitrogen deposition. Data analysis was based on the application of the exponential N-utilization-model for fish (3). Results were submitted to ANOVA using the SAS.

Results and conclusions: The results of daily N-deposition (table 1) give an indication that it was allowed to make further conclusions for the lysine efficiency (N-deposition >55 mg N/ $BW_{kg}^{0.67}$) within the used exponential N-utilization-model (1). The calculated lysine efficiencies were in the range of the former study (1). With regard to the present values, the efficiency of lysine among the diets was not significantly different ($p > 0.05$), indicating that the lysine efficiency of the single pea protein sources differed also not significantly. This means, that neither the origin of the batch nor the applied heat-treatments had any significant effect on the lysine efficiency of the pea proteins.

Table 1: Daily N-deposition (mg N/$BW_{kg}^{0.67}$) and corresponding lysine efficiency in the diets

Diets	A	A(T_1)	A(T_2)	B	C	D	E	F
N-intake	270	310	365	263	269	271	265	253
± SD	± 19	± 29	± 36	± 38	± 10	± 10	± 38	± 15
N-deposition	95	128	147	96	106	106	100	96
± SD	± 13	± 11	± 15	± 19	± 8	± 3	± 16	± 9
Lys-efficiency	578[a]	606[a]	569[a]	622[a]	623[a]	618[a]	613[a]	608[a]
(bc^{-1}_{Lys}) ± SD	± 34	± 23	± 31	± 59	± 22	± 14	± 35	± 34
$NPU_{stand.}$ [%]	66.1[a]	67.4[a]	65.9[a]	67.1[a]	67.8[a]	67.7[a]	67.5[a]	67.9[a]
± SD	± 1.5	± 1.0	± 1.4	± 2.7	± 0.9	± 0.6	± 1.4	± 1.5

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35. Comparison of NRC and GfE amino acid recommendations in diets for broilers on growth and slaughter performance (Vergleich von NRC- und GfE-Aminosäurenempfehlungen im Alleinfutter für Broiler im Hinblick auf die Mast- und Schlachtleistung).

R. Leitgeb, F. Hutterer, J. Bartelt, Nanda Zeh*, F. Ertl and Christine Iben – Wien/Cuxhaven

In the present trial the influence of NRC and GfE recommendations of amino acid requirements with higher and lower protein levels in diets for broilers on growth and slaughter performance, carcass composition and blood parameters was investigated. NRC and GfE differ manly in the relation of methionine and threonine to lysine.

Method: The trial was carried out on poultry trail station, Äussere Wimitz 3, A-9311 Kraig. 350 broiler chicks (Ross 13) were divided randomly into 24 pens (6 pens per group). All birds were fed ad libitum. As diets were used starter, grower and finisher feed. The diets relations were: 10 % starter, 47 % grower and 43 % finisher. The content of ME and lysine in starter, grower and finisher feed was 12.4, 12.8 and 12.9 MJ/kg and 1.38, 1.30 and 1.15 %, respectively. Amino acid recommendations in feeding group 1 and 3 were used of NRC and in feeding group 2 and 4 of GfE. The growing period was 36 days. The protein content in starter, grower and finisher diets of FG1 and 2 was 23, 22 and 21 % and in FG3 and 4 21, 19.5 and 18.5 %.

Results: Diets with high protein levels caused significant higher live weight, better feed conversion rates and slaughter performances than diets with lower protein levels. The recommendations of NRC and GfE for amino acid relation showed equal results in diets with higher and lower protein levels. For a stronger reduction of protein content in diets of broilers, supplementation with lysine, methionine, threonine and tryptophan is not sufficient for high production levels. With regard to the proportion of breast meat on carcass, the amino acid recommendations of GfE are beneficial. This investigation shows, that diets with lower protein content require more than the usual four amino acids for supplementation to ensure high production levels.

Traits	FG1	FG2	FG3	FG4	P
Live weight, g	2074[a]	2084[a]	1918[b]	1988[ab]	<0,01
Feed conversion rate	1.72[a]	1.75[a]	1.91[b]	1.84[ab]	<0.01
Eviscerated carcass warm	1626[a]	1619[a]	1518[b]	1523[b]	<0.01
DM, %	33.3[a]	33.7[ab]	34.4[b]	34.6[b]	<0.01
Protein, %	18.8[a]	18.5[ab]	18.2[b]	18.2[b]	<0.01
Fat, %	11.8[a]	12.3[ac]	13.2[bc]	13.6[b]	<0.01
Heart, g	9.2[a]	9.1[ab]	8.3[cd]	8.6[bd]	<0.01
Roast carcass (RC), g	1482[a]	1462[ab]	1367[b]	1359[b]	<0.01
Breast meat with skin in % of RC	26.9[ab]	28.0[a]	25.1[b]	26.8[ab]	<0.01
Total protein, g/dl	3.01	3.02	3.05	3.04	0.99
Albumin, g/dl	1.40	1.39	1.44	1.38	0.64
Uric acid, mg/dl	7.83	6.87	6.70	6.00	0.42

Conclusion: Broiler needs 10 essential amino acids for a good growth performance. For feed supplementation, only 4 amino acids are available. Therefore, it is very difficult to reduce the content of protein in diets to a lower level. With the availability of more essential amino acids to a saleable price further improvement of the protein quality and lower protein levels in diets for broilers are possible.

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36. Effects of chloride and sulfate ions on the surface pH of rumen epithelium (Wirkungen von Cl^- und SO_4^- auf den pH-Wert der Epitheloberfläche im Pansen).

Sabine Leonhard-Marek*, Bernd Schröder and Roger Busche - Hannover

Chloride and sulfate salts are given to lactating cows either pre partum as a strategy to prevent hypocalcemia or post partum to adjust electrolyte imbalances. At the epithelial level chloride is able to stimulate the ruminal absorption of Ca ions (1). The underlying mechanism of this stimulation are not clear. The current concept of chloride absorption across the rumen tissue involves a Cl^-/HCO_3^- exchange mechanism at the apical membrane of rumen epithelial cells (2). Sulfur has been shown to be rapidly absorbed from the rumen as sulphide (3). Data on the ruminal absorption of sulphate are scarce. In other tissues sulphate is transported via anion antiporters in exchange for HCO_3^- or OH^- (4). The hereby induced changes in HCO_3^- or OH^- secretion might have an effect on local pH values. We therefore investigated whether Cl^- or SO_4^- were able to change the pH-microenvironment at the ruminal surface.

For this purpose isolated epithelia from sheep rumen were incubated in a microperfusion chamber. The surface pH was measured with 5-N-hexadecanoyl-aminoflorescein (HAF), a pH-sensitive fluorescent dye which inserts in the outer leaflet of plasma membranes (5).

The mean surface pH of rumen epithelium amounted to 7.47 ± 0.10 (N = 8) in the presence of bicarbonate. It was increased by 0.167 ± 0.050 (N = 6, P <0,001) when the luminal buffer solution was changed from a low (10 mmol/l) to a high chloride (68 mmol/l) solution. A comparable increase in surface pH (0.107 ± 0.007, N = 3, P = 0,002) could be seen, when the luminal sulfate concentration was changed from 0 to 29 mmol/l (in the presence of 10 mmol/l chloride).

The data suggest that a chloride (sulfate) / bicarbonate antiporter and/or a sulfate (chloride) / hydroxyl antiporter might contribute to the uptake of chloride and sulfate across the luminal membrane of rumen epithelial cells. Due to these mechanisms chloride and sulfate salts that are included in the diet could change the ruminal absorption of other electrolytes via an alteration of the rumen surface pH.

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37. Effects of IGF-1 on Na^+ transport across rumen epithelium and on Na^+/H^+ exchanger (Effekt von IGF-1 auf den ruminalen Na+-Transport und den Na^+/H^+-Austauscher).

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In goats consuming an energy- and protein-rich diet elevated plasma IGF-1 levels and an increased number of IGF-1 receptors in rumen papillae were observed (1). Furthermore, the ruminal Na^+ absorption was stimulated (1). IGF-1 is known to participate in the regulation of growth and differentiation of several tissues and cells, but so far a direct action of IGF-1 on ruminal electrolyte transport has not been documented. It was the aim of the present study to examine the nature and potential mechanism of IGF-1 on sheep rumen epithelium Na^+ transport.

Methods: Experiments were carried out with isolated ruminal epithelial tissues of sheep (N=12) using the conventional Ussing-chamber technique to determine short circuit current (I_{sc}), tissue conductance (G_t) and unidirectional flux rates of Na^+. The effect of IGF-1 on Na^+ transport and Na^+/H^+ exchanger (NHE) activity was tested by serosal application of LR^3-IGF-1 in a dosage of 20 µg/l and amiloride (1 mmol/l), respectively. In another series of experiments a dose-response curve was determined by exposing the serosal side of the epithelium to increasing dosages (0, 12.5, 25, 50, 100 and 200 µg/l) of LR^3-IGF-1. Additional experiments were performed with cultured ruminal epithelial cells, REC (prepared as described previously). Na^+/H^+ exchanger activity of REC was estimated from the rate of intracellular pH (pH_i) recovery after acid-loading, which was done by exposure to butyrate. pH_i was measured using the fluorescence probe BCECF. The amiloride-sensitive component of pHi recovery was determined in the absence (control) or in the presence of 20 µg/l of IGF-1 in the extracellular buffer.

Results: The presence of IGF-1 in the serosal buffer solution significantly ($p \leq 0,05$) increased the mucosal to serosal Na^+ flux (Jms^{Na}) and consequently the net Na^+ flux ($Jnet^{Na}$) in a dose-dependent manner. Independently of the presence of IGF-1, amiloride led to a significant 59% and 76% inhibition of Jms^{Na} and $Jnet^{Na}$, respectively. Neither IGF-1 nor amiloride altered G_t or I_{sc}. Application of IGF-1 increased the rate of pH_i recovery following acidification in REC, but this effect was completely abolished by amiloride. The data are summarised in Table 1 (mean ± SEM).

Table 1: Effect of IGF-1 and of amiloride on Na^+ transport across rumen epithelium and on REC pH_i recovery after an acid load

	Control	IGF-1	Amiloride	IGF-1 + amiloride
Jms^{Na} ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)	4.02 ± 0.89	5.54 ± 1.47*	2.01 ± 0.34*	2.5 ± 0.79*
Jsm^{Na} ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)	1.44 ± 0.39	1.31 ± 0.36	1.31 ± 0.23	1.35 ± 0.20
$Jnet^{Na}$ ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)	2.59 ± 1.04	4.23 ± 1.14*	1.21 ± 0.61*	1.15 ± 0.83*
pH_i (pH units)	0.26 ± 0.02	0.33 ± 0.02*	0.14 ± 0.07*	0.11 ± 0.05*

*$p \leq 0,05$ vs. control

Conclusion: The results confirm that IGF-1 stimulates Na^+ absorption across rumen epithelium. A role for Na^+/H^+ exchange activity in mediating this effect was suggested by the findings of increased amiloride-sensitive Jms^{Na} and as well as by the potentiation of Na^+-dependent, amiloride-sensitive pH_i recovery of REC after acid loading.

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38. Functional and molecular characterisation of the vacuolar H^+-ATPase in sheep rumen epithelial cells (Funktionelle und molekulare Charakterisierung einer vakuolären H^+-ATPase in ruminalen Epithelzellen des Schafes).

Monika Schweigel*, Elisabeth Froschauer, B. Etschmann and H. Martens – Berlin/Wien

Recently we found functional evidence for a vacuolar-type H^+-adenosine triphosphatase (vH^+-ATPase) to be expressed in ruminal epithelial cells, REC (1). Such electrogenic H^+-ATPases have been shown to energize the apical membrane of several epithelia by generating an inside negative membrane potential (2). The existence of an active H^+-extruding mechanism in REC has been explored in the present study.

Methods: The presence of vH^+-ATPase was investigated by RT-PCR, Western blotting and immunofluorescence microscopy. H^+ secretion was estimated from the rate of intracellular pH (pH_i) recovery after acid-loading in cultured REC (prepared as described previously). Acid-loading was achieved by exposure of REC to butyrate and/or CO_2/HCO_3^- and pH_i was measured using the fluorescence probe BCECF. Different types of H^+-extruding systems were identified by using Na^+-containing or Na^+-free media and by pharmacological means.

Results: Using specific primers, fragments of the mRNA of vH^+-ATPase subunits E and B were amplified from the total RNA of rumen epithelium cells by RT-PCR. The regions amplified from the coding regions of the subunit B and subunit E mRNAs reached from nt 498 to nt 1307 and from nt 17 to nt 624, respectively. Furthermore, a 60-kDa immunoreactive band contributing to vH^+-ATPase subunit B was detected in protein lysates of REC by immunoblotting. Using immunocytochemistry, REC stained positive with an antibody directed against the B(60-kDa)-subunit of the vH^+-ATPase. The rate of pH_i recovery following acidification was reduced by lowering the temperature and by the vH^+-ATPase inhibitors bafilomycin A_1 and foliomycin (Table 1). After inhibitor application the pH_i-recovery rate is lowered by about 23 %. The residual H^+-extrusion activity can be blocked by amiloride and is therefore generated by a Na^+/H^+ exchanger.

Table 1 Effect of vH^+-ATPase inhibitors on pHi recovery from an acid load

solution		$\Delta pH_i \pm$ S.E.M.	n
HEPES-buffered	control	0.23 ± 0.01	3
with butyrate	bafilomycin A_1 (5 µmol/l)	0.17 ± 0.02*	3
CO_2/HCO_3^--containing	control	0.22 ± 0.02	3
	foliomycin (2 µmol/l)	0.17 ± 0.01*	3

* $P < 0.05$ vs. control

Conclusion: The findings clearly confirm that a vH^+-ATPase is present in REC. As has been shown for Mg^{2+}-absorption, the proton-motive force generated by such electrogenic H^+-ATPases in the cell membrane can be utilized as a driving force for numerous transport processes. The activity of ruminal vH^+-ATPase seems to be modulated by bacterial fermentation products, explaining there effects on the transport of several solutes (e.g. Na^+, Cl^-, Mg^{2+}) across the rumen epithelium.

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39. Effects of short chain fatty acids on nerve mediated ruminal motility in vitro (Wirkung kurzkettiger Fettsäuren auf die nerval vermittelte Pansenmotorik in vitro).

Helga Pfannkuche and G. Gäbel - Leipzig

The ovine forestomach expresses distinct motility patterns which are assumed to be controlled by myenteric neurones of the enteric nervous system. Intrinsic gastrointestinal motility is strongly influenced by composition of the digesta. In some studies short chain fatty acids (SCFA) are reported to enhance forestomach motility. Other studies point to inhibition of ruminal motility by intraluminal SCFA.

It was the aim of our study to investigate the effect of SCFA on ruminal motiliy and in particular the involvement of the enteric nervous system in this effect.

Methods: Motility patterns induced by stimunlation of the myenteric plexus were recorded on circular and longitudinal muscle layers of the ruminal wall. For this purpose, specimen (5x5 cm) from the ventral rumeninal sac were pinned flatly in an organ bath. Force tranducers were fixed in direction of both, the longitudinal and the circular muscle layers. For electrical field stimulation (EFS), platinum electrodes were fixed above and below the specimens. Parameters for EFS were 0.5ms pulses, 20 Hz frequency, 10s duration. By this approach depolarisation of myenteric neurones but not of smooth muscle cells can be induced. Effects of acetate or butyrate on nerve mediated motility and the involvement of motorneurones were analysed by incubating the tissues with 36 mM acetate or butyrate during EFS.

Results: Electrical field stimulation of the myenteric plexus induced an atropine-sensitive contraction of the circular as well as of the longitudinal muscle layer. Addition of atropine demasked a nitrergic mediated relaxation of both muscle layers during electrical field stimulaton. Preincubation with 36mM SCFA resulted in an enhanced contractile response of both muscle layers. This increased excitatory response included an increase in contraction amplitude and duration. Consequently the area under curve during contraction was significantly enhanced in the presence of SCFA. It was 200% and 135% of the control values for circular and longitudinal muscle, respectively. Same effect was seen in the presence of β-hydroxybutyrate, a catabolite of intracellular SCFA degradation. Nerve mediated relaxation was not influenced by the presence of SCFA. Hexamethonium, a ganglion blocking substrate, was not able to prevent the effect of SCFA on nerve mediated contraction. Therefore, the effect of SCFA was at least partly driven by motorneurones directly innervating the smooth muscle layers.

Conclusions: We conclude that under physiological condition SCFA increase ruminal motility. The effect is mediated by excitation of enteric neurones. Especially, cholinergic motorneurones of the myenteric plexus might be involved in this effect.

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40. Functional and structural parameters of Ca absorption from pig jejunum as affected by vitamin D_3 (Vitamin-D_3-Abhängigkeit funktioneller und struktureller Parameter der Ca-Absorption im Schweinejejunum).

M. Brandenburger, K. Huber and B. Schröder* - Hannover

It is well known that active Ca^{2+} absorption in pig jejunum requires a definite amount of cytosolic calbindin-D_{9k} since reduction due to low plasma calcitriol levels completely abolished active Ca^{2+} absorption whereas treatment with vitamin D_3 could reconstitute this process (1). From the data, however, no conclusions could be drawn with respect to the potential role of brush-border membrane Ca^{2+} channels such as TRPV5 (former ECaC1, vanilloid receptor subfamily of the transient receptor potential protein superfamily of ion channels) and TRPV6 (former ECaC2) as a rate-limiting step of the overall transepithelial Ca^{2+} transport. In a calcitriol deficient mouse model upregulation of duodenal TRPVs by calcitriol could be observed at least at mRNA levels (2). However, for conventional animals only rare information is available regarding the potential interrelationship between mucosal Ca^{2+} transport and TRPV expression. Therefore, TRPV mRNA levels in jejunal samples of weaned piglets which were either be treated with vitamin D_3 or not were compared with kinetic parameters of Ca^{2+} uptake into isolated brush-border membrane vesicles (BBMV) as well as transepithelial active Ca^{2+} transport.

Methods: Weaned piglets (30-45 d of age, n=6) received 100,000 IU vitamin D_3 intramuscularly 3 d prior to experiments. Tissue samples of the jejunum were obtained for analysis. Results were compared with correspondent data from non treated littermates (n=6). Unidirectional flux rates of Ca^{2+} were measured in Ussing chambers (1) in the presence or absence of Ruthenium Red as a Ca channel blocker (mucosal side, 10 $\mu mol \cdot l^{-1}$). Ca^{2+} uptake into BBMV was investigated as a function of extravesicular free Ca^{2+} (0.015-4 $mmol \cdot l^{-1}$) to calculate V_{max} values as a measure of Ca^{2+} transport activity on the basis of the Michaelis-Menten equation (3). Relative quantification of TPRV5, TRPV6 and calbindin-D_{9k} mRNA levels in mucosal cells (based on β-actin expression) was performed by real-time RT-PCR with SYBR green I, using the Stratagene Mx4000 thermal cycler PCR system.

Results: Active Ca^{2+} absorption was significantly increased by about 50% in vitamin D_3 treated animals. Almost 50% of the vitamin D_3 effect could be blocked by Ruthenium Red indicating the involvement of TRPVs in activated Ca^{2+} transport. No effect of the Ca channel blocker was observed in control animals. In contrast, V_{max} values of BBM Ca^{2+} uptake were not significantly different between the treated and non-treated situation. Similarly, TRPV5 and TRPV6 mRNA levels were not affected.

Conclusions: Parameters of single steps of overall transepithelial Ca^{2+} transport (brush-border membrane transport capacities, TRPV mRNA levels) could not be related to Ca^{2+} fluxes across the intact epithelium. On the one hand this may be due to lack of still unknown regulatory factors of the living cell. But on the other hand time dependent changes in term of downregulation of TRPV mRNA (and protein) due to the 3 d exposure to vitamin D_3 (and increased plasma Ca^{2+} availability) cannot be excluded. To further elucidate this possibility time course experiments recording vitamin D_3 short time effects at the mRNA level could be helpful.

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41. Catabolic pathways of histamine in the porcine proximal colon (Verstoffwechslungswege von Histamin im Dickdarmepithel des Schweins).

Vivica von Vietinghoff*, G. Gäbel and J.R. Aschenbach – Leipzig

Histamine is responsible for the disease pattern histaminosis, resembling acute shock. We recently demonstrated that the enzymes histamine-N-methyltransferase (NMT) and diamine oxidase (DAO) play an important role in detoxification of histamine in the colonic epithelium of pigs (1). However, this previous study was lacking analytic differentiation of the catabolites. Therefore, a HPLC-method for the detection of 1-methylhistamine and histamine was established to gain more information on the catabolic pathways of histamine.

Methods: Slide-stripped epithelia of the porcine proximal colon were incubated in Ussing-chambers. Histamine (100 μM) spiked with ^{3}H-labelled histamine was added to the buffer solution on either the mucosal or the serosal side. Amodiaquin and aminoguanidin (100μM) were added on both sides. These substrates inhibit the catabolic enzymes histamine-N-methyltransferase and diamine oxidase, respectively, which are responsible for the degradation of histamine. To analyze the native substances, histamine and 1-methylhistamine, an on-line and on-column derivatization method was used employing a C18 reversed phase column with a mobile phase containing the ortho-phtaldialdehyd (OPA) reagent. Detection of the OPA derivatives of histamine and 1-methylhistamine was performed using a fluorescence detector. The calculations of histamine flux rates were based on the translocation of radioactively labelled histamine across the epithelium. They include the radioactively labelled catabolites and are therefore named rad-hist flux rates. Flux rates of native histamine and 1-methylhistamine were determined on the same epithelia and were corrected for the spontaneous release of endogenous histamine and 1-methylhistamine.

Results: In control epithelia, the rad-hist flux rate in the mucosal-to-serosal (ms) direction was as great as its flux rate in the serosal-to-mucosal (sm) direction, so there was neither secretion nor absorption across the colonic epithelium. Flux rates of native histamine were small in both directions. They indicated catabolism of about 95.5 % of rad-hist. 1-Methylhistamine appeared only on the serosal side. After application of 100 μM histamine on the mucosal side, the appearance of 1-methylhistamine was less than its appearance after serosal histamine application ($P<0.05$). After applying amodiaquin to inhibit the histamine-N-methyltransferase, native histamine flux rates incremented up to rad-hist flux rates, whereas the appearance of 1-methylhistamine ceased. In epithelia treated with aminoguanidin, native histamine flux rates augmented only in sm direction becoming as great as the rad-hist flux rate in the sm direction. 1-Methylhistamine appeared again only on the serosal side with significantly greater appearance after serosal addition of histamine ($P<0,01$). Flux rates of rad-hist did not change after the treatments with either amodiaquin or aminoguanidin.

Conclusions: Histamine is more efficiently taken up on the serosal than on the mucosal side. It is then almost completely catabolized by NMT. The produced 1-methylhistamine is either exported on the serosal side or further catabolized by DAO.

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42. Influence of dietary protein source on metabolism of nucleic acids and alkaline phosphatase activity in the small intestinal mucosa of preruminant goats (Einfluss der Proteinquelle in der Diät auf den Nukleinsäurestoffwechsel und die Aktivität der alkalischen Phosphatase in der Mukosa des Dünndarms bei jungen Ziegen).

Ulrike Schönhusen*, R. Žitňan, S. Kuhla, Korinna Huber, G. Breves, R. Pabst and J. Voigt - Dummerstorf/Košice/Hannover

Abnormal mucosal growth and reduced digestion and absorption of nutrients is widely reported when preruminants are fed milk replacers containing a high percentage of soy bean protein products (1). Impaired intestinal synthesis ability appears to be associated with morphological changes in the intestinal structure. Little quantitative and qualitative information is available on nucleic acid (NA) synthesis in intestinal epithelial cells, as well as on the activity of degradative enzyms in NA metabolism. The study was conducted to measure the effect of a partial substitution of casein by soy protein in a milk diet for lambs on RNA and DNA concentrations, as well as on the level of alkaline phosphatase (AP) activity in small intestinal mucosa. AP is involved in nucleic acid breakdown. Likewise, the influence of the dietary protein composition on the pathways of intestinal RNA biosynthesis by incorporation of breakdown products of dietary ^{15}N-RNA into RNA of epithelial cells was investigated.

Methods: Two groups, each of 6 male preruminant goats (Zwergziege, Deutsche weiße Edelziege, aged 7 d) were used. One group was fed for 35 d with cow milk, lactose and casein (160 ml/kg BW), in the other group 35% of milk protein were substituted by soy protein. Five h before slaughter 13 mg yeast ^{15}N-RNA (73 at%-exc.)/lamb were administrated with the milk drink.

Results: The influence of the dietary protein source on villus hight at the jejunum, RNA/DNA ratio, RNA synthesis capacity and AP activity of jejunal epithelial cells is shown in the table (Mean ± SD).

	Casein group	Soy group	*P*
Villus hight *(µm)*			
Jejunum proximal	691 ± 54	577 ± 39	***0.004***
Jejunum medial	631 ± 42	547 ± 38	***0.007***
RNA/DNA			
Jejunum proximal	2.0 ± 0.2	2.0 ± 0.4	*0.785*
Jejunum medial	1.7 ± 0.4	1.8 ± 0.3	*0.869*
Jejunum distal	1.6 ± 0.5	1.5 ± 0.2	*0.446*
^{15}N-RNA *(at% -exc.)*			
Jejunum proximal	0.1101 ± 0.0104	0.1152 ± 0.0133	*0.459*
Jejunum medial	0.1086 ± 0.0182	0.1075 ± 0.0240	*0.940*
Jejunum distal	0.0976 ± 0.0615	0.0309 ± 0.0079	***0.042***
AP *(U^1/mg RNA)*			
Jejunum proximal	16.9 ± 6.8	14.3 ± 4.6	*0.418*
Jejunum medial	9.6 ± 2.8	7.0 ± 4.6	*0.272*
Jejunum distal	3.9 ± 2.0	2.0 ± 0.5	***0.012***

[1]1U enzyme activity catalyses the hydrolysis of 1 µmol p-nitrophenylphosphate per min at 37°C

Conclusion: Morphological changes in intestinal mucosa induced by substitution of 35% milk protein in the diet by soy protein are not accompanied by changes of the RNA/DNA ratio in mucosal cells. In the soy group the lower ^{15}N-RNA enrichment might indicate an increased de novo synthesis of RNA, whereas the decreased activity of AP suggests a decreased nucleic acid breakdown. This points to an altered RNA turnover in the jejunum which needs to be further examined.

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43. Could dietary protein source influence the expression of nutrient and electrolyte transport in the small intestines of preruminant goats? (Könnte die diätetische Proteinquelle die Expression von Nährstoff- und Elektrolyttransporten bei präruminierenden Ziegen beeinflussen?)

Korinna Huber*, Ulrike Schönhusen, S. Kuhla, R. Žitňan, J. Voigt and G. Breves – Hannover/Dummerstorf/ Nitra

Feeding preruminant goats with soy bean protein as a protein source in milk replacers could result in disturbances of intestinal mucosa functions especially absorptive transport processes. Therefore, it was the aim of this study to examine the expression of glucose and peptide transport as nutrient transporter candidates and of phosphate transport as a candidate of electrolyte transporters influenced by dietary soy bean protein. In goats, glucose transport was mediated by SGLT1 (sodium linked glucose transporter), peptide transport by PEPT1 (peptide transporter) and phosphate transport by NaPi IIb (sodium coupled phosphate transporter type IIb, renamed as SCL34A2). The expression was determined on mRNA and protein level in the enterocytes of mid and distal jejunum.

Methods: Control goats (n=5) were fed with a milk replacer containing cow milk, lactose and casein (160 ml/kg BW). 35 % of milk protein was substituted by soy protein in the experimental group (n=7), whereas amino acid composition was adjusted to the control milk replacer. Phosphorus and calcium content was equal in both, control milk replacer and soy protein containing milk replacer. Transporter mRNA and protein expression was determined by semiquantitative northern and western blot analyses using β-actin as an internal standard for relative quantification. Specific DNA probes were created by RT-PCR and were validated by sequencing. Antibodies were get either commercially or as kind gifts of other work groups. Signal detection was done with Quantity One software (BioRad).

Results and Discussion: SGLT1 and PEPT1 mRNA expression was not disturbed in mid and distal jejunum by soy protein application. But mid jejunal NaPi IIb mRNA was not expressed in four of seven goats of the experimental group implicating an influence of soy protein on this transporter mRNA expression. In distal jejunum no effect was observed regarding NaPi IIb mRNA expression. Furthermore, SGLT1 protein expression seemed to be decreased or lack in the apical membranes of mid jejunal enterocytes of soy protein fed goats indicating also an effect of protein source on transporter expression on protein level. But until now, these effects could not be explained on a mechanistical level. Studies of the functional properties of nutrient and electrolyte transport are necessary to determine the physiological consequences of this disturbed transporter expression.

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44. Long-chain polyunsaturated fatty acids in milk produced in lowland and alpine systems

(Langkettige mehrfach ungesättigte Fettsäuren in Milch aus Tal- und Alpproduktionssystemen).

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Milk produced in alpine systems appears to contain more conjugated linoleic acid (CLA) and α-linolenic acid (α-LA) than milk from lowland systems (1). However, it is still unclear, whether this systemic difference reflects uniquely alpine effects or whether it is the result of a more general effect of extensive grazing-based dairy production which also could occur in extensively managed lowland farms (2). Specific alpine effects on milk composition could be caused by the particularly high biodiversity of the pastures as well as by hypoxia associated with altitude (3). The aim of this study was to compare a typical intensive lowland diet for lactating cows with a forage-only diet at a lowland and at a high alpine site with regard to changes in the fatty acid composition of the milk.

Methods: Eighteen Brown Swiss cows in second lactation and on average at the 82nd lactation day were allocated to three treatment groups of six cows each. Group I was kept at 400 m a.s.l. and fed a mixed diet (MD) throughout the experiment. Groups II and III were kept at 400 m during April and May and at 2000 m a.s.l. during June and July. These two groups received fresh herbage either on pasture or indoors. At each site two intensive sampling weeks were carried out. The respective first sampling week took place when the herbage was in the early, intensive growth phase, the second at the flowering stage. Measurements were carried out after 10 to 14 days of adaptation to altitude and/or feeding conditions. Herbage quality in the four measurement weeks was as described in (3). Herbage diversity was significantly higher in the alpine pastures. Feed and milk samples were analysed for fatty acid content and composition by gas chromatography. Herbage fatty acids were separated on a supelcowax-10 column, milk fatty acids on a CP-sil88 column.

Results and Discussion: Green forages differed only slightly in content and composition of fatty acids. The most pronounced change was a decline of total fatty acids and, particularly, of α-LA in the alpine forage during the flowering stage. Concentrates and maize silage of the MD contained only traces of α-LA but more saturated fatty acids and C18:2n-6. Grass silage had the same fatty acid profile as fresh lowland grass. Feeding fresh forages elevated CLA and its precursor C18:1t11 in milk (Group I: 0.31 Groups II and III: 0.79 g CLA/kg milk; $P<0.001$), but there was no difference between milk from the lowland or the alpine site. In contrast, α-LA increased only at the alpine site (from 0.31 to 0.53 g/kg milk; $P<0.001$), as also the other C18-cis fatty acids. Long chain n-3 fatty acids constantly increased during the experiment in groups II and III. The increase of CLA was clearly caused by the fatty acid profile of the green forages, while the alpine specific increase of α-LA cannot be related only to the feed. More likely, and in accordance with (2), it was caused by body fat mobilisation, which is also indicated by significantly increased plasma levels of β-hydroxybutyrate during the alpine sojourn (652 μmol/l in lowlands vs. 1020 μmol/l at the alpine site; $P<0.001$).

Conclusion: Milk from alpine origin contains a dietetically advantageous fatty acid profile. However, CLA contents of milk seem to be elevated by pasture-only conditions rather than by any specific alpine influence. In contrast, there seems to be a clear alpine effect on α-LA, which could be caused by alpine-specific energy deficiencies, due to poorer nutrient contents in the forage and to hypoxia, while fatty acid composition of the alpine herbage does not seem to be a major determinant.

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45. Effects of different isomer ratios and dose-response relationship of CLA in finishing pigs

(Wirkung verschiedener Dosierungen und Isomerenverhältnisse von CLA in Mastschweinen).

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Conjugated linoleic acids (CLA) gained increasing interest in animal and human nutrition mainly due to its potentially beneficial effects reported in studies using animal models and *in vitro* techniques. Results of human studies have been less convincing, which may question the value of strategies to increase CLA in food of animal origin. Nevertheless, CLA was reported to reduce body fat in pigs while increasing intramuscular fat (IMF) and lard firmness, making it an interesting feed additive. Here we briefly summarise the results of two experiments conducted to examine (i) the dose-response of a commercial CLA mixture mainly consisting of the *cis*-9, *trans*-11 (c9/t11) and the *trans*-10, *cis*-12 (t10/c12) isomers, and (ii) the effects of different ratios of these CLA isomers, when used as feed supplements for finishing pigs.

Methods: In the dose-response trial, 97 female and castrated male Large White and Swiss Landrace pigs were allocated to five experimental groups, balanced according to gender, breed, litter and initial weight, and fattened from 66 to 106 kg live mass on a control diet containing 2 % palm oil or the same diet, containing 0.25, 0.49, 0.72, or 0.95 g CLA/100g feed, partly replacing the palm oil. In the second experiment 96 Duroc × (Landrace × Large White) castrates were fed a common finisher diet supplemented with either 0.5 % sunflower oil or one out of three oils containing the two CLA isomers c9/t11 and t10/c12 in the ratios 3:1, 1:1 or 1:3. These animals were fattened in two consecutive series from about 66 to 120 kg and 68 to 124 kg, respectively. All animals had *ad libitum* access to the experimental diets. Analyses of variance were performed with feeding treatment, gender, their potential interaction, and sire (i) as fixed effects. Liner contrasts were calculated to test the dose-response relationship of CLA or the CLA isomers and the measured characteristics of growth performance, carcass composition, meat quality and backfat composition and firmness.

Results: Growth performance and feed conversion efficiency was not affected by CLA in any of the experiments. Backfat thickness of the CLA fed animals tended to be lower than of control animals ($p=0.05$) in all CLA treatments of the dose-response trial, whereas no consistent effect on lean percentage was found in any of the experiments. This is in contrast to other studies but consistent with our earlier findings. Meat quality in terms of pH, colour, drip loss, cooking loss, and texture was not affected by CLA. The IMF was only numerically higher in the *m. longissimus dorsi* of CLA fed animals (i). The proportion of both CLA isomers in backfat (i, ii) and muscle neutral as well as phospholipids (ii) increased linearly with increased intake. The *t*10/*c*12 isomer, however, was incorporated to an appreciably lower degree than the *c*9/*t*11 isomer. Additionally, a very clear dose dependent increase of saturated fatty acids at the expense of monounsaturated fatty acids (MUFA) with increased CLA intake was found in backfat. Concomitantly, lard firmness was raised remarkably up to values four times higher than in the control. The isomer experiment (ii) clearly revealed that this effect may be attributed to the *t*10/*c*12 isomer, which also tended to decrease the proportions of arachidonic acid (C20:4n-6) in meat and lard.

Conclusion: The *t*10/*c*12 CLA isomer reduces MUFA in backfat very efficiently and markedly improves lard firmness, which is desirable in terms of processing quality aspects and may give some space for a higher proportion of valuable polyunsaturated fatty acids in pig feed. Already 2.5 g/kg feed of the 1:1 isomer mixture proved to be effective. Thus, manufacturing CLA-oils containing predominantly one of the CLA-isomers may not be necessary when it is intended to be used as feed supplement in order to improve backfat firmness of finishing pigs.

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46. The effect of dietary conjugated linoleic acids on the metabolism of α-linolenic acid and the formation of eicosanoids in rats (Einfluss konjugierter Linolsäuren auf den Stoffwechsel der α-Linolensäure und die Bildung von Eicosanoiden bei Ratten). K. Eder, Nadine Slomma and Karin Becker – Halle (Saale)

It has been shown that dietary conjugated linoleic acids (CLA) impair the desaturation of linoleic acid to arachidonic acid, and lower the release of eicosanoids deriving from arachidonic acid. The fact that the desaturation of α-linolenic acid (ALA) to eicosapentaenoic acid (EPA) proceeds by the same pathway, makes it probable that CLA also impairs the desaturation of ALA. The effect of dietary CLA on the metabolism of ALA, however, has not yet been studied in detail. The objective of this study was to investigate the effect of dietary CLA on the metabolism of ALA and the formation of eicosanoids in rats. We planned to study the effect of dietary CLA in comparison to fats rich in linoleic acid or oleic acid. Therefore, we used two control fats, namely sunflower oil (SFO) which is rich in linoleic acid and high oleic-acid sunflower oil (HO-SFO) which is rich in oleic acid.

Methods: An experiment with 45 male growing rats was performed which were divided into three groups of 15 each. They were fed diets with 30 g/kg of either a CLA oil (containing 32.2 g *cis-9, trans-11* CLA and 33.3 g *trans-10, cis-12* CLA per 100 g), or SFO or HO-SFO for 4 wk. Thereafter, 5 rats of each group were killed and used for analysis. In the 5th wk, the remaining 10 rats of each group received the same diets supplemented with 50 g of linseed oil/kg as a source of ALA. During both feeding periods, diets were administered at restriced amounts to stadardize the diet intake. At the end of the 5th week, the remaining 10 rats of each group were used for analysis. To study the effect of CLA on the metabolism of ALA, the amounts of individual (n-3) polyunsaturated fatty acids (PUFA) in liver phospholipids [phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE)] were determined; to study the effect on eicosanoid formation, the concentrations of various eicosanoids in liver and plasma, the activity of the secretory phospholipase A_2 ($sPLA_2$) and the relative mRNA concentrations of cyclooxygenases-1 and -2 in the liver were measured.

Results: Body weights at the end of the experimental period did not differ between the three groups of rats. Supplementation of the diets with ALA caused a strong increase of the amounts of total (n-3) PUFA in liver PC und PE of the rats. This effect, however, was greater in rats fed CLA (increase from 5.6 to 24.3 g/100 g fatty acids in PC and from 3.0 to 30.1 g/100 g fatty acids in PE) than in rats fed SFO (increase from 3.0 to 9.8 g/100 g fatty acids in PC and from 2.5 to 17.8 g/100 g fatty acids in PE) and rats fed HO-SFO (increase from 4.6 to 18.6 g/100 g fatty acids in PC and from 3.3 to 27.5 g/100 g fatty acids in PE). Rats fed CLA had also a higher mRNA concentration of Δ6-desaturase in the liver, higher concentrations of total desaturation products of ALA [EPA + docosapentaenoic acid + docosahexaenoic acid], a higher ratio between (n-3) and (n-6) PUFA, a higher ratio of (n-3) PUFA with 22 carbon atoms to EPA and lower concentrations of total C20-PUFA in liver phospholipids than rats of the other two groups. The concentration of arachidonic acid in liver phospholipids was lower in rats fed CLA than in rats fed SFO or HO-SFO. Rats fed the CLA diet had also lower concentrations of various eicosanoids in liver and plasma and a lower activity of $sPLA_2$ in the liver than rats of the two other groups; the relative mRNA concentrations of cyclooxygenases were not different between the three groups of rats.

Conclusion: The study suggests that dietary CLA stimulates the formation and the incorporation of long-chain (n-3) PUFA into liver phospholipids at the expense of (n-6) PUFA. Reduced concentrations of eicosanoids in the liver of rats fed CLA may be the result of reduced concentrations of C20-PUFA in liver phospholipis and a reduced release of C20-PUFA by phospholipase A_2.

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47. Gene expression profile in the liver of rats fed diets with an oxidized fat and various concentrations of vitamin E (Genexpressionsprofil in der Leber von Ratten bei Diäten mit oxidierten Fetten und unterschiedlichen Vitamin E-Konzentrationen). K. Eder, Andrea Sülzle and F. Hirche – Halle (Saale)

The consumption of oxidized fats containing high concentrations of lipid peroxidation products affects the animal metabolism in several ways. The effects of oxidized fats on the animal metabolism, therefore, might be due to an altered expression of many genes. The present study was undertaken to identify a comprehensive collection of genes in the liver whose expression is influenced by dietary oxidized fats. To investigate which effects of oxidized fats can be prevented by an increased supply of antioxidants, diets with different vitamin E concentrations were used.

Methods: An experiment with 40 male rats was performed which were allotted to four groups of 10 each. They were fed semisynthetic diets with 100 g fat/kg. The diets differed in their type of fat (fresh fat vs. oxidized fat) and the vitamin E concentration (25 vs. 250 mg vitamin E/kg). The four diets were: fresh fat, 25 mg vitamin E/kg (FF25), fresh fat, 250 mg vitamin E/kg (FF250), oxidized fat, 25 mg vitamin E/kg (OF25), oxidized fat, 250 mg vitamin E/kg (OF250). The oxidized fat was a mixture of sunflower oil and lard (1:1) which was heated for 38 d at 50°C. The fresh fat was a mixture of sunflower oil and lard in the ratio of 31:69. The fatty acid composition of both fats was similar but the oxidized fat had much higher concentrations of peroxides (918 vs. 5 mEq O_2/kg), thiobarbituric acid reactive substances (22 vs. 0.01 mmol/kg) and conjugated dienes (277 vs. 14 µmol/kg) than the fresh fat. To standardize the feed intake diets were administered in restricted amounts. Diets were fed for 9 weeks. Total RNA was extracted from the liver. The gene expression profile of the liver was determined with use of a cDNA array containing 1176 unique cDNA spotted on a nylone membrane (Hybond Atlas rat 1.2 array, Clontech, Heidelberg, Germany). Gene expression of FF250, OF25 and OF250 rats was compared with that of FF 25 rats which served as a control. A difference in the expression of a gene was considered significant at a ratio threshold of 1.4 or more.

Results: Rats fed the oxidized fat diets had only slightly lower body weight gains but a markedly lower vitamin E status than rats fed the fresh fat diets. Approximately 400 of the 1176 genes on the array yielded signal intensities above background levels. In the OF25 rats, the expression of 48 genes was altered (39 increased, 9 decreased), in the OF250 rats the expression of 37 genes was altered (27 increased, 10 decreased) and in the FF250 rats, the expression of 22 genes was altered (19 increased, 3 decreased) as compared to FF25 rats. In both, OF25 and OF250 rats, there was overexpression of a series of genes involved in mitochondrial and peroxisomal ß-oxidation and microsomal ω-hydroxylation of fatty acids. Most of them were target genes of the peroxisome proliferation activating receptor α (PPAR α). Determination of gene expression of acyl CoA oxidase and activity of catalase, both indicator enzymes of peroxisomes, confirmed that oxidized fats caused peroxisome proliferation, irrespective of dietary vitamin E concentration. OF25 and OF250 rats had also reduced gene expression and activities of lipogenic enzymes and an increased gene expression of Δ5- and Δ6-desaturase. OF25 and OF250 rats had reduced concentrations of triglycerides and cholesterol, increased concentrations of phospholipids and reduced concentrations of highly unsaturated (n-3) PUFA in liver phospholipids. These alterations are consistent with the altered expression of genes involved in lipid metabolism. In OF25 and OF250 rats, there was also overexpression of 12 and 5, resp., genes involved in xenobiotic metabolism and stress response, of 7 and 7, resp., genes involved in protein metabolism, of 5 and 2, resp., genes encoding intracellular effectors or modulators and of 5 and 6, resp., genes encoding activators or repressors of transcription or translation.

Conclusion: This study shows that a dietary oxidized fat influences the expression of many genes in the liver which encode proteins involved in several metabolic pathways. The expression profile of genes involved in lipid metabolism shows that dietary oxidized fats activate the PPARα and enhance the catabolism of lipids in the liver, irrespective of the vitamin E supply.

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48. Effects of long chain ω3 fatty acid supplementation on fatty acid profiles and lung function in horses affected with RAO (Recurrent Airway Obstruction) (Effekte von langkettigen ω3 Fettsäuren auf Pferde mit RAO).

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Equine Recurrent Airway Obstruction (RAO) is the most important respiratory disease in horses. Different investigators have compared RAO to human asthma, both of which are characterised by mucosal inflammation, airway obstruction, and airway hyperreactivity. Long term use of long chain ω3 fatty acids (LCω3FA) has been shown to have beneficial effects on human asthma, due to their immunomodulatory effects. The present study aimed to test the hypothesis, that incorporation of LCω3FA, derived from seal oil, modulates plasma and leucocyte-phospholipid fatty acid composition in equines, has an impact on inflammatory processes induced by RAO and therefore has a favourable effect on lung function.

Methods: Using a cross-over study design, a feeding trial with nine horses affected by RAO was performed. Each animal received seal oil (STO) at a dose of 320 mg/kg bodyweight (BW) and day, providing about 70 mg/kg BW of LCω3FA as test substance or the same amount of sunflower oil (SBO) as control substance for ten weeks each. Fatty acid compositions of plasma and leucocyte-phospholipids were analysed by GC and calculated as normalised percent peak area. Lung function measurements, bronchoalveolar lavage fluid (BALF) cytology and detailed clinical examinations were performed. Data were analysed using Mann-Whitney- as well as ANOVA- and Scheffé-tests.

Tab. 1: Fatty acid characteristics of oils (% of total fatty acids)

	C16:0	C16:1	C18:0	C18:1ω7	C18:1ω9	C18:2	C20:4	C20:5	C22:5	C22:6
SBO	5,23	0,06	4,30	0,67	24,90	64,07	n.d.	0,08	n.d.	0,22
STO	7,72	17,36	0,97	4,63	24,34	2,02	0,55	8,48	6,57	8,57

n.d.= not detectable

Results: Different diets clearly affected fatty acid composition of plasma and leucocyte-phospholipids. A significant decrease in linoleic acid (C18:2ω6) percentage could be observed after STO diets, compared to SBO ($p<0.001$). Differences also were observed between STO and no oil supplementation in plasma ($p<0.001$) and between STO and SBO in leucocyte-phospholipids ($p<0.05$), STO showing a marked decrease in C18:2ω6. STO induced a highly significant increase ($p<0.001$) in plasma C20:5ω3, C22:5ω3, and C22:6ω3. In leucocyte-phospholipids elevation of C20:5ω3 and C22:6ω3 ($p<0.01$ and <0.05, respectively) could be observed. Accumulation of supplemented fatty acids by increasing feeding time was evident. Total BALF leucocytes were reduced significantly by STO supplementation ($p= 0.035$), neutrophil granulocytes were reduced tendentially ($p=0.1$). Neither lung function nor clinical signs could be influenced significantly by STO or SBO diets.

Conclusion: Supplemented LCω3FA were effectively incorporated by horses, showing a time dependent tendency for accumulation. This study did not investigate the influence of LCω3FA on the concentration of lipid mediators derived from C20:4ω6 or C20:5ω3, but reduction of BALF cell counts supports their antiinflammatory properties. Nevertheless the severeness of RAO could not be influenced in our trial, possibly unstable environmental conditions or too short feeding periods being responsible for this. Incorporation and conversion of polyunsaturated fatty acids is a slow acting process, therefore further studies are required to investigate long term effects of LCωFA on inflammatory response in horses.

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49. Performance, carcass quality and fatty acid composition of trout (Salmo gairdnerii) as affected by dietary conjugated linoleic acid and vitamin E supplementation (Zootechnische Parameter, Schlachtkörperqualität und Fettsäuremuster von Forellen nach Zulage von CLA und Vitamin E).

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Conjugated linoleic acids (CLA) are of major interest in fatty acid research, largely because of their potential health benefits. In order to produce human food enriched with CLA fish (trout) seems to be of special importance because fish is already thought to be healthy food and fish feed contains high fat portions up to more than 20%. Therefore the aim of this study was to determine the effects of graded levels of CLA and different vitamin E supplementation on performance, carcass quality and fatty acid composition of trout (Salmo gairdnerii).

Methods: Three levels of CLA (as methylester with the isomers of 47.7% C18:2 t10, c12 and 50.8% C18:2 c9, t11 – supplied by BASF) in portions of 0%, 1% and 3% and two levels of α-tocopherylacetat (150 mg and 600 mg/kg) were used as supplements in a total of six different diets. The diets consisted of 41.3% fish meal , 18.5% soybean protein concentrate, 9.0% soybean oil, 4.0% linseed oil, 3.0% sunflower oil (in exchange with CLA) 4.2% vitamins and minerals, 100 mg BHT and 400 mg astaxanthin per kg (in average: 45.2% XP, 20.9% total fat, 0.5% XF, 11.2% XA i. FM of the diet). Each diet was fed to two tanks (18 m^3 water, 9.2°C, 9.1 mg O_2/ l) with 500 trout per tank (initial weight of 120 g) in amounts of 1.2% (week 1-8) and 1.0% (week 9-14) of live weight. Trout were slaughtered after a feeding period of 6 (S1), 10 (S2) and 14 weeks (S3) and one group was kept for an additional period of 6 weeks without feed (week 20, S4). For further analytical procedures the trout were divided in fillet, viscera and remaining carcass. Besides the fresh samples additional fillets of S3 and S4 were deep frozen stored for 6 month.

Results: The trout had a high specific growth rate of 1.21%, reached a mean final live weight of 383 g and showed a mean feed efficiency of 0.78 kg feed per kg growth. The average fat content of fillet (total carcass) increased from 4.6% (11.4%) (S1) up to 6.5% (15.0%) (S3) and decreased to 4.2% (12.8%) (S4), whereas the protein content differed only slightly (fillet: S1: 18.6%, S3 and S4: 19.4%; total carcass: S1: 16.9%, S3 and S4: 17.2%). There was no significant effect of the different diets on performance or carcass composition. Also the fatty acid composition was not influenced by the vitamin E content of the diets, the deprivation of the feed or the frozen storage. However, CLA supplementation significantly increased the CLA content of the fillet depending on the duration of feeding and portion in the diet up to an average of 7.0% i. FAME (S3, 3% CLA in the diet). With rising CLA content the sum of SFA slightly increased and the sum of MUFA decreased, whereas the PUFA (including CLA) stayed nearly constant. Only in the treatments with 3% CLA a small increase of PUFA was measured. Over all treatments the CLA isomers showed a constant composition of an average of 38.9% C18 t10, c12 and 56.2% C18 c9, t11. The sensory characteristics (odour, flavour, texture) of trout fillet were not affected by CLA or vitamin E supplementation. Surprisingly the flavour of all deep frozen fillets with the treatments of 3% CLA and 600 mg vitamin E per kg was evaluated distinctly positive.

Conclusion: This study has shown that trout is capable to accumulate dietary CLA in fillet without altering performance and carcass quality.

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50. Comparison of CLA methyl esters and sunflower seeds in their effects on milk fat content and fatty acid profile in cows (Vergleich von CLA-methylestern und Sonnenblumenkernen hinsichtlich ihres Einflusses auf den Milchfettgehalt und das Milchfettsäurenmuster bei Kühen)

Frigga Dohme*, M. Collomb and G. Bee – Posieux/Bern

Feeding protected conjugated linoleic acids (18:2*c*9*t*11/18:2*t*10*c*12, CLA) or seeds rich in linoleic acid (18:2*c*9*c*12) increases the CLA content in the milk fat of cows. However, little is known whether similar effects can be expected from unprotected CLA.

Methods: The experiment was conducted with 16 dairy cows (milk yield: 29.8 ± 0.4 kg/d; days in milk: 137 ± 6 d) which were randomly assigned to one of three supplementation treatments: 1) 1.0 kg/d ground sunflower seeds (SF; n=5), 2) 0.5 kg/d CLA (CLA; n=6) or 3) 0.75 kg/d of a mixture of sunflower seeds and CLA (SFCLA; n=5). The supplements CLA and SF had the same amount of 18:2 either as CLA or as 18:2*c*9*c*12. The CLA-oil (BASF-AG, Offenbach, Germany) was supplied as methyl ester, with a total CLA concentration of 60% equally composed of 18:2*c*9*t*11 and 18:2*t*10*c*12 isomers. The cows had free access to hay and fodder beets whereas the energy and protein concentrates were adjusted according to the milk yield and live weight. After being accustomed to the same basal diet for 11 d, the cows were fed the respective supplements for 18 d. During the last 4 d of the experimental period feed intake, live weight and milk yield were daily recorded and milk samples were collected at each milking. Blood samples were collected from the jugular vein on d 15 and 18 of the experimental period. Feeds were sampled throughout the experiment and analysed by standard procedures. The analysis of fatty acid composition of the milk fat was carried out according to the method described by Collomb and Bühler (1).

Results: Daily intake of dry matter (21.5 kg), total fatty acid (744 g), 18:2*c*9*c*12 and CLA (404 g) as well as live weight (664 kg) and milk yield (28.5 kg) were not affected by the supplements. Although treatments had no influence on milk protein content and yield, fat content (SF: 3.6%; CLA: 2.1%; and SFCLA: 2.0%) and yield (SF: 1101 g; CLA: 548 g; and SFCLA: 586 g) were depressed ($P < 0.001$) in the CLA and SFCLA compared to the SF treatment. The lactose content differed ($P < 0.01$) between treatments being highest in the SFCLA (5.0%), lower in the SF (4.8%) and lowest in the CLA (4.6%) group. The concentration of saturated fatty acids (< 14:0) was increased ($P < 0.01$) in the milk of cows fed SF compared to those fed CLA or SFCLA. The highest CLA concentration was found in the CLA and the lowest in the SF group ($P < 0.001$). The 18:2*c*9*t*11 concentration was similar in the CLA and SFCLA treatments but higher ($P < 0.001$) than in the SF treatment. As expected the 18:2*c*9*c*12 content of milk from cows fed either SF or SFCLA was higher ($P < 0.001$) compared to milk from cows fed CLA. Although the fat content was lowest in the CLA group, the amount of daily excreted 18:2*t*10*c*12 was higher compared to the SF and SFCLA groups, whereas no differences among treatments were found in the amount of excreted 18:2*c*9*t*11. Compared to the SF and CLA treatment the 16:1/16:0, 18:1/18:0 and CLA/18:1*t*10/11 ratios were higher ($P < 0.05$) in the SFCLA treatment. In the plasma the free fatty acid concentration of cows fed CLA or SFCLA was higher than of cows fed SF ($P < 0.05$).

Conclusions: Although the dietary concentration of 18:2*t*10*c*12 was highest in the CLA treatment, the partial replacement of CLA by sunflower seeds had the same inhibitory effect on milk fat synthesis. The dietary CLA supply caused an increase of CLA isomers in the milk fat but due to the low milk fat content the daily excretion of CLA in the milk was markedly higher with sunflower seeds.

1) COLLOMB, M. and BÜHLER, T., (2000): Mitt. Lebensm. Hyg. 91: 306-332.

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51. Influence of the dietary fat content on the oral bioavailability of quercetin-3-*O*-glucoside and quercetin in pigs (Einfluss des Fettgehalts der Diät auf die orale Bioverfügbarkeit von Quercetin-3-*O*-Glukosid und Quercetin beim Schwein).

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A recent study reported that the bioavailability of quercetin-3-*O*-glucoside (Q3G) in pigs was dependent on dietary factors. Oral administration of the flavonol together with ground beef led to a significantly higher bioavailability than administration with a commercial pig diet based on wheat, barley and soybean meal (1). We speculated that the different fat content of these two diets could have been the reason for the observed difference in bioavailability. Thus, we performed this study in order to investigate the effect of the dietary fat content on the bioavailability of Q3G and of quercetin aglycone (Q).

Methods: Male castrated growing pigs (30-35 kg body weight (b.w.)) equipped with a permanent jugular catheter were used. They were restrictively fed (80% of the ad libitum feed intake) twice daily with a commercial pig diet based on wheat, barley and soybean meal (17 % crude protein, 2.7 % crude fat, 4.6 % crude fibre and 12.8 MJ ME/kg diet). Each animal received in consecutive experiments either Q or Q3G (30 µmol/kg b.w.) mixed into a test meal. The test meal consisted either of 200 g of the regular commercial diet or of isocaloric meals containing the same diet and 15% or 30% lard. Blood samples were drawn over a period of 24 h and aliquots were analyzed by HPLC with prior β-glucuronidase/sulfatase treatment. Total bioavailability (AUC_{total}) was calculated by adding up bioavailabilities of all metabolites with an intact flavonol structure.

Results: After treatment with β-glucuronidase/sulfatase, metabolites of Q or Q3G found in plasma were identified as quercetin and the methylated quercetin derivatives isorhamnetin and tamarixetin. Total bioavailability of Q was significantly higher from the meal containing 15% lard (AUC_{total} = 184.5 ± 19.8 min µmol l^{-1}, n=6; $p < 0.05$) than from the regular diet low in fat (AUC_{total} = 117.3 ± 18.5 min µmol l^{-1}, n=7). AUC_{total} of Q administered together with the meal containing 30% lard (176.0 ± 18.5 min µmol l^{-1}, n=7) was not different, though, from AUC_{total} after the 15% lard meal. Similar to Q, bioavailability of Q3G from the 15% lard meal (AUC_{total} = 270.9 ± 19.8 min µmol l^{-1}, n=6) was significantly higher than bioavailability from the regular diet (AUC_{total} = 205.5 ± 19.8 min µmol l^{-1}, n=6; $p < 0.05$). Q3G bioavailability after the 30% lard meal (249.7 ± 19.8 min µmol l^{-1}, n=6) was not different from that after the meal containing 15% lard. When bioavailability between Q and Q3G were compared at the various diets, Q3G bioavailability was always significantly higher ($p < 0.05$).

Conclusion: In accordance with previous results, this study showed that bioavailability from Q3G in pigs was always higher than that from Q. Furthermore, the bioavailability of the flavonols quercetin and quercetin-3-*O*-glucoside was influenced by the dietary fat content. Increasing the fat content of a regular pig diet by addition of lard (15%) improved bioavailability of both flavonols significantly. However, increasing the lard content to 30% had no further effect. It is concluded that the intestinal absorption of the lipophilic flavonol quercetin is enhanced by dietary fat.

1) CERMAK, R. et. al. (2003): J.Nutr. 133: 2802-2807.

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52. Effect of dietary sodium bicarbonate supplementation on the toxicokinetics of ochratoxin A and B in pigs (Zum Einfluss einer Natriumhydrogencarbonat-Zulage zur Diät auf die Toxikokinetik von Ochratoxin A und B beim Schwein).

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Ochratoxin A (OA) is a mycotoxin produced by several species of Aspergillus and Penicillium. It is regarded as a causative agent for endemic nephropathy in farm animals and humans. Due to extensive reabsorption along the nephron, OA accumulates in the kidney. This is of toxicological importance because renal reabsorption contributes to the long half-life of the mycotoxin in the body. Microinfusion studies in rats showed, that reabsorption of OA results from nonionic diffusion and by carrier-mediated mechanisms indicating that urine alkalinization may help to accelerate OA excretion and thus reduce its toxicity. The aim of the present study was to investigate the effect of a dietary sodium bicarbonate supplementation as a means to increase urinary pH on the systemic availibility and excretion of OA in pigs.

Methods: Seven pigs (36.7 ± 2.0 kg), equipped with a permanent jugular vein catheter were assigned to the following two treatments. Treatment 1 (control group, n=3) received a commercially avaible pig diet with 13.5 MJ ME and 17.4 % crude protein, while treatment 2 (sodium bicarbonate, n=4) received the same diet supplemented with 2 % sodium bicarbonate. Dietary electrolyte balance (Na+K-Cl) of the control and sodium bicarbonate group were 170 and 400 meq/kg, respectively. The animals were fed twice daily and daily feed intake was 1400 g/d. After an adaptation period of 4 d, all animals received a single dose of 150 g wheat containing 2.42 mg OA, 0.36 mg OB, and 0.03 mg Oα. After exposure to OA, jugular blood samples, urine and faeces were collected over a period of 96 h. Ochratoxin A, OB and Oα were analyzed in blood serum, urine and faeces by HPLC.

Results: Daily gain and feed efficiency were not influenced by treatment. Supplementation of sodium bicarbonate increased ($P < 0.05$) urinary pH (5.68 ± 0.20 vs. 8.25 ± 0.12) and daily urine volume (1108 ± 276 vs. 2479 ± 912 ml). Sodium bicarbonate reduced ($P < 0.05$) the systemic availibility of OA and OB based on AUC (area under the curve) values to 75 and 68 % compared to the control group. This effect was mainly due to an increased elimination of OA and OB in the urine (Table: Excretion of OA, OB and metabolites in urine and faeces in percent of its respective intake).

		Control	Sodium bicarbonate		Control	Sodium bicarbonate
Urine	OA	5.5 ± 1.9^{A}	16.5 ± 4.2^{B}	OB	26.9 ± 5.1^{A}	60.5 ± 16.0^{B}
	Oα	1.7 ± 0.3^{A}	3.6 ± 0.9^{B}	Oβ	n. a.	n. a.
	Sum	7.2 ± 2.0^{A}	20.1 ± 4.3^{B}	Sum	26.9 ± 5.1^{A}	60.5 ± 16.0^{B}
Faeces	OA	0.8 ± 0.2	1.1 ± 0.8	OB	7.2 ± 0.8^{A}	1.5 ± 3.2^{B}
	Oα	45.4 ± 2.7	41.0 ± 4.5	Oβ	n. a.	n. a.
	Sum	46.2 ± 2.9	42.1 ± 4.0	Sum	7.2 ± 0.8^{A}	1.5 ± 3.2^{B}
	Total	53.3 ± 4.7^{A}	62.2 ± 1.5^{B}	Total	34.1 ± 5.8^{A}	62.0 ± 13.1^{B}

n. a.: not analyzed; $^{A, B}$ Means in the same row with different superscripts in each table differ ($P < 0.05$)

Conclusion: The results of this study show that alkalinization of urinary pH accelerates the renal excretion of OA and OB. The faster renal elimination might be explained by a reduced reabsorption of OA by nonionic diffusion, because at higher urinary pH OA mainly exists in its ionized form. It is further concluded, that urinary alkalinization may be an efficient means to partially reduce the toxic effects of OA in pigs.

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53. Fiber-related digestive processes in modern crossbreed pigs and old pig breeds (Faser bezogene Verdauungsprozesse bei Schweinen moderner Gebrauchskreuzungen und alter Landrassen).

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Consequent selection on high daily body weight gains and high feed utilization with feeding concentrated feedstuff may has led to a loss of the ability for fiber degradation in modern pigs.
In a 2 factorial experiment with three different breeds, (Schwäbisch Hällisches Schwein (SH), Buntes Bentheimer Schwein (BB) and crossbreed pig (CB)) each with 12 animals and two different diets (basal diet and basal diet + sugar beet pulp (SBP)) fiber-related digestive processes were studied. Basal diet was calculated to meet 80% of energy and 100% of N-requirement for average daily body weight gains of 700g/d. Pigs had an average weight of 37,9± 5,3kg and were first used for a metabolic trial. At the end of this, pigs were slaughtered and their caecum content was used for the Colon Simulation Technique (COSITEC). Fiber digestibility, fecal N fractions and pH value in the caecum content were determined as indicators for microbial activity in the hindgut of pigs.

Results: There was no influence of the breed considering digestion of NDF. This was very different if crude fiber that consists of slower degradable fiber fractions was regarded. Breed and diet did interact with each other. When feeding SBP BB were digesting significantly more crude fiber than CB. Partition of fecal N showed an interaction between diet and breed when undigested dietary N (UDN) and bacterial endogenous debris N (BEDN) were taken in account. When SBP diet was fed UDN fraction was highest in CB (26,46%) and lowest in BB (12,37%). The portion of BEDN was significantly higher in SH and BB (60,48 and 68,76%) compared to CB (47,55%). pH value measured in caecum content also showed an interaction of diet and breed. The diet-related changes in pH values were associated with SCFA production rates observed during COSITEC. When feeding high fiber diet, pH values were lowest and SCFA production rates highest for BB and SH whereas pH was not influenced by diet considering CB pigs.

	Diet		Breed			Inter-action
	Basal	SBP	SH	BB	CB	
DM intake	$69,70^{a}$	$85,49^{b}$	78,09	77,34	77,35	
NDF intake	$9,25^{a}$	$15,81^{b}$	12,68	12,48	12,45	
CF intake	$2,27^{a}$	$4,97^{b}$	3,65	3,6	3,6	
NDF digested	$5,39^{a}$	$10,13^{b}$	7,57	7,85	7,87	0,548
CF digested	0,73	2,71	1,65	1,82	1,69	0,033
Fecal N ($g/kg^{0,75}$*d)	$0,30^{a}$	$0,44^{b}$	$0,39^{a}$	$0,46^{b}$	$0,46^{b}$	0,350
UDN (% of fecal N)	9,47	18,43	11,52	12,63	17,68	0,000
WSN (% of fecal N)	21,17	22,64	$18,28^{a}$	$20,55^{a}$	$26,90^{b}$	0,205
BEDN (% of fecal N)	69,36	58,93	70,19	66,82	55,42	0,028
pH	6,60	6,51	6,53	6,53	6,59	0,000

Means in the same row with different superscript are significantly different (P<0.05)

Conclusion: The ability of digesting high fiber feedstuff and the contribution of SCFA as an energy source differs among the three breeds being used. Different microbial activity in the hindgut affects fecal N- excretion and total N-metabolism in pigs of different breeds. However there is no clear indication for higher fiber degradation in old pig breeds. SH showed even lower fiber digestion than CB, which were expected to have a reduced capacity of fiber degradation caused by selection process.

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54. Influence of particle size of grass silage fed as TMR on chewing activity and rumen fermentation on high-yielding dairy cows (Einfluss der Partikellänge einer TMR auf Kauaktivität und Fermentation im Pansen bei Hochleistungskühen).

M. Tafaj*, Q. Zebeli, B. Junck and W. Drochner – Stuttgart-Hohenheim

Objective: Fibre effectiveness of rations becomes more important in feeding of high-yielding dairy cows and is strongly influenced by the forage particle size. This study aimed to investigate the effects of the particle size (**PL**) of grass silage (**GS**) on fibre effectiveness of a Total Mixed Ration (**TMR**) using the parameters of fermentation in the rumen and chewing activity as indicators.

Methods: The experiment was carried out with 4 Holstein cows (BW 640kg) in the early lactation (36kg Milk/d) fitted with ruminal cannulas. **GS** (31.3% DM, 13.2% CP and 46.5% NDF in DM, degradation rate of DM [**DM-k_d**] 0.087) that was harvested at 25mm theoretical length of cut provided the coarse silage for the diets. Middle and finely chopped silage were obtained by re-cutting the ensiled GS in a self-propelled forage harvester (CLAAS-Jaguar 675) at 11mm and 5.5mm, respectively. The GS of three levels of particle size was fed as TMR (45.9% DM, 15.1% CP and 35% NDF in DM, **DM-k_d** 0.105) with a concentrate (**C**) mixture (18.7% CP, 19.3% NDF, **DM-k_d** 0.127) and coarse fibre-rich hay (**H**) in a ratio of GS:H:C=45:5:50. Cows were fed *ad lib* twice daily at 0800 and 1600 h. After an adaptation period of 11d, the chewing activity was recorded continuously for 8d (at least 3d per animal) using a PC-based program. Digesta samples were taken on three cows from different rumen digesta layers (**D**) in 4 sampling times (**T**: 0730, 1030, 1500 and 1900h). Each sampling was repeated two times. The time interval between samplings was at least 8h. Approx. 300g digesta was taken from dorsal (**SRF-T**) and ventral (**SRF-B**) rumen (TAFAJ et al., 2001), manually squeezed and filtered through cloth bags. Approx. 300ml free liquid (**FRL**) from ventral rumen was collected by a sucking-pump. The pH, concentration of VFA, NH_3, bicarbonate and buffer capacity were measured. The effects of PL and D on fermentation were tested using a model for double (D, T) repeated measures (MIXED PROC, SAS, 8.2). The effect of PL on chewing activity was tested by analysis of variance (GLM PROC). Least significant difference (**LSD**) was computed.

Parameters	Time	Particle size (PL), mm			LSD	Digesta layers (D)			LSD	Significance
		25	11	5.5	(α=0.05)	SRF-T	SRF-B	FRL	(α=0.05)	P < 0.05
pH	0700	6.12	6.26	5.76	0.33	5.76	6.13	6.25	0.15	PL, D
	1030	5.83	5.90	5.77	0.28	5.57	5.75	6.16	0.21	D
VFA, mmol/l	0700	125.2	118.7	141.0	9.37	139.3	125.2	120.4	9.37	PL, P, PL*D
	1030	133.5	135.5	137.9	16.45	145.8	139.2	121.5	11.46	D, PL*D
DMI, kg/d		20.2	22.3	22.2	2.21		-			ns
Rumination, h/d		8.14	7.93	8.22	1.20		-			ns
Eating, h/d		6.65	5.90	4.51	2.13		-			PL

Results: A significant influence of PL was observed at 5.5mm level and was more pronounced on the total VFA and ammonia concentration as well as buffer capacity of rumen digesta. pH-value, A/P-ratio and concentration of bicarbonate were affected significantly by PL only at 1h before morning feeding (0700). At 5.5mm PL the pH was below 6.0 across the day, also before morning feeding. The chewing and eating time were reduced significantly at PL of 5.5mm, while the rumination time was not affected by PL. There were significant differences in the parameters of fermentation between top and bottom rumen digesta layer independent from PL.

Conclusion: As far as a tendency of negative influence of PL on rumen conditions was observed only by the finely chopped GS (5.5mm), it could be concluded that chopping GS (at least) up to 11mm did not negatively affect the fibre effectiveness of GS-based TMR diets containing 5% coarse fibre-rich hay and 50% concentrate with high degradation rate.

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55. Ruminal nutrient degradation of untreated and chemically treated wheat grain (Nährstoffabbau im Pansen von unbehandeltem und chemisch behandeltem Weizen).

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The objective of this study was to compare ruminal nutrient degradation of untreated wheat grain (dry matter basis: 67.2% starch, 13.4% crude protein [CP]) as compared to a wheat grain that was chemically treated with xylose in an aqueous calcium-magnesium lignosulphonate solution at elevated temperatures (WeiPass®; 55.6% starch, 14.8% CP).

Methods: A standardized *in situ* procedure was applied using three ruminally cannulated mature steers. Water-soluble feed fractions were determined and feed samples were incubated in the rumen for 0, 2, 4, 8, 16, 24, 48, 72 and 336 h to estimate rate and extent (effective degradability at assumed rumen outflow rates of 2, 5 and 8%/h) of ruminal degradation of CP and starch. The proportions of ruminally undegraded CP (**RUP**) and ruminally undegraded starch (**RUS**) were estimated as (100 – effective degradability [%]).

Results: The water-soluble fraction of CP (11.1 versus 16.7%) and starch (18.2 versus 18.4%) and the lag phase before the commencement of degradation (all values < 0.5 h) were not different (P > 0.15) between wheat and WeiPass®. However, the sizes of the insoluble but degradable CP and starch fractions and their rates of degradation (data not shown) were higher (P < 0.05) for wheat than for WeiPass®. Because the magnitude of the difference in rate of degradation was much greater than the difference in the size of the insoluble but degradable fraction, wheat was more extensively degraded ruminally than WeiPass®, thus resulting in higher (P < 0.01) effective degradability values of CP (72 vs. 42% at 8%/h outflow rate) and starch (82 vs. 64% at 8%/h outflow rate) for wheat than for WeiPass®. Consequently, WeiPass® had RUP values which were 20 to 30 percentage units higher (P < 0.01) than those of ground wheat, depending on the assumed rumen outflow rate (see Table). An increase in RUS values for WeiPass® compared to wheat was also observed, though not as pronounced numerically as for RUP. The RUS values of WeiPass® were between 10 and 20 percentage units higher than the respective values for the wheat. If the increase in RUP and RUS for WeiPass® above the wheat values is expressed relative to the wheat values, the proportions of RUP and RUS of WeiPass® at an assumed rumen outflow rate of 8%/h had doubled compared with the wheat, i.e., it increased from 28 to 58% of CP for RUP and from 18 to 36% of starch for RUS.

Table: Values for ruminally undegraded protein (RUP) and starch (RUS) of wheat and WeiPass® at assumed rumen outflow rates of 2, 5 and 8%/h[a].

Outflow rate (/h)	RUP (% of crude protein)		RUS (% of starch)	
	Wheat	WeiPass®	Wheat	WeiPass®
2%	13	34	7	18
5%	22	50	13	29
8%	28	58	18	36

[a] RUP and RUS values were calculated as (100 – effective degradability).

Conclusion: Treating wheat with xylose in an aqueous calcium-magnesium lignosulphonate solution at elevated temperatures yielded increases in the proportions of RUP and RUS. It could be demonstrated that WeiPass® had elevated levels of both RUP and RUS compared with wheat. From this data it can be concluded that WeiPass® can be a valuable source of ruminally undegraded protein and starch in rations of ruminant animals.

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56. Comparison of methods to predict supply of ketogenic and glycogenic precursors from starch in dairy cows (Vergleich von Methoden zur Einschätzung des Angebotes an ketogenen und glycogenen Nahrungsstoffen aus Stärke bei Milchkühe).

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Starch digestion in the rumen and large intestine yields volatile fatty acids that are either ketogenic (acetate, butyrate) or glycogenic (propionate) precursors, whereas starch digestion in the small intestine yields mainly glycogenic precursors. In this study the site of digestion of wheat, maize and potato starch was estimated by in vitro methods, i.e. the gas production technique and an enzymatic technique, and the nylon bag technique. Results were validated in an in vivo experiment with dairy cows.

Methods: Three starch sources were tested: wheat meal (WM), maize meal (MM) and native potato starch (PS). Rumen fermentation was simulated with the gas production technique (2); digestion in the small intestine with a 4-h incubation in a pancreatin solution (1). The *in situ* rumen incubations were performed in three rumen-cannulated cows. In a Latin square design experiment, four dairy cows fitted with a rumen cannula and T-piece cannulæ in the distal duodenum and distal ileum, received either a starch-free diet, containing 43 %DM grass silage, 27 %DM ensiled sugar beet pulp (SBP) and 30 %DM of a starch free concentrate mixture with 70 % dried SBP or diets in which the dried SBP was replaced either by WM, MM or PS. Diets were fed during four weeks, with the third week for faecal and the fourth week for digesta collections.

Results: Differences in digestion were observed in gas production between sources at 5 and 10 h incubation, but after 20 h incubation, approx. 90% of the starch had been digested irrespective of source (Table 1). Digestion with pancreatic enzymes was low for PS. Based on the in situ results, the estimated proportion of effectively rumen degradable starch was high for WM and low for MM. A lag time of 10.6 h was observed for PS. The *in vivo* experiments showed that for all three starches, the rumen was the main site of digestion. No disappearance of PS was recorded in the small intestine, which was in accordance with the in vitro digestibility using pancreatin. Almost all starch entering the large intestine was digested, resulting in a faecal starch output of less than 0.10 kg/d.

Table 1 Starch content and *in vitro, in situ* and *in vivo* degradation parameters of different starch sources

Parameter	Starch source			S.E.D.
	WM	MM	PS	
Starch content, g per kg of dry matter	686	590	997	-
Gas production technique:				
Starch fermented after 5 h, %	21	16	7	-
Starch fermented after 10 h, %	68	62	38	
Starch fermented after 20 h, %	89	93	90	-
Starch degraded after 4 h pancreatin, %	47	61	11	-
Effectively rumen degradable starch from *in situ* parameters, %	86	53	66	
Rumen, % of intake	90[a]	75[b]	84[a]	2.5
Small intestine, % of duodenal flux	70[a]	65[a]	0[b]	5.9
Large intestine, % of ileal flux	72[b]	72[b]	86[a]	4.7

Conclusions: Potato starch had a low rumen digestibility in the first 10 hours *in vitro* and *in situ*. However, final *in vitro, in situ* and *in vivo* rumen digestibility of PS was high. This suggests compensation for the low initial rate of digestion by a low ruminal passage rate of PS. Incubation with pancreatin predicted the low *in vivo* digestibility of PS in the small intestine, but for the three samples investigated no relationship in ranking according to source was found for *in vitro* and *in vivo* digestion in the small intestine.

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57. Effect of level of feed intake and of a *Fusarium* contaminated wheat on rumen fermentation in cows (Einfluss der Futteraufnahme und eines *Fusarium* kontaminierten Weizens auf die Pansenfermentation in Kühen).

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The presence of *Fusarium* toxins in animal feedstuffs was shown to affect the health under experimental and practical conditions. However, ruminants appear to be relatively insensitive to the *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON), which can be attributed to the metabolization by microbes in the rumen. Therefore it is possible, that the feed intake (passage rate), as indicator for the residence time of the feed in the rumen, can influence the ruminal metabolization of the toxins. A rapid passage rate may limit the metabolization and the effect of the mycotoxins as well as the microbial fermentation of nutrients in the rumen. The objective of the present study was to investigate the coherence between varying levels of feed intake by using contaminated or not contaminated wheat on fermentation in the rumen.

Methods: 14 dairy cows fitted with rumen and duodenal cannulae, were used in four periods. Diets contained 60% concentrate (on dry matter basis) including 55% wheat (contaminated or not) and were completed with maize- and grass silage. All animals were fed the same diet, only the daily amount was adjusted according to the current lactation stage (total daily dry matter intakes ranged from 5.7 to 20.5 kg). Each cow was fed both the control wheat (control period) and the contaminated wheat (mycotoxin period, 8.21 mg DON/kg wheat and 0.08 mg ZON/kg wheat on dry matter basis).
Each period consisted of 4 weeks. Duodenal chyme was collected in 2 hours intervals over 5 days in week 4. To estimate the digesta flow Cr_2O_3 was used as a marker. Microbial N in the duodenal NAN was analysed by NIRS.

Results: The intake of organic matter (OMI) ranged between 5.2 and 19.1 and 5.3 and 19.1 kg/d per cow during the mycotoxin and control period, respectively. Microbial fermented organic matter (FOM) and flow of microbial N from the rumen increased linearly with OMI independently of dietary treatment (Figure 1).

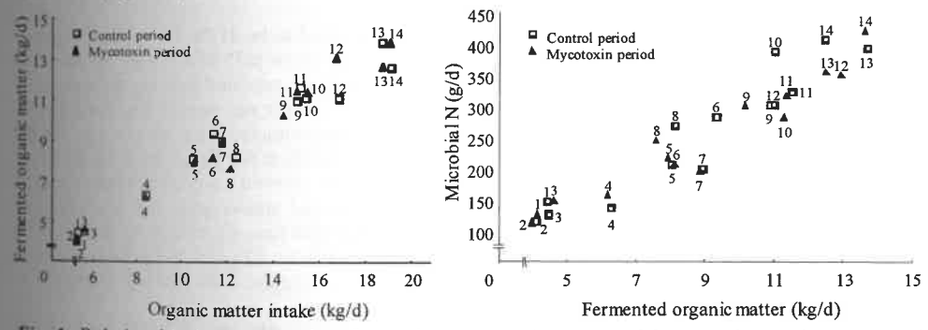

Fig. 1: Relationship between intake of organic matter and passage of fermented organic matter and microbial N to the duodenum of dairy cows in the mycotoxin and control period (numbers over the symbols denote individual cows).

Conclusions: Contrary to the assumption that a shorter retention time of feed in rumen could decrease the microbial fermentation, portion of organic matter fermented in the rumen was independent of level of feed intake. These relationships were not or only to a minor extent influenced by the *Fusarium* contamination of the wheat.

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58. Praecaecal amino acid digestibility of field beans in broilers (Praecaecale Aminosäurenverdaulichkeit von Ackerbohnen bei Broilern). Annette Simon - Berlin

The aim of the experiments was to investigate the praecaecal amino acid digestibility of field beans in broiler chickens depending on the variety and the cultivating location.

Material and Methods: The praecaecal digestibility (pcD) of amino acids was estimated for two varieties of field beans (*Music* and *Scirocco*), which were grown at two different locations (Berge and Blumberg), where Berge was characterized by a better soil quality. In total 768 broiler chickens at the age from 14 to 21 days were fed with 9 experimental diets. The pelleted diets contained 0 % (1 diet), 15 % (4 diets) and 30 % (4 diets) of the respective field beans. The field beans were supplemented at the expense of maize starch, which caused an increasing CP and amino acid content. All diets contained 0.5 % TiO_2 as indigestible marker. The animals were kept in groups of 12 in floor pens and received feed and water *ad libitum*. At day 21 animals were slaughtered and the ileum content was obtained and pooled within each replicate (n = 7 or 8 replicates per treatment). The samples were stored at – 18°C until analyses. The pcD was calculated for each diet and each replicate. The pcD for amino acids from field beans was determined with linear regression analysis.

Results: The crude protein content of the different batches of field beans ranged between 27.7 and 30.4 % in DM, with higher values at the Blumberg location for both varieties. Generally, the mean amino acid content of all batches was within the range given in literature. The mean content of sulphur-containing amino acids amounted to 1.64 g/16 g N. In most cases the differences in the amino acid content of the field beans were marginal. But, with only few exceptions, the amino acid content (g/16 g N) of the *Music* variety was lower compared to the *Scirocco* variety. Only for Cys, Arg and the branched chained amino acids the CV of the amino acid content exceeded 5 %. In the course of experimental feeding, animals which received diets with 15 or 30 % field beans (21.5 to 26.1 % CP on as-fed basis) gained between 68 and 76 g/d. The respective FCR ranged between 1.48 and 1.64 kg feed per kg BWG. As expected, broilers receiving the basal diet with 0 % field beans (17.5 % CP in the diet) showed a lower performance (54 g BWG per day and 1.88 kg feed per kg gain). It is noticeable that the feed intake of animals receiving 30 % field beans was always slightly below animals with 15 % field beans in the diet.

The mean pcD for CP for all investigated field beans was estimated to be 81 %. For pcD of sulphur-containing amino acids only preliminary data are available. The mean pcD of all other amino acids ranged between 72 % (Glu) and 90 % (Lys). The statistical analyses revealed that the differences in pcD of CP and amino acids caused by the main effect "location" were not significant. For CP, Glu and Pro a significant interaction between location and variety was estimated. That means, *e.g.*, for the pcD of CP that highest values were measured for the *Scirocco* variety at the Berge location (84 % *vs.* 77 % at Blumberg) and at the Blumberg location the *Music* variety showed a significant better pcD of CP (85 % *vs.* 79 % at Berge). Among essential and semi-essential amino acids the *Music* variety showed a better pcD for Thr, Val, Ile, Gly, His, Lys, Tyr, Phe and Leu. Compared to the *Scirocco* variety the significant differences of digestibility ranged from 10 % for Thr to 4 % for Leu. This was for all mentioned amino acids associated with a tendency of lower content in this variety expressed as g/16 g N.

Conclusions: Different varieties of field beans can express a significant different pcD of CP depending on the location they are grown. On the other hand, in this study a better soil quality was not accompanied with better pcD of CP, but depends on the cultivated variety. Furthermore, the results indicated that a lower content of essential amino acids can at least partly be compensated by a significant higher digestibility, as was shown for the *Music* variety. For the explanation of these effects further investigations are necessary.

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59. Genetic profiles of rumen microbial communities: A cultivation independent technique to study the effect of feeding (Genetische Profile von Mikroorganismengemeinschaften aus dem Pansen: Eine kultivierungsunabhängige Technik zur Untersuchung von Fütterungseinflüssen). E. Strobel*, J. Wesolowski, G. Flachowsky and C. C. Tebbe - Braunschweig

Objective: The objective of the study was to develop and apply a method for mapping the microbial diversity independent of cultivation to analyze the effects of different feeding conditions, e.g., structure, food additives and contaminants, on microbial communities inhabiting the rumen ecosystems. The method is based on generating genetic profiles (fingerprints) of PCR-amplified ribosomal RNA genes of the small subunit (SSU rDNA).

Methods: SSU rDNA occurs in all living organisms and are valuable indicators for their identity as they can be used to find phylogenetic relatives. SSCP (single strand conformation polymorphism) was used as a genetic profiling technique (1). Total DNA was separately extracted from both the liquid and the solid phase of rumen substrate collected from fistulated muttons and dairy cows. Each species was analyzed with three individuals on the same diet. The DNA was diluted and used as a template in PCR. Universal primers ("com") were applied to amplify a 400 bp partial sequence with the SSU rDNA. The diversity of products was analyzed on SSCP-gels (electrophoresis on non denaturing polyacrylamide gels). In addition, primer pairs with specificities for Eubacteria, Archaea, Actinobacteria, "non-bacteria", Alpha-Proteobacteria and members of the genus Pseudomonas were applied. PCR products obtained with these primers were used in a nested PCR approach with com-Primers. The SSCP patterns were compared with each other by digital image analysis using the GelCompar software package (Applied Biomaths, Kortrijk, Belgium).

Results: The fingerprinting technique allowed to directly compare microbial communities of different samples, collected from the rumen. Depending on the primers each profile obtained from both cows or sheep consisted of 6 to 50 bands which could differentiate. With primers targeting Eubacteria or Actinobacteria, respectively, many bands occurred which could not be differentiated because they accumulated in the same regions of the gel. On the other hand, SSCP-patterns from Alpha-Proteobacteria and Archaea were composed of up to 30 bands that could clearly be differentiated. With Pseudomonas specific primers only few bands were found, but these bands were very strong. All primers allowed to differentiate the microbial communities from cows and sheep. These differences were most pronounced with the patterns of Alpha Proteobacteria and Archaea, but less with Actinobacteria and Pseudomonas. The patterns among individuals of the same species were similar but not identical. For sheep, these minor differences occurred consistently when samples taken during three days within a week were compared to each other. The variability between different days was very low.

Conclusions: PCR-SSCP was established for the analysis of microbial communities in the rumen ecosystem without the need of cultivating the single microorganisms. Primers of different specificities make it possible to focus on selected groups of microorganisms, as demonstrated for Archaea or Alpha-Proteobacteria. Differences as detected by digital image analysis can be further identified by DNA-sequencing of selected bands from these profiles and comparison to sequences in the public databases. The method can be used to study how different structures, food additives or contaminants affect the rumen ecosystem.

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60. Effects of Brown Midrib 3 Mutation in Silage Corn on Ruminal Degradability, Digestibility and Performance of Beef Cattle (Einfluss einer "Brown Midrib 3"-Mutation von Silomais auf ruminale Abbaubarkeit und Verdaulichkeit der Maissilage sowie auf Leistungsparameter bei Mastbullen).

H. Kurtz*, Friederike Flaßhoff and F.J. Schwarz – Freising-Weihenstephan

The brown midrib-3 (bm_3) gene mutation has been incorporated into corn plants to potentially improve fiber digestibility. The objective of this work was to determine the effects of a bm_3 corn silage (BMCS) on ruminal degradability (RDG) and on performance of beef cattle.

Methods: A bm_3 hybrid and its isogenic normal counterpart (Helix, KWS) were cultivated and harvested under identical conditions (harvest date: 1st October, 2002). The normal hybrid (CS) had a very good DM yield/ ha of 160 dt whereas the DM yield of BMCS was about 24% less. The DM (%) and crude nutrients (% i. DM) of both silages were similar (CS vs. BMCS): DM: 38.1, 37.4; XA: 3.85, 4.29; XP: 8.58, 8.83; XL: 3.69, 4.01; XF: 23.5, 23.6. However, CS contained more starch (26.4% i. DM) in comparison to BMCS (21.3% i. DM). The effective RDG of both hybrids was studied with fresh kernels and the residual plant, harvested at different dates (29th August, 25th September, 1st October), as well as with the whole plant corn silage (WPCS) using the in sacco-method. Furthermore a digestibility trial using 10 wethers was carried out feeding 1.3 kg DM of WPCS. Finally, in a feeding experiment over 9 weeks 72 Simmental bulls received CS or BMCS (36 animals per treatment) ad libitum together with 1.5 kg of concentrate in an individual feeding system (CALAN). Mean starting weight of the animals was 244±15 kg.

Results: Table 1 shows the mean RDG of the kernels and residual plant (fresh material) at different harvest dates and of the WPCS. The RDG of both plant materials (kernels and residual plant) and of both hybrids decreased with increasing stage of maturity. The kernels of the normal hybrid tended to higher RDG in comparison to the bm_3 hybrid, whereas the residual plant of the bm_3 hybrid showed a distinct increase in RDG (harvest date 9/25 and 10/1). Therefore, the RDG of the BMCS-DM was 71% at a passage rate of 8%/h in comparison to nearly 65% of the CS-DM. However, the in vivo-digestibility of the OM didn't differ with values of 76.1% (CS) and 75.8% (BMCS). Only the DXF of BMCS with 66.5% was numerically higher than that of CS with 63.3%.

Table 1: Effective ruminal degradability (RDG) of DM at different dates (passage rate 8%/h)

Harvest date		Kernels[1,2]		Residual Plant[1,2]		WPCS[2]	
		DM (%)	%	DM (%)	%	DM (%)	%
8/27/02	CS	49.1	78.2	21.2	48.8		
	BMCS	53.9	74.0	20.0	48.5		
9/25/02	CS	67.2	57.9	20.9	36.6		
	BMCS	66.2	54.7	19.1	42.6		
10/1/02	CS	67.3	54.7	26.1	34.9	37.6	64.7
	BMCS	68.4	50.6	20.0	40.6	38.2	71.1

[1]fresh material, [2]freeze dried

In the feeding trial the mean final live weight of the Simmental bulls fed BMCS was higher (341 kg vs. 336 kg) and, therefore, these bulls reached an ADG of 1554±175 kg in comparison to 1469±182 kg CS-fed bulls. This trend of an improved ADG (+5.8%) was combined with a higher feed intake for the BMCS-fed animals of 0.68 kg DM/ animal, d. The mean total daily feed intake per bull was 6.28±0.45 kg DM (BMCS-fed) and 5.60±0.46 kg DM (CS-fed).

Conclusion: The results of this work have shown a clearly higher feed intake and a trend for better ADG of the bulls fed WPCS of an hybrid with a higher ruminal degradability of the residual plant (BMCS). Therefore, in order to optimize the feed value of corn silage besides the starch content the ruminal degradability of the residual plant has to be considered intensively as well.

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61. Influence of grass silage particle size fed as TMR on the *in vitro* digestive characteristics of rumen digesta in dairy cows (Einfluss der Partikellänge der Grassilage einer TMR auf *in vitro* Charakteristika der Fermentation des Panseninhaltes bei Milchkühen).

Q. Zebeli*, M. Tafaj, B. Junck and W. Drochner – Stuttgart-Hohenheim

Objective: Particle size reduction might affect the ruminal fermentation and hence the digestive capacity of digesta. The purpose of the present study was to investigate, by means of the gas production profiles, the effect of particle size (**PL**) of grass silage (**GS**) on the *in vitro* digestive characteristics of the ruminal digesta collected in different sampling sites (**D**).

Methods: Two Holstein cows (BW 640kg) in early lactation (36kg Milk/d) fitted with ruminal cannulas (Ø=100 mm) were used as donors. The GS of three levels of theoretical particle size (25mm, 11mm and 5.5mm) was fed as TMR with a concentrate mixture (**C**) and coarse fibre-rich hay (**H**) in a ratio of GS:H:C=45:5:50. Diets were offered *ad lib* twice daily at 08.00 and 16.00h. After an 11d adaptation period, the ruminal digesta were collected 1h before the morning feeding. Approx. 200 ml free rumen liquid (**FRL**) was sucked through a tube from the ventral sac. Then 250-350g solid digesta from top (**SRF-T**) and bottom (**SRF-B**) rumen were collected (1). The samples from both animals were pooled. FRL was buffered according to Gas-Test method, blended by Waring Blender (2 min, low speed) and incubated afterwards. A defined portion of solid digesta was manually squeezed in order to obtain the required inoculum volume, then diluted with the required buffer solution, homogenised by Waring Blender (2min, low speed), squeezed again and filtered with a cloth bag and incubated in three replicates. 200mg dry matter (**DM**) of TMR *(15.1% CP, 35% NDF)*, GS *(13.2% CP, 46.4% NDF)*, H *(7.4% CP, 64.1% NDF)*, C *(18.7% CP, 19.3% NDF)* and Molasses chips *(9.2% CP, 30.8% NDF)* were incubated and gas production (**GP**) was recorded on 2, 4, 8, 12, 24, 32 and 48h. The incubation was repeated on three subsequent days. The effects of PL, D, PL*D were tested by MIXED PROC (SAS, 8.2) using a model for double (D, Day) repeated measures.

Tab.1. Gas production rate (*Lsmeans - ml/h/200 mg DM*) (BV was subtracted for substrates)

Substrates	Time Interval (h)	Particle size (PL), mm			Digesta layers (D)			LSD
		25	11	5.5	SRF-T	SRF-B	FRL	(α=0,05)
Blank value (BV)	0 - 4	3.2	4.1	4.5	6.2	4.9	0.9	1.17
	4 - 12	1.3	1.3	1.2	1.9	1.6	0.4	0.21
TMR	0 - 4	5.6	5.1	5.5	5.3	5.9	4.9	0.72
	4 - 12	2.0	2.1	1.8	1.5	1.8	2.6	0.26
Grass Silage (GS)	0 - 4	4.2	4.1	4.3	4.3	4.6	3.7	0.63
	4 - 12	1.6	1.7	1.5	1.2	1.5	2.0	0.28
Concentrate (C)	0 - 4	5.9	4.5	5.8	5.1	6.0	5.0	1.11
	4 - 12	2.6	3.0	2.6	2.0	2.5	3.7	0.37

Results: With reduction of the PL of GS up to 5.5mm the fermentation rate increased within first 4h of incubation by BV, while an opposite trend was observed by all substrates during the interval from 4 to 12h of incubation compared to 11mm PL. Following 12h of incubation no influence of PL on the fermentation rate was observed. SRF-B showed a higher fermentation rate than SRF-T during the interval from 4 to 12h of incubation. FRL showed a significantly lower fermentation rate for all substrates only within the first 2h of incubation, later it indicated significantly higher fermentation capacity compared to SRF-B and SRF-T.

Conclusion: The reduction of PL of GS, fed as TMR, up 11mm PL seems to stimulate the digestive capacity of rumen digesta. The squeezed liquid from solid rumen digesta showed a higher digestive capacity compared to free liquid only at the start (up to 4h) of incubation.

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62. Comparison of grass and lucerne silages in situ degradability in the rumen of dairy cows.

(Vergleich der in situ Abbaubarkeit von Gras-und Luzernesilage bei Milchkühen.). Vivian Ariyo*, P. Lebzien, U. ter Meulen and G. Flachowsky - Braunschweig/Göttingen

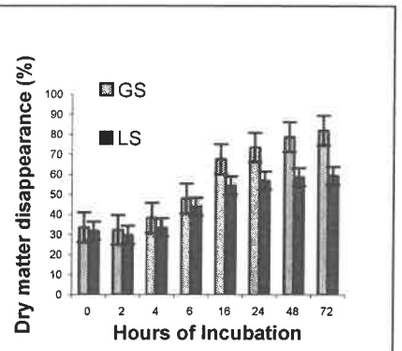
The nylon bag technique is an experimental procedure to evaluate degradability of feedstuffs in situ. Applying the equation of Ørskov et al (1979), $p = a + b\,(1-e^{-ct})$, the constants a, b and c can be calculated to characterise degradation behaviour of feedstuffs. A number of substantial differences in the morphology and cellular structures of grasses and luzerne affects patterns of digestion, rumination and particle size reduction. The objective of this study was to compare the parameters for grass silage (GS) and lucerne silage (LS)to get more information on the differences.

Methods: Luzerne and grass silages were harvested at the first and second cutting and ensiled . The dry matter (DM) degradation was determined by weighing 3.5g - 4g of milled (3mm) freeze dried samples into nylon bags with a dimension of 10*15cm and pores size of 53±10µm each. Two replications (2 bags) of each sample were carried out for each incubation time and repeated in two fistulated cows. Rumen incubation periods of 2,4,8,16,24,48 and 72hrs were considered. Bags were suspended in the rumen prior to the morning feeding and removed after each incubation time, machine washed and dried at 60°C for 48hrs and the washing losses from non incubated material (zero hour) was also determined in the same way. The course of degradation was described by fitting the data to the equation of Ørskov et al (1979) and the effective degradability was calculated.

Results: The dry matter degradability (DG) was low at the beginning of incubation, but rapidly increased up to 24 hours for both silages. DG rate (c%/h) obtained for LS (14%) was higher than that of GS (9%) but the potential degradability (a+b) for GS (81,3%) was higher than that of LS (59,7%).Therefore at passage rates of 2%/h (ED2), GS has a high effective degradability, but at a higher passage rate of 8% there was no much difference between the ED values of both silages.

Tab.1: Degradability parameters of GS- and LS-DM

	Degradability parameters					
	a	b	a+b(	c	ED2	ED8
	(%)	(%)	%)	(h^{-1})	(%)	(%)
GS	32.9	48.3	81.3	0.09	72,2	58,1
LS	30.8	28.8	59.7	0.14	56,1	49,2
SE	1,1	9,7	10,8	0,03	5,7	4,5



DM=dry matter, a=soluble fraction, b=degradable fraction a+b=potential DG, c=rate constant for b, ED=effective degradability at passage rates of 2 (ED2)and 8 (ED8)%/h,

SE=standard error of means

Figure 1 (left): DM disappearance of grass and luzerne silages in the rumen of dairy cows

Conclusion: Based on this study, GS had a lower rate of degradation and a higher potential degradability. Therefore cows with a high feed intake (high passage rate) can utilize lucerne more effectively than grass silage while on the other hand cows with low feed intake (low passage rate) can make use of grass silage better than of luzerne silage.

ØRSKOV, E. R. and McDONALD , I. (1979): J. Agric. Sci. (Camb.) 92: 499-503

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63. Influence of a linseed oil supplementation at different concentrate/roughage ratios on some rumen parameters in lactating dairy cows

(Einfluss einer Leinölzugabe bei unterschiedlichen Kraftfutter/Rauhfutter-Verhältnissen auf einige Pansenparameter von laktierenden Kühen). Kristin Erdmann*, P. Lebzien and G. Flachowsky - Braunschweig

Introduction: To meet the energy requirements of high yielding dairy cows with a limited feed intake capacity, rations with a high portion of concentrates and/or fat have to be fed. Nevertheless, increasing amounts of concentrates and too much dietary fat are known to affect ruminal fermentation. The aim of our experiment was to investigate if there are interactions between the concentrate/roughage ratio and the supplementation with 200g linseed oil/day with regard to rumen parameters.

Methods: Seven multiparous lactating dairy cows, fitted with cannulae in the rumen, were randomly assigned in a incomplete 2 x 2 Latin Square Design with four experimental periods. The following rations were tested:

- **CON 70:** *roughage/concentrate ratio 70% : 30%* (9,1 kg hay-DM and 3,9 kg concentrate-DM)
- **FAT 70:** CON 70 with additional 2x100g linseed oil (spread on top of the hay)
- **CON 30:** *roughage/concentrate ratio 30% : 70%* (4,0 kg hay-DM and 9,0 kg concentrate-DM)
- **FAT 30:** CON 30 with additional 2x100g linseed oil (spread on top of the hay)

The hay (+ oil) was given twice daily and the conentrates in four equal portions. After three weeks of adaptation to the diet, rumen fluid was taken from every cow via the rumen fistula at different times. In samples of the rumen fluid we analyzed: *pH*-value (*pH*), ammonia nitrogen (NH_3-*N*) and volatile fatty acids (*VFA*).

Results: Concentrations of NH_3-*N* in the rumen fluid and *pH* at different times are given in Table 1 and 2.

Table 1: *pH* in the rumen fluid at different sampling times (CON: n = 4; FAT: n = 5)

Time after 1st feeding (min)	CON 30	CON 70	FAT 30	FAT 70
0	6,88 a	6,55 b	6,81 a	6,49 b
180	5,72 a	5,98 a	5,62 a	5,86 a
300	5,71 a	6,03 b	5,72 a	5,90 ab

Table 2: NH_3-*N* (mg /100ml) in the rumen fluid at different sampling times (CON: n =4; FAT: n = 5)

Time after 1st feeding (min)	CON 30	CON 70	FAT 30	FAT 70
0	9,56 a	4,35 a	7,68 a	5,26 a
180	6,88 a	9,64 a	10,09 a	10,75 a
300	1,64 a	3,86 a	2,43 a	4,76 a

Different superscripts in one line indicate significant differences ($p < 0,05$)

Concerning the *pH*-value only the concentrate/roughage ratio had significant effects, but there was no significant effect of linseed oil supplementation before as well as three and five hours after the first feeding time with both concentrate/roughage ratios. The concentration of total *VFA* was also not affected and linseed oil did not affect *VFA* patterns and the NH_3-*N* concentration in the ruminal fluid at any time.

Conclusions: The supplementation of 200g fat in the form of linseed oil had no impact on the ruminal fermentation if rations with high or low amounts of concentrates were fed.

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64. Composition of unextracted and extracted *Moringa oleifera* leaves and their effects on *in vitro* rumen fermentation (Zusammensetzung von unextrahierten und extrahierten *Moringa oleifera*-Blättern und ihre Wirkung auf die Pansenfermentation *in vitro*).

Carla R. Soliva*, M. Kreuzer, N. Foidl, G. Foidl, Andrea Machmüller and H. D. Hess – Zurich/Caslano

Several studies demonstrated that the tropical plant *Moringa oleifera* and its products may be useful for various purposes including application as an animal feed (1, 2). The high crude protein content of the leaves suggests that they could become a substitute for common protein supplements in ruminants. Increasing the protein content by extraction processes might even enhance the applicability of Moringa leaf as feed. Ruminal protein degradation of the Moringa leaves and their effects on non-ammonia N production in the rumen as important criteria for metabolic protein supply in ruminants are still unknown and further characteristics determining their feeding value have not yet been analysed in detail.

Methods: The chemical composition and energetic values of dried unextracted and extracted Moringa (*Moringa oleifera* Lamarck) leaves, and their effect on ruminal N turnover and other rumen fermentation traits were investigated in comparison with soybean meal and rapeseed meal either isolated or included in complete diets. The *in vitro* techniques used comprised the Hohenheim gas test apparatus, applying the standard protocol given for the determination of the net energy content of feedstuffs and diets, and the rumen simulation technique (Rusitec; only complete diets). An 8-fermenter Rusitec apparatus was used with incubations lasting for 10 days in four replicates each. The daily supplied amounts of complete diets were incubated for 48 h each by the use of two nylon bags per fermenter. An enzymatic standard method using *Aspergillus* spp. enzymes was used for the estimation of the rumen-degradability of the protein of isolated feeds and complete diets.

Results: The main compositional differences between the batches of unextracted and extracted Moringa leaves investigated were those in crude protein (CP) as well as in ether extract and total fatty acid contents (the latter two as high as 49 and 35 g/kg DM in unextracted leaves, respectively). Contents of CP in the unextracted and extracted leaves were 329 and 467 g/kg DM, respectively, relative to 555 and 374 g/kg found in soybean meal and rapeseed meal. The corresponding contents of net energy lactation, as calculated from gas production in the Hohenheim gas test, were 6.6, 7.5, 8.1 and 6.8 MJ/kg, and the enzymatically determined CP degradabilities were 629, 594, 751 and 677 mg/g, respectively. Unexpected was the lower degradability of rapeseed meal protein compared to soybean meal protein which might reflect recent technological changes in the production of rapeseed meal. Fermenter fluid ammonia concentration, determined in the Rusitec experiment, was particularly low in the diet with unextracted Moringa leaves. In both diets with Moringa products, CP apparently degraded and not recovered in ammonia was high suggesting a high production of microbial protein. *In vitro* degradabilities of organic matter and fibre of the complete diets containing unextracted or extracted Moringa leaves were similar or higher compared with diets containing soybean meal or rapeseed meal as protein sources. Methane emission tended to be lower from diets containing Moringa leaf products compared with diets based on soybean meal or rapeseed meal.

Conclusion: The results show that the feeding value of unextracted and extracted Moringa leaves in total is similar to that of the commonly used soybean and rapeseed meals. It seems that Moringa leaves might enhance the metabolic protein supply by supporting the synthesis of microbial protein in the rumen due to its substantial contents of readily fermentable N and energy. Therefore, Moringa leaves as products from tropical origin seem to have a potential as protein source in ruminants.

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65. Accounting for marker detachment in studies of particulate passage through the gastrointestinal tract – a mathematical approach (Die Berücksichtigung von Markerablösung in Studien zur gastrointestinalen Passage von Partikeln – ein mathematischer Ansatz).

Eva Schlecht, H. Richter – Stuttgart-Hohenheim

Introduction: Rare earth elements used as particulate marker are frequently observed to detach from the mordanted fibre in the gastrointestinal tract (GIT). This is attributed to changes in the cation exchange capacity of the labelled component in different compartments (1). Since detached marker migrates to the liquid phase and leaves the GIT earlier than fibre-bound marker, time delay between dosing and first appearance of fibre-bound marker (TT) is underestimated and particle half life (T_{50}) is overestimated if the detachment is not accounted for. We propose mathematical modelling of liquid and fibre passage to overcome this problem.

Methods: For trials conducted with cattle fed millet leaves and described earlier (2), data of 10 animals was re-analyzed, since continuous marker detachment was observed after visual inspection of Yb excretion curves. Passage of fibre-bound and detached Yb was modeled as illustrated in Figure 1A; formulae will be given in the poster.

Results and Conclusion: A typical multi-humped curve of Yb excretion caused by continuous marker migration to the liquid phase is illustrated in Figure 1B, together with the modelled excretion curves for fibre-bound and detached Yb. For particles, TT and T_{50} averaged 27.4 h (±2.8) and 71.7 h (±9.6) when Yb-detachment was accounted for as compared to 14.2 h (±4.6) and 85.5 h (±18.3) if all excreted Yb was attributed to the particle flow. It is concluded that the mathematical adjustment for marker migration from the fibre to the liquid phase improves the determination of key parameters of particulate passage.

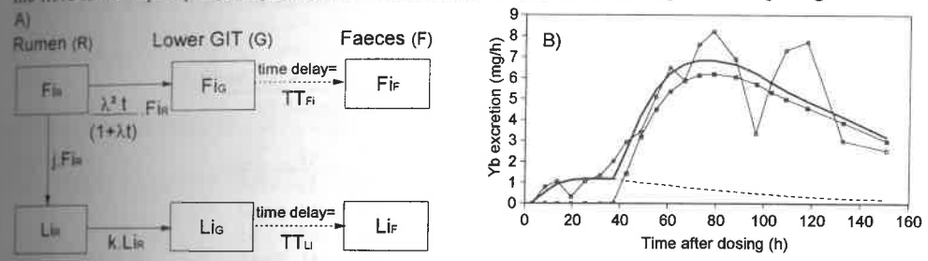

Figure 1. A) Conceptual framework of marker migration from the fibre to the liquid phase in the rumen, based on a one-compartment model. λ and k are fractional flow rates of fibre (Fi) and liquid (Li) from the rumen, j is the fractional rate of marker detachment from the fibre, TT is the time until first recovery of marker (Li or Fi) in faeces. B) Measured faecal Yb excretion (–o–) and modelled excretion curves for fibre-bound (–■–), detached (– –) and total Yb (—) in cow #6.

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66. Digestion studies in captive Indian rhinoceroses (*Rhinoceros unicornis*) (Verdauungsversuche beim Indischen Panzernashorn in Menschenobhut).

M. Clauss *, Ellen Kienzle, Carmen Polster, H. Wiesner, Katrin Baumgartner, Friedericke von Houwald, Ellen Dierenfeld – München/Nürnberg/Basel/New York

The Indian rhino is an endangered species with an estimated free-living population of only 2500 animals. Captive management plays an important role for the survival of the species. In captivity, several, potentially diet-related problems have been reported. Traditionally, the horse has been used as a model animal for designing diets for this species; however, this procedure has so far not been evaluated comparatively.

Methods: 11 Indian rhinos from 4 institutions were submitted to 2 feeding trials each, one on the regular zoo diet (roughage and concentrates, diet RC) and one on a roughage-only diet (diet R). Feeds consumed and faeces voided were weighed *in toto*, and representative subsamples analysed by Weender analysis and bomb calorimetry; Ca was measured after wet digestion by photometry. Volatile fatty acids (VFA) were determined in preparations from fresh faeces using gas chromatography. Water intake was measured, and urine was sampled sporadically and analysed for Ca and creatinine. Passage rates were detmined using Co-EDTA as a fluid and Cr-mordanted fibre (< 2 mm) as a particle marker.

Results: The average mean retention times for fluids and particles were 41 and 60 h, respectively. Dry matter intake ranged from 0.8-1.3 % BW on diet RC and 0.5-1.3 % BW on diet R. Achieved dry matter digestibility ranged from 53-63 % on diet RC and 38-61 % on diet R, respectively; in general, digestibility coefficients achieved were similar to reported values for domestic horses on rations of similar composition.

Examples for achieved digestibility coefficients (averages of animals per facility)

Ration	Ration content (%DM)		Apparent digestibility (%)			
	XP	XF	DM	XP	XF	GE
Grass hay/concentrates	9	27	57	52	41	54
Grass hay	7	36	46	54	34	42
Grass silage/grass hay	8	37	59	46	63	55
Straw/concentrates	9	30	54	54	41	50

The average faecal concentration of VFA and the C2:C3-ratio were 39 mmol/l and 5.5 on diet RC and 25 mmol/l and 8.2 on diet R. Total water intake averaged 4.1 l/100 kg BW and did not differ between rations. The urinary Ca:creatinine-ratio increased with increasing Ca intake. Digestible Energy intake ranged from 0.51-0.99 MJ/kg $BW^{0.75}$ on diet RC and 0.27-0.68 MJ/kg $BW^{0.75}$ on diet R.

Conclusion: The discrepancy between longer ingesta retention times and similar digestive efficiency when compared to the domestic horse warrants further investigations into the negative effects of large body size on digestive processes. In terms of animal husbandry, the results indicate that the domestic horse is a suitable model animal for the nutrition of Indian rhinos. The traditional feeding regime of produce, concentrates and hay *ad libitum* can lead to potentially excessive energy intakes.

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67. Dose response of selected carbohydrate-rich feed additives to chyme fermentation in vitro (Einfluss der Dosierung auf die in vitro Fermentationseigenschaften kohlenhydratreicher Substrate).

Eva Bauer*, Barbara A. Williams, Christina Voigt, R. Mosenthin and M.W.A. Verstegen – Stuttgart-Hohenheim/Wageningen

Fermentation characteristics of chyme from pigs, alone and in combination with carbohydrate-rich feed additives, were assessed in vitro, to determine which additive might benefit animal health. Additives were used at different concentrations to determine the dose-response and any interactions in fermentation characteristics due to changes in concentration. Virginiamycin was added to chyme to investigate the effect of an antibiotic on fermentation characteristics.

Methods: Test substrates were transgalacto-oligosaccharide (TOS), arabic gum (AG), sugarbeet pulp pectin (SBP), and virginiamycin (ANT). Ileal chyme was collected from four cannulated pigs, freeze-dried and used as basal substrate in combination with the test substrates. To 0.5 g of chyme, the test substrates were added at concentrations of 10, 30 and 50%, except for the antibiotic, that was added at a level of 5, 25 and 50 ppm. Fermentation kinetics were determined using the cumulative gas production technique (1). End-products measured were total gas, NH_3 and volatile fatty acids (VFA). The branched chain ratio (BCR) was calculated. The variables obtained were tested for significance by analysis of variance using the Tukey multiple range test.

Results: Mean values of fermentation characteristics of the different additives at different concentrations fermented with chyme, and for chyme alone are shown in Table 1. At a concentration of 30 and 50%, TOS and SBP led to a significant higher gas production than chyme alone. For TOS, $T_{1/2}$ was significantly earlier than fermentation of chyme alone. Addition of arabic gum and TOS led to a significantly faster R_M at 30% and 50%. Arabic gum produced less NH_3 at higher concentrations. Except for the antibiotic, for all the additives, the BCR decreased with increasing concentration (30 and 50%).

Table 1 Mean values of the fermentation characteristics of the different additives

Substrates		DMCV[1] [ml/g DM]	$T_{1/2}$[2] [h]	R_M[3] [ml/h]	NH_3 [mg/bottle[4]]	Total VFA [mgAAE[5]/gDM]	BCR[6]
CHY + ANT,	5 ppm	172^{defg}	12.6^{abcd}	10.4^{de}	40.1^{a}	654^{a}	0.161^{a}
	25 ppm	165^{fg}	14.2^{a}	9.0^{e}	39.0^{ab}	645^{abc}	0.146^{abc}
	50 ppm	175^{defg}	12.4^{abcd}	10.8^{de}	38.7^{ab}	641^{abc}	0.163^{a}
CHY + AG,	10%	164^{g}	13.0^{abc}	10.6^{de}	38.4^{ab}	620^{cd}	0.159^{ab}
	30%	202^{cdefg}	10.5^{def}	16.4^{bc}	33.6^{de}	617^{cd}	0.139^{bcd}
	50%	209^{cdef}	10.8^{bcde}	19.6^{b}	31.5^{ef}	596^{d}	0.124^{def}
CHY + SBP,	10%	198^{cdefg}	11.0^{bcde}	13.3^{cde}	38.2^{ab}	621^{bcd}	0.163^{a}
	30%	215^{bcd}	11.0^{bcde}	14.4^{cd}	36.8^{bc}	628^{abc}	0.130^{cde}
	50%	240^{abc}	10.9^{bcde}	16.2^{bc}	37.4^{abc}	619^{cd}	0.114^{efg}
CHY + TOS,	10%	203^{cdefg}	10.7^{cde}	13.9^{cde}	37.3^{abc}	652^{a}	0.150^{ab}
	30%	239^{abc}	8.3^{fg}	19.8^{b}	37.1^{abc}	634^{abc}	0.120^{defg}
	50%	255^{ab}	6.7^{g}	25.4^{a}	34.8^{cd}	644^{abc}	0.105^{fg}
CHY		167^{efg}	13.1^{ab}	9.8^{de}	37.1^{bc}	626^{abc}	0.161^{a}

[1]total gas; [2]half-time of asymptotic gas production; [3]maximum rate of gas production; [4]bottle containing 87 ml; [5]acetic acid equivalents; [6]branched-chain ratio; [a,b,c]superscripts which differ within the same column differ significantly ($p<0.05$)

Conclusion:

If supplemented dietary carbohydrates reach the terminal ileum, it could be expected that fermentation in the large intestine would be stimulated, resulting in more gas and faster rate of gas production, and a decreased BCR. Carbohydrate fermentation, compared with protein fermentation (as suggested by the decreased BCR) could be beneficial to the host animal.

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68. Comparison of caecal chyme and faeces of pigs as inoculum for the fermentation of hay, oat bran and gelatine with the modified Hohenheim gas test (Vergleich von Chymus und Kot von Schweinen als Inokulum für die Fermentation von Heu, Haferkleie und Gelatine mit dem modifizierten Hohenheimer Gas Test).

B. Zacharias*, Dagmar Duda, B. Buske, M. Tafaj, H.-P. Piepho, W. Drochner – Stuttgart-Hohenheim

Faecal samples are often used as inoculum for the simulation of fermentation processes in the large intestine of the pig in vitro. Faecal sampling is less labour intensive and the disturbance to the animal is minimized as compared to the sampling of chyme. The use of faeces as inoculum requires that the microbial population in the faeces is comparable to the microbial population in the large intestine. The aim of the present study was to evaluate wether faeces can be used instead of caecal chyme for the inoculation of different feedstuffs in vitro.

The gas production and the formation of total volatile fatty acids of hay, oat bran and gelatine were determined with faeces and chyme as inocula in an in vitro system in which fermentations were conducted in 100 ml gas-tight glass syringe barrels. An amount of buffering substances was added to the system which was comparable to the amount of buffering substances in the chyme of pigs. Chyme was collected from 6 pigs fitted with T-cannulas at the caecum. Faeces were collected from the rectum of the animals. 100 mg of the samples were incubated at 39 °C for 12, 24 and 48 h.

			12 h	24 h	48 h	SEM
hay	gas production, ml	chyme	8.36 ^{aA}	10.4 ^{abA}	13.1 ^{bA}	0.62
		faeces	6.87 ^{aA}	11.0 ^{bA}	14.4 ^{bA}	0.65
	volatile fatty acids, mmol/l	chyme	9.94 ^{aA}	12.8 ^{abA}	16.4 ^{bA}	1.04
		faeces	8.82 ^{aA}	11.5 ^{aA}	14.0 ^{aA}	1.08
oat bran	gas production, ml	chyme	22.1 ^{aA}	25.1 ^{abA}	26.3 ^{bA}	0.60
		faeces	18.4 ^{aB}	27.5 ^{bA}	27.6 ^{bA}	0.60
	volatile fatty acids, mmol/l	chyme	18.9 ^{aA}	21.7 ^{aA}	20.1 ^{aA}	1.01
		faeces	12.0 ^{aB}	20.8 ^{bA}	21.3 ^{bA}	1.01
gelatine	gas production, ml	chyme	8.69 ^{aA}	12.8 ^{bA}	13.5 ^{bA}	0.62
		faeces	5.78 ^{aB}	11.9 ^{bA}	12.6 ^{bA}	0.60
	volatile fatty acids, mmol/l	chyme	21.5 ^{aA}	30.2 ^{bA}	30.6 ^{bA}	1.04
		faeces	18.8 ^{aA}	27.9 ^{bA}	29.1 ^{bA}	1.01

A, B means of the different inocula with no common superscript differ at $p<0.05$
a,b means of the different incubation times differ at $p<0.05$

In all samples the gas production and the concentration of volatile fatty acids showed lower values within 12 h when faeces was used as inoculum as compared to chyme. Only for hay the gas production and the amount of volatile fatty acids increased further within 24 h to 48 h with both inocula. No differences in the production of gas and in the amount of volatile fatty acids between the two inocula were obtained after 24 h and 48 h.

The fermentation starts more rapid when chyme is used as inoculum. After 24 h in oat bran and gelatine the fermentation is finished in both inocula. The further fermentation of hay with both inocula might be caused by the higher amount of cellulose in this sample. As there were no significant differences between the two inocula after 24 h and 48 h, faeces can be used instead of chyme as inoculum for the simulation of fermentation processes in the large intestine of the pig using 24 h of incubation time.

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69. Investigations on the effects of splitting meals and/or enzyme doses in pancreatic duct ligated pigs on nutrient digestibility (Untersuchungen zu Auswirkungen einer gesplitteten Fütterung und/oder Gabe von Enzymen auf die Rohnährstoffverdaulichkeit bei pankreasgangligierten Schweinen).

Kirsten Stemme*, Elisabeth Kammlott, Jeannine Karthoff, P. C. Gregory, J. Kamphues - Hannover

Exocrine pancreatic insufficiency (EPI) is known to be a common cause of severe disturbances of nutrient digestibility. In human as well as in veterinary medicine it is generally recommended that EPI patients take food / feed and enzyme preparations in multiple meals / doses over the day.

Aim of this study, therefore, was to investigate the effect of splitting feed intake and/or enzymes over the day with regard to the improvement of nutrient digestibility rates in EPI pigs.

Material and methods: The studies were performed in 20 female, adult Göttingen minipigs. In 16 the pancreatic duct was ligated (EPI), the other 4 were controls. The animals were fed with 255g of a diet (% in DM: 19.1 crude fat, 18.3 crude protein, 6.62 crude fiber, 96.6 organic matter, 40.1 starch). In successive trials the animals were fed twice a day and received either none, 2, 4 resp. 6 enzyme doses (per day: identical total amount of enzyme dose). In further trials the diet and enzymes were divided and given in 4 small portions over the day (per day: identical diet amount and enzyme dose). Faeces were collected for 5 days (5x24 h). Standard methods were used for analysis of crude nutrients (Weende analysis).

Results: Ligation of the pancreatic duct led to severely reduced digestibilities. Substitution of pancreatic enzymes remarkably improved digestibilities. Compared to an application of two enzyme doses, distributing enzymes in small doses over the day did not result in significantly higher digestibility rates. The digestibility rate of crude fat and nitrogen-free extracts even tended to decrease the more doses of enzymes were administered. Feeding the animals four times (instead of only twice) a day did not result in a significant increase of the digestibility of organic matter (OM), crude fat (XL), crude protein (XP), and nitrogen-free extracts (XX), neither with nor without enzyme application.

Tab. 1: Digestibility rates (%) of organic matter (OM), crude fat (XL), crude protein (XP) and nitrogen-free extracts (XX) in EPI pigs fed the diet and/or enzymes twice daily or in small meals/doses over the day

	Controls	Pancreatic duct ligated (EPI) pigs					
	2 meals	2 meals / without enzymes	4 meals / without enzymes	2 meals and enzymes application in 2 doses	4 doses	6 doses	4 meals / 4 enzyme doses
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
OM	92.5 ± 1.12	72.2 ± 4.18	72.9 ± 3.87	87.8 ± 2.40	89.2 ± 1.24	88.3 ± 1.58	86.9 ± 2.29
XL	97.4 ± 0.43	32.8 ± 12.9	34.2 ± 11.0	81.3 ± 7.32	80.5 ± 4.96	77.2 ± 4.87	78.1 ± 7.84
XP	92.2 ± 1.15	62.4 ± 6.52	63.5 ± 8.34	85.7 ± 1.83	86.3 ± 1.97	84.5 ± 2.67	84.7 ± 1.42
XX	94.2 ± 1.10	92.2 ± 2.00	93.0 ± 0.61	93.9 ± 1.65	81.9 ± 2.08	81.1 ± 2.57	93.6 ± 0.96

Conclusion: The oral substitution of pancreatic enzymes remarkably improved the digestibility. Yet, the values did not reach those of controls. When the animals were fed twice a day, administering the enzymes in small dosages over the day (4 resp. 6 doses instead of only two) had no influence on the digestibility of organic matter, crude fat, and crude protein. In EPI pigs, feeding the diet and enzymes in 4 meals / doses (instead of only two) also had no favourable effects on the digestibility of organic matter, crude fat, crude protein, and nitrogen-free extracts. It remains to be determined if patients with a residual exocrine pancreatic secretion, in contrast to pigs with total exocrine pancreatic insufficiency, might benefit from spreading enzyme doses over the day.

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70. Effects of feeding different types of carbohydrates (cellulose, pectin, starch and fructans) on breath hydrogen and methane responses in healthy horses (Effekte der Fütterung verschiedener Kohlenhydrate (Cellulose, Pektine, Stärke und Fruktane) auf die Wasserstoff- und Methanexhalation beim gesunden Pferd). Anne Mößeler, Ingrid Vervuert and M. Coenen - Hannover

The aim of the study was to investigate the effect of feeding highly fermentable carbohydrates on the microbial activity in the intestinum of horses by measuring the methane and hydrogen exhalation with special emphasis on fructans which are involved in the microbial dysfunction in equine laminitis.

Material and methods: In a cross-over design 6 horses were fed in a randomised order: grass meal (cellulose), topinambur (50 % fructans, inulin-type), oats (starch) or sugar beet pulp (SBP, pectin). All diets were formulated to provide 1.5 g hydrolyzable carbohydrates/kg BW/day from the different sources over a feeding period of ten days. Additionally, 6 kg hay/day were given to the horses. At day one, three, eight and ten of the feeding trials, hydrogen and methane exhalations were measured for the respective test meals. A wash-out period of ten days was done in-between the different test diets by feeding exclusively hay. Breath samples were collected with a face mask and a connected air tight tube. Breath samples were withdrawn from the tube and hydrogen and methane were measured using a gas chromatograph (GC 14 A, Fa. Shimadzu). All results were presented as means ± SD.

Results: In comparison to oats or topinambur (fructan of inulin-type) horses showed a higher methane exhalation after grass meal and SBP feeding (treatment P<0.05, Table). In contrast to methane, the hydrogen exhalation was significantly higher after oats or topinambur intake. The hydrogen exhalation peaked ~330 min postprandial (ppr) at >120 ppm for the topinambur (inulin) treatment, whereas oats intake revealed a mean maximum of 70 ppm (210 min ppr., treatment P<0.05).

Table : CH_4 and H_2 exhalation [ppm] after feed intake [min ppr, N =6, mean over day 1, 3, 8 and 10]

	minutes postprandial						
treatment	30	90	150	210	270	330	600
	methane exhalation [ppm]						
grass meal	496±161^a	526±215^a	557±172^a	571±184^a	572±176^a	616±202^a	589±222^a
SBP	522±160^a	531±169^a	523±168^a	562±161^a	545±165^a	569±167^a	628±180^a
oats	491±141^a	449±149^b	458±152^b	431±179^b	442±183^b	463±149^b	464±179^b
topinambur	525±115^a	460±123^b	469±118^b	458±139^b	447±119^b	464±124^b	449±111^b
	hydrogen exhalation [ppm]						
grass meal	18±10^a	18± 7^a	15± 7^a	16±10^a	19±15^a	22±17^a	16±12^a
SBP	20± 9^a	18± 8^a	16±10^a	14± 7^a	15±10^a	18±16^a	18±12^a
oats	22±14^a	28±22^a	57±44^b	74±44^b	70±36^b	64±32^b	23±13^a
topinambur	20±16^a	55±32^b	89±46^b	103±51^b	117±56^b	130±67^b	45±35^b

means with varying small letter superscripts are different (P<0.05)

Conclusion: The hydrogen expiration after starch or fructan intake indicated an intensive fermentation. The rapid increase in hydrogen exhalation within 90 minutes after feed intake emphasised a certain contribution of fermentative processes in the small intestine.
In addition from the differences in methane exhalation, it can be concluded that beside the intensity of fermentation the involvement of methanogenic bacteria in the microbial digestion of cellulose or pectin is quite different from the fermentation of starch or fructans. Results from in vitro studies confirmed the most dynamic fermentation by fructans. These intensive fermentation and individual factors (e.g. microbial community in the small intestine) are of great interest in the aetiology of laminitis in horses.

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71. Comparison of total faecal collection (standard method) and the indicator method with 4 N HCl insoluble ash to determine the digestibility of proximate nutrients in horses (Vergleich von Gesamtkotsammlung [Standardmethode] und der Indikatormethode mit 4 N HCl-unlöslicher Asche zur Bestimmung der scheinbaren Verdaulichkeit der Rohnährstoffe an Pferden). Annette Zeyner*, R. Fuchs, Helga Kaske and M. Hoffmann – Leipzig

According to the nature of equines, moderately intense movement seems to promote the digestibility of nutrients compared to leisure time (5). Therefore, digestion trials with horses should include an appropriate exercise protocol. However, it is difficult to guarantee total faecal collection during exercise. In this way, an indicator method would be helpful. Because of the high amount of roughage in horse diets an innate marker is to prefer. 4 N HCl insoluble ash has always been successfully tested as innate indicator in digestion trials with roe deers (1) and pigs (1,2,3). The marker showed also good results in digestion trials to determine the digestibility of nitrogen in male horses (4). The recent study was conducted to assess the applicability of 4 N HCl insoluble ash to determine the digestibility of proximate nutrients in a typical diet for horses compared to total faecal collection as standard method.

Methods: Four adult Warmblood-type geldings (body weight 535-644 kg) were fed a diet containing 42.7% oats, 42.7% meadow hay and 14.6% wheat straw. According to a cross over design, the digestibility (D) of dry matter (DM), organic matter (OM), crude protein (CP), acid ether extract (AEE), crude fibre (CF) and nitrogen-free extracts (NFE) was determined comparatively with the standard method of total faecal collection and 4 N HCl insoluble ash as indicator, respectively. Each period lasted 23 days with included 6 days faecal collection. Throughout the entire experimental period the horses were daily ridden according to a 45 minutes lasting moderate exercise protocol (5). Analysis of variance (SPSS 11.5) was used to investigate effects of the methods and individual animals, respectively. The level of statistical significance was pre-set at P<0.05.

Results: The D of DM, OM and proximate nutrients determined by the indicator method was not different (P>0.05) from that estimated by the standard method (Tab.). However, in spite of the absence of a significant method effect and in general, D determined by the indicator method tended to be somewhat higher. The animal influenced (P<0.05) the D of DM, OM and all investigated nutrients.

Conclusion: The results of the study indicate that the digestibility of proximate nutrients in horse diets can be determined with high security by using 4 N HCl insoluble ash as indicator. Nevertheless, further trials should finally prove whether the indicated minor difference between standard and indicator method is repeatable and therefore systematic or not.

Method	D_{DM} [%]	D_{OM} [%]	D_{CP} [%]	D_{AEE} [%]	D_{CF} [%]	D_{NFE} [%]
Standard method	69.9^a	72.4^a	76.9^a	72.7^a	42.1^a	79.7^a
Indicator method	72.3^a	74.5^a	78.0^a	72.0^a	47.5^a	81.4^a
±pooled s.d.	*3.26*	*3.81*	*2.07*	*3.95*	*6.88*	*2.52*

^{a}Means with common superscript do not differ significantly (P>0.05).

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72. Fructans in horse nutrition: Occurrence in pasture grasses and fermentation kinetics

(Fruktane in der Pferdefütterung: Vorkommen im Weidegras und Fermentationskinetik). Ingrid Vervuert, M. Coenen, Sandra Dahlhoff, Sophie Plumhoff and W. Sommer - Hannover/Münster

In the temperate forage grasses, the water soluble carbohydrates sucrose and fructan (oligo- and polyfructosyl sucrose) are the primary carbon reserves in the vegetative tissues. In recent studies it is proposed that fructans might be responsible for pasture-induced laminitis in the horse. The aim of this study was to analyse fructan concentrations in mixed pasture grasses for horses during grazing season. Furthermore, the fermentation kinetics of fructans, using the gas production technique, were in the focus of interest.

Fructans in pasture grasses: Ten horse pastures from 10 farms in Germany were included. Grass samples were collected monthly from May to November 2002. Freeze-dried grass samples were analysed for fructan concentrations (Megazyme®), crude nutrients, and minerals. Ambient data were available by the German meteorological office for the whole sampling period from 01.05.-30.11.02.

Fermentation kinetics: In a cross-over design 6 horses were fed in a randomised order: grass meal, topinambur (50 % fructans, inulin-type) or sugar beet pulp (SBP). All diets were formulated to provide 1.5 g hydrolyzable carbohydrates/kg BW/day from the different sources over a feeding period of ten days. Additionally, 6 kg hay/day were given to the horses. The gas production technique of Theodorou et al. (1994) was used to measure the rate of gas accumulation as a marker of in vitro fermentability for the respective diets. One gram of each feed was incubated with the respective faecal inoculum from the horses at day zero and day ten of the different feeding periods. Gas pressure and volume readings were taken every 2 hours for 12 hours post-inoculation.

Results: All pastures consisted of a grass legume mix. The mean fructan concentration in grass varied between 18 g/kg DM in August and 57 g/kg DM in May (Table 1). There was a close negative relationship between protein (CP) and fructan concentration in grass samples (fructan [g/kg DM]= -0.2135*CP+75.29, r=-0.52, P<0.01, N=70). Beside protein, the K and P concentrations in grass as well as the ambient and ground temperature seemed to be negatively related to the fructan contents.

Table 1 Fructan concentrations (g/kg DM) in grass legume mixtures and in L. perenne aurora

Fructan, g/kg DM	May	June	July	August	Sept.	October	Nov.
Grass legume mix	57 ± 17^{a}	38 ± 17^{bd}	30 ± 20^{b}	18 ± 8^{be}	31 ± 18^{db}	42 ± 10^{d}	42 ± 11^{d}
L. perenne aurora*	340	330	420	360	360	290	220

means with varying small letter superscripts are different (P<0.05), *Longland et al. (1999)

Topinambur (fructans from inulin-type) revealed the highest gas production, followed by SBP and grass meal with no differences between the first and tenth day of the feeding trial and the respective faecal inoculum (Table 2).

Table 2 Area under the curve (AUC, over-pressure accumulation [Bar] 2-12 h post-inoculation) for the different feedstuffs

Gas pool AUC	faecal inoculum, 1st day of feeding trial			faecal inoculum, 10th day of feeding trial		
	Grass meal	SBP	Topinambur	Grass meal	SBP	Topinambur
	7.1 ± 0.9^{A}	10.9 ± 1.5^{B}	17.9 ± 3.4^{C}	7.4 ± 1.1^{A}	11.5 ± 0.9^{B}	20.6 ± 3.5^{C}

means with varying letter superscripts are different (P<0.01)

Conclusions: The analysed fructan concentrations in the grass legume mixtures seemed to be moderate when compared to single high-fructan accumulation varieties. However, the rapid fermentation kinetics of the fructans among the hydrolyzable carbohydrates should be pointed out and highlight the role of fructans in the aetiology of laminitis in horses.

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73. The use of near-infrared reflectance spectroscopy to predict the protein, fat and gross energy contents of broiler excreta

(Einsatz von NIRS zur Vorhersage von Protein-, Fett-, und Bruttoenergiegehalten in Broiler-Excreta), Petra Philipps*, Raffaella Aureli, B. Cochin de Billy, Anna-Maria Klünter - Village-Neuf

For the determination of apparent nutrient digestibility in balance studies with broiler chickens, analyses of the nutrient content of excreta are required. However, the classical reference analytical methods are both laborious and time consuming. One alternative to determine the composition of broiler excreta could be the use of near-infrared reflectance spectroscopy (NIRS). The objective of this study was to evaluate the feasibility of a NIRS-calibration to predict the protein, fat and gross energy content of broiler excreta from chickens fed diets based on maize starch and soybean meal.

Methods: A total of 350 broiler excreta samples from several balance studies were utilized to develop an appropriate NIRS calibration. The chickens were housed in wire-floored battery cages and were fed diets based on maize starch and soybean meal. Excreta samples were collected once per day from day 14 to 17, pooled per cage, and frozen directly after collection. After thawing, the excreta samples were homogenized. For gross energy, total fatty acid content and NIR determinations a part of the samples was freeze dried. The content of protein, fat and energy was determined using the corresponding reference method: Crude protein by the Kjeldahl method, gross energy using an adiabatic bomb calorimeter and total fatty acids after extraction using a gas chromatograph. The near-infrared measurements were carried out at room temperature with a Foss NIRSystem 6500. 45 samples were randomly selected for validation of the calibration. The remaining samples were used to develop the calibration. The spectral analysis program WINISI II was used to process the data and to develop the mathematical models. The method of modified partial least-squares residues was used to establish the correlation between the reference values and the NIRS spectra. The calibration was characterized by the coefficient of correlation (RSQ) and the standard error of calibration (SEC). The validation was described by the standard error of prediction (SEP) and R2.

Results: The descriptive statistics for both calibration and validation are shown in table 1. It is essential for any calibration procedure to ensure that the range of variation found in the whole population is represented by the samples selected for the calibration development. In this case the contents of crude protein, crude fat and gross energy varied widely. In the validation set, crude protein showed a R2 of 0.94 and a standard error of prediction (SEP) of 0.69. A high R2 and a relatively low SEP were also obtained for gross energy (R2=.86 ; SEP= 0.18) and for total fatty acids (R2= 0.92 ; SEP= 0.23).

Table 1: Descriptive statistics of the NIRS calibration set and validation for broiler excreta

	Calibration						Validation		
	n	mean	min	max	SEC	RSQ	Slope	SEP	R2
Crude Protein (% DM)	290	31.2	23.4	39.0	0.51	0.96	0.95	0.69	0.94
Total fatty acids (% DM)	288	2.7	0.3	5.1	0.18	0.95	1.057	0.23	0.92
Gross Energy (KJ/g DM)	296	15.8	14.4	17.2	0.15	0.91	1.004	0.18	0.86

Conclusion: The results of the present study showed that, with the developed NIRS-calibration it is possible to accurately predict the contents of crude protein, crude fat and gross energy in freeze-dried samples of broiler excreta.

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74. Studies with alfalfa silage as a forage source for high yielding dairy cows (Untersuchungen mit Luzernesilage als Grundfutterkomponente für Hochleistungskühe). M. Bulang*, J. Boguhn, T. Engelhard, H. Kluth and M. Rodehutscord - Halle (Saale)/Iden

The suitability of alfalfa silage as forage part in total mixed rations (TMR) for high yielding dairy cows was investigated with studies on feed intake and milk yield, on nutrient digestibility, and on *in vitro* synthesis and amino acid pattern of bacterial crude protein (BCP).

Methods: Three TMR were used calculated for a milk yield of >35 kg/d, containing as the forage part (% of DM) either maize silage (50), maize/grass silage (20/30), and maize/alfalfa silage (20/30). Further ingredients were moist ensiled corn, brewer's grains, barley, extracted meals from soybean and rapeseed, and hay. Calculated nXP contents were >168 g/kg DM. Each TMR was fed to 28 cows for 168 d beginning 6 wk after parturition. Intake was recorded for individuals, and cows were milked three times a day. Statistical evaluation was made under inclusion of the test day-model. Nutrient digestibilities for the TMR were determined with wether sheep according to the official methods. A RUSITEC system was used to study *in vitro* OM degradation and BCP net synthesis. Two of the TMR were tested in triplicate for 8 d after an adaptation period of 7 d. BCP net synthesis was measured using the isotope dilution technique after continuous infusion of ^{15}N labelled NH_4Cl with the artificial saliva. Isotope enrichment was measured in reference bacteria obtained by centrifugation. Amino acid content of bacterial protein was analysed.

Results: DM intake of cows was on a high level, and cows fed alfalfa had a significantly higher intake than cows fed the grass ration (see table). As compared to the maize-based ration, milk yield was lower but milk fat as well as milk protein content were higher in the two mixed-forage rations. Digestibility and, consequently, NEL content was lowest in the alfalfa diet. As judged by the *in vitro*-data, the grass and alfalfa diets were significantly different in OM fermentation but not in efficiency of BCP net synthesis. In bacteria isolated from the liquid phase, the contents of arginine, glycine, glutamic acid, and lysine were significantly higher, and the proline content was significantly lower with the grass diet as compared to the alfalfa diet.

	TMR forage		
	Maize	Grass/maize	Alfalfa/maize
Dry matter intake, kg/d	22.5[a] *±0.40*	21.1[b] *±0.40*	23.2[a] *±0.40*
Energy-standardised[1] milk, kg/d	39.2[b] *±0.78*	37.0[a] *±0.75*	37.9[ab] *±0.75*
Milk fat, %	3.59[b] *±0.09*	3.94[a] *±0.09*	3.95[a] *±0.09*
Milk protein, %	3.34[a] *±0.03*	3.39[a] *±0.03*	3.37[a] *±0.03*
NEL, MJ/kg DM[2]	7.1[a] *±0.11*	7.2[a] *±0.07*	6.8[b] *±0.07*
Organic matter digestibility, %	78.4[a] *±0.40*	80.0[b] *±0.40*	76.6[c] *±0.40*
Crude fibre digestibility, %	64.7[a] *±0.90*	70.7[b] *±0.90*	59.9[c] *±0.90*
In vitro OM fermentation, %	n.d.	42.2[a] *±0.40*	41.0[b] *±0.26*
BCP/fermented OM, g/kg	n.d.	187[a] *±5.0*	182[a] *±4.8*

[1] kg milk × (0.38 × % fat + 0.21 × % protein + 1.05) / 3.28 [2] based on digestibility measurements

[a,b] letters show significant treatment effects (p<0.05)

Conclusions: In spite of its relatively low nutrient digestibility, alfalfa silage may be a suitable ingredient for dairy cows with high milk yield. Low digestibility is partly compensated for by higher dry matter intake of alfalfa diets. The amino acid pattern of microbial protein may be different between grass and alfalfa-based diets. In maize-based rations, alfalfa silage appears suitable forage to improve structure in the diet as well as ruminal nitrogen balance.

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75. Recovery of ^{13}C in breath CO_2 of young bulls (German Holstein x Charolais) after a bolus dose of $NaH^{13}CO_3$ (^{13}C-Wiederfindung im Atem-CO_2 bei Jungbullen (German Holstein x Charolais) nach einer Bolusgabe von $NaH^{13}CO_3$). P. Junghans*, J. Voigt, W. Jentsch, C.C. Metges and M. Derno - Dummerstorf

The recovery of ^{13}C is needed as correction factor for measurements of substrate oxidation (1) and energy expenditure based on the ^{13}C dilution technique (2, 3). The objective of this study was to quantify the recovery of ^{13}C in young bulls after a bolus dose of $NaH^{13}CO_3$ and to explore effects of ^{13}C tracer dose on the recovery of ^{13}C.

Methods: The experiments were performed with bulls of the German Holstein x Charolais F_2 generation (n = 12, age: about 4.5 months, live weight: 332±16 kg). For measuring of gas exchange animals were adapted to the diet and the respiration chambers for 2 weeks. Thereafter, they received an intravenous bolus of $NaH^{13}CO_3$ (group A: 1 µmol/kg (n=2 animals), group B: 2 µmol/kg (n=4), group C: 6 µmol/kg (n=6), 99 atom % ^{13}C) into the jugular vein during their stay in the respiration chamber. After the ^{13}C dose the breath gas was collected from the respiration chamber during a 12h-period (7.00-19.00 h) and transferred into breath bags. Thereafter, the ^{13}C enrichment in breath CO_2 was measured by means of an infrared ^{13}C analyzer (IRIS II, WATV, Bremen).

Results: The 12h-recovery of 13C in breath CO_2 (% of 13C dose) was not affected by the different doses of $NaH13CO_3$(A: 54.2±4.5, B: 54.4±3.5, C: 60.6±6.3; P>0.05). The recovery of 13C, averaged over all animals, was 57.2±5.7 %. This 13C recovery is very close to our values found previously in German Holstein and Charolais to be 60 and 65, respectively. The CO_2 production measured by indirect calorimetry amounted to 17.1±2.2 (L CO_2 / (12h * kg0.75)). The excretion of ^{13}C via urine and faeces was less than 3 and 1 % of ^{13}C dose, respectively. Other potential losses (e.g. as urea via sweat) are considered to be negligible.

Conclusion: Future studies on energy expenditure in bulls (German Holstein x Charolais) can be carried out by the ^{13}C bicarbonate dilution method (3) using the bicarbonate correction factor (= ^{13}C recovery/100) of 0.57. Thus, the ^{13}C bicarbonate dilution method can be used alternatively to indirect calorimetry.

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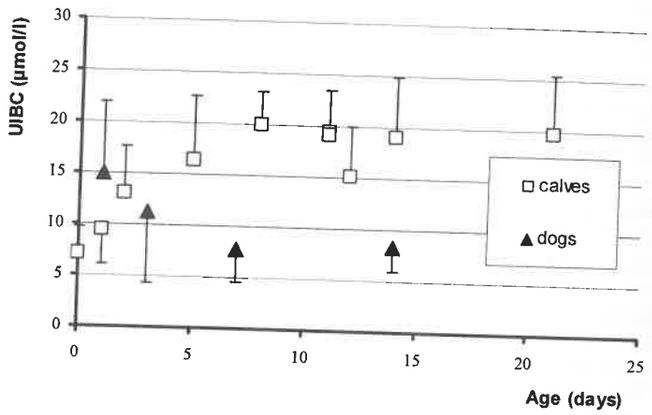
76. Impact of iron status on antioxidant status of newborn animals (Einfluss des Eisenstatus auf den antioxidativen Status von neugeborenen Tieren). M. Stohrer*, S. Porzig, J. Schwarzer, W. Rambeck, M. Stangassinger - München

Antioxidant status is known to be a significant predictor of disease and mortality in infants, especially premature infants. Oxygen radicals are involved in bronchopulmonary dysplasia, retionopathy of prematurity, necrotising enterocolitis and intracerebral hemorrhage. The cause of the impaired antioxidant status of newborns is not known. The objective of this study was to prove the hypothesis that liberation of iron during degradation of fetal hemoglobin causes substantial antioxidant consumption.

Methods: Iron status and antioxidant status of species with fetal hemoglobin (horse, cattle) and without fetal hemoglobin (dog) was investigated. In order to characterize antioxidant status trolox equivalent antioxidant capacity (TEAC) was measured in blood serum starting immediately after birth and continued over several weeks. Iron status was characterized by measurement of unsaturated iron-binding capacity (UIBC), total iron and free iron using a sigma test kit.

Results: TEAC levels were equal in blood sera of mares (0.38 ± 0.15 mmol/l, n = 67) and newborn foals (0.38 ± 0.06 mmol/l, n = 35). However, antioxidant status was significantly impaired during the first week of life (0.24 ± 0.03 mmol/l, n = 18 at 7th day) and remained at low levels during the investigated period. The same pattern was found in cows (0.27 ± 0.07 mmol/l, n = 19), newborn calves (0.28 ± 0.08 mmol/l, n = 19) and calves after one week (0.20 ± 0.05 mmol/l, n = 19 at 5th day). In contrary, newborn puppies had almost the same antioxidant status (0.30 ± 0.04 mmol/l, n = 11) as after one week (0,27 ± 0.03 mmol/l, n = 11).



Postnatal UIBC was low in animals with fetal hemoglobin and significantly higher in animals without fetal hemoglobin.

Conclusion: These results support the hypothesis, that postnatal iron liberation during degradation of fetal hemoglobin contributes significantly to the impaired antioxidant status of species with fetal hemoglobin.

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77. Comparison of different protein feedstuffs for growing-fattening turkeys (Vergleich von verschiedenen Eiweißträgern in der Putenmast). S. Hintenaus*, Christine Iben, W. Zollitsch - Vienna

Introduction: Diets for growing turkeys up to about 6 weeks of age usually contain animal protein sources. As a result of the BSE-crisis, meat and bone meal has been excluded from diets for all livestock species. Due to this concern, in Austria fish meal and certain milk by-products, together with increased levels of soybean meal and synthetic amino acids are used to meet the animals' requirements. In the following experiment different protein feedstuffs were used to find out, whether diets containing no animal protein sources are suitable for grower diets during the first weeks of the fattening period.

Materials and Methods: 96 male and female one day-old turkey chicks were divided into four groups of 24 birds each and placed into litter pens. The diets of all groups were based on soybean meal. For the first three weeks (phase 1), the feed mixture also contained 5 % fishmeal (group 1), 3 % fishmeal and 4 % cheese-scraps (group 2). The diet of group 3, only contained soya as the main protein source, as it was the case for group 4 which was supplemented with 0.004 % carnitin. Between week four and seven the diets contained 3% fishmeal (group 1), 1.5 % fishmeal and 2 % cheese-scraps (group 2), only soybean meal (group 3), and only soybean meal with carnitin-supplementation of 0.00375 % (group 4). In weeks eight to fifteen for the female turkeys and eight to nineteen for the male turkeys all groups received an identical diet without animal protein sources, except group four, which was supplemented with carnitin for the hole fattening-period. For all treatments, varying percentages of soybean meal and synthetic amino acids werde added (l-lysine and dl-methionine).
Turkeys were weighed after each phase. Blood samples were collected and analyzed for selected physiological parameters (Urea, Phosphorus) Mobility, animal welfare and animal health were evaluated regularly during the phases. After fifteen and nineteen weeks the female and male turkeys were slaughtered, respectively.

Results: No differences were observed concerning mobility, animal welfare and animal health in the various litter pens. No significant differences were found for female and male turkeys at the end of week 14 and 19, respectively. There was no significant difference in feed conversion in any stage of life, during the first three weeks the feed conversion was between 1.59 in groups 2 (SD=0.09) and 4 (SD=0,16) and 1.64 (SD=0.13) in group 1 and 1.6 (SD=0,03) in group 3. During the second phase feed conversion ranged between 1.75 (group 4) and 1.92 (group 3). Serum Phosphorus and Urea levels were in the same range throughout all groups.

Conclusion: The removal of animal protein from turkey diets did not result in growth depression at an early stage if diets were properly supplemented for amino acids. Health related problems frequently reported as beeing connected to feeding diets based solely on plant protein were not observed in this experiment.

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78. Influence of high altitude on bone metabolism of growing sheep (Einfluss der Alpung auf den Knochenstoffwechsel bei heranwachsensen Schafen).

Annette Liesegang*, D. Hüttenmoser, F. Leiber and H. Leuenberger - Zurich/Hunenberg

Bone metabolism in ruminants can be monitored in vivo by measuring enzymes and other protein products released by osteoblasts and osteoclasts, respectively. Bone mineral density (BMD) and bone mineral content (BMC) can be determined by peripheral quantitative computer tomography (pQCT). The purpose of this study was to investigate, if high altitude with other feed conditions, different weather and light situations and other exercise (on hillside) has an influence on bone metabolism and bone mineral content and density of grazing growing sheep.

Twenty-four 5-month-old sheep (Jura breed) were randomly assigned into two groups. One group with 12 animals was kept at an high altitude (HA group) (Alp Weissenstein, ETH, 2000 m a.s.l.) for 3 months, and the other group remained on an average swiss altitude (control group = C) at the research station Chamau of the ETH Zurich (400 m a.s.l.). At the beginning of the experiment blood samples were taken from all animals. In addition, the sheep were weighed and the left metatarsus (50%) of each animal was measured with pQCT. Each group was housed outdoors and grazing on the pasture. Salt was added on the pasture. After one month adaptation period blood samples were taken again and weight was controlled. Thereafter, every 2 weeks all animals were weighed and blood was taken until the end of the high altitude period. Then, the animals were slaughtered and tibia and metatarsus were taken. Serum crosslaps (SCL) was used as bone resorption marker and bone-specific alkaline phosphatase (bAP) as bone formation marker. To determine SCL and bAP commercially available ELISA testkits were used. In addition, Ca, Mg and P were determined in serum by colorimetry with an autoanalyzer (COBAS MIRA® Roche-Auto-analyzer, Basle, Switzerland). Since the animals of the high altitude group already had higher weights prior to the beginning of the experiment, the weight was set as 100 % at the first measurement. The relative weight and weight gain was calculated thereafter depending on the weight at the beginning.

In both groups mean Ca, Mg and P concentrations remained in the normal range during the whole experimental period of three months. The development of the mean weight of the two groups was in a normal range and no significant difference between the two groups occured. Mean SCL concentrations and mean bAP activities were significantly higher in the HA group compared to the C group during the whole experiment except at the last sampling time where this difference disappeared. Mean BMC in the metatarsus and tibia of the HA group increased during the experimental period and was also higher than the BMC of the C group. But neither the increase with the time nor the group effect was significant. Mean BMD remained unaffected with time in both groups.

In conclusion, it was shown, that growing sheep kept at high altitude showed a higher bone turnover which was demonstrated with higher bone formation and resorption markers. Also the bone mineral parameter showed a tendency towards higher mineral content in the HA group. The reason for this difference may be the more intensive motion of the animals in the slopes of the mountains, but could also be related to the increase of UVB radiation at high altitude which has an influence on the production of vitamin D in the skin. Mean BMD did not vary, since bone formation and resorption were coupled and in balance. However, more specific information needs to be collected and evaluated before a definite conclusion can be drawn from the presented results. Since the weather conditions and the feedstuffs are not evaluated until now, no specific comments can be given at this time.

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79. Effects of a gradual replacement of grain by dried sugar beet pulp in calve diets on feed intake, digestion, apparent fermentation in the forestomach, N-containing fractions in the duodenal fluid and body weight gain (Effekte eines schrittweisen Austauschs von Getreide durch Trockenschnitzel in Rationen für Kälber auf Futteraufnahme, Verdaulichkeit, scheinbare Fermentation im Vormagen, N-haltige Fraktionen im Duodenalfluid und Lebendmasse-zunahme).

E. Boldt*, T. Hanna and M. Hoffmann - Leipzig

Effort exists to evaluate energy supplying feeding stuffs other than grain for calves. This study was conducted to assess the effects of a stepwise exchange of grain by dehydrated sugar beet pulp (SBP) in calve diets on feed intake, digestion, apparent fermentation in the forestomach, N-containing fractions in the duodenal fluid and body weight gain (BWG).

Methods: A total of 56 calves received diets containing limited amounts of concentrates plus grass hay *ad libitum* for 112 days (27th to 139th day of life [LD]). Additionally, the calves were fed 800 g of a commercial milk replacer (27th to 68th LD) or 150 g dried skim milk (69th to 95th LD) per day. The concentrate contained in every case 34% of a basal mixed feed and in the diets SBP-0, SBP-22, SBP-44 and SBP-66 furthermore 66, 44, 22 and 0% barley+wheat (1:1) plus 0, 22, 44 and 66% dehydrated sugar beet pulp, respectively. The 4 diets were fed in parallel to 14 calves each group. BWG, feed intake and digestibility (D) of organic matter (OM), crude protein (CP) and crude fibre (CF) were determined in all animals per group. Contrary, selected parameters in the forestomach (apparent fermentation [AF] of OM and cellulose) and duodenal fluid (DF; total amino acids [AA], NAN [non-AA nitrogen], bacterial N, bacterial N/NAN) were investigated in 4 calves per group and while feeding diets SBO-0 and SBP-66 only. For this, the 2x4 animals were fitted with a duodenal cannula.

Results: The concentrate intake was not significantly affected by the diet. Contrary, the voluntary hay intake increased while the consumption of SBP rose. D_{OM} and D_{CP} decreased, but D_{CF} increased with higher amounts of SBP in the diet (Tab.). In the same way, $AF_{Cellulose}$ was higher in diet SBP-66 compared to diet SBP-0. In the duodenal fluid, all N-fractions measured tended to be higher when the diet contained SBP. The BWG was not rectilinear influenced by SBP consumption.

Conclusion: The results indicate that grain in calve diets can be replaced by SBP up to 66% of the concentrate without obviously detrimental effects. Further trials should address the water balance.

Parameter			SBP-0	SBP-22	SBP-44	SBP-66
Intake	Dry matter	[g/d]	1995	2043	2074	2040
		[g/kg $BW^{0.75}$xd]	73.5	74.6	75.4	74.9
	Crude protein	[g/d]	381	381	379	367
	AA	[g/d]	365	363	354	337
	Crude fibre	[g/d]	299	346	397	430
D	Organic matter	[%]	74.0	74.9	73.2	71.8
	Crude protein	[%]	68.1	66.6	62.4	61.0
	Crude fibre	[%]	58.7	66.2	68.8	71.9
AF	Organic matter	[%]	49.9	-	-	48.8
	Cellulose	[%]	63.7	-	-	76.9
DF	NAN	[g/kg OMx24 hrs]	27.2	-	-	30.6
	Bacterial N	[g/kg OMx24 hrs]	14.7	-	-	17.1
	Bacterial N/NAN	[%]	53.6	-	-	55.9
	AA	[g/kg OMx24 hrs]	141	-	-	159
BW	initial	[kg]	44.8	44.3	43.0	44.1
BWG		[g/d]	672	689	715	675

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80. Recording of metabolic parameters during the first twelve weeks post partum in dairy cows: Interrelationships with milk yield, body condition and age (Aufzeichnung von Stoffwechselparametern während der ersten zwölf Laktationswochen: Beziehungen zu Milchleistung, Körperkondition und Alter). Sabrina Hachenberg*, Claudia Weinkauf, Stephanie Hiss, Ute Müller, H. Spiekers and Helga Sauerwein - Bonn

Metabolic imbalances during the first weeks post partum (p.p.) are characteristic for high yielding dairy cows. The relative deficits in energy supply frequently occurring during this stage are related to health impairments and to reduced reproductive performance. Among other reasons, limitations in the metabolical adaptation to lactation may be secondary to poor feed intake and/or inadequate or suboptimal feed management. The present study was undertaken at an experimental farm (Riswick, Kleve) in which different management practices (organic and conventional farming) as well as feeding regimes are realised. Different blood parameters were recorded in cows during the first 12 weeks post partum and the data for ß-hydroxybutyrate (ß-OH-B), glutamate dehydrogenase (GLDH) and urea presented herein were tested for potential relationships with milk yield, body condition and lactation number.

Methods: 42 Holstein Friesian cows, being in their first to eighth lactation were studied in three groups. Two groups (OI; n=15 and OII; n=14) were kept in the free-stable barn at the organic section of the farm. OI-animals were fed with a mixed ration diet covering a milk yield of 20 kg/day. For group OII, concentrates were added to this diet to cover 26 kg/day. The third group (C; n=13) with a genetic background comparable to groups OI and OII, was kept in a similar housing system at the conventionally managed section of the farm and received another mixed ration diet covering 30 kg milk yield. In addition, all groups received concentrates on an individual feeding basis according to their actual milk production. Body condition score (BCS) and milk yield (energy corrected milk, ECM) values used herein were recorded during the first month p.p.. Blood samples were collected weekly by jugular venipuncture from week 1 to 12 p.p.. Serum ß-OH-B, urea and GLDH were determined by a veterinary diagnostic laboratory (VLK, Köln). Statistical analyses were done using the repeated measure approach with the SPSS program.

Results: For all parameters recorded, no group related differences were observed during the recorded times. ECM, BCS and age were not co-related. The post partal time course of ß-OH-B concentrations was affected by BCS values, as indicated by the interaction of time x BCS (p=0.061). Age, i.e. number of lactations, also affected ß-OH-B serum concentrations (p=0.054), i.e. older cows had higher ß-OH-B concentrations in average than younger ones. Urea concentrations were affected by ECM (p=0.064), but not by BCS or age. GLDH activity in serum was unaltered by time, but was affected by age and ECM (p=0.020 and p=0.022, respectively).

Conclusions: The differences in energy intake aimed at in the groups OI versus OII and C were not reflected in detectable differences in either parameter recorded. At the present state of data assessment, the actual intakes could not yet be considered. Until now, the significant relations established for some blood parameters with age, milk yield and BCS indicate that these factors might be more relevant effectors of the metabolic parameters. Whatever the relative contribution of either factor might be, the meaning of the blood parameters in terms of health, welfare and reproductive performance still requires consideration and further investigation.

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81. Interindividual and intraindividual variation of feed intake in fresh cows (Die interindividuelle und intraindividuelle Variation der Futteraufnahme bei frisch laktierenden Milchkühen). Karen Horstmann*, Sabine Seggewiß, J. Rehage, U. Meyer, G. Flachowsky and M. Kaske – Hannover/Braunschweig

The fresh cow period represents the phase with the highest risk for infectious and non-infectious health problems during the reproductive cycle of a dairy cow [1]. Especially metabolic disturbances have been attributed to the negative energy balance (NEB) in early lactation due to the delayed increase of feed intake in face of high milk production. Although NEB is evident for nearly all high-yielding dairy cows during the first weeks post partum [p.p.], only a relatively small portion of these cows develop health problems. We hypothesized that this phenomeon is due to a large interindividual variance in the capability of cows to handle such a situation by endocrinological adjustments of the metabolism. As the first step to verify this hypothesis, the variation of feed intake within a herd of high-yielding dairy cattle was investigated.

Methods: Twenty pluriparous German Holstein Friesian cows (606 ± 44 kg body weight; mean ± SD) randomly selected from a herd of 60 cows were included in the study. All cows were housed in a free stall with 58 cubicles, 51 computerized feeder stations allowing the automatic registration of individual silage intake, 4 automatic concentrate feeders and four water stations. A mixture of corn silage and alfalfa silage (65:35) was fed ad libitum, concentrates were offered according to milk yield. In each cow, body weight, milk yield and intake of concentrates and silage were registered daily from parturition until week [w] 15 post partum [p.p.] to characterize the individual energy balance on a daily basis. Venous blood samples were obtained weekly and liver biopsies were taken two weeks prior to calving at w 2, 4, 6 w p.p., respectively. In addition, at day [d] 13, d 25, and d 43 p.p., 10 kg concentrates were withdrawn; silage was still available ad libitum; blood samples were obtained at the day prior to energy restriction, the day of feed restriction and the following day. The intraindividual coefficients of variation [CV_{intra}] were calculated for each cow from all weekly results, the interindividual coefficients of variation [CV_{inter}] were calculated for each week from all cows being investigated.

Results: Mean dry matter [DM] intake increased gradually from w 1 to w 4 p. p. up to a level of 23 - 25 kg DM/d during w 5 to w 15 p.p., while average milk yield peaked in w 5 p.p. (40.4 ± 4.7 kg FCM/d). Intake of silage reached a plateau already in w 3 p.p. and averaged thereafter at 11 – 12 kg DM/d. Intake of concentrates increased up to w 6 and averaged thereafter at 11-12 kg DM/d. CV_{inter} for silage intake was markedly higher (27 %) than that for concentrate intake (19 %). CV_{intra} of silage intake did not depend on the week p. p. and averaged at 18 %. By contrast, CV_{intra} of concentrate intake was higher during w 1 – w 4 p.p. (12-28 %) as compared to the following weeks (4-12 %). From w 6 to w 15, intraindividual variation of concentrate intake was smaller than that for silage intake. The intraindividual variation of energy intake was considerably higher (mean CV 10-15 % for w 1 to w 15 p.p.) as compared to the variation of energy output according to milk production (mean CV 5 %, respectively. Despite of a high interindividual variability of feed intake especially during the first weeks p.p., nearly all cows exhibited a negative energy balance during this period of lactation. It is concluded that the higher intraindividual variation of dry matter intake during w 1 to w 4 compared to the following weeks may reflect the increased risk for health problems in this sensible period of lactation. Further data analyses are on the way.

1) FLEISCHER, P., METZNER, M., BEYERBACH, M., HOEDEMAKER, M., KLEE, W. (2001): The relationship between milk yield and the incidence of some diseases in dairy cows. J. Dairy Sci. 84, 2025-2035

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82. Milk composition in fresh cows: the influence of energy balance and week of lactation (Die Milchzusammensetzung bei frisch laktierenden Milchkühen: der Einfluss der Energiebilanz und der Laktationswoche).

Sabine Seggewiß*, Karen Horstmann, J. Rehage, U. Meyer, G. Flachowsky and M. Kaske – Hannover/Braunschweig

The objective of the study was (a) to evaluate how fast changes in the energy supply of fresh cows become evident in the composition of the milk and (b) to characterize the interindividual differences in respect to the manifestation of changes in milk composition due to an energy restriction.

Methods: Twenty pluriparous German Holstein Friesian cows (606 ± 44 kg body weight; mean ± SD) were randomly selected from a herd of 60 cows. All cows were housed in a free stall with 58 cubicles, 51 computerized feeder stations allowing the automatic registration of individual silage intake, 4 automatic concentrate feeders and four water stations. A mixture of corn silage and alfalfa silage (65:35) was fed ad libitum; concentrates were offered according to milk yield. Milk yield was obtained daily. The composition of the milk was determined (Milkoscan FT 6500) twice weekly for the morning and evening milking from week [w] 1 to w 12 post partum [p.p.]. The energy balance was calculated on a daily basis for each animal. In addition, at day [d] 13, d 25, and d 43 post partum [p.p.], 10 kg concentrates were withdrawn; silage was still available ad libitum. Milk composition was assessed in 12 h intervals at the day prior to energy restriction, the day of feed restriction and the following day.

Results: The evaluation of the data has not been completed until now. First of all, clusters of all results within the respective weeks post partum (Table 1) were constituted; these results were compared with those where the energy balance was used for the classification.

Table 1: Means (± SD) of milk yield and energy balance and medians (25/75 percentiles) of milk composition for 20 fresh cows; EB – energy balance; n – number of milk samples

Week p.p.	n	Milk yield [kg/d]	EB [MJ NEL/d]	Milk fat [%]	Milk protein [%]	F/P-ratio
1/2	28	32.6 ± 4.3	-38 ± 21	4.7 (3.9 / 5.3)	3.6 (3.5 / 4.1)	1.26 (1.13 / 1.46)
3/4	38	37.7 ± 4.6	-20 ± 25	4.1 (3.3 / 4.5)	3.3 (3.1 / 4.1)	1.23 (0.82 / 1.37)
5/6	38	39.2 ± 4.5	-9 ± 29	3.7 (3.2 / 4.5)	3.2 (3.1 / 4.6)	1.17 (0.72 / 1.39)
7/8	28	39.3 ± 4.9	2 ± 32	3.5 (3.0 / 4.1)	3.3 (3.1 / 4.7)	1.00 (0.64 / 1.29)
9/10	57	38.4 ± 4.4	-9 ± 30	3.5 (2.9 / 4.1)	3.3 (3.1 / 4.8)	0.99 (0.67 / 1.27)
11/12	40	37.8 ± 4.9	-10 ± 33	3.5 (3.2 / 4.2)	3.4 (3.2 / 4.8)	1.04 (0.66 / 1.29)

Results reveal high milk fat contents in the first weeks of lactation probably due to increased lipolysis being the consequence of the negative energy balance which was evident in almost all cows in week 1 and 2 p.p.. However, milk protein was not decreased and the fat/protein-ratio [FPR] did not indicate a lack of energy. This observation was substantiated by the results of the evaluation according to the extent of energy supply: milk samples taken from those cows with the most pronounced energy deficit (< -40 MJ NEL/d; n = 39) showed higher protein contents than the milk of cows in a positive energy balance (> 20 MJ NEL/d; n = 29) (M 3.6 vs. 3.3 %). The FPR was not affected by the energy balance (1.12 vs. 1.06); however, this parameter revealed a lack of structure in the ration. It is concluded that the proportion of structure in the ration (see paper of Horstmann et al.) affected milk composition more pronounced than the energy balance per se. Interindividual variation was large as well as the reactions on milk composition provoked by the restriction of concentrates for one day; these results will also be presented after final analysis.

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83. Investigations on water intake of fattening bulls (Untersuchungen zur Wasseraufnahme von Mastbullen).

Wiebke Stahl*, U. Meyer and G. Flachowsky – Braunschweig

Water is the most important nutrient. A sufficient supply of clean water is generally accepted as essential to prevent negative effects on animal health, performance and welfare. The goal of this experiment was to investigate relationships between water intake and influencing factors such as average ambient temperature, humidity, average daily life weight gain, body weight, dry matter intake, dry matter content of the roughage, dry matter intake from the roughage and roughage portion of the ration.

Methods: In the experiment 62 German Holstein bulls with an initial mean body weight of 193 kg were kept on slatted floor in group boxes (7 or 8 bulls per box) in a thermally non regulated stable. The experimental ration consisted of corn silage and concentrate mixtures with wheat, dried beet pulp, minerals, soybean oil and soybean meal or peas. Corn silage was offered ad libitum and the amount of concentrate was increased according to the growth intensity in the course of the experiment (from 2.1 to 3.2 kg/day). The bulls had free access to water at any time. Feed intake and daily live weight gain as well as daily water intake were registered individually. The experiment was terminated with a life weight of approximately 550 kg.

Results: The subjection of the data to multiple regression analysis results in the following equation:
Water intake (kg/day) = - 3.85 + 0.507 * average ambient temperature (°C) + 1.494 * dry matter intake (kg/day) – 0.141 * roughage fraction (%) + 0.248 * dry matter content of the roughage (%) + 0.014 * body weight (kg).
The average ambient temperature caused the highest r^2-increase (0.17). For each °C temperature increase, water intake rose by 0.51 kg/day. Applying dry matter intake as additional variable r^2 was enhanced by 0.10. Each kg of dry matter intake needs additionally 1.49 kg drinking water; each percent more roughage in the ration reduces the need of drinking water by 0.25 kg. The inclusion of the dry matter content of the roughage and body weight into the equation improved r^2 by 0.02 (Table 1).

Table 1: Results of the stepwise regression analysis (n = 17762)

Step	Variable	r^2	Increase in r^2
1	Average ambient temperature	0.1697	0.1697
2	Dry matter intake	0.2736	0.1039
3	Roughage fraction	0.3158	0.0422
4	Dry matter content of the roughage	0.3370	0.0212
5	Body weight	0.3520	0.0150
6	Average daily life weight gain	0.3553	0.0033
7	Maximal ambient temperature	0.3581	0.0028
8	Atmospheric humidity	0.3601	0.0020

Conclusions: It is supposed that the presented equation considers the most significant factors for predicting the water consumption of fattening bulls under housing and feeding conditions existing in Central Europe.

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84. Influence of various anionic salts on rumen fermentation, blood and urine parameters and their possible relevance in the prevention of milkfever (Einfluss verschiedener Anionenergänzungen auf die Pansenfermentation, Blut-, und Harnparameter und ihre mögliche Bedeutung in der Prophylaxe der Gebärparese). Meike Engel*, S. Frömer, Leonie Löffler - Berlin

The parturient paresis is still one of the oldest and most common metabolic diseases of early lactating cows. In recent years the supplementation of strong anions ("acid salts") 2 to 3 weeks a.p. has become more important due to the hardly practicable Calcium intake of less than 40 g of Ca per day. The objective of the presented study was to examine the effects of several pure or mixed anionic salts on rumen VFA (volatile fatty acids), on urinary electrolyte concentration and acid-base balance.

Methods: The experiment was conducted with 11 rumen-fistulated Holstein Frisian female cattle. All of them were non-pregnant, non-lactating and had more than two lactation periods. The cows (650-750 kg) received a maintenance diet of 8 kg of hay and 2.5 kg of concentrates. The daily ration included 120-150 g Ca in total.
8 pure acid salts ($CaCl_2$, $CaSO_4$, $CaSO_4$-D10, $MgCl_2$, $MgSO_4$, NH_4Cl, $(NH_4)_2SO_4$, NaCl), 2 mixed anionic salts ($CaCl_2 + MgSO_4$, $NH_4Cl + CaSO_4$) - resolved in water - and water as negative control were applied randomly (11 x 11 Latin square) over 14 days. This period of supplementation was followed by a 14 day "wash-out" phase in which parameters should reach their bench marks. The daily dose of 2 equivalents per animal was administered directly into the rumen during feeding time at 7 am and at 2 pm.
Twice a week samples of blood, urine and rumen fluid were taken during the whole experimental period and stored at -20 °C until analysis.

Results: The applied salts and salt-combinations had no significant influence on rumen fluid pH (values between 6,5 and 6,9) and only small effects on rumen VFA contents (120-140 mmol/l rumen fluid) at different times of sampling. Also small effects had been observed with regard to the single analysed fatty acids.
The potassium and phosphate concentration in urine were not affected during the treatment with anionic salts. According to the expectations, application of sodium, magnesium and chloride anionic salts caused a significantly higher urinary concentration of these elements. With the exception of NaCl and $CaCl_2 + MgSO_4$ the calcium concentration of urine was increased significantly in all treated cows. All anionic salts caused a significant decrease of urinary pH by -0.4 to -1.7 pH units as well as NAE (net acid excretion) by -80 to -200 mmol/l. Similar effects were observed for the BE (base excess).

Conclusion: The treatment with anionic salts had only small effects on microbial fermentation characterised by pH and VFA in the rumen. The efficiency of the different anionic salts was characterised by increased calcium concentration and decreased NAE and pH in the urine.

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85. Methane emission of dairy cows fed easily and hardly fermentable roughages with and without concentrate (Methanemission von Milchkühen beim Einsatz von leicht- und schwerfermentierbarem Grundfutter mit und ohne Kraftfutter). Ida K. Hindrichsen*, H.-R. Wettstein, Andrea Machmüller and M. Kreuzer - Zurich

Feeding increasing amounts of concentrate as assumed to reduce methane energy loss in ruminants (1). The decrease in methanogenesis is either due to a decrease in pH, which reduces fibre digestibility and therefore hydrogen production, and/or due to a higher propionate production, which serves as a sink for hydrogen. Furthermore, the quality of the roughage has an important influence on methane emission, since it influences the fermentability (1). This would suggest that feeding cows with diets based on high quality roughage and few concentrate might result in massively higher methanogenesis than to offer low-quality roughages supplemented with high amounts of concentrate. The present study was conducted to determine these effects under the condition of nutrient and energy being supplied according to requirements.
Methods: Four different diets (A, B, C, D) were fed to 24 dairy cows (n=6). Group A had a low milk yield and was fed grass silage and low quality hay without concentrate, while group B had a medium milk yield and was fed the same roughage but together with concentrate in a ratio of 1:1. Group C had the same milk yield as B, but was fed grass silage and maize silage mixture without concentrate. Group D with high genetic merit in milk yield was fed the same roughage as C together with concentrate in a ratio of 1:1. The diets were calculated to meet requirements for maintenance and milk production. After 2 weeks of adaptation to the diets, total faeces, urine and milk outputs were recorded and sub-samples collected over a period of eight days. On days 4 and 5, gaseous exchange was measured in open-circuit respiration chambers.
Results: The intended differences in diet composition and milk yield were widely realised (Table 1). The NDF and ADF digestibility of group D was significantly ($P<0.05$) lower compared to diet A. The methane emission in g/kg of milk from cows receiving diet A (40) was significantly ($P<0.05$) higher compared to diets B, C and D (20, 21 and 15, respectively). The gross energy loss through methane emission (% of gross energy) was significantly ($P<0.05$) lower for the two groups of cows receiving concentrate (6.0 and 6.0 in B and D) compared to the two groups receiving no concentrate (7.1 and 7.3 in A and C). Energy expenditure (EE) of cows receiving no concentrate (A and C) was significantly ($P<0.05$) lower compared to cows supplemented with concentrate (B and D).

Table 1. DM intake and diet composition, milk yield, methane emission and energy expenditure.

Diet/group	Group A	Group B	Group C	Group D
DM intake, kg/day	13.6	19.6	14.3	20.2
NDF, g/kg diet DM	569	496	509	424
ADF, g/kg diet DM	356	251	309	217
Milk yield, kg/day	9.3	21.2	18.2	28.0
Methane, g/day	326^{b}	416^{a}	367^{ab}	415^{a}
EE, MJ/day	96^{b}	118^{a}	100^{b}	126^{a}

Conclusion: The present results illustrate that diets relying on high quality forages are not necessarily environmentally hazardous from the viewpoint of methane emission. Concentrate supplementation nevertheless reduces relative methane emission to a certain extent.

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86. Evaluation of daily requirement of ME for fattening lambs of the genotype German Merino landsheep using different ME systems (Überprüfung des täglichen Bedarfes an Umsetzbarer Energie für Mastlämmer der Rasse Merinolandschaf bei Verwendung unterschiedlicher ME-Bewertungssysteme). G. Bellof* and J. Pallauf – Freising-Weihenstephan/Giessen

The purpose of this study was to evaluate the daily requirement of ME for fattening lambs using three different systems of ME prediction. The current British (AFRC, 1993) and German (GfE, 1996) ME systems should be compared with a model which was suggested by BELLOF and PALLAUF (2003).
Methods: Data from a lamb fattening experiment (BELLOF, 2003) were used to evaluate the different ME systems. Twenty male lambs of the genotype German Merino landsheep were fed different hay and concentrate in the daily rations. Whereas in the first group the animals were offered concentrate (pelleted) ad libitum and a limited amount of hay, the second group was given hay ad libitum but was offered only a limited amount of concentrate (about 70 % of ad lib.). The feeding of the animals at special demand-feeding stations made it possible to record the daily feed intake of both feedstuffs for each animal and at the same time that of the group.
The ME concentration in the dietary dry matter increased from 11.4 MJ/kg (average of second group) to 12.2 MJ/kg (av. first group). In the first group the average daily weight gain was 401 g, while in the second group it was 328 g. For each animal and each weight group, the daily ME expenditure was compared to the daily ME requirement. ME requirement was determined according to:
a) the model (2) recommended by BELLOF and PALLAUF (2003), based on fattening experiments and comparative slaughter techniques as described by BELLOF et al. (2003);
b) the German ME system (GfE, 1996), using given energy value of tissue gain (no sex differences);
c) the British ME system (AFRC, 1993), modified by using the energy value of tissue gain in the investigation of BELLOF et al. (2003).
The data pairs obtained were evaluated by a regression analysis.
Results: The evaluation results for the ME requirement are shown in Figure 1.

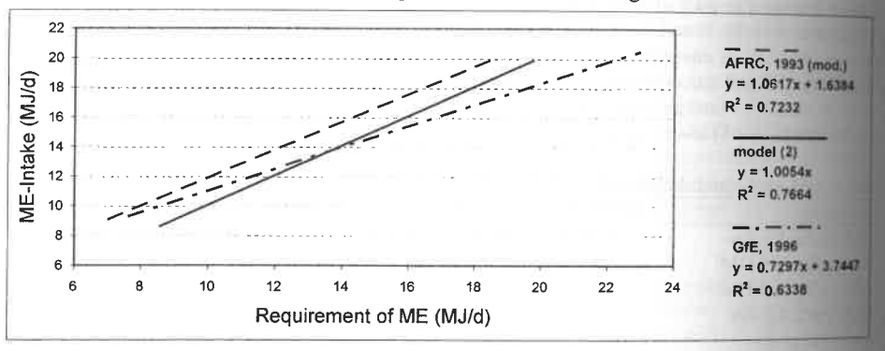

Figure 1 *Evaluation of ME requirement by different models: – – – AFRC, 1993 (modified); ——— own study (model 2); –·–·– GfE, 1996*

Conclusion: These study indicates that the energy utilization factors and maintenance requirement values derived from the model (2) should be favoured under practical conditions with the genotype investigated.
Literature: AFRC (1993): CAB Intern., Wallingford, UK. BELLOF, G. (2003): Züchtungskunde, 75, 274 - 283. BELLOF, G. and PALLAUF, J. (2003): Proc. Soc. Nutr. Physiol., 12, 23. BELLOF, G. et al. (2003): Züchtungskunde, 75, 53 - 68. GfE (1996): Proc. Soc. Nutr. Physiol., 5, 149 - 152.

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87. Prediction of energy digestibility based on total dietary fiber in complete dry food for dogs and cats (Zur Abschätzung der Energieverdaulichkeit in Trockenalleinfuttern für Hunde und Katzen anhand des Gehaltes an Gesamtfaser). Ellen Kienzle*, V. Biourge, Alexandra Schönmeier – Munich/Aimargues

It has been repeatedly demonstrated that there is a negative relationship between fibre in dry matter and energy digestibility in pet foods. This is true for any method of fibre determination that measures the major percentage of cellulose (Kienze et al. 1998). So far, however, there are only few observations on total dietary fibre (TDF) and energy digestibility.

Methods: In the present study 610 digestion trials with dog food and 261 digestion trials with cat foods were evaluated in retrospect. Digestion trials had been carried out with 6 out of 18 dogs of different breeds or 10 out of 30 cats, respectively. Each trial lasted for 10 days (5 days pre-period, 5 days total faecal collection, according to AAFCO-protocols. Energy in food and faeces was determined by bomb calorimetry. TDF was determined by the method of Prosky et al. (1985). In addition to TDF the crude fibre (CF) content was determined in 495 dog foods.

Results: The following regression equation between energy digestibility (%) and TDF in % dry matter (DM) was obtained for dog food: energy digestibility = 96.6 –0.96 * TDF (% DM); r=0.94**. A similar equation was obtained for cat food: energy digestibility = 95.6 – 0.89 * TDF (% DM); r=0.88**. For crude fibre in dog foods the regression equation was: energy digestibility = 92.9 – 1.6 * CF (% DM); r=0.87**. This confirms earlier results. The correlation was even closer for TDF than for CF, and for TDF there were no outliers.

Conclusions: Prediction of energy digestibility in dry food by TDF as a basis for prediction of ME may be even more accurate then prediction of energy digestibility by CF.

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88. Investigations on effects of high doses of vitamin K_3 in complete diets for small parrots (*Agapornis spp.*) (Untersuchungen zu Effekten höherer Gehalte an Vitamin K_3 im Futter für Kleinpapageien – *Agapornis spp.*).

Petra Wolf*, Carolin Hupfeld, G. Dorrestein und J. Kamphues - Hannover/Utrecht

Objectives: Background to this study is a discussion that is currently going on in magazines for pet birds, on internet, as well as in veterinary journals. Focus of this discussion is a purported toxic effect of high doses of vitamin K_3 on pet birds. Feather biting, a reduced laying activity in hens, and liver diseases, as well as carcinogenic effects have been brought up in this context. However, some of the opinions and perspectives presented in contributions to this context are not or only barely substantiated - at least not through scientific investigations. So far health problems in different species that have been reported in the scientific literature pertain almost exclusively to consequences of a parenteral application of vitamin K_3. The typically manifested symptoms occur mostly in form of a megaly in kidney, spleen, and liver; an icterus of the cernels in the brain as well as Heinz-bodies, or other anomalies of the blood-count, changes in the electrolyte balance, as well as changes in the parameters of the blood chemistry. The few cases in which clinical symptoms after orally giving K_3 occured were related to an over-dose of up to 5000 to 45.000 times the correct dose.

Aim of this study was to test, whether and upon which dosage (given orally for a longer period) vitamin K_3 had negative effects on pet birds` health.

Methods: The experiments were carried out with 36 adult lovebirds, which were allotted to three groups, each of them containing 12 birds (group C: control; group V1 and V2: experimental groups). The pelleted diet used in this study consisted of seed mixtures with commonly used ingredients. In test group V1 40 mg of menadion-sodium-bisulfate were added to their diet, test group V2 received 400 mg. The analysis of the vitamin K_3 contents resulted in levels of less than 1 and 25, or 170 mg/kg. Compared to generally recommended dosages of 1-2 mg/kg diet the test groups received dosages that were 12.5 to 25, or 85 to 170 times higher than the recommended levels.

At the beginning of the study two blood samples were taken with a time difference of one week. This established base line values of individual lovebirds. In the following period the general well being and condition of the birds were checked. Once a week a blood sample was taken and examined according to changes in packed cell volume (hematocrit), total white blood cell counts and hemogram. Clinical biochemistry of the blood plasma included total protein, albumin, globulin, uric acid, calcium, ionized calcium, sodium and potassium as well as for kidney or liver typical enzymes. At the end of the trials 4 birds from each group were sacrificed (chosen randomly) for necropsy and histopathological examination. Additionally, vitamin K_3 levels in their livers were examined.

Results: During the trial all birds were in a well condition; behaviour, feed/water intake or excrement quality were not influenced by treatment. A systematic, i.e. direct impact of K_3 on the hematological and blood-chemical parameters, respectively, was not observed at any point in time on any of the birds. Necropsy did not show any pathological macroscopic lesions related to vitamin K_3 oversupply. By histopathological examination, birds of all three groups had depositions of iron in different organs and protein exudates were found. However, Heinz-bodies were not found in any of the groups. None of the findings indicated any dependance on vitamin K_3 intake according to frequency or intensity. Vitamin K_3 levels in livers (estimated in pooled samples) showed no accumulation and suggest a rapid metabolism of absorbed vitamin K_3.

Conclusions: Supplementing the diet for six months with vitamin K_3 (up to 25 or 170 times higher than recommended) had no negative effects neither on general condition of the lovebirds nor on their hematopoesis or any of their organs. According to these findings no doubts exist about the tolerance of normal K_3 doses (e.g. recommended doses) in diets for pet birds.

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89. Influence of phytoestrogens, polyunsaturated fatty acids, vitamin D and K on bone metabolism: investigations on intact and ovariohysterectomized beagle dogs (Der Einfluss von Phytoöstrogenen, mehrfach ungesättigten Fettsäuren, Vitamin D und K auf den Knochenstoffwechsel: Untersuchungen am intakten und ovariohysterektomierten Beagle).

U. Wehr, Susanne Kern and W.A. Rambeck - München.

Osteoporosis has a major impact on health care costs in the European Union. Hospital costs for the therapy of osteoporotic fractures produce an annual financial burden of more than four billion euros. But this is not the only reason for the necessity of research on effective therapy and early prophylaxis for osteoporosis. Hormone replacement therapy was the treatment of choice until recently. But there is not only a low compliance of the affected women. More and more risks of this treatment have been proven, e.g. a higher incidence of breast and ovarian cancer.

Thus, research on alternatives in prophylaxis and therapy is of current interest.

In the present study effects of a combination of genistein, polyunsaturated fatty acids, and vitamin D and K on bone metabolism and microarchitecture of bone in intact and ovariohysterectomized dogs were investigated. Ovariohysterectomized beagle dogs are a large animal model for postmenopausal osteoporosis, administered by the Food and Drug Administration.

A total of 12 intact female Beagle dogs, aged 18 months, were randomly allocated to receive either a combination of genistein (15 mg/kg BW), PUFA (12 g/kg BW), vitamin D_3 (1500 IU/kg BW), vitamin K (2000 µg/kg BW) or vehicle (n=6 per group). 16 weeks after the start of the treatment, all dogs were ovariohysterectomized. During the experiment serum and urine samples were collected every 4 weeks over a 32 weeks period. Serum bone specific alkaline phosphatase (bALP) was measured as a marker of bone formation with a commercial enzyme immunoassay. Pyridinoline (PYD) was measured as a marker of bone resorption by high performance liquid chromatography.

In the therapy group supplemented with phytoestrogens, PUFA, vitamin D and vitamin K, the increased bone resorption and bone formation (high turnover) caused by the ovariectomy was effectively reduced.

weeks	Bone alkaline phosphatase in IU/l		Pyridinoline in nmol/mmol Crea	
	control group	therapy group	control group	therapy group
week 0	14,25 ± 5,4	13,41 ± 4,3	144,2 ± 27,0	154,3 ± 22,5
week 4	16,06 ± 8,4	14,24 ± 4,0	138,3 ± 25,2	144,0 ± 17,7
week 8	13,73 ± 8,9	11,60 ± 3,0	156,7 ± 30,6	167,7 ± 29,1
week 12	12,16 ± 6,6	11,13 ± 1,6	158,3 ± 43,1	146,0 ± 28,2
week 16 (OHX)	12,18 ± 6,8	12,10 ± 1,2	146,3 ± 31,0	136,3 ± 10,3
week 20	13,82 ± 8,1	13,35 ± 2,1	161,0 ± 39,2	145,0 ± 8,7
week 24	19,26 ± 6,5[a]	12,03 ± 2,1[b]	355,0 ± 100,6[a]	249,3 ± 49,0[b]
week 28	15,23 ± 6,4	12,93 ± 1,8	289,0 ± 67,6[a]	199,7 ± 50,0[b]
week 32	16,25 ± 5,0	13,34 ± 1,9	214,3 ± 79,4	173,3 ± 32,5

a,b: Values without the same superscripts differ significantly

The results of this study show, that a combination of genistein, polyunsaturated fatty acids, and vitamin D and K can have a protective effect on bone in the intact as well as in the ovariohysterectomized beagle. This combination could be a possible alternative to hormone replacement therapy in postmenopausal osteoporosis.

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90. Influence of ensiling on the DNA-degradation in isogenic and transgenic corn (Einfluss der Silierung auf den DNA-Abbau in isiogenem und transgenem Mais).

Karen Aulrich* G. Pahlow and G. Flachowsky - Trenthorst/Braunschweig

The public discussion concerning the safety of genetically modified plants (GMO) and the safety of food of animal origin when animals were fed with GMP is not completed up to now. One question regarding the safety of GMP is the stability of DNA during feed processing. The objective of the study was to determine the influence of ensiling on DNA fragmentation and to evaluate the quality of the silages.

Methods: The isogenic and transgenic (herbicide resistant: equipped with phosphinotricinacetyltransferase gene) corn varieties were grown on experimental fields in the FAL. After harvesting the corn varieties were ensiled under laboratory conditions as corn cob mix (CCM) and as whole plant silages (WPS). At different times during the ensiling process samples were taken (Table 1). DNA was extracted with a commercial kit and different transgenic fragments (1016, 680, 194 bp) were determined by PCR based methods. In addition parameters of the silage quality were assessed. The corn varieties were analysed for contents of starch, sugar, protein, ELOS, EULOS, CDOM and buffering capacity. Samples taken during the ensiling process were analysed for pH, ammonia-N and volatile fatty acids.

Results: The large fragment of 1016 bp were detected in all starting materials, up to 5 days after ensiling in CCM and up to 28 days in WPS (Table 1: data only shown for fragments of transgenic corn). The fragment of 680 bp was detected up to 21 days in CCM and up to 35 days in WPS. From day 35 up to the end of ensiling the DNA was degraded to fragments smaller than 680 bp. It was only possilble to detect fragments of 194 bp.

The parameters of silage quality indicated that the resulting silages were of a good fermentation quality. Both CCM silages reached 84 points according to the DLG-score (maximum = 100 points). The WPS silages reached even this optimum of 100 points by the same evaluation criteria.

Table 1: Determined DNA-fragments in transgenic CCM and WPS as influenced by the ensiling period

Duration of ensiling (days)	Fragment 1016 bp		Fragment 680 bp		Fragment 194 bp	
	CCM	WPS	CCM	WPS	CCM	WPS
0	+	+	+	+	+	+
2	+	+	+	+	+	+
5	+	+	+	+	+	+
7	-	+	+	+	+	+
14	-	+	+	+	+	+
21	-	+	+	+	+	+
28	-	+	-	+	+	+
35	-	-	-	+	+	+
70	-	-	-	-	+	+
100	-	-	-	-	+	+
200	-	-	-	-	+	+

Conclusion: The forage conservation by ensiling caused the degradation of DNA to small fragments of about 200 bp. The degradation in CCM occured faster than in WPS.

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91. Gene chip analysis reveals α-tocopherol sensitive gene in rat liver (Identifikation α-Tocopherol sensitiver Gene mittels Genechip Analyse)

G. Rimbach*, Elisabeth Stöcklin – Kiel/Basel

The recent discovery that vitamin E (VE) regulates gene activity at the transcriptional level indicates that VE may exert part of its biological effects by mechanisms which are independent of its well-recognised antioxidant function. In order to obtain a comprehensive understanding of the mechanisms of action of VE, a global gene expression profile in rat liver was obtained using Gene chip technology. Various time-point measurements were introduced in the experimental design in order to elucidate whether or not differences in the gene expression profile were manifested consistently over a prolonged period of time.

Methods: Two groups of 30 male albino rats (Fisher 344, Charles River) with an initial average weight of 50-60 g were randomly assigned to either a vitamin E containing diet (VE^+) or to a, vitamin deficient diet (VE^-) for 290 days. The VE^+ diets contained 60 mg/kg RRR-α-tocopheryl acetate. High-density oligonucloetoide microarrays (Affymetrix) comprising ~7,000 genes were used to define the transcriptional response in the liver of vitamin E deficient rats. Differential gene expression was monitored at 4 individual timepoints, and rats were individually profiled. Differences in gene expression in response to VE deficiency were substantiated by real time PCR. Metabolic endpoint measurements were chosen in order to determine whether or not changes in mRNA levels were associated with any functional changes.

Results: Feed intake was not significantly different between the two experimental groups. Differences in dietary VE supply were reflected by significant differences in hepatic VE concentrations. Liver VE levels increased time-dependently in the VE^+ group. Out of the 7,000 genes represented on the gene chip only fourteen were consistently differentially expressed in response to VE deficiency for at least three consecutive time-points. Seven genes were up and seven genes were down-regulated in the VE^- rats as compared to controls. VE deficiency up-regulated coagulation factor IX (FIX) and 5-α-steroid reductase type 1 mRNA levels. Furthermore VE deficiency resulted in a significant down-regulation of hepatic gamma glutamyl-cysteinyl synthetase, the rate limiting enzyme of glutathione synthesis. A significant increase in FIX mRNA levels in VE^- rats was accompanied by a significant change in activated partial thromboplastin time. Rats receiving the VE^- diets had a significant higher plasma 5-α-dihydrotestosterone/testosterone ratio as compared to VE^+ animals. VE^- rats exhibited 20 % lower hepatic GSH levels as compared to VE^+ rats.

Conclusion: Overall, the current study reveals that dietary VE has important long-term effects on liver gene expression with potential downstream effects on extra-hepatic tissues. Gene arrays are important platforms to examine the molecular targets of vitamin E.

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92. The rhythm of melatonin in cattle milk and the possible role of "night milk" as "functional food" (Die Melatoninrhythmik in Kuhmilch und die Rolle von `Nacht-Milch' als Nahrungsergänzungsmittel).

Anna-Caroline Wöhr*, J. Birkle, J. Unshelm and M. H. Erhard – München

Melatonin is the major product of the pineal gland and released into the circulation in dependency of the light/dark cycle. The distinct circadian secretion pattern of melatonin could be found in most mammalian species and suggests that one function of the pineal gland is to adjust the biological clock by increasing the melatonin release over 10-fold and higher at night.
Since several months in Finland and Great Britain consumers could buy `night milk', which comes from cows milked at darkness or at night and which should calm and support the sleep of old people and children. According to the producers `night milk' has a melatonin-concentration 10- and more-fold higher than `normal' Milk. It is well accepted that melatonin modulates the sleeping behaviour by a calming effect on the organism as well as there is a functional relationship between melatonin and the immune system in mammals. Thus, `night milk' could be a product which possibly full-fill the standards for `functional food' and act on the body to enhance physiological functions and reduce the risk of diseases. The **purpose of the present work** was to investigate whether the levels in bovine milk exhibit a similar daily rhythm as plasma levels and whether a correlation exists between plasma and milk concentrations. Furthermore to determine the maximum night levels of the melatonin concentration in milk and if different melatonin levels in milk have any influence on the plasma concentrations of nursing calves.
Methods: The study population consisted of 9 cows and their calves from a cow-calf herd of a beef-cattle farm. The plasma samples of the cows were taken over a period of 3 consecutive days by a central jugular vein catheter every 12 h (6 p.m. versus 6 a.m.). Milk samples were collected at 1-hourly intervals for 48 h directly from the udder. In a first step the calves received no milk at night and plasma samples were collected over a period of 48 hours at 11 p.m., 4 a.m., 12 a.m. and 6 p.m. In a second step the calves received night milk from their mother ½ hour before each sampling. Plasma and milk melatonin levels were measured in duplicate using an RIA method.
Results: A significant diurnal rhythm of melatonin levels was seen in the plasma of adult cows and calves as well as in the milk of the cows. The mean night-time melatonin concentrations were 197.7 pg/ml in cow plasma, 108.5 pg/ml in calf plasma and 18.7 pg/ml in milk. The mean values during the daytime were 14.7 pg/ml in cow plasma, 7.0 pg/ml in calf plasma and 1.9 pg/ml in milk. The results show distinct differences between the melatonin concentrations of individual cows and between the values obtained for a given animal on different nights. In the milk a significant rhythm with a variable 1-2 hour transition period in the morning and the evening was visible. Regression analysis revealed a non-linear correlation between plasma and milk melatonin levels. Melatonin levels in the milk did not significantly influence the plasma melatonin levels of the claves.
Conclusions: In contrast to many other countries, in Germany synthetical Melatonin is defined as a drug which one can obtain only by prescription. So `night-milk' could be an easy way to support the sleeping behaviour of children and elderly people as well as to reduce the risk of diseases. Our data demonstrate that at night the concentration of melatonin in milk is about 10-fold higher than the daily values, but feeding `night-milk' to calves did not influence the melatonin rhythm. he calves displayed their own, independent diurnal variation in plasma melatonin concentrations. Also very high inter- and intraindividual variations of the melatonin concentrations in milk and plasma are detectable. Our assumption is, that with `night-milk' it is difficult to guarantee for a product of non-varying quality and that the melatonin concentration at night in milk is not high enough to act on the body as a pacemaker. Therefore further investigations are necessary for the consumer's protection.

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93. Investigations on nutritional factors related to the Bavarian BSE epidemic (Untersuchungen zu ernährungsbedingten Faktoren im Zusammenhang mit der Bayerischen BSE-Epidemie).

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The ingestion of feedstuffs that are contaminated with infectious animal material is commonly regarded to be the main vector for BSE. This concept is based on laboratory studies as well as the fact that the number of British and Swiss BSE cases decreased after the introduction of feed bans. Additionally, the epidemiological evidence indicates that infection occurs mainly at a very young age. So-called "alternative hypotheses", such as a contribution of Mn oversupplementation or Cu deficiency, have been mentioned but not conclusively been investigated.
Methods: Three data sources were evaluated for this study: (1) data on the feeding practice for animals that later developed BSE was available for the first 82 Bavarian cases. (2) Data on the occurrence of animal material in Bavarian ruminant feeds was available for the years 1996-2002, including data on the contamination of single feeds (soy and grain products) from 2001 and 2002. (3) Product information on the mineral content of mineral feeds, milk replacer and concentrates for dairy cows was available from 103 archieved food labels from 1978-2002.
Results: (1) Among the first 82 Bavarian BSE cases, in 19 % of the cases the animals that were tested positive for BSE had received only milk replacer when young but no other proprietary feedstuffs. In half of these cases, no feeds for other animals had been purchased on the respective farms, and only in 20 % of these cases, a mineral supplement had been given. In two of all cases, no milk replacer but other proprietary feeds had been used. (2) Between 1996-2000, about 75 % of all investigated mixed feeds for ruminants (excluding milk replacer and mineral supplements) contained traces (< 1 or 0.5 %) of animal material. In 2001, this applied to only 7 % of all investigated samples, and in 2002 to 0.3 %. In 2001, about 5 % of investigated grain products were contaminated. In 2003, this proportion was 0.3 %. (3) In the declaration of mineral feeds for cattle, an increase in the ratio of Mn:Cu was evident from 1987 on; in milk replacers, this increase was evident from 1991. Of the four labels of concentrates for dairy cows that contained information on mineral content, the two from 1989-1990 both had a lower ratio than the two from 1994/96.
Conclusion: (1) The results on the calf feeding practices demonstrate that calves that did not receive any proprietary feeds other than milk replacer were infected with BSE. Statistically, milk replacer is the largest common denominator of the cases for which information is available, and it should be taken into consideration as a potential vector for the infection. (2) The data on cross-contamination of ruminant feeds with animal material before and after the comprehensive feed ban underline that as long as animal material is allowed for one species, feeds for other species are likely to be contaminated under the current production practices, and that the complete feed ban effectively reduced the occurrence of cross-contamination. Whether cross-contamination of ruminant feeds with animal material played a role in the German BSE epidemic can only be assessed by comparing data from different regions. (3) There is no temporal link between the increase of Mn levels in ruminant feeds and the German BSE epidemic.

This work is part of the Bavarian BSE Risk Analysis.

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94. Investigations concerning the effect of glyphosate residues in feed on rumen fermentation parameters and *in sacco* dry matter degradation (Untersuchungen zum Einfluss von Glyphosatrückständen im Futter auf pansenphysiologische Parameter und auf den *in sacco* Trockensubstanzabbau).

Liane Hüther*, Svenja Drebes and P. Lebzien – Braunschweig

Glyphosate is the active substance in a number of herbicides such as Roundup, Roundup Ultra or Touchdown. Its mode of action is to inhibit a substantial enzyme of the shikimic acid pathway, what consequently prevents the plant from synthesizing essential aromatic amino acids. Beside plants the shikimate acid pathway is also present in all bacteria and fungi and thus also in rumen microorganisms. Since herbicide treated feed grain, grass and weeds are permitted to use as feed for ruminants, the aim of the study was to investigate the effect of glyphosate contaminated feed on rumen fermentation parameters and on *in sacco* dry matter degradation of grass hay and ground corn grain.

Methods: In four consecutive periods of 28 days four wethers equipped with a rumen fistulae were daily fed with 1 kg corn silage on dry matter base supplemented with an urea-vitamin-mineral premix. During each period two animals received a daily amount of 1.07 g Roundup Ultra on a piece of bread, corresponding to 0.77 g glyphosate. The amount of the applied glyphosate was calculated in a worst case approach from the highest glyphosate residues determined in grass after approved application of the herbicide (1). Furthermore the ration was fed either with or without supplementation of aromatic amino acids. The first 14 days of each period served for adaptation to the respective rations whereas the remaining 14 days were used for rumen fluid sampling and *in sacco* trials according to ØRSKOV and McDONALD (2).

Results: There was no significant effect of glyphosate on rumen fermentation parameters (pH value, volatile fatty acids, ammonia) and on *in sacco* dry matter degradation of grass hay and ground corn grain independent from aromatic amino acid supplementation. The Table shows the results of the regression analysis relating the time of nylon bag incubation in the rumen to the dry matter degradation of grass hay for the different treatments according to (2).

ration	soluble fraction a	degradable fraction b	rate constant of b c	ED 2*)	ED 8*)
control	23,7	58,1	0.030	58.5	39.5
glyphosate	23,7	59,7	0.033	60.1	40.7
amino acids	23,7	59,7	0.030	59.6	40.0
glyphosate and amino acids	23,7	59,8	0.031	59.7	40.2

*) effective degradability at a passage rate of 2 resp. 8 %/h

Conclusion: From this study it can be concluded that grass, weeds or fodder corn contaminated with glyphosate residues due to intended uses of the plant protection products can be used as feed for ruminants without having any concern to rumen fermentation.

1) Monograph Glyphosate, December 1998
2) ØRSKOV, E. R., McDONALD, I., (1979): J. Agr. Sci. 92, 499-503

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95. Effect of feeding ergot on piglets and chickens (Einfluss von Mutterkorn im Futter auf Ferkel und Masthähnchen).

Simone Mainka*, S. Dänicke, S. Matthes, H. Böhme, G. Flachowsky - Braunschweig/Celle

The toxicity of ergot (*Claviceps purpurea*) is primarily due to its alkaloid content. Two dose response trials were conducted with piglets and chickens to study both the influence of feeding ergot with a known alkaloid content and pattern on growth performance and clinical-chemical blood parameters.

Methods: The sclerotia of *Claviceps purpurea* used in both experiments were analysed with the HPLC- technique as described by (1). The total alkaloid concentration was found to be 279 µg / 100 mg, including 8 % ergometrine, 5 % ergotamine, 3 % ergocornine, 2 % α- ergocryptine, 15 % ergocristine and 67 % residues. Three groups of 16 weaned piglets (BHZP; 8 barrows and 8 gilts) each were kept in pens with four animals each over a period of five weeks in which their live weight ranged from 8 to 22 kg. In the trial with chickens, a total of 84 one-day old male animals (LOHMANN MEAT) were allotted to three feeding treatments over three weeks in the live weight range of 43 to 719 g and housed in cages of four animals each. Piglet and chicken diets were in accordance with the current GfE recommendations and were supplemented with ergot at levels of 0, 0.1 and 0.4 %. Feed and water were available *ad libitum*. Live weight per animal and feed consumption per pen / cage were recorded once a week. On the last day of the trial, blood samples of all piglets were taken from the vena jugularis externa. The chickens were slaughtered by bleeding to death on the day after finishing the trial. Blood was collected from all animals. Clinical-chemical blood parameters (Table) were determined in serum.

Results: Feed intake and weight gain were significantly affected in the experiment with piglets but only when the diet with the highest ergot content (0.4 %) was fed. The serum albumin concentration decreased with increasing ergot supplementation, both in piglets and in chickens. AST increased in piglets fed the diet with the highest ergot supplementation (0.4 %) whereas no consistent effect of ergot was observed in chickens. In contrast, γ- GT was not affected in piglets while a significant increase occurred in chickens from the lowest ergot inclusion level (0.1 %). GLDH was influenced in neither piglets nor in chickens.

Table: Mean daily feed intake, live weight gain and clinical-chemical blood parameters

Trial	PIGLETS			CHICKENS		
Group	I	II	III	I	II	III
% ergot in the diet	0	0.1	0.4	0	0.1	0.4
Feed intake (g / d)	676 [a]	635 [a]	556 [b]	46.1	45.3	46.9
Weight gain (g / d)	413 [a]	420 [a]	347 [b]	32.6	31.7	32.2
AST (IU / l)	28.8 [a]	26.7 [a]	37.1 [b]	96.7 [a]	109.3 [b]	97.4 [a]
γ- GT (IU / l)	21.7	22.9	23.1	21.8 [a]	25.2 [b]	26.4 [b]
GLDH (IU / l)	0.72	1.02	1.04	5.20	4.79	4.45
Albumin (g / l)	36.8 [a]	32.3 [b]	31.4 [b]	17.0 [a]	15.3 [b]	15.2 [b]

[a,b] different letters in one trial and line indicate significant differences between means of each group at $p < 0.05$

Conclusions: Under the applied conditions (dietary ergot alkaloid concentration and pattern) piglets responded more sensitively to ergot than chickens as performance parameters indicate. Ergot seemed to affect some serum-clinical parameters.

1) WOLFF, J., NEUDECKER, Ch., KLUG, Ch., WEBER, R., 1988: Z. Ernährungswiss. 27, 1 – 22.

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96. Effects of chlorinated paraffins on performance of laying hens and on carry over (Einfluss von Chlorparaffinen auf die Leistung von Legehennen und den Carry over).

K.-H. Ueberschär* and S. Matthes - Celle

Chlorinated paraffins (CPs) are world wide heavily used as sealants, as additives in plastics and paints and as flame retardants. By open applications considerable amounts of CPs are released into the environment. Due to the lipophilic and persistent properties the chemical is accumulated in aquatic and terrestrial animals. The aim of the present study was to examine the effects of CPs on performance of laying hens.

Methods: Sixty laying hens (week 24 to 32 of age) were divided into 6 groups (12, 9, 9, 9, 9 and 12 hens). Diets were supplemented with 0, 2, 20, 45, 70 and 100 mg CPs per kg diet. Recovery of the CP additions was on average 77 (61-84) % as determined by GC using the same technical mixture of chlorinated paraffins (C10-C13, 60 % chlorine, Fluka, Germany) as analytical standard, which was added to the diets.

Results: No bird died during the 8 weeks of the experiment. No influence of CPs on the health of the hens was noted. Gross macroscopical inspection of inner organs and glands gave no indications of lesions, inflammation or other pathological impairment. No significant differences ($p = > 0.1$) were detected for live weights and organ weights (relative to live weight) (liver, kidneys, thyroid gland, spleen, heart und gizzard) between the control group and the group fed 100 mg CPs/kg diet. However, the mean relative weight of pancreas (0.18 % ± 0.03) was significantly reduced ($p = 0.046$) in comparison to the control (0.21 % ± 0.01).

A linear correlation ($R^2 > 0.8$) between the CP concentrations of the diet and those found in abdominal fat, yolk, liver, kidneys and muscle was found. The highest concentrations, about 14 % and 7% related to the feed, were determined in fat and yolk. No CPs were analysed in albumen.

Table 1 Effects of chlorinated paraffins (CPs) on the performance of laying hens (8 weeks of experiment)

mg CPs/kg feed	Laying intensity (%)	Daily egg mass (g/hen)	Egg weight (g)	Daily feed intake (g/hen)	Feed conversion (g/g egg mass)
0	96.1	58.2	60.7	115	2.00
2	92.1	60.5	60.5	118	1.97
20	97.6	61.7	61.7	113	1.84
45	96.0	63.6	63.6	123	1.94
70	91.7	59.0	59.0	116	1.97
100	96.4	58.3	59.7	116	2.00
ANOVA (p)	0.734	0.197	0.286	0.256	0.426

No negative effects on performance data were observed after feeding CP contaminated diets for 2 and 4 weeks or for the whole experiment (Table 1).

Conclusions: The experiment showed no influence of CPs on the performance of laying hens. At CP concentrations of 100 mg/kg a low impact on digestive organs seems to be possible. Taken together, the effects of feeding CP concentrations up to 100 mg/kg diet to laying hens seems to be negligible. A carry over of CPs especially into the lipophilic tissues (abdominal fat and yolk) was found.

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97. Faba Beans (*Vicia Faba L.*) with Reduced Pyrimidine Glucosides as a Feed Component for Broiler Breeders (Ackerbohnen mit reduzierten Gehalten an Pyrimidinglucosiden als Futterkomponente für Broiler-Elterntiere)

B. Lenz*, Martina Gerken, Hj. Abel - Göttingen

The pyrimidine glucosides, vicine and convicine, present in conventional faba beans (*Vicia faba*) exert negative effects on egg quality, thus limiting their suitability in breeder diets. The present study was designed to evaluate a recently developed variety of faba bean with genetically reduced pyrimidine glucosides in broiler breeders and their progeny.

Ross broiler parents, between their 20th and 40th weeks of life, were fed one of three feed mixtures containing 0% (Control) or 15% faba beans, with either a high pyrimidine glucoside content (High Vicine: 8104 µg vicine + 6198 µg convicine/kg DM) or a reduced content (Low Vicine: 288 µg vicine + 23 µg convicine/kg DM). Data on egg production (weekly), feed consumption (weekly) and egg weight (30, 35 and 40 weeks) were obtained, and hatchability was determined in eggs collected at 40 weeks. The possible effect of the parent diet on the broiler chickens was studied in the progeny. The broiler chickens received a well-balanced feed mixture ad lib. and were slaughtered at 42 days. The table shows the mean effects of the dietary treatments on selected performance traits in both the parents and their progeny.

	Dietary treatment [1]		
	Control	Low Vicine	High Vicine
Broiler breeders			
Feed consumption (g/d)	159	158	159
Laying intensity (%)[2]	59.0	59.2	60.0
Hatchability (%)[3]	98.6	95.5	95.3
Egg weight (g)	64.4[b]	63.8[b]	60.2[a]
Broiler chickens			
Hatching weight (g)	48.5[b]	48.6[b]	45.8[a]
Final weight (g)	2429[b]	2398[b]	2318[a]
Feed/gain (kg/kg)	1.56	1.66	1.58
Carcass weight (g)	1770[b]	1759[b]	1674[a]
Valuable cuts (%)[4]	66.7[b]	65.4[ab]	64.9[a]

[1]Each diet was fed to 2 groups of 40 hens and 5 cocks/group [2] Hen day [3]Based on fertile eggs [4]Breast and thighs [a,b] Means with no common superscript differ significantly (P<0.05)

The egg production was not affected by the diets. There was a non-significant reduction in hatchability in breeders fed either one of the two faba bean diets. The egg weight was significantly reduced in hens receiving the high vicine variety, whereas it was not affected in birds on the low vicine diet. The lower egg weights led to reduced hatching weights in the high vicine progeny. This adverse effect persisted throughout growth and was also detrimental to the slaughter performance.

Conclusion: In contrast to conventional faba beans, varieties with reduced pyrimidine glucosides can be fed in considerable amounts to broiler breeders without negative effects on the animals' performance.

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98. Bioavailability of nonpurified acrylamide from a heated potato product – a pilot study with Japanese quails.

(Acrylamid-Verfügbarkeit aus einem erhitzten Kartoffelprodukt - eine Pilotstudie mit Japan. Wachteln).

D. Ranz *, M. Jezussek, Claudia Thielen, P. Schieberle, Ellen Kienzle – München/Garching

Acrylamide (AMD) which originates incidentally when carbohydrates and proteins are heated together above a temperature of 170°C has recently received attention as a carcinogen in human food but also in animal feed. Questions such as bioavailability of nonsynthetic AMD produced by heating food or feed and the transfer from feed to human food are still open. Therefore, in the present study a pilot experiment in Japanese quails with a heated potato product was undertaken to give a better estimate of the practical relevance.

Methods: Thirty egg-laying Japanese quails (Coturnix coturnix japonica), three months old, were randomly assigned to three groups of 10 each. The three groups were allotted to three dietary treatments: the control group and two AMD-treated groups with 10 % and 40 % of potato meal (PM) in the experimental diets, respectively. The PM contained naturally originated AMD and was detected in an official feed control. The feeding study lasted 30 days with a preperiod of six days and an experimental period of 24 days.
The determined AMD content of the potato meal (PM) was 6400 µg/kg feed which led to a measured content of 663.0 µg AMD/kg and 2472.5 µg AMD/kg in the 10 % PM and 40 % PM diet, respectively. The measured AMD content of the control diet was 16.7 µg/kg feed. Feed and water were provided ad libitum to the quails. Every day excreta and eggs from each group were collected and weighed. AMD was determined in the pooled excreta and egg samples on day 1 and day 5. At the end of the study the birds were slaughtered and organ samples (liver, breast muscle, serum) were removed from each bird. AMD was determined by high-resolution gas chromatography – mass spectrometry (HRGC-MS) using extraction with 1-propanol according to JEZUSSEK et al. (J. Agr. Food Chem. 2003).

Results: The measured AMD content in the pooled egg samples on day 1 and day 5 was 7.80 µg/kg wet weight and 7.73 µg/kg w.w. for the 10 % PM group and 24.3 µg/kg w.w. and 24.8 µg/kg w.w. for the 40 % PM group, respectively. The AMD content of the pooled excreta samples on day 1 and day 5 was 49.3 µg/kg w.w. and 47.4 µg/kg w.w. for the 10 % PM group and 132.7 µg/kg w.w. and 124.0 µg/kg w.w. for the 40 % PM group, respectively. The measured AMD concentrations in the pooled egg and excreta samples represent 0.4 % of AMD intake in eggs and 5.5-6.0 % of AMD intake in the excreta samples in both AMD-treated groups. All AMD values of the control group were not detectable (< 3.5 µg/kg w.w.) for the pooled egg and pooled excreta samples. The content of AMD in all liver samples was not detectable, too. A slight increase of AMD concentrations was observed in the serum and in the breast muscle samples.

Conclusion: This pilot study showed for the first time that nonpurified AMD which was formed spontanously during the manufacturing process of potato meal is bioavailable from feed to eggs, serum and breast muscle of Japanese quails. Compared to foodstuffs high in AMD (e.g. potato chips and French fries) the measured AMD contents in eggs and breast muscle are relatively low. Therefore, AMD in feedstuffs are likely to represent a low human health risk.

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99. Effects of dry matter content and pH-value on the development of clostridia in silages

(Einfluss von Trockensubstanzgehalt und pH-Wert auf die Clostridienentwicklung in Silagen)

Ehrengard Kaiser*, Kirsten Weiß and Polip Iv - Berlin

The fast decreasing of pH-value by lactic acid fermentation resp. the increasing of dry matter (DM) content in ensiling material are considered as the most important measures to prevent clostridia activity during fermentation process. In the case of nitrate-free ensiling material clostridia activity could not completely suppressed in spite of increasing DM content and intensification of acidification (Iv and Kaiser, 1997). The aim of present paper is to check the clostridia activity resp. the clostridia spore content depending on inhibitory factors, acting during fermentation process. In the first instance it is reported on the effect of DM-content of the ensiling material, including lactic acid fermentation.
Materials and methods: An ensiling experiment with *Dactylis glomerata,* low in nitrate, was carried out under laboratory conditions. The DM-content of the ensiling material was adjusted to appr. 20, 30, 40 und 50 %. At each DM level the material was ensiled both without and with several additives. Here is reported beside of the control variant on variants with lactic acid bacteria (LAB appr.10^5 cfu/g FM) additive and with LAB plus 2% glucose in fresh matter (FM). Volume of laboratory silos was 1L. Silages was analysed concerning their contents of fermentation products and their clostridia spores (Cl-spores) content after 3, 7, 14, 28, 56 und 180 days. To show the effects of inhibitory factors, all silages were additional contaminated with Cl-spores (appr. 10^4 Cl-Spores/g FM). The FC-values of ensiling material amounted from 47 to 65.
Results: In the variants without additives the extend and the rate of lactic acid (LA) formation decreased with increasing DM content. But the critical pH-value was reached in all DM levels on the 7th day. Nevertheless, butyric acid (BA) was formed in the first three DM levels, namely the earlier and in the extend higher the lower the DM content. Only silages of the highest DM level (51,5 %) were BA-free. In the dry matter level of 21,6 % the Cl-spores content shortly decreased after beginning of ensiling, but clearly increased up to very high level with the beginning of BA formation. In the 2nd and 3rd level of DM content the beginning of BA formation and the increasing of spore content were retarded and the reached extend was limited. Only in the level of 51,5 % of DM a continuous decrease of Cl-spore content occurred in the fermentation process.
As a consequence of LAB-additive the LA formation was increased in all DM levels and thus the decreasing of pH-value was faster. Thereby the occurrence and the extend of BA formation were limited clearly. Nevertheless in the experiment with 21,6 % DM the same extremely high Cl-spore content was present like in the control variant after 56 days of storage period. In contrast to this in the level of 32,5 % DM the renewed increase of CL-spore content was strongly delayed. But only with DM contents of more than 40 % and 50 % the BA formation was limited and the Cl-spore content decreased slowly in fermentation process.
Fazit: In the lower range of DM the naturally acidification is not sufficiently to suppress BA fermentation and formation of Cl-spores when nitrate as an inhibitor of clostridia is absent in fermentation substrate. With increasing of DM content in green forage the reduced availability of water becomes effective as an inhibitor of clostridia development, additionally to the effect of acidification.

POLIP IV and KAISER, E. (1997): 51. Jahrestagung der Gesellschaft für Ernährungsphysiologie 4.-6. 3. 1997, Proc. Soc. Nutr. Physiol., 6, 107

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100. The anti-oxidative state before and after a diagnosed abomasal displacement (DA) in cows (Antioxidativer Status vor und nach diagnostizierter Labmagenverlagerung [DA] bei Kühen). M. Fürll*, M.N. Dabbagh, H. Kirbach, C. Nauruschat, T. Sattler, H. Wilken – Leipzig/Hama (Syrien)

Objective: DA is one of the most important non-infection diseases in high yielding dairy cows. Aetiological factors are mainly disturbances in energy metabolism beginning in part in the dry period. Oxidative stress is involved in the pathogenesis of these diseases. But there is a lack on knowledges.
Aims: In three complexes investigations were performed to answers to the following questions: 1) Significance of antioxidants in the aetiology of DA, 2) the antioxidative state at the time of diagnosis of DA, 3) development of antioxidative state after reposition of displaced abomasum.

Experimental design: The antioxidative state was checked in 20 healthy and 137 cows suffering from DA: 1) 8 cows on 3rd day after parturition (ap) before DA and at the diagnosis of DA; 2) 87 cows at the diagnosis of DA; 3) 21 cows before and in the course of 24 hours after DA reposition; 4) 21 cows before as well as on first and third day after DA reposition. In the blood were analysed superoxide dismutase (SOD), glutathione peroxidase (GPX), TEAC, CK and AST as well as further haematological and clinical chemical parameters (Hitachi 704, Technicon 1).

Results: The SOD-activity increased from 6729 ±1048 U/ml one week (w) up to 7506 ±1208 U/ml four w ap in erythrocyte lysate (el) of healthy cows resp. from 10 U/ml (0/50) 140 U/ml (270/340) in serum. The same trend was observable in TEAC with 226±42 to 310±30 µmol/l. At the second day ap were not TEAC differences between healthy and cows with later DA (237±37µmol/l) as well as at the diagnosis of DA (250±74µmol/l). Cows with DA had significant higher CK activities at second day ap (416 U/l [157/771]) and at the diagnosis of DA (313 U/l [166/414]). The SOD activity was in 87 cows at diagnosis of DA 7125 U/ml el (6154/ 8625) and did not differ from healthy cows. Cows with left side DA had slightly lower SOD activities than cows with right side DA. 44,8% of the cows had SOD activity <7000 U/ml el and are suffered from massive metabolic stress. The GPX activity in cows with DA was 385 ± 103 U/mg Hb. Between SOD and GPX was found a negative correlation (r=-0,40, p<0,01). After reposition of left side DA SOD activities decreased shortly, in contrast in cows with right side for more than 24 h (p<0.05). Cows with left side DA had higher TEAC concentrations compared with right side DA. After reposition of DA the TEAC concentration didn't differ significantly.

Conclusions: The antioxidative capacity (SOD, TEAC) increases in healthy cows after parturition. The TEAC concentration is not changed before DA, but the CK activity is higher (p<0.05). Approximately 45% of the cows with DA have a stressed antioxidative system (SOD-activity <7000 U/ml el). This is mostly the case in cows with a left side DA. Shorter and massive metabolic stress is equivalent to GPX-activities >500 U/mg Hb. In these cases the SOD activity is only slightly decreased. The SOD activities decrease special after reposition of right side DA.

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101. The influence of light physical activity on postprandial lipemia in healthy but not endurance trained young people (Der Einfluss von leichter physischer Aktivität auf die postprandiale Lipämie bei gesunden aber nicht ausdauertrainierten jungen Personen). Martina Pfeiffer*, Lida Brich, C. Wenk and P. C. Colombani - Zürich

Delayed postprandial lipemia is an independent risk factor for coronary heart disease. Regular endurance exercise improves blood lipids in the fasted as well as postprandial state. However, information on the influence of single bouts of light physical activity corresponding to the minimal recommended duration of daily 30 min is scarce. We therefore investigated if 30 min of light cycling reduced postprandial lipemia in normal weight and healthy but not endurance trained young people.

Subjects & Methods: Eight men aged 26 (6) (mean (SD)) y with a body mass index (BMI) of 22 (1) $kg \cdot m^{-2}$ and 7 women aged 23 (2) y with a BMI of 21 (2) $kg \cdot m^{-2}$ participated in this crossover study, each one performing an activity (A) trial (30 min cycling) and a control (IA) trial (no physical activity). They were non smokers, had less than three hours of sports activities per week and were not aware of any known metabolic disorders. After an overnight fast and measurement of fasting capillary triacylglycerol (TAG), the subjects either cycled for 30 min on an ergometer (perceived exertion of 9 according to Borg RPE scale) in the A trial or rested in the IA trial. Then they received breakfast (fat 0.5 $g \cdot kg^{-1}$ body mass (BM), carbohydrates 1.7 $g \cdot kg^{-1}$ BM and protein 0.3 $g \cdot kg^{-1}$ BM) and 3 h later lunch (fat 0.4 $g \cdot kg^{-1}$ BM, carbohydrates 1.6 $g \cdot kg^{-1}$ BM and protein 0.4 $g \cdot kg^{-1}$ BM). TAG was measured hourly for 6 h after the first meal to assess postprandial TAG course. Respiratory exchange ratio (RER) was measured in the fasted state and 3 and 6 h after the first meal. To compare TAG and RER between trials repeated measures ANOVA was used.

Results: All subjects had normal fasting TAG (men: 1.38 (0.65) $mmol \cdot L^{-1}$; women: 1.44 (0.41) $mmol \cdot L^{-1}$). Heart rate during exercise was 102 (9) min^{-1} and 114 (16) min^{-1} for men and women, respectively. Men cycled at 65 (10) Watt and women at 54 (6) Watt. Postprandial lipemia was not statistically affected by the 30 min of low intensity cycling (men: P=0.72; women: P=0.95). The 6 hours incremental area under the curve (dAUC) for postprandial TAG in the A trial was 24 % and 42 % lower compared to the IA trial for men (P=0.32) and for women (P=0.14), respectively. Maximal TAG value was consistently observed 1.5-2 h after lunch: it corresponded to 2.95 (1.27) $mmol \cdot L^{-1}$ and 3.27 (1.53) $mmol \cdot L^{-1}$ (P=0.66) for men and to 2.29 (0.84) $mmol \cdot L^{-1}$ and 2.28 (0.79) $mmol \cdot L^{-1}$ (P=0.97) for women in the A and IA trial, respectively. The maximal increase in TAG during the 6 h observation period was non-significantly lower in the A trial than in the IA trial in men (1.53 (0.82) $mmol \cdot L^{-1}$ compared to 1.85 (0.96) $mmol \cdot L^{-1}$; P=0.49) and in women (0.77 (0.44) $mmol \cdot L^{-1}$ compared to 0.92 (0.46) $mmol \cdot L^{-1}$; P=0.54). RER was not significantly affected by activity neither in men (P=0.65) nor in women (P=0.24).

Conclusion: A single bout of 30 min low intensity cycling reduced dAUC after two meals, corresponding to current recommendations for macronutrient composition, in healthy but not endurance trained young people. However, the reduction was not significant, likely due to either the short duration and/or low intensity of the activity, and/or the moderate fat content of the meals.

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102. Four generations feeding of GMO-corn to breeder quails (Fütterung von gentechnisch verändertem Mais an Zuchtwachteln über vier Generationen). Ingrid Halle*, Karen Aulrich and Gerhard Flachowsky - Braunschweig

There exist many feeding experiments with diets which contained feeds from genetically modified plants as recently summarized by Aulrich et al. (1). But nevertheless the public asks for results of long term experiments and studies over several generations on different animal species. The objective of the present experiment was to feed genetically modified corn (Bt 176) as high content in diets about ten generations on breeder and growing quails. The paper reports on the first four generations of the experiment.

Methods: Basal diets were formulated to contain 40 % (chicken) or 50 % (growers, hens) corn (isogenic corn or Bt-corn) per kg. Feed and water were provided for ad libitum consumption. Eggs were collected at a quail hen age of 10 weeks. Hatched quails were reared over 6 weeks before the hens started the laying period.

Results: Results after four generations are given in Tables 1 and 2. Insignificant differences between the two corn groups and also the four generations were observed for some parameters ($p>0.05$).

Conclusions could be given after finishing the ten generations experiment.

Table 1: Feed intake (FI), laying intensity (LI) (at the age of 7 to 11 wk) and hatchability (Hatch) (at the age of 10 wk) of breeder quails (n=32 hens, Hatch = 300 eggs)

	Corn, isogenic			Bt-corn		
Generation	FI g/uail/day	LI %	Hatch %	FI g/quail/day	LI %	Hatch %
1	23,3	81,4	90,5	23,4	78,7	82,8
2	20,4	87,8	90,5	22,2	86,7	81,0
3	22,6	81,9	76,2	22,6	79,4	83,2
4	22,0	75,9	83,0	22,2	79,4	79,2
$\bar{x}$	22,1	81,8	85,0	22,6	81,06	81,6

Table 2: Body weight (BW) of growing quails at the age of 6 weeks and feed intake (FI) (n=100 male and female each)

	Corn, isogenic		Bt-corn	
	BW, g/quail	FI, g/quail/day	BW, g/quail	FI, g/quail/day
1st generation				
Male	146,7 ±12,0	15,6	142,5 ± 9,9	15,7
Female	172,6 ±25,7		171,9 ±24,8	
2nd generation				
Male	142,1 ±10,3	15,2	144,0 ±12,4	16,0
Female	182,3 ±14,9		181,7 ±16,1	
3rd generation				
Male	149,0 ±12,3	15,6	141,6 ±13,2	14,8
Female	173,1 ±15,1		175,0 ±18,5	
4th generation				
Male	147,2 ±10,3	17,2	145,5 ± 9,9	17,7
Female	180,8 ±13,0		181,3 ±13,2	
$\bar{x}$ Male	146,2	15,9	143,4	16,0
$\bar{x}$ Female	177,2		177,5	

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103. Effect of organic acids on dietary self-selection by the piglet (Einfluss von organischen Säuren auf die Futterauswahl von Ferkeln). T. Ettle, K. Mentschel and F.X. Roth – Freising-Weihenstephan

Several studies have demonstrated that the inclusion of organic acids in the diets of weaned piglets can enhance performance criteria. However, especially positive effects on feed intake seem to depend on the level and type of the respective organic acid used. For this reason a first choice experiment (exp) investigated if there is a preference of piglets for diets with different levels of a potassium (K-) diformate supplementation. In a second choice feeding experiment the possible preferences for diets supplemented either with formic or sorbic acid were investigated.

Methods: Both trials were carried out over a period of 6 weeks and used 48 piglets each. Female as well as castrated male piglets with an initial body weight of 8.0 ± 0.9 (exp I) and 7.3 ± 0.8 kg (exp II) were randomly subdivided into four (exp I) or 5 (exp II) groups. In exp I two reference groups were fed either an unacidified diet (treatment (treat) 1) or a diet containing 2.4 % of potassium diformate (Formi®, BASF) (treat 4) with no choice for selection. The other groups had the possibility to choose from either the unacidified diet or a diet supplemented with 1.2 % potassium diformate (treat 2) or the unacidified diet or the diet supplemented with 2.4 % potassium diformate (treat 3). In exp II, animals of the reference groups were offered exclusively an unacidified diet (treat 1) or a diet supplemented with 1.2 % formic (treat 4) or sorbic acid (treat 5), respectively. The animals of the organic acid choice groups had the possibility to choose from either the unacidified diet or a diet supplemented with 1.2 % formic acid (treat 2) or 1.2 % sorbic acid (treat 3).

Results: In exp I average daily feed intake, daily gain and feed to gain ratio were 751 g, 458 g and 1.64 kg/kg, respectively. Daily gain in treat 2, 3 and 4 was about 2 %, 9 % and 5 % higher than in treat 1 (440 g/d) and feed to gain ratio was 0.6 %, 1.8 % and 3.6 % better than in treat 1 (1.67 kg/kg) but differences between the means were not significant. In the choice feeding groups there was no evidence for a preference for the diets supplemented with potassium diformate. In contrary, chosen diets in treat II and III contained on average 53.7 and 50.9 % of the unacidified feed, respectively.

In exp II average daily feed intake was 849 g with no significant differences between the treatments. However, animals of treat 2 – 5 consumed 6 %, 6 %, 3 % and 1 % more feed than animals of the negative control group (822 g/d, respectively). Animals of both organic acid choice groups showed a significant preference for the unacidified diets. Over the whole experimental period, animals of treat 2 and 3 consumed on average 31.7 and 27.7 kg of the unacidified diets but only 4.9 and 9.0 kg of the diets supplemented with 1.2 % formic or sorbic acid. Thereby, animals of both organic acid choice groups chose a diet containing a significantly higher portion of the unacidified feed in each experimental week. Whereas in treat 2 the portion of the unacidified diet as a percentage of the total diet increased from 72.8 % in the first experimental week to 94.2 % at the end of the experimental period, in treat 3 the mean portion of 76.5 % remained rather unchanged in the course of the experiment. Average daily gain in the negative control group (506 g) was 6 %, 2 %, 9 % and 6 % lower than in treat 2 – 4, but differences between treatments were not significant. Feed to gain ratio in treat 4 (1.53 kg/kg) was significantly better than in treat 1, 2 and 3 (1.62, 1,62 and 1.69 kg/kg, respectively) and animals of treat 5 had a significantly better feed to gain ratio than animals of treat 3.

Conclusions: When given a choice, piglets avoid a feed acidified with formic or sorbic acid, but not the feed acidified with a buffered acid (potassium diformate). In this case the chosen portions of the acidified and the nonacidified diets were almost equal. Nevertheless, the inclusion of any of the organic acids on test lead to an improved feed to gain ratio compared to animals fed unacidified diets at least in numerical terms.

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104. The effect of gluconic acid on growth and intestinal microflora of weanling pigs (Einfluss von Gluconsäure auf Wachstum und intestinale Mikroflora von Absetzferkeln). G. Biagi*, E. Vezzali, A. Piva and F.X. Roth – Bologna/Freising-Weihenstephan

The finding that antibiotics fed to farm animals as growth promoters can be responsible for the spreading of resistant bacteria and the consumer demands for a food chain free of drugs determine the need to study alternative strategies to control microbial activity in the gastrointestinal system of monogastric animals. Gluconic acid (GA) has been shown to reach the large intestine in rats where it can be fermented by the microflora (1). Aim of this study was the evaluation of the effect of feeding GA on piglet growth and intestinal microflora.

Methods: Immediately after weaning, 48 piglets were divided into 4 groups (12 animals per group, housed in individual cages) for a 6 week trial. Treatments were a commercial diet with a) no addition (control diet) or with b) 0.3%, c) 0.6%, and d) 1.2% of GA. Feed and water were provided *ad libitum*. Animals were weighed every week and feed consumption was recorded. At day 10 and 31, faecal samples were collected from 6 animals per group and cultured for viable bacteria (coliforms, clostridia and lactobacilli). At the end of the trial, 4 animals per group were killed. Samples of jejunum and caecum content were cultured for viable bacteria and their ammonia and volatile fatty acids (VFA) content as well as pH were determined.

Results: Feeding 0.3 and 0.6% of gluconic acid increased average daily gain (ADG) compared to control ($P < 0{,}10$), while gain to feed ratio (GF) was not influenced by treatments (Table 1). Faecal bacterial counts after 2 weeks did not show any differences. At week 5, coliforms were markedly reduced by the 0.3% GA treatment compared to control ($P < 0.10$) and all GA diets showed a tendential increase of the number of lactobacilli. In the caecum, clostridia were significantly reduced by GA at 0.6 and 1.2% ($P < 0{,}10$). As previously observed in the faeces, lactobacilli were tendentially increased in the caecum by GA. Ammonia concentration in the intestinal samples was not influenced by treatments. VFA in the intestine were increased by GA, but the differences observed did not reach the significance level due to the high variability. Interestingly, GA mainly increased acetic acid in the jejunum and butyric acid in the caecum.

Table 1. Live weight, average daily gain (ADG), daily feed intake and feed to gain ratio (FG) of piglets in the 6 weeks after weaning. Values are means of 12 animals ± SD.

	Final live weight (kg)	ADG (g/d)	Daily feed intake (g/d)	FG
Control	25.08 ± 3.09	423 ± 67	704 ± 121	1.67 ± 0.06
Gluconic acid 0.3%	26.73 ± 2.73*	464 ± 55*	754 ± 108	1.62 ± 0.07
Gluconic acid 0.6%	26.85 ± 2.95*	466 ± 71*	749 ± 94	1.62 ± 0.08
Gluconic acid 1.2%	25.25 ± 2.22	428 ± 52	696 ± 68	1.63 ± 0.06

*Different from control by $P < 0.10$.

Conclusion: The present results show how feeding GA can improve the growth performances of piglets after weaning. Nevertheless, when used at a high concentration (1.2 %) the positive effect of GA was not observed. The bacterial counts and the VFA analyses show that GA may influence the composition and the activity of the intestinal microflora. Further studies will be needed to achieve a better understanding of the mode of action of GA in pigs.

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105. Effect of Echinacea purpurea L. (MOENCH) on performance and immune status in pigs (Der Einfluss von Echinacea purpurea L. (MOENCH) auf Leistungsparameter und Immunstatus beim Schwein). Barbara M. Böhmer*, Brigitte R. Paulicks, Dora A. Roth-Maier – Freising-Weihenstephan

The aim of the present investigation was to examine the inclusion of the herb Echinacea purpurea (L.) MOENCH as feed additive in diets of sows, piglets, and grower/finisher pigs on growth performance, blood picture, plasma enzymes including proliferation of lymphocytes, antibody status, and protein and immune globulin content of colostrum. The dosage of Echinacea was adapted according to the recommendations for human. The application form of Echinacea was grounded cobs derived from dried aerial parts of the plant. The control groups were supplemented with alfalfa meal. Analyzed contents of cichory acid and alkamids ranged from 290 - 420 mg/100 g and from 10.7 - 67.5 mg/100 g, respectively. In another charge used for grower/finisher pigs, the analyzed contents were170 mg/100g and 44.1 mg/100 g, respectively.

Sows: 36 sows received diets with0, 1.2, or 3.6 % Echinacea cobs from d 85 to d 110 of gestation and 0, 0.5, or 1.5 % Echinacea cobs up to d 28 of lactation. No significant differences were found for growth performance, weight loss, blood picture, plasma enzymes, and colostrum composition. Performance of the sucking piglets was not impaired either during lactation or during a 4week observation period after weaning. The health status was similar in all treatment groups.

Piglets: In a second experiment, lasting 6 weeks, with 36 piglets (5.8 – 22.1 kg body weight), the treatment groups were control, 1.8 % Echinacea supplementation, or 20 mg/kg feed Flavomycin®. No significant differences were found for the recorded parameters. Feed efficiency of the Echinacea group was slightly (4 %) improved (1.54 vs. 1.60).

Grower/finisher pigs: In a third trial 48 grower/finisher pigs were used during a 9week experimental period in the live weight range of 32 –83 kg with 2 supplementation phases (week 1-3 and week 7-9). The experimental groups received 0, 1.5 % cobs or 4/6 ml (grower/finisher) pressed juice (commercial standard), respectively. Vaccination with Swine erysipelas was implemented in week 1 and 5 to determine the specific immune response.

Growth performance and blood picture for all groups were similar, however, feed efficiency of the Echinacea groups was significantly better (2.43 vs. 2.51, see table). In addition, the Swine erysipelas antibodies showed a marked significance in regard of altitude and duration of reaction in both groups supplemented with Echinacea (see table).

Effects of Echinacea supplementation in diets of grower/finisher pigs with inoculation (Swine erysipelas vaccine, Intervet)

Echinacea supplementation	**0**	**Juice 4/6 ml[1]**	**Cobs 1.5 %**
Feed efficiency (kg feed/kg gain)	2.51^{a}	2.43^{b}	2.45^{ab}
Swine erysipelas antibodies (Extinction x 10^{-3})	74.5^{b}	142.0^{a}	134.3^{a}

[a b] significance according to Student-Newman-Keuls-test (P<0.05) [1] per day in grower/finisher period, resp.

Conclusion: It can be concluded, that Echinacea purpurea can be used as a feed additive to increase feed efficiency and to achieve specific and unspecific immune stimulating effects in pig production. The efficiency of cobs is comparable to a commercial juice product. The effect of Echinacea depends not only on the main ingredients cichory acid and alkamids but should be attributed to varying chemical interactivities.

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106. PEG does not fully prevent the formation of tannin-protein complexes *in vitro* (PEG verhindert die Komplexierung von Tanninen mit Proteinen nicht vollständig). S. Muetzel, K. Becker – Stuttgart-Hohenheim

The high affinity of PEG to tannins is used to prevent the formation of tannin protein complexes *in vitro* and *in vivo*. The inclusion of PEG into *in vitro* incubation systems is used to estimate the biological effect of tannins on the rumen fermentation (Makkar *et al.*, 1995). Here we show that PEG does not fully prevent the complexation of tannins with BSA.

Methods: Leaf samples of *Juglans regia, Rhus typhina, Aesculus hippocastanum* and *Salix alba* were separated in two portions and either freeze dried or oven dried (60°C). Four treatments were incubated *in vitro* using the Reading Pessure Technique (Mauricio *et al.*, 1999). Leaves were either incubated without any addition, with 10mg/ml PEG 6000, with 1mg/ml bovine serum albumin (BSA) or with PEG+BSA. Samples were collected after 3, 6, 12 and 24 hours of incubation. Gas production was recorded by a pressure sensor, short chain fatty acid (SCFA) concentrations were determined by gas chromatography and NH_4 was quantified by the alkaline nitroprussid method. The soluble and insoluble proteins were determined by a dot blot assay (Hoffmann *et al.*, 2002) and visulalised by polyacrylamide gel electrophoresis (PAGE).

Results: Addition of PEG increased gas production by 14.8 ml/g and SCFA concentration by 6.0 µmoles/ml. This so called PEG effect was in the same order when BSA was included into the incubation, indicating that the addition of further protein does not decrease the negative effects on tannins on the rumen fermentation. Soluble protein was only detected in the incubations containing BSA, and followed a nearly linear degradation kinetic. With no PEG added, a part of the BSA was precipitated by the tannins and detected within the insoluble fraction of the incubation residue. This precipitation was most apparent at early hours of incubation. The leaf sample that contained exclusively hydrolysable tannins (*Rhus thypina*) showed a very strong affinity to the BSA right after adding the rumen fluid, but the BSA was released form the tannins rather rapidly within the following 3 hours of incubation. Interpretation of the insoluble protein has to be done very carefully, since it is the sum of the degradation of feed proteins and the changes in microbial protein concentrations. However, the insoluble protein was always higher in the treatments that did not contain PEG and it was also higher in the treatments containing BSA, indicating that some portion of the BSA was coprecipitated with the tannin even in the presence of PEG. This was confirmed later on by PAGE, where BSA can be visualised as a distinct band. Since the pH *in vitro* is around 6.8, where the precipitation of BSA (pI 4.9) is very low (Hagerman and Butler, 1978), a much higher precipitation of leave protein (Rubisco, pI 6.8) would be expected even with PEG present under *in vitro* conditions.

Conclusion: The use of PEG clearly improved rumen fermentation but it apparently does not fully prevent the formation of tannin-protein complexes and therefore, a negative effect of tannins on the rumen microbial community cannot be excluded.

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107. Effects of supplemented probiotic Lactobacillus acidophilus strain on digestive parameters and health status in German Shorthair Pointers (Effekte eines zugefütterten, probiotischen Lactobacillus acidophilus-Stammes auf verdauungsphysiologische Parameter und allgemeinen Gesundheitsstatus bei Hunden der Rasse Deutsch Kurzhaar). M. Pascher*, J. Zentek – Wien

Our hypothesis was, that oral administered Lactobacillus acidophilus has beneficial effects on general health status, immune system and the intestinal microbial metabolism in terms of stabilising the equilibrium between the lactogenic, acid tolerant flora and the undesirable, toxinogenic and putrefactive bacteria.

Methods: A consecutive feeding trial was designed. In the control period a dry diet (92.53% DM, 29.4 % crude protein, 9.5 % crude fat, 2 % crude fibre, 7.1 % crude ash) was compared to the identical dry diet in probiotic period, supplemented with a probiotic Lactobacillus acidophilus strain. The probiotic Lactobacillus acidophilus was suspended in an oil and fat matrix and mixed to dog dry diet at ambient temperature and by keeping the water content as low as possible. Daily dosage of 10^6 cfu/g Lactobacillus acidophilus was fed to 6 adult, healthy German Shorthair Pointers. After the experimental diet dogs were fed with the control diet again. Frequency of defecation, feces consistency, dry matter, free water, pH value, ammonia, lactate, IgA levels and microbial counts of Clostridium perfringens, E. Coli, Lactobacillus spp. and Bifidobacteria spp. were investigated in fecal samples. Additional parameters were IgG level, and acute phase proteins in blood samples. Statistics: ANOVA, probability values of less than 0.05 were taken as significant.

Results: The frequency of defaecation did not change significantly (control mean 2.56, probiotic 2.04, control 2.11) although a tendency towards first control period ($p<0,1$) was evident (table 1). Fecal consistency was significantly lower ($p<0.05$, mean 3.54) in the first control period compared to following probiotic feeding period (mean 3.34). Fecal dry matter was significantly ($p<0,01$) higher in the probiotic period compared to first control and second control period. Fecal pH value did not show any differences and tendencies between feeding periods. Ammonia concentrations in first control period were significantly higher compared to probiotic and second control period. Lactate concentration was significantly ($p<0.05$) lower in probiotic period.

Table 1: Fecal characteristics in the experimental dogs

Diet	DM, %	free water, %	pH	Ammonia, g/kg	Lactate, g/kg
control	26.2 +/- 0.93	30.7+/- 3.24	6.23 +/- 0.11	0.99 +/- 0.14	0.41 +/- 0.13
probiotica	29.3 +/- 1.06	25.5 +/- 2.26	6.19 +/- 0.12	0.83 +/- 0.005	0.17 +/- 0.05
control	26.1 +/- 1.62	27.5 +/- 2.18	6.14 +/- 0,14	0.87 +/- 0.11	0.28 +/- 0.08

The bacterial counts of Clostridium perfringens, Lactobacillus spp., E.coli and Bifidobacteria spp. did not show any significant changes, though the mean cfu/g of Clostridia and E.coli decreased and mean cfu/g Lactobacillus and Bifidobacteria spp. increased in probiotic period (table 2).

Table 2: Bacterial counts in the feces of the experimental dogs (log/g)

Diet	Clostridium	E.coli	Lactobacillus	Bifidobacteria
control	7.92 +/- 0.32	7.31 +/- 0.27	8.72 +/- 0.66	7.83 +/- 1.24
probiotica	7.61 +/- 1.03	7.21 +/- 0.56	9.26 +/- 0.76	8.35 +/- 0.81
control	7.98 +/- 0.31	6.72 +/- 0.69	8.91 +/- 0.95	8.00 +/- 0.41

C-reactive protein (CRP) was significantly lower in probiotic period.No significant changes in feces IgA levels and Haptoglobin concentrations in blood serum.

Conclusions: The Lactobacillus acidophilus feed additive did have significant effects on fecal quality, especially improved fecal consistency, increased dry matter and decreased ammonia concentrations.

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108. Impact of a probiotic *Enterococcus faecium* preparation on animal performance and selected microbial pathogenicity markers in piglets (Beeinflussung von Leistungsdaten und ausgewählten mikrobiologischen Pathogenitätsmerkmalen bei Ferkeln durch das Probiotikum *Enterococcus faecium*). D. Taras*, M. Macha, W. Vahjen, O. Simon - Berlin

Following the definition of a probiotic, an improvement of the intestinal microbial balance and consequential beneficial effects for the host animal characterize its mode of action. Therefore, determination of animal performance was accompanied by intestinal microbial analysis in two groups of sows and their respective litters either receiving or not receiving *Enterococcus faecium NCIMB 10415*.

Methods:
Twenty pregnant sows and their litters were randomly assigned to two treatment groups (*E. faecium*/control). Piglets were weaned at 28 days of age and penned pairwise. Feed and water were offered *ad libitum*. Body weight and feed intake were measured once a week. Fecal consistency of weaned piglets was recorded daily. Additional, precaecal amino acid digestibility analysis was performed with ground, freeze-dried digesta and diet samples of ten piglets of each treatment group. Fecal *E. coli* isolates were screened by a multiplex PCR assay for 9 relevant porcine pathogenic factors (4 fimbriae, 5 toxins). Furthermore, digesta and fecal samples were tested for the presence of β-toxin gene carrying *C. perfringens* strains.

Results:
Probiotic supplementation of diet had no statistical significant influence on overall live weight, weight gain and feed conversion ratio in piglets up to the age of 70 days ($p>0.05$). A tendency towards reduced animal losses during the suckling period due to probiotic treatment was observed. Furthermore, compared to controls, probiotic fed weaned piglets showed a significantly reduced incidence of watery feces. Similarly, the occurrence of fecal pathogenic *E. coli* isolates were decreased in the offspring of probiotic fed mothersows even before administration of solid feed. On the other hand, isolates with the fimf41a gene, coding for an adhesin or its combination with the shiga-like toxin were detected in the feces of probiotic fed piglets at the age of 14 days while absent in feces of control piglet at this stage. Age group related analysis revealed further differences in abundance of pathogenic factors, but which were inconsistent in relation to treatment.

Conclusion:
Although Enterococcus faecium NCIMB 10415 represented only a small proportion of the intestinal population, it nevertheless exerted influence on the composition of the intestinal population, which in return may have had an impact on fecal consistency. As also suspected by others in previous reports, it may be speculated that the probiotic impact on animal performance is less obvious under good sanitary conditions. Anyhow, the transfer of the probiotic strain and a possibly already modified microbiota from maternal feces to piglets seems to be an important factor in the establishment of a beneficial microbiota in the suckling phase.

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109. Influence of *Enterococcus faecium* on the immune system of sows and piglets (Einfluss von *Enterococcus faecium* auf das Immunsystem von Sauen und Ferkeln). Lydia Scharek*, Barbeline Altherr, M.F.G. Schmidt - Berlin

Since the use of antibiotics as growth promotors is already restricted or banned in many countries, research on probiotic feed supplements is a hopeful alternative to compensate for the possible decrease in production. In numerous studies it has been shown that bacterial colonisation influences the function of immune cells of the gut-associated lymphoid tissue (GALT) and even can affect systemic immunity (1; 2). In an animal study with swine, we tested the influence of the probiotic *Enterococcus faecium* NCIMB10415 as a feed supplement on the development of the intestinal immune system.

Methods: *E. faecium* was fed to sows starting with the second day of pregnancy, as well as to the piglets. Test animals were sacrificed on days 14, 28, 35, and 56 after birth and immune cells from the piglets' intestinal mucosa were isolated from both the intestinal epithelium (proximal jejunum) and from the continuous Peyer's patch. Cells were prepared using percoll gradients according to Solano-Aguilar *et al.* (2). Fluorescent antibodies against several surface antigens (CD4, CD8, CD21) were used to quantify the development of different lymphocyte populations using flow cytometry. ELISA were used to measure antibody levels in feces and blood of sows and piglets. Independently, the occurrence of pathogenic bacteria in the feces was monitored.

Results: A significant decrease in CD8+ intraepithelial lymphocytes (IEL) in probiotic-treated piglets during the first seven weeks of life was observed. The numbers of B cells (CD21+) tended to be lower in the Peyer's patches of treated piglets at the age of 14 days compared to the control group. After weaning, seral IgG levels tended to be lower in probiotic-treated piglets. No differences were observed in the amounts of fecal IgA prior to day 70, at which time treated piglets showed less IgA. Enteropathogenic bacteria such as *E. coli* O141 and *Chlamydia* spp. were reduced in the probiotic treated piglets.

Conclusions: The reduction in enteropathogenic bacteria may explain the decline in cytotoxic T cells as well as the tendency of the lower IgG and IgA levels. The presence of *E. coli* or *Chlamydia* in the control group however, does not appear to result in significant changes in the CD4+ or CD8+ cells in the continuous Peyer's patches of the piglets. Alternatively, the reduction in CD8+ IEL in the probiotic group may reflect an increase in tolerance, aiding in a more rapid establishment of the normal gut flora.

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110. *Bacillus cereus* as a probiotic supplement - Effects on absorption and secretion in pig jejunum (*Bacillus cereus* als probiotischer Futterzusatz – Wirkung auf Absorption und Sekretion im Jejunum vom Schwein).

Ulrike Lodemann*, Anke Finnah, Barbara Lorenz, H. Martens - Berlin

In piglet rearing a probiotic preparation of *Bacillus cereus* showed positive effects on daily weight gain and feed conversion (1). Very little is known about the underlying mechanisms of action of probiotics. Baum et al. (2) observed an increase in villus length in the small intestine in piglets, which were fed a diet supplemented with *Bacillus cereus*. Furthermore, Breves et al. (3) showed positive effects on the glucose transport rates. *Bacillus cereus* also seems to have a growth-retarding effect on enterobacteria in the digesta of the small intestine in piglets.

Methods:

Sows were assigned randomly to a control group and a probiotic group which was fed with a diet supplemented with 4,4-8,8 x 10^8 CFU/kg of *Bacillus cereus var. toyoi* from the 91st day before birth. The supplemented diet was fed to the piglets from the 15th day after birth. Tissue samples (epithelia) were taken from the mid jejunum of the piglets at day 14, 28, 35 and 56. The mucosa was stripped and mounted into conventional Ussing chambers.

Glucose transport was stimulated by 0.5, 1, 4 and 10 mmol/l of glucose and secretion was stimulated by different concentrations of PGE_2 (0.05, 0.1, 0.5 and 1 µmol/l). The tissues were incubated under short circuit conditions and the changes in short circuit current (ΔI_{sc}) were recorded.

Results:

The response of I_{sc} on addition of increasing concentrations of glucose showed saturation kinetics. The mean of ΔI_{sc} was lower in the probiotic group compared to the control group on day 14 and 56. The ΔI_{sc} was inconsistent on day 28 and 35. The variation between the animals was high.

Serosal PGE_2 caused a dose-dependent change in I_{sc}. On the 14th day the response to PGE_2 was reduced in the probiotic group at higher PGE_2 concentrations. The effect of PGE_2 on I_{sc} significantly varied with age.

Conclusions:

The working hypothesis of this study is the assumption that the positive effect of *Bacillus cereus* on daily weight gain and feed conversion can be explained by altered transport properties of the intestine. The obtained data of the present study partly support this hypothesis (reduced ΔI_{sc} after treatment with PGE_2). However, the reduced response of I_{sc} after mucosal addition of glucose is in contrast to previous findings (3) and obviously age dependent.

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111. Modification of intestinal bacterial communities by a probiotic *Enterococcus faecium* strain in piglets (Modifizierung intestinaler Bakteriengemeinschaften durch einen probiotischen *Enterococcus faecium* Stamm in Ferkeln).

W. Vahjen*, D. Taras, M. Macha, O. Simon – Berlin

Methods:

Total nucleic acids were extracted from digesta and faeces samples of piglets (14 – 56d) and their respective mother sows fed a diet with or without 4x10^8 viable *Enterococcus faecium* NCIMB10415 per kg diet. Purified DNA extracts were PCR amplified with eubacterial 16S ribosomal DNA primer. PCR products were separated with the denaturing gradient gel electrophoresis (DGGE) and resulting band profiles were analyzed with respective software. Calculation of pairwise similarity indices and construction of corresponding dendrograms allowed anylysis of DGGE profiles so that relationships between bacterial communities in individual samples could be compared.

Specific Digoxigenin-labelled 16S RNA oligonucleotide probes were hybridized against total nucleic acid extracts (50 ng total nucleic acid) spotted on nylon membranes in a dot-blot procedure. After antibody detection of the Digoxigenin label, chemoluminescence signals were recorded with a light sensitive camera. Calibration curves consisting of an equimolar mix of all sample extracts were employed to quantify ribosomal activity of selected bacterial genera expressed as specific ng ribosomal RNA (rRNA).

Results:

DGGE:

Band pattern of DGGE profiles of colon digesta grouped according to treatment and exhibited highest similarity in samples of 14 day old piglets. In general, species diversity in colon samples decreased from day 14 to day 28 of age, but reached its maximum at 35 days of age. Several bands representing operational taxonomic units (OTUs) could be tracked throughout ageing, while other vanished or emerged at different time points. Species diversity in these samples of preweaned piglets tended to be higher in the control group than in the probiotic group.

RNA:

Hybridizable rRNA (all rRNA with Univ519 probe) in total nucleic acid extracts was similar in the stomach and proximal jejunum on all sampling days, however central jejunum rRNA displayed higher amounts in the control group. Total rRNA was always highest in the colon. Eubacterial rRNA generally followed a trend for lower amounts before, but higher amounts after weaning. *Enterococcus* rRNA was higher in the control group preweaning, however parallel to high specific colony counts of the probiotic *E. faecium* strain (colony hybridization with specific probe), elevated *Enterococcus* rRNA were recorded on the 28th day of life in the probiotic group.

Lactobacillus spp. rRNA in the stomach and proximal jejunum was always higher in the probiotic group preweaning, however no differences were found after weaning. The reverse picture was seen for central jejunum, while colon segments remained unchanged. *Lactobacillus* spp. was higher or similar in the control group preweaning, but increased after weaning. *Bifidobacteria* rRNA was also enhanced by probiotic supplementation. Especially in stomach and colon samples, high differences were observed.

Conclusion:

The modification of intestinal bacterial communities by the probiotic *E. faecium* strain seems to be a result of an already modified microbiota of the mother sow. Cell numbers of the probiotic strain were not sufficient to increase *Enterococcus* spp. activity. However, constant uptake of probiotic feed after weaning further modified the intestinal microbiota.

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112. Molecular analysis of the effects of probiotics on bacterial pathogens in swine (Molekulare Analyse der Wirkungsmechanismen von Probiotika auf bakterielle Darmpathogene beim Schwein). P. Schierack*, M. Nordhoff, Marion Pollmann, P. Schwerk, Christiane Tölke, K. Tedin und L. H. Wieler - Berlin

Most beneficial effects of probiotics were empirically described in veterinary medicine, However, the basic mechanisms underlying these effects are known scarcely. We have concentrated our efforts on determining the effects of probiotic bacteria in defense against intestinal pathogens, taking our lessons from the field of cellular microbiology, as invasive and non-invasive intestinal pathogens interact intimately with the host cells they infect. Therefore, we are performing *in vitro*- and *ex vivo* infection models to clarify the effects of probiotics on these interactions. In this paper two aspects are investigated more intensively: (a) the influence of the probiotic *Enterococcus faecium* strain NCIMB10415 on the interaction between *Salmonella typhimurium* as a model for invasive prokaryotic pathogen and a permanent porcine intestinal cell culture (IPEC-J2); (b) the influence of this probiotic on the infection of piglets with the obligate intracellular *Chlamydia*.

Methods: Sows and piglets were fed under controlled conditions with 10^9 CFU *Enterococcus faecium* NCIMB10415/g food. *Chlamydia* were isolated through cultivation in BGM cells from feces and tissue, and the shedding rate of piglets and sows through nested PCR, utilizing genus and species specific primers (*C. suis, psittaci, pecorum* and *trachomatis*) targeting *ompA*. *Salmonella* invasion was quantified in IPEC-J2 cells by gentamycin protection assay.

Results: In gentamycin protection assays we could show, that a factor produced by the probiotic bacteria was able to significantly decrease the invasion by *Salmonella*. Also, feeding experiments revealed that piglets fed with the probiotics shed less Chlamydia than control animals.

Conclusions: The probiotic *Enterococcus faecium* NCIMB10415 was able to reduce *Salmonella* invasion *in vitro* and Chlamydia shedding rate *in vivo*. The cell culture infection approach does not only reflect the interactions of the pathogen with the host cells, but also now gives us the chance to unravel the cellular mechanisms underlying the protective effect of this probiotic.

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113. Influence of an *Enterococcus faecium* probiotic on the alkaline phospatase activity of the small intestine in piglets (Einfluss eines *Enterococcus faecium* Probiotikums auf die Aktivität der alkalischen Phosphatase im Dünndarm von Ferkeln). B. Seelig, K. D. Weyrauch*, W. Vahjen, Barbara Drewes and M. Rieß - Berlin

Probiotic bacteria have been shown to modify many functions within the intestinal tract. It has been documented that the resorptive tissue of animals fed probiotic bacteria differs in immunological as well as in physiological behaviour from tissues of control animals. Alkaline phosphatase (AP) which can be found in many resorptive active tissues, plays an important part as one of the so called brushborder hydrolases and also as lymphatic enzyme following ingestion of lipids. The aim of the study was the qualitative and quantitative determination of AP activity in small intestinal epithelial tissues of piglets fed a diet with or without a probiotic *Enterococcus faecium* strain.

Methods: A total of 40 piglets aged 14, 28, 35 and 56 days were used for the study. The animals were fed a diet either with or without $4x10^8$ viable *E. faecium* NCIMB10415 cells/kg diet. Per diet group and sampling day 5 animals were slaughtered. Tissue samples were taken immediately after slaughter from the duodenum and the proximal jejunum. Samples were fixed in 4% paraformaldehyde for qualitative histochemical investigations and frozen in liquid nitrogen for quantitative measurements. Qualitative determinations of AP activity were carried out with tissue sections and light microscopy modified after Gomori (1952). A quantitative photometric assay (p-Nitrophenylphosphat, Sigma) based on wet weight was employed for the quantitative determination of AP activity in supernatants of homogenized tissues.

Results: The topographic evaluation of AP activity in tissues with light microscopy showed that the highest activity was generally seen in the apical cell membranes of the duodenum and jejunum. No or very weak activity was noted in lateral and basolateral membranes. In the remaining areas no activity was found. - Similar results could be shown for the quantitative determination of AP activity in supernatants of tissue homogenates, however standard deviations were high. The duodenum of control animals displayed higher values on the 35th day of life (table 1). The duodenum generally showed higher AP activity with decreasing activity as the animal aged. The AP activity in the jejunum was always higher in probiotic fed animal by a factor of 1.3 – 2.2.

Table 1: Alkaline phosphatase activity in the small intestine of piglets [U/ mg wet weight]

Duodenum	**14 day**	**28 day**	**35 day**	**56 day**
Contol group	- *	756 ± 72	728 ± 603	371 ± 83
Probiotic group	- *	816 ± 337	288 ± 179	634 ± 233
Jejunum	**14 day**	**28 day**	**35 day**	**56 day**
Contol group	204 ± 61	184 ± 39	112 ± 33	209 ± 86
Probiotic group	364 ± 164	238 ± 70	186 ± 89	446 ± 197

* = no samples

Conclusions:
The supplementation of the Enterococcus faecium probiotic to piglet feed generally led to a slight increase of alkaline phosphatase activity in the duodenum and jejunum.

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114. Performance, ileal digestibility of nutrients, amino acids and microbial status of large intestine in broiler fed diets with increased yellow lupine content and carbohydrases supplement (Auswirkungen des Einsatzes von steigenden Mengen von Lupine und des Zusatzes von Carbohydrasen auf die Mastleistung, ileale Verdaulichkeit der Nährstoffe, Aminosäuren und mikrobiellen Status des Dickdarminhalts). D. Jamroz, J. Orda, A. Wiliczkiewicz, T. Wertelecki, J. Skorupińska - Wrocław

Lupine seeds contain in dry matter about 27-35 % of non-starch polysaccharides and about 8 % of oligosaccharides, which are undegradable by endogenous enzymes in poultry, have antinutritive effect and decreases the performance but also can perform function of prebiotica in poultry gut.
Methods: 1200 one-day old chickens, divided to six dietary treatments (each with four replications) fed wheat+barley diet without (I-II) or with yellow lupine var. Juno – 5 or 10 % (III-IV) in starter and 10 or 20 % in grower mixtures (V-VI). At the age of 28 days from each treatment 12 chickens were selected, killed and the content of intestine from Meckel's diverticulum to ileum-caecal junction was collected for the ileal both nutrients and amino acids digestibility determination (using Cr_2O_3 as indicator). In content of large intestine the number of Lactobacillus and E. coli FCU were estimated. On day 42 the performance and carcass quality of broiler chickens were determined.
Results: The basal results are presented in Table 1.

	Lupine (%)			Enzymes (ppm)	
	0	5/10	10/20	0	400
Body weight at day 42. (g) males	2861Aa	2656b	2553Bc	2622a	2733b
females	2404	2396	2329	2350	2406
Feed conversion in 1-42 days (kg/kg BW)	1,87	2,01	1,95	1,95	1,94
Apparent ileal digestibility coeffs (%)					
Crude protein	78,6a	81,3b	81,7b	79,3A	81,8B
Met	85,9a	85,9a	88,9b	85,6a	88,2b
Cys	74,9a	73,4a	79,2b	74,4a	77,3b
Lys	72,9a	76,8b	76,0b	74,1a	76,4b
Breast muscle in empty body (%)	23,9A	22,7AB	21,7B	22,6	22,9
Concentration in large intestine content (log_{10} CFU/g)					
Lactobacillus spp.	6,95A	5,99B	6,13B	6,17A	6,55B
E. coli	6,41A	5,39B	4,62C	5,65A	5,30B

Conclusion: The rise of lupine share in diet significantly decreased the body weight in male birds (by 7.2 or 10.2 %), in female in group III only. Application of carbohydrases increased the body weight by 2 % (♂) and by 2.4 % (♀). In feed conversion insignificant differences were stated. Higher level of lupine increased the protein and numerous amino acids ileal digestibility. Similar but significantly better effect was obtained by enzymes application. Reduction of E. coli and Lactobacillus was caused by increased share of lupine, however carbohydrases increased the Lactobacillus spp. number and decreased E. coli FCU.

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115. Influence of inulin on total bacteria and bifidobacteria in the intestine of growing pigs as determined by *Fluorescent In Situ Hybridization* (Einfluss von Inulin auf die Gesamtbakterien und Bifidobakterien im Dünndarm wachsender Schweine nachgewiesen durch *Fluorescenz In Situ Hybridisierung*). G. Loh*, M. Eberhard, R. M. Brunner, U. Hennig, M. Kwella, C.C. Metges – Dummerstorf

Prebiotics such as inulin can not be digested by mammalian enzymes and are substrates for selected bacteria. Inulin was reported to increase numbers of *Bifidobacterium spp.* and *Lactobacillus spp.* in the intestinal chyme and/or faeces of rats, pigs and humans. These bacterial groups are considered to be beneficial because they are related to a mild stimulation of the host immune system and direct antagonistic effects against pathogenes. To some extent these bacteria contribute to the energy and protein/amino acid supply. According to the prebiotic concept, inulin should reach the large intestine without previous degradation in the small intestine. Thus most studies focus on the effects of prebiotics in the caecum and colon. However, it is of interest whether the bacterial community in the small intestine is also affected. To avoid the known biases of classical microbial culturing techniques, *Fluorecence In Situ Hybridization* (FISH) was used to explore possible changes in the bacterial population due to the addition of inulin to practical diets.
Methods: 24 piglets were divided into two groups at the age of 42 days and were fed a conventional wheat and barley (36 % each) based diet. One group received inulin at a level of 30 g/kg feed. In the other diets glucose and other easily fermentable sugars present in the inulin preparation were compensated by an equivalent of saccharose. Animals of each group were killed after 21 or 42 days of feeding the experimental diets (groups 1-4; n = 6/group) and total contents of the jejunum, caecum and colon were collected. An aliqout (0.5 g wet weight [ww]) was fixed with 4 % paraformaldehyde. Bacterial suspensions (10 µl) were spotted on glass slides and hybridized with 5'-Cy3 labelled 16S rRNA oligonucleotide probes specific for total bacteria and for members of the *Bifidobacterium spp.*, respectively. After hybridization for 16 hours at 46 °C and washing at 48 °C the slides were examined with an epifluorescence microscope.
Results: The addition of inulin had no effect on luminal bacterial counts. Total bacteria per gram ww were present at a level of 10^8 in the chyme from the jejunum and 10^9 in the caecum and colon, respectively, with no influence of diet or age observed. Surprisingly, numbers of luminal bifidobacteria were below the threshold of detection (about 10^4/g ww) of FISH in 40 % of all animals. When bifidobacteria were detected, they were present at levels of 10^6 in the jejunum and 10^7 in the caecum and colon, respectively. Again, no influence of diet or duration of feeding was found. Proportion of bifidobacteria to total bacteria for individual animals ranged from 0.5 to 5.5 % in the jejunum, from 0.1 to 3.3 % in the caecum, and from 0.1 to 2.2 % in the colon, showing a wide variation between animals.
Conclusion: The bifidogenic effect of inulin is often pointed out but could not be shown in this study. One reason for these findings might be the content of soluble arabinoxylans derived from wheat and soluble ß-glucans derived from barley in the experimental diets. These NSP are known to affect the intestinal microflora and may mask a possible effect of inulin. Another reason could be that an already very stable luminal microflora has developed in the pig's intestine until day 42 of age which does not respond to a moderate addition of inulin. Ongoing studies with NSP-free diets will show the impact of the wheat and barley based diets.

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116. Investigation on the precaecal and faecal digestibility of lactulose and inulin and influence of these substances on nutrient digestibility and microbial characteristics (Untersuchungen zur praecaecalen und faecalen Verdaulichkeit von Lactulose und Inulin und die Auswirkungen dieser Substanzen auf Nährstoffverdaulichkeit und mikrobielle Kenndaten). Gertraud R. Branner*, Barbara M. Böhmer and Dora A. Roth-Maier – Freising-Weihenstephan

The objective of the present study was the determination of the precaecal and faecal digestibility of lactulose and inulin. Furthermore, the influence of these substances on nutrient digestibility and microbial characteristics were examined. The pig was used as the animal model because it was validated in extensive studies as a good experimental model for studying aspects of digestive physiology in humans and monogastric animals.

Methods: In metabolic trials 3 of 6 growing pigs (German Landrace x Pietrain) were fitted with an ileo-rectal anastomosis (IRA) in end-to-end technique (EEA). The remaining 3 pigs were used as intact partners (IN) for the EEA pigs. The experimental diets were based on corn, wheat, barley and soybean meal supplemented with either 1,5% lactulose or 2% inulin in replacement of diatomaceous earth. During the final 5 days of a 12day feeding period the digesta and urine were collected quantitatively. Diets, freeze-dried chyme and faeces were analysed for the supplemented substances and the nutrients. Fresh digesta was used for analysis of volatile fatty acids (VFA), pH and bacterial population (Gram-positive and Gram-negative anaerobes, Lactobacilli, Bifidobacteria, Enterococci and E. coli). The experimental design was a 3x2x3 Latin square that means each of the EEA pigs and IN pigs had consecutively each substance. The treatment effects were analysed by two-factorial analysis of variance.

Results: The precaecal digestibility of lactulose and inulin was assessed to be 79% and 98%. Both substances were completely digested faecally (Table). The supplementation of lactulose and inulin to the diet had only minor effects on the precaecal and faecal digestibility of nutrients. Significant differences in nutrient digestibility were obvious between EEA and IN pigs whereas the EEA pigs showed lower digestibility values except of ether extract. The pH of pigs digesta was widely unaffected by the different supplemented diets. Bacterial population in the digesta of EEA and IN pigs tended to a modified composition when the supplemented diets were fed. Concentration of VFA in precaecal chyme decreased significantly when EEA pigs received the lactulose supplemented diet whereas VFA in faeces were unaffected.

Supplementation	Lactulose		Inulin	
Procedure	EEA	IN	EEA	IN
Prebiotic intake (g/d)	22.5	22.5	31.5	31.5
Digesta prebiotic excretion (g/d)	4.6	0	0.7	0
Digestibility (%)	79.4	100	97.7	100

Conclusion: This study has shown that lactulose and inulin were digested mainly precaecally and only 21% of lactulose reached the colon. This may have caused the decreasing concentration of VFA in precaecal chyme of the lactulose group. In pigs nutrition these prebiotics did only partly fulfill the expectation to be undigestible in the small intestine as it is confirmed for humans. Nevertheless, lactulose and inulin tended to show positive effects on precaecal and faecal microbial characteristics.

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117. Effect of drying procedure on a tannin bioassay and it's relation to various chemical tannin assays (Der Effekt der Trocknungsmethode in einem Tannin Bioassay und die Beziehung zu verschiedenen chemischen Tannin Assays). S. Muetzel, K. Becker – Stuttgart-Hohenheim

The incubation of tanniferous substrates in the presence of PEG is used to determine the biological effect of the tannins on the rumen fermentation *in vitro* and *in vivo* (Makkar *et al.*, 1995;McSweeney *et al.*, 2001).

Methods: Leaf samples of *Juglans regia, Rhus typhina, Aesculus hippocastanum* and *Salix alba* were separated in two portions and either freeze dried or oven dried (60°C). Total tannins and the protein precipitation capacity (PPC) were determined as described in the accompanying paper. Samples were incubated *in vitro* with and without PEG using the Reading Pressure Technique (Mauricio *et al.*, 1999). Gas production and short chain fatty acid (SCFA) concentrations were determined after 24 hours of incubation. The effect of tannins is expressed either as absolute differences or relative to the PEG free incubation. True protein content of the leaves and the samples was determined as described in Hoffmann *et al.* (2002).

Results: Oven drying decreased solubility of NDF by 15% and of leaf protein by 50%. Consequently, the amount of gas and SCFA produced after 24 hours of *in vitro* incubation was decreased by 11 and 9% respectively. However, no differences in gas production due to the addition of PEG were observed, showing that drying had no effect on the reactivity of tannins *in vitro*. In contrast, the addition of PEG tended to increase the SCFA concentrations by 8.8 and 8.1µmoles/ml for the freeze dried and oven dried samples, respectively, thus suggesting that the oven drying lead to a decreased activity of the tannins. On average the increase of gas production due to the inhibition of tannins by PEG was 17.4% and much lower than the response of the SCFA production which increased by 23.9%. Changes in molar proportions of the SCFA produced due to the higher availability of proteins for fermentation can only explain a part of this difference. PEG increased the amount of propionate produced by 0.2 mmole/g of substrate.

No significant correlation was observed between any one of the chemical assays (total phenols and total tannins) using Folin Ciocalteau or Ferric Chloride Method was observed (for details see accompanying paper). The PPC, which is meant as a biological indicator of the activity of tannins also did not show any significant correlation with the activity of the tannins *in vitro*. This lack of a relationship between chemical and biological assays is most likely due to the fact that chemical methods and the PPC are determined from extracts of tannins while in vitro all the tannins present in the substrates act on the fermentation. In addition, the PPC assay is carried out at pH 4.9, which is around the pI of BSA, but does not necessarily predict the activity of tannins *in vitro* where pH is around 6.8.

Conclusion: Drying procedure influences the solubility of the NDF and of leaf protein, but does not affect the biological activity of tannins *in vitro*. No correlation was found for any of the chemical tannin assays and the PPC with either gas or SCFA production *in vitro*.

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118. Drying procedure does not affect the extractability and activity of tannins (Die Trockungsmethode beeinflusst nicht die Extrahierbarkeit und die Aktivität von Tanninen).

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The ability of tannins to form complexes with polymers is used in various tannin assays to separate tannins from other phenolics and to determine their protein precipitation capacity. Extractability of tannins is an important issue in the quantification of this components. Besides different solvent systems, the drying procedure of the plant material was reported to have an significant impact on the values obtained (Ahn *et al.* 1997).

Methods: Leaf samples of *Juglans regia, Rhus typhina, Aesculus hippocastanum* and *Salix alba* were separated in two portions and either freeze dried or oven dried (60°C). Finely ground samples (1g) were extracted twice in 100ml aqueous acetone (70% v/v) in a ultrasonic waterbath. Total phenols and total tannins in the extracts were determined by the Folin Coicateau method (Makkar *et al.* 1993) and the ferric chloride method (Hagerman and Butler 1978) using PVPP and BSA respectively for the precipitation of tannins. Total and specific protein precipitation capacity was determined as described in Hoffmann *et al.* 2002.

Results: A higher phenol content of the plants was determined using the folin method compared to the ferric chloride method. Freeze drying significantly increased the amount of total phenols determined by folin method but not by ferric chloride method. Drying procedure did not affect the amount of total tannins determined by either of the methods, although a somewhat higher total tannin content in the oven dried samples was observed using the Ferric chloride method. However the proportion of phenols that were precipitated and subsequently determined as tannins was 77.4% of the total phenols for the Folin and 23.9% for and the Ferric chloride method, thus indicating that different classes of phenols are precipitated by PVPP and BSA.

Oven drying increased the amount of BSA precipitated significantly over freeze drying corresponding to the elevated total tannins extracted. However the specific activity of the tannins extracted from oven or freeze dried material did not differ significantly, showing that their biological activity was not negatively affected by the drying procedure.

Conclusion: Oven drying does not negatively affect extractability or biological activity of the tannins tested.

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119. Effects of a feed additive enriched with nucleotides on faecal microbiota of calves (Einfluss eines nukleotidhaltigen Futterzusatzes auf die fäkale Mikrobiota von Kälbern).

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Our understanding of the complex nature of microbial ecology (microbiota) of calves is still far from complete. While the better studied gastrointestinal pathogens are of obvious importance, the role of commensal bacteria in host nutrition and colonic health is becoming increasingly recognized. Factors such as diet composition or feed additives are of particular interest with respect to their effects on microflora and microbial fermentation products in the gastrointestinal tract of calves. A preliminary study was conducted to evaluate the influence of a nucleotide-containing feed additive (NA) (80 % natural mineral silicates, Clinoptilolite of sedimentary origin; 20% inactive yeast extracts with a high nucleotide content) on faecal microflora and faecal volatile fatty acids (VFAs) of young calves. The present work is the first attempt to identify microbial groups in faeces of calves by culture-independent fluorescent *in situ* hybridization (FISH) technique using 16S rRNA-targeted probes.

Methods: Female calves (Holstein-Friesian, age: 12 days) were randomly assigned to experimental (+ NA) and control (- NA) groups. NA (20g/d) was supplied via milk replacer per calf in a 35-d study. Additional solid feed was available from d 10. Effects on total faecal *Bacteria* and microbial population groups, e.g. *Bifidobacterium* (Bif), *Lactobacillus* (Lac), *Enterococcus* (Ecoc), *Clostidium coccoides-Eubacteium rectale* (Erec), *Atopobium*, *Coriobacterium* and *Eggerthella lenta* (Ato), *Bacteroides* (Bac), *Fusobacterium prausnitzii* (Fprau), *Enterobacteriaceae* (Entero), *Clostridium histolyticum* (Chis), *C. lituseburense* (Clit) and *Archea* (Arch) in response to NA administration were investigated by FISH at the end of the study. VFAs were determined by gas chromatography.

Results: NA administration resulted in higher numbers of Bif ($P<0.05$) and Ato in the faeces than control (Table) and in a trend to lower counts of Bac and Fprau. The faecal concentration of total VFAs tended to be higher in NA-treated calves than control (110,3 ± 9,6 vs. 96,4 ± 11,0 µmol/g, P=0,056), but the mean molar ratios (%) of the faecal VFAs (acetic : propionic : butyric : valeric : iso-acids) did not differ between the groups: (+ NA) 72,4 : 15,2 : 7,9 : 2,9 : 1,3 vs. (- NA) 72,4 : 16,2 : 6,1 : 3,2 : 1,2.

Table: **Composition of the faecal microflora of calves [mean, pooled SD]**

	Bacteria	Arch	Bif	Lac	Ecoc	Erec	Ato	Bac	Fprau	Entero	Chist	Clit
Control -NA	9,3	7,3	5,9	6,4	6,4	9,1	7,6	8,5	7,7	7,5	5,9[b]	5,4[c]
Treatment +NA	9,3	7,7	7,6	6,8	7,0	9,0	8,3	8,0	7,2	7,7	5,7[b]	n.d.
Pooled SD	0,2	0,6	0,5	0,4	0,3	0,2	0,4	0,5	0,5	0,5	0,4	

[a] Counts expressed as $\log_{10}$ cells/ gram of wet weight (6 calves per group)

n.d. no bacteria detected

[b] Bacteria only detected in 2 calves.

[c] Bacteria only detected in one calf.

Conclusion: FISH with 16S rRNA-targeted probes is well suited for the characterization of flora modulation in calves by additives. This study demonstrates that small changes in the feed (NA in milk replacer) can alter the balance of faecal bacteria in calves towards a potentially healthier microflora.

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120. Influence of Living and Autoclaved Yeast Cultures (Yea-Sacc®) on in vitro Ruminal Microbial Metabolism

(Einfluss lebender und autoklavierter Hefekulturen (Yea-Sacc®) auf den mikrobiellen Pansenstoffwechsel *in vitro*).

Hakan Öztürk*, Anke Werner and G. Breves – Ankara/Hannover

Dietary supplements of living yeast cultures, based on dried Saccharomyces cerevisiae, have been reported to improve health and productivity of ruminants. In comparison with antimicrobial agents, yeast cultures offer a natural alternative to manipulate animal performance. However, in practice the response due to supplementation with yeast culture has been contradictory and in some studies no response has been reported. Therefore, it was the aim of the present study to investigate the effects of this yeast culture on ruminal microbial metabolism.

Methods: The study was carried out using the long-term **Ru**men **Si**mulation **Tech**nique (Rusitec) according to Czerkawski and Breckenridge (1977). It consisted of nine incubation vessels with a volume of about 1 l each. The study lasted 16 days. The first 8 days presented an adaptation period to achieve steady state conditions and were followed by a collection period of 8 days. From day 8 on living and autoclaved yeast cultures were added to the fermentation vesssels daily. In the collection period the nine vessels were divided into three groups. The first group served as control, 1500 mg of living (L-YC) and autoclaved (A-YC) yeast cultures were added to the second and third group daily, respectively. In each vessel pH was measured daily. Liquid effluents were collected daily and samples were taken for measurement of short chain fatty acids (SCFA), NH_3-N and ^{15}N analyses. Organic matter digestibility was measured in nylon bags which had been incubated for 48 h.

Results: The effects of living and autoclaved yeast culture on the in vitro parameters of rumen metabolism are shown in the following table ($\overline{X}$ ±SD, n = 3).

	Control	L-YC	A-YC
pH	6,81±0,05	6,82±0,02	6,78±0,04
Total SCFA (mmol/d)	31,9±1,8	34,4±1,6	33,7±0,9
Acetate (mmol/d)	17,9±0,8	18,9±0,8	18,7±0,5
Propionate (mmol/d)	6,4±0,6	7,2±0,4	6,9±0,2
Butyrate (mmol/d)	5,4±0,5	5,8±0,3	5,6±0,2
i-Valerate (mmol/d)	0,6±0,1	0,9±0,2	0,8±0,2
Valerate (mmol/d)	1,7±0,1	1,7±0,1	1,7±0,1
NH_3-N (mmol/l)	9,3±0,2	10,7±0,5	10,0±0,4
^{15}N, atom % in bacterial fraction	0,678±0,007	0,650±0,005	0,667±0,003
Organic matter digestibility (%)	60,7±3,8	59,1±2,1	59,3±2,0

Conclusion: Both living and autoclaved yeast cultures stimulated rumen microbial metabolism. Application of living and autoclaved yeast cultures resulted in an increase in SCFA production as well as in increased NH_3-N concentrations. From lower ^{15}N enrichment in microbial fractions in response to yeast treatment it can be assumed that microbial protein synthesis was higher compared with control vessels. Since organic matter digestibility was unchanged by yeast cultures the increases in SCFA as well as in NH_3-N concentration and higher microbial protein synthesis are indicators that the microflora has used the supplied yeast culture as additional substrates. However, the stimulatory effect in response to living yeasts tended to be higher than in the autoclaved yeasts.

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121. Yeasts as a substitute of soy bean protein in complete diets for weaner piglets: Investigations of N-metabolism

(Hefen als partieller Ersatz von Sojaprotein im Mischfutter für Absetzferkel: Untersuchungen zum N-Stoffwechsel).

M. Spark*, H. Paschertz and J. Kamphues – Hannover

The supply of young animals with high quality protein sources is a big challenge for the feed production. Due to the presumption of high RNA-contents in yeasts and regarding their negative effects on the N-balance (1,2), there were only few efforts to use single cell proteins (e.g. yeasts) as regenerative protein sources for growing animals in the past. The ban of meat meal and the restricted use of fish meal in diets of food producing animals demand an opening of further high quality protein sources. The aim of this study was to characterise several different yeasts as protein sources in complete diets for weaner piglets.

Methods: Three different diets, in which 40 % of the soy bean protein were exchanged with different yeast proteins, were tested in comparison with a control diet (C) on a wheat basis [20 % soy bean extraction meal (SBM), 216 g crude protein/kg dry matter (DM), 14.5 g lysine/kg DM]. The three experimental diets were BY (8 % beer yeast and 12 % SBM), MY (12 % milk yeast and 12 % SBM), and WY100 (11.2 % whey yeast and 12.6 % SBM). In a further experiment, the whey yeast was tested with diets WY50 and WY150, in which soy bean protein was replaced by the yeast to 20 % and 60 %, respectively. Trial and control diets all had similar values for crude protein (XP) and lysine (lys). The trials were set up in a way that every piglet was fed every kind of diet. The contents of RNA in yeasts and of urinary allantoin, the final product of the purine metabolism, were examined and the digestibility, excretion and retention of nitrogen were estimated/calculated for all the trials. Further, the five diets with yeasts were evaluated by the difference method.

Results: All yeasts had a relatively low RNA-matter. The highest absolute amounts of crude protein, true protein (TP), and lysine were found in the beer yeast. The whey yeast stands out due to its relatively high level of true protein (84 %).

Yeast	DM in g/kg	XP in g/kg DM	TP in g/kg DM	Lys in g/kg DM	RNA in g/kg DM
Beer yeast	959	473	361	33.6	48.0
Milk yeast	921	331	261	22.6	44.0
Whey yeast	978	392	329	25.8	35.0

While an apparent N-digestibility (N-dig.) of 86.5 ± 0.38 % was observed for the control diet (C), the value for the diet WY150 was 88.2 ± 0.07 %. Using the difference technique, a N-dig. of 92.0 ± 0.23 % and a N-ret. of 67.4 ± 1.08 % were calculated for protein of the whey yeast alone.

	C	BY	MY	WY 50	WY 100	WY 150
N-dig. (%)	86.5 ± 0.38	86.7 ± 0.06	85.8 ± 0.16	87.1 ± 0.10	87.7 ± 0.08	88.2 ± 0.07
N-ret. (%)	59.6 ± 0.88	60.0 ± 0.12	59.3 ± 0.16	60.1 ± 0.13	60.9 ± 0.11	62.0 ± 0.33
N-excr. (%)#	1.70	3.58	3.00	2.69	3.14	4.28

via urinary allantoin of total urinary N

Although the rations contained high levels of yeast, high N losses (1,2) via urinary allantoin that were expected did not occur. The N-excretion (N-excr.) with allantoin increased from 1.70 % (C) up to 4.28 % (WY150) of total urinary N, feeding the highest yeast concentration.

Conclusions: It was determined in these experiments that the substitution of up to 60 % of the soy bean meal with these yeasts had no expected negative effects on the N-balance, but positive effects on the N-dig. and N-ret. The use of such regenerative protein sources produced under controlled conditions is welcome under the aspect of food safety, but is still related to the costs of altervative protein sources, e.g. soy bean extraction meal.

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122. Investigations the probiotic effects of Escherichia coli strain Nissle 1917 after oral infection with Salmonella Enteritidis in chickens

(Untersuchungen zur probiotischen Wirkung des Escherichia coli Stammes Nissle 1917 bei Küken nach oraler Infektion mit Salmonella Enteritidis PT4).

I. Mohamed*, W. Schrödl*, H. Hafez and M. Krüger - Leipzig/Berlin

E. coli strain Nissle 1917 has been known for more than a century, it was very effective in treatment of chronic intestinal diseases. It had antagonistic effect against different bacteria (enterotoxic *E. coli*, enterohaemorrhagic *E. coli*, *S.* Enteritidis, *S.* Typhimurium) and yeast. The oral application of *E. coli* Nissle 1917 significantly stimulates specific humoral and cellular responses and simultaneously induces nonspecific natural immunity. The study was conducted to estimate the effects of *E. coli* strain Nissle 1917 on reduction of *S.* Enteritidis PT4 colonization in the caecum of infected chickens and serum levels of IgY against *S.* Enteritidis PT4.

Methods: One day old specific pathogen free lohmann chickens were divided into groups of 20 animals. Group A was received 5 x 10^8 viable cells per ml at first day directly into the crop by gavage, while chickens were challenged at second day with *S.* Enteritidis 2.5 x 10^5 viable cells per ml. After that the chicken were received *E. coli* Nissle 1917 5 x 10^8 to 7.5 x 10^9 viable cells per ml from second day to the last day of experiment every day in drinking water. Group B acts as positive control, chickens were challenged with *S.* Enteritidis 2.5 x 10^5 viable cells per ml at second day of experiment directly into the crop by gavage. Bacteriological and immunological examination were conducted at 7 days, 14 days, 21 days and 28 days of age.

Results: Our results showed a significant reduction in the *S.* Enteritidis colonization in caecum of chickens specially at 21 and 28 days old ($P<0.05$) in trial group A in comparison to positive control group B (fig. 1). No significant differences were observed in the level of IgY against *S.* Enteritidis in the trial group in comparison to the positive control group at 7, 14 and 21 days old, but the level of IgY against *S.* Enteritidis was significantly increased in trial group ($P<0.05$) in comparison to positive control group at 28 days old (fig. 2)

Figure 1 Effects of *E. coli* strain Nissle 1917 on the isolation of *S.* Enteritidis in the caecum of experimentally infected chickens

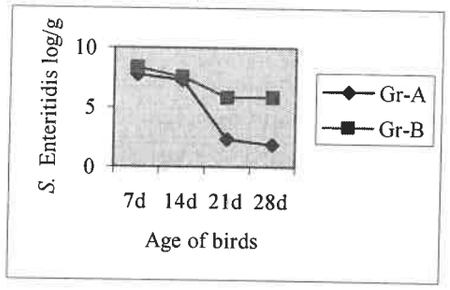
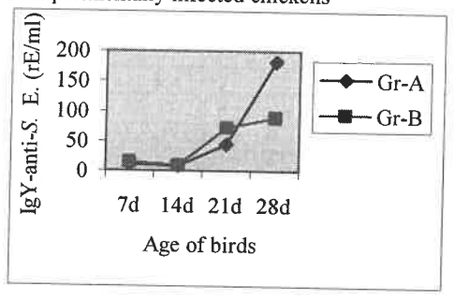


Figure 2 Effects of *E. coli* strain Nissle 1917 on the serum IgY-anti-*S.* Enteritidis concentration of experimentally infected chickens



Conclusion: From our results we can conclude that *E. coli* Nissle 1917 had antagonistic effects against *S.* Enteritidis in vivo. The immune system of chickens was improved after oral application of *E. coli* strain Nissle 1917 and increased the antibodies production (1).

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123. Influence of different dietary Zn additives on intestinal parameters and zinc metabolism in piglets

(Einfluss von verschiedenen Zinkzusätzen auf Parameter des Darms und auf den Zinkstoffwechsel bei Ferkeln).

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Introduction: Post-weaning diarrhoea associated with Escherichia coli is a major disturbance in pig husbandry during the first 2 weeks post-weaning. To avoid this condition the addition of zinc oxide in high dosages to feed is common but not meeting the legal regulations. One option to deal with the problem might be to use specific preparations of zinc oxide that have lower solubility in the stomach. Coating of zinc oxide might increase the stability in the stomach and by this could influence the zinc levels in the gut. On the other hand, zinc supply has to be maintained especially in young piglets for zinc is an essential component of several enzyme systems. In this trial the uptake and distribution of zinc in tissues (liver, kidney and muscle) and the effects of different dietary zinc additives on the intestinal concentrations of E. coli were evaluated in weaned pigs.

Materials and Methods: For this trial 30 weaned piglets were split into 3 groups, all received a complete diet for 3 weeks with different Zn additives: The control group, (15 mg Cu of $CuSO_4$ and 125 mg Zn of zincoxide), group 1 (15 mg Cu of $CuSO_4$ and 125 mg zinc coated) and group 2 (15 mg $CuSO_4$, 25 mg organic Zn and 100 mg zinc coated). Food intake, bodyweight, proximate analysis in feed, E. coli in the gut, lactate, zinc, copper in chyme, plasma, liver, kidney and muscle were measured. Data are expressed as means and standard deviations (SD). Group differences were evaluated by Student t-test.

Results: The zinc content in the small intestine was different ($p<0.05$) between group 1 and group 2. The zinc and copper contents in the large intestine were significantly higher in group 1. No changes of E. coli counts were noted. The lactate content in the gut was variable (table 1). No differences were seen in kidney, liver and muscle zinc and copper concentrations (table 2).

Table 1: Zinc and copper, E.coli and lactate contents in the gut; $p<0.05$

		small intestine		large intestine		small intestine		large intestine		small intestine	large intestine
		mg/kg DM				log/g intestinal contents				mg/kg fresh matter	
		zinc	copper	zinc	copper	E. coli	häm. E. coli	E. coli	häm. E. coli	lactate	lactate
control	mean	423.29[ab]	56.71[b]	758.12[b]	127.31[b]	6.4	3.5	6.9	2.4	1048	31
	SD	146.8	8.0	130.0	9.7	1.5	1.4	1.3	0.9	307	22
group 1	mean	539.7[a]	68.92[a]	1055.74[a]	146.75[a]	6.6	3.7	7.1	2.8	1240	24
	SD	160.3	20.6	306.8	29.3	2.1	1.7	1.2	1.4	621	14
group 2	mean	365.05[b]	43.45[b]	770[b]	100.74[b]	6.9	3.3	7.2	2.6	944	17
	SD	164.1	8.8	253.6	16.7	1.0	1.3	0.8	1.0	407	13

Table 2: Zinc and copper contents in kidney, liver and muscle (mg/kg DM)

		kidney		liver		muscle	
		zinc	copper	zinc	copper	zinc	copper
control	mean	163.4	39.4	300.7	89.8	117.7	15.4
	SD	30.8	24.6	104.2	15.3	55.2	11.5
group1	mean	154.7	38.7	321.2	68.6	107.3	12.7
	SD	40.1	9.5	170.6	23.0	30.0	8.0
group2	mean	154.7	38.7	321.2	68.6	107.3	12.7
	SD	40.1	9.5	170.6	23.0	30.0	8.0

Conclusion: According to our results it seems to be possible to influence the zinc levels in the gut when zinc oxide is administered in coated form.

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124. Thermostability and activity of a phytase added to a broiler diet (Thermostabilität und Wirkung einer zugesetzten Phytase in einer Broilerdiät). Ruth Messikommer und C. Wenk* - Zürich

Introduction: In processed feed for farm animals the activity of added enzymes depends beside other factors on their thermostability during pelleting. In this study a non GMO phytase derived from *Aspergillus niger* by solid substrate cultivation technique was studied for its thermostability and the suitability in a broiler diet.

Material and methods: A maize/soybean meal diet was formulated to cover the nutrient requirements for growing chickens but to contain only 2.2 g available phosphorus per kg. By adding phytase (800 PU/kg feed) an enhancement of the available P up to 3.2 g/kg feed was intended. The feed blend was split and steam pelleted at 60, 75 and 90 °C, resulting in the three treatment diets P60, P75 and P90, respectively. After one week adaptation to a commercial starter diet 72 male broiler chickens (ROSS 308), kept in 12 cages of six birds each, were assigned to the three treatments (four replicates per treatment). Feed and water were offered *ad libitum*. Body mass and feed intake were measured weekly, water consumption was recorded for four days per week. In the third and fifth week excreta samples were collected on three consecutive days for the determination of dry matter and metabolizability parameters. All animals were slaughtered at 36 days of age, after overnight fasting. Carcass weight was measured and the tibia bone was resected for subsequent bone characterization: mineral density, mineral content, breaking strength, bone length, weight and ash percentage. Immediately after the first experiment, a second one, identical in design and procedure, was conducted. Data represent mean values over both experiments.

Results: Nutrient analyses showed a high accordance between treatment diets. The hardness of the pellets was increased in P90. Abrasion losses (according to PFOST) were only 50% in P90 compared to P60. If the phytase activity of the unpelleted diet was taken as 100 %, the heat treatment at 60 °C reduced the activity by 30 %. At 75 °C only 20 to 30 % of the phytase activity remained. At 90 °C activity was close to the detection limit and thus equal to the native phytase activity of the unsupplemented feed.

After the adaptation period birds started the experiment with a well-balanced mean body weight of 171.5 g and achieved after four weeks treatment an equal body weight of 1825 and 1817 g in treatment P60 and P75, respectively. Birds in treatment P90 showed a significantly lower end weight (1692 g). This can be explained with the significantly lower feed intake in this group (-6.2 %). Feed efficiency ratio in P60 and P75 (1.594 vs 1.590) differed significantly from P90 (1.646). Water intake per bird and feed unit tended to be higher in P90 leading to a higher excreta water content. Metabolizability of energy and N-utilization were within normal ranges and were not influenced by the different treatments. With increasing pelleting temperature P-utilization decreased by 15 % from 0.682 (P60) over 0.616 (P75) to 0.578 (P90), resulting in a dietary net-P content of 3.07, 2.77 and 2.60 g/kg, respectively. The P-retention amounted to 4.89 (P60), 4.41 (P75) and 4.28 (P90) g/kg body weight gain which was in P75 and P90 clearly below the basic value of 5 g needed to meet requirements. Bone characterization data which are not yet completed seem to reflect the difference in available P in the three treatment groups.

Conclusions: In its actual form and application the examined phytase showed activity losses of 30, 75 and nearly 100 % at pelleting temperatures of 60, 75 and 90 °C. Nevertheless the heat loaded diets up to 75 °C achieved a good growth performance in broilers. In respect of the action of the added phytase in the present diet, per 100 analytically verified PU/kg an improvement of both the P-utilization by 0.012 units and the net-P by 0.06 g, respectively was calculated.

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125. Effects of Savoury, *Nigella sativa L.* and Cacao husks on growth and carcass composition of broiler chickens (Einfluss von Bohnenkraut, Schwarzkümmelsaat und Kakaoschalen auf Wachstum und Ganzkörperzusammensetzung von Broilern). Ingrid Halle* and R. Thomann – Braunschweig/Berholz-Rehbrücke

Herbs, parts of plants or plant extracts can beneficially affect feed intake, secretion of digestive juices and the immune system of animals. A variety of these phytogenic additives have been used already in broiler nutrition. The effects varied from a marked enhancement in performance and health to none or even a negative result. The objective of this study was to determine the effect of a diet supplemented with Savoury, *Nigella sativa L.* and Cacao husks on growth and carcass composition of broiler chickens.

Methods: All diets were tested in the performance experiment which was carried out in cages with male broilers of the LOHMANN MEAT strain from day 1 to 35 of age. A total of 560 day-old male chicks were randomly distributed in dietary treatments with 7 chicks per cage and 10 cages per group. Feed and water were provided for ad libitum consumption. Live weight was recorded for each broiler individually whereas feed was weighed back on a cage-basis weekly. Basal diet was formulated to contain 0 g, 3 g, 4 g, 10 g, 20 g Savoury or 10 g *Nigella sativa L.*, 10 g *Nigella sativa L.* plus 10 g Savoury or 10 g Cacao husks and 10 g Cacao husks plus 5 g *Nigella sativa L.* per kg.

Results: Summarised results of the experiments are given in Table 1. Feed intake was improved in all supplemented groups in comparison to the control group. *Nigella sativa L.* and Cacao shells showed clear growth promoting effects. The broilers of supplemented groups were 2.5 - 7 % heavier at 35 days of age. Feed conversion didn't significantly effect. No effects on the carcass quality of broilers could be shown.

Table 1: The influence of Savoury, *Nigella sativa L.* and Cacao husks on feed intake (FI), final body weight (FBW) and feed conversion (FC) of male broilers (Means, Student-Newman-Keuls Test, $P \leq 0.05$)

Group	FI, g/day/broiler	FBW, kg/broiler	FC, kg/kg
Control	89.2	2.092 b	1.521
3 g Savoury	91.5	2.180 ab	1.497
4 g Savoury	93.0	2.177 ab	1.522
10 g Savoury	91.4	2.156 ab	1.514
20 g Savoury	91.1	2.146 ab	1.515
10 g *Nigella sativa L.*	95.1	2.241 a	1.512
10 g *Nigella sativa L.* + 10 g Savoury	92,7	2189 ab	1.507
10 g Cacao husks	93.3	2204 a	1.512
10 g Cacao husks + 5 g *Nigella sativa L.*	94.9	2214 a	1.525

Conclusion: This study demonstrated that the most pronounced effect of *Nigella sativa L.* and Cacao husks supplementation was improved final body weight. In addition, the results indicated that the influence of *Nigella sativa L.* and Cacao husks on growing broilers were stronger than that of Savoury.

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Workshop I

Ernährung, Stoffwechsel und Krankheitsanfälligkeit der Hochleistungkuh

Workshop II

Ernährung der Milchkühe im organischen Landbau

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W1. Zur Fütterung von Hochleistungskühen (Feeding of high performance cows).

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Die Milchleistungen sind in den zurückliegenden Jahren erheblich angestiegen, so dass 50 kg Milch je Tag oder 10.000 kg je Laktation keine Seltenheit mehr sind. Bei diesen Leistungen werden täglich ≈ 2 kg Milchfett, ≈ 1,6 kg Protein, ≈ 2,4 kg Laktose und ≈ 0,5 kg Mineralstoffe über die Milchdrüse sezerniert. Daraus resultieren hohe Anforderungen an die Futteraufnahme, die Umsetzungen im Verdauungstrakt sowie an Leber und Milchdrüse.

Versorgungsempfehlungen

Sowohl GfE (2001) als auch NRC (2001) haben kürzlich überarbeitete Versorgungsempfehlungen für Milchkühe publiziert. Darin wird neben dem Bedarf an Energie und den wichtigsten Nährstoffen auch auf bisher in derartigen Dokumenten nicht übliche Größen eingegangen, wie z.B. Empfehlungen zur Versorgung mit strukturiertem Grundfutter (GfE 2001), Anforderungen an Tränkwasser (NRC 2001) oder Zusammenhänge zwischen Ernährung und Umwelt (NRC 2001).

Versorgung mit „strukturiertem" Grundfutter

Die Aufnahme ausreichender Mengen von „strukturiertem" Grundfutter ist die Voraussetzung für einen ungestörten Fermentationsablauf im Pansen und damit für eine hohe Futter- und Energieaufnahme sowie für Gesundheit und Leistung der Kühe. Die zur Strukturfutterversorgung gegebenen Empfehlungen von Rohr und Kaufmann (1975; > 18 % Rohfaser in der Trockensubstanz (T) im ersten Laktationsdrittel) sowie von Piatkowski und Voigt (1990) bzw. Hoffmann (1990; > 400 g strukturwirksame Rohfaser je 100 kg LM und Tag) sind als eher konservativ zu betrachten, da vor allem bei Angaben zum Mindest-Rohfasergehalt in der T die Höhe der Futteraufnahme begrenzt wird. In einem von De Brabander et al. (1999) unterbreiteten Vorschlag wird ein Strukturwert von ≥ 1 je kg T gefordert. Dieser dynamisch erscheinende Ansatz offenbart vor allem beim Einsatz von Grassilagen (hoher Strukturwert) als alleinigem Grundfutter gewisse Schwachstellen (z.B. sind Rationen mit 6 kg T aus Grassilage und 14 kg T aus Kraftfutter rechnerisch möglich), wie Meyer et al. (2001, 2002) demonstrieren konnten. Zur besseren Bewertung der Strukturwirksamkeit verschiedener Futtermittel besteht demnach Forschungsbedarf, um die Rationen von Hochleistungskühen pansenphysiologisch sicher gestalten zu können.

Energie

Ein hoher Verzehr energiereicher Rationen ist die Voraussetzung für die Deckung des Energiebedarfes von Hochleistungskühen. Trotz hoher Verzehrleistungen (z.B. > 25 kg T je Tier und Tag bzw. > 4 kg T/ 100 kg LM) ist es kaum möglich, auch bei Verabreichung energiereicher Rationen den Energiebedarf einer Milchkuh mit > 50 kg Milch je Tier und Tag zu decken (Tab. 1). Daraus resultiert eine negative Energiebilanz (Tab. 2), die durch die Mobilisation von Körperreserven kompensiert werden muss.

Die Lebendmasse und teilweise auch der Body Condition Score (BCS; Tab. 2) sind wenig geeignete Indikatoren, um katabole Prozesse im Tier zu erkennen, da nach der Abkalbung die Futteraufnahme deutlich ansteigt. Je kg höhere T-Aufnahme ist ein Anstieg der ermittelten Lebendmasse von 2,5 bis 4,0 kg zu erwarten. Bemerkenswert sind diesbezüglich auch tierindividuelle Unterschiede (Tab. 3), die als Ursache für unterschiedliche Reaktionen von Einzeltieren anzusehen sind.

Tabelle 1: Einfluss der Milchleistung (kg/Tier und Tag; 650 kg LM der Kuh) auf die erforderliche T-Aufnahme (kg/Tier und Tag in Abhängigkeit von der Energiekonzentration der Ration) sowie die Passagerate des Futters (%/h)

Milch[1] (kg/Tag)	Energiebedarf (MJ NEL/Tag)	Erforderliche T-Aufnahme (MJ NEL je kg T)			Passagerate[3] (%/h)
		6,8	7,2	7,6[2]	
30	137	20,1	19,0	18,0	7
40	170	25,0	23,6	22,4	9,5
50	203	29,8	28,2	26,7	12
60	236	34,7	32,8	31,1	14

1) Milch mit 4 % Fett und 3,4 % Protein, Bedarf: 3,3 MJ NEL/kg Milch
2) in praxisnahen Rationen sind derartige NEL-Konzentrationen kaum realisierbar
3) bezogen auf eine T-Aufnahme bei 7,2 MJ NEL/kg T

Ansteigende Futteraufnahme bewirkt eine höhere Passagerate (Tab. 1) und folglich eine geringere Verdaulichkeit der Futterinhaltsstoffe (2 bis 4 % je Ernährungsniveau). Dieser Rückgang wird jedoch durch geringere Methan- und Harnenergieverluste (1 bis 3 % je Ernährungsniveau) weitgehend kompensiert, so dass die durch GfE (2001) vorgenommene Korrektur (+ 0,1 MJ NEL/kg Milch) die noch vorhandene Differenz annähernd ausgleicht. Entsprechende Bilanzversuche mit Hochleistungskühen erscheinen jedoch erforderlich.

Tabelle 2: Kennzahlen des Energieumsatzes bei Hochleistungskühen (n=8, Holstein-Friesian, Lebendmasse nach Abkalbung: 691 kg/Tier, BCS: 2,7; Hattan et al. 2001)

Laktationswoche	6	12	18	24	30
Lebendmasse (kg)	658	670	683	687	700
BCS	2,3	2,4	2,4	2,5	-
Milchleistung (kg/Tier und Tag)	51,7	47,7	43,9	39,6	35,4
T-Aufnahme (kg/Tier und Tag)	24,1	25,3	25,1	23,7	23,3
Energieansatz (% der ME-Aufnahme)	- 7,5	- 2,1	2,7	4,2	5,4

Tabelle 3: Variation zwischen Einzeltieren im ersten Laktationsdrittel (10.-120. Laktationstag, Ration: Mais-/ Luzernesilage und Kraftfutter; Kraftfutteranteil ≈ 55 %, Meyer et al., unveröffentlicht)

Tier-Nr.:	T-Aufnahme (kg/Tag)	Energie-aufnahme (MJ NEL/Tag)	Milch-leistung (kg FCM/Tag)	Lebendmasse-änderung (kg/Tier)	Energie-bilanz (MJ/Tag)
1	26,4	193	39,7	33	+ 26
2	25,1	177	44,0	23	+ 2
3	24,2	177	36,9	54	+ 18
4	23,1	163	34,2	11	+ 18
5	21,5	147	40,2	28	- 21

Die Möglichkeiten der Fütterung sind begrenzt, durch erhöhte T-Aufnahme bzw. energiereichere Rationen die negative Energiebilanz im ersten Laktationsabschnitt auszugleichen:

- Einsatz von Futtermitteln, die im Pansen schneller abgebaut werden und eine hohe Passagerate aufweisen (z.B. Leguminosen)
- „Vorverdauung" von Grundfuttermitteln durch Zusatz zellwandspaltender Enzyme
- Erhöhung des Kraftfutteranteils, daraus resultiert aber die Gefahr verminderter Strukturfutteraufnahme und von Pansenacidose sowie verminderter Futteraufnahme
- Mehr Bypass-Stärke, aber die Stärkeverdaulichkeit im Dünndarm ist auf ≈ 1,5 kg/Tier und Tag begrenzt (Matthé et al. 2001), und die Gefahr der Ansiedlung pathogener Mikroorganismen im Dickdarm nimmt zu (Gollnisch 2000)

- Erhöhter Fetteinsatz; aber nachteilige Einflüsse auf Pansenfermentation und Erhöhung des Anteils an t-Fettsäuren im Milchfett sind möglich (maximal 4-6% Fett in der Rations-T abhängig vom Anteil ungesättigter Fettsäuren)

Protein- und Aminosäuren

Für die Versorgung der Milchkuh mit Aminosäuren am Dünndarm ist vor allem eine ausreichende und möglichst kontinuierliche Versorgung der Pansenmikroben mit Energie und Stickstoff erforderlich. Hierzu sollte die Ration aus einer Mischung sowohl schnell- als auch langsam- abbaubarer Kohlenhydrat- und N-Quellen zusammengesetzt sein wobei die durch eine optimale Synchronisation von XP- und Energielieferung zu erwartenden Effekte nicht überbewertet werden sollten (Kaswari et al. 2001). Die Frage nach dem Einsatz von Bypass- Protein ist erst zu stellen, wenn die mikrobielle Proteinsynthese aufgrund begrenzter Futteraufnahmekapazität nicht mehr mit dem bei höheren Leistungen steigenden nXP-Bedarf Schritt hält. Dabei ist jedoch auf hochwertiges Protein mit guter Dünndarmverdaulichkeit zu achten. Bedingungen, unter denen geschützte Aminosäuren einen positiven Effekt auf Leistung und/oder Gesundheit der Tiere haben, sind bisher nur unzureichend definiert. Auf jeden Fall ist ein Proteinüberschuss zu vermeiden, da dieser unökonomisch ist und zu einer unnötigen Belastung von Tier und Umwelt führt.

Mengen- und Spurenelemente sowie Vitamine

Bei Hochleistungskühen werden teilweise die Versorgungsempfehlungen für Mineralstoffe und Vitamine wissenschaftlicher Gesellschaften mit dem Hinweis der besonderen Anforderungen in Frage gestellt. Dabei wird übersehen, dass sowohl Dosis-Wirkungsstudien als auch die faktorielle Ableitung zu Bedarfswerten führen, bei denen anschließend vor Überführung in die praktische Fütterung noch Sicherheitszuschläge erfolgen. Aber auch bei der Bedarfsableitung wird meist mit einer gewissen Sicherheit herangegangen. Beispielsweise hat GfE (2001) bei der faktoriellen Ableitung des P-Bedarfs unvermeidliche Verluste von 1,0 g/kg T-Aufnahme sowie 1 g P/kg Milch und eine Gesamtverwertung des P von 70 % unterstellt. Der neueren Literatur sind Hinweise zu einer Verwertung von 80 – 90 % zu entnehmen, der P-Gehalt der Milch wird mit 0,8 – 0,9 g/kg angegeben (Pfeffer 2003). Daraus resultiert auch ohne Sicherheitszuschlag bereits eine erhebliche „Sicherheit" der gegenwärtigen Bedarfsangaben.

Der mikrobielle Abbau von fettlöslichen Vitaminen im Pansen, eine nicht ausreichende mikrobielle B-Vitaminsynthese und ein höherer Vitaminbedarf der Tiere sind Themen, die zur Vitaminversorgung von Hochleistungskühen hinterfragt werden.

Nicht-essentielle Zusatzstoffe

In die zu dieser Stoffgruppe zusammengefassten Substanzen (z.B. Mikroorganismen, Enzyme, Kräuter, ätherische Öle) werden teilweise auch bei der Hochleistungskuh große Erwartungen gesetzt. Dabei wird sowohl eine Erhöhung der Futteraufnahme als auch eine Steigerung der Verdaulichkeit bzw. eine gleiche Verdaulichkeit bei erhöhter Passagerate (Tab. 1) im Pansen angestrebt. Sowohl beim Einsatz von Probiotika, wie z.B. lebende Hefezellen (Newbold 1996), als auch nach Zusatz zellwandspaltender Enzyme (Beauchemin et al. 2001) wird über derartige Befunde berichtet, wobei nicht klar ist, ob diese Ergebnisse reproduziert werden können und wie hoch der Anteil nicht publizierter Befunde ist, bei denen kein Effekt eintrat.

Forschungsbedarf

Aus der Auflistung der verschiedenen Aspekte ergeben sich u.a. folgende Forschungsansätze mit Hochleistungskühen, wobei die dabei erzielten Befunde auf Tiere mit geringeren Leistungen übertragen werden können:

- Einfluss der Kälber- und Jungrinderaufzuchtfütterung auf Leistung, Gesundheit, Fruchtbarkeit und Langlebigkeit der Kühe
- Einfluss der Fütterung während der „Transit"-Periode auf Leistung und Gesundheit der Kühe

- Interaktionen zwischen Fütterung (Energie- und Nährstoffbedarf) auf der einen Seite und Tiermaterial (Genetik), Haltung der Tiere (Stall, Auslauf) und weiterer Umweltfaktoren auf der anderen Seite
- Erfassung tierindividueller Besonderheiten (Futteraufnahme, Umsetzungen im Verdauungstrakt und Stoffwechsel, „Referenzwerte" u.a.)
- Einfluss der Futterdarbietung (Futtermittel, TMR, Fütterungsfrequenz und –sequenz u.a.) auf die Höhe der Futteraufnahme und die Umsetzungen im Pansen
- Einfluss der Höhe der T-Aufnahme auf den Energieumsatz bei der Hochleistungskuh
- Unter welchen Bedingungen ist der Einsatz geschützter Proteine / Aminosäuren sinnvoll?
- Wie kann die mikrobielle Proteinsynthese maximiert werden?
- Welche RNB ist bei Hochleistungskühen optimal?
- Anforderungen der Hochleistungskuh an Mengen- und Spurenelemente sowie Vitamine (Einfluss anderer Applikationsformen, B-Vitaminsynthese im Pansen u.a.)
- Bewertung der Wirkung nicht-essentieller Zusatzstoffe
- Einfluss der Tierernährung auf die Produktqualität (Tansfer erwünschter und unerwünschter Stoffe vom Futter in die Milch u.a.)
- Möglichkeiten der Tierernährung zur Reduzierung der Ausscheidungen (Umweltbelastung) bei den Milchkühen (z.B. N, P, Methan, Zn, Cu)
- Ökobilanzen (Life Cycle Studien) der Milcherzeugung unter Berücksichtigung unterschiedlicher Aufzuchtintensität und der Leistungshöhe der Kühe

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W2. Adaptation and regulation of epithelial functions in the gastrointestinal tract (Adaptation und Regulation epithelialer Mechanismen im Gastrointestinaltrakt). G. Gäbel*, S. Wolffram - Leipzig/Kiel

Adaptation of absorptive processes in the gastrointestinal absorptive tract is forced by a lot of challenges. Those challenges can result from altered demands of the animal as well as from altered supply of nutrients.

Nature of adaptive responses may be non specific or specific. Non specific responses are mainly mediated by quantitative changes of intestinal epithelial and adjacent tissues. Specific responses are represented by an up- or down-regulation of a certain transport process and/or a transport protein.

As regards high yielding cows, we have to focus on the adaptive responses to a higher supply of nutrients, i.e., short-chain fatty acids, glucose, amino acids and peptides. The nutrients can stimulate or repress their transport processes or leave them unchanged. Following Ferraris and Diamond [16], several functional considerations have to be taken into account from a teleological point of view:

1. A transport process should be repressed if its cost exceeds the benefits that the transporter provides; the benefits could be either metabolizable calories or an essential nutrient.
2. Metabolizable nutrients yield calories in proportion to the amount of nutrients; the transporters should be up-regulated by the substrates. SCFA and glucose would be expected to induce this type of regulation.
3. For essential nutrients, the daily requirements can be fixed. Transporters of such nutrients should be down – regulated by their substrates if the supply exceeds the demand. This type of regulation might be expected in the case of essential amino acids and peptides.

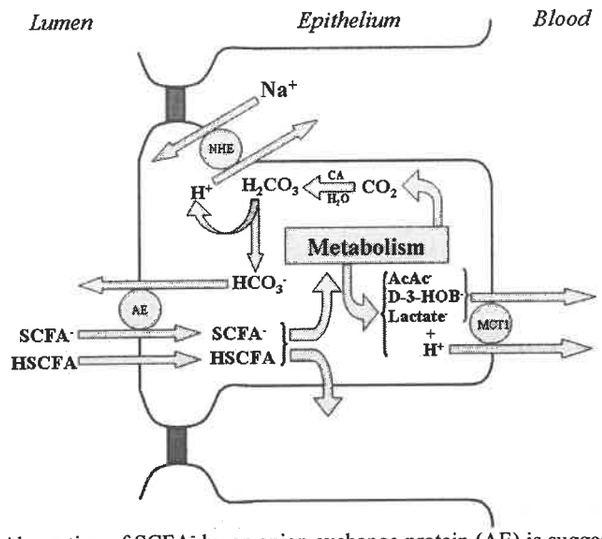

Fig. 1: Absorption of $SCFA^-$ by an anion exchange protein (AE) is suggested by in-vivo [3; 19] and by in-vitro studies [33; 57; 59]. After taken up, SCFA are partially metabolized through oxidative or anaerobic pathways. Carbon dioxide from the oxidative break down can be further transformed by the activity of carboanhydrase (CA) to H_2CO_3 which in turn dissociates into HCO_3^- and H^+. Ketone bodies ($AcAc^-$, acetoacetate; $D\text{-}3\text{-}HOB^-$, D-3-hydroxybutyrate) and lactate derived from the anaerobic breakdown can be extruded on the basolateral side by the proton-linked monocarboxylate transporter MCT1. NHE: sodium/proton exchanger. Modified from Gäbel et al. [18].

Absorption of SCFA

SCFA are mainly absorbed from the forestomach and the colon. As for the forestomach, numerous quantitative and qualitative investigations have been conducted. As for the colon, most of the data have been achieved in laboratory animals. Moreover, adaptive responses of the colon to a higher supply of soluble carbohydrates have not been investigated so far.

Mechanism of SCFA absorption in the forestomach

Using various experimental approaches (washed reticulorumen, intraruminal infusion of SCFAs and determination of net disappearance, portal recovery), the ability of the forestomach to absorb SCFA very rapidly has been demonstrated in a number of in-vivo studies [14; 36; 46; 47; 64]. Comparing SCFA production with absorptive capabilities, absorption rates up to 90 % can be calculated [18].

SCFAs exist in a protonated form (HSCFA) and an ionized form ($SCFA^-$). HSCFA are highly lipid-soluble and, consequently, may permeate biological membranes directly [37; 66]. As reviewed by Gäbel et al. [18], experimental evidence for an effective absorption of $SCFA^-$ by the rumen epithelium also exists. Mechanisms of $SCFA^-$ and HSCFA absorption are outlined in Figure 1.
Comparing in-vitro and in-vivo results, Bergman [9] concluded that SCFA taken up are largely metabolized within the epithelium. Intraepithelial metabolism of SCFA was confirmed in subsequent studies [36; 57; 58]. The findings on the extent of intraepithelial breakdown of acetate and propionate are controversial. So far, only for n-butyrate the studies agree that a high portion is broken down. The metabolism of n-butyrate may reach 95% [9; 36; 44; 57; 58]. As shown in Figure 1, SCFA breakdown can be both aerobically to CO_2 and anaerobically to acetoacetate, D-3-hydroxybutyrate and lactate.

Adaptation of ruminal SCFA absorption

Adapting ruminants to energy rich diets can induce an up to four-fold absorption in the net absorption/disappearance rate of SCFAs [10; 19; 64; 55; 56]. The increase in absorption rate can be caused by various mechanisms, which are outlined in the following.

Extra epithelial changes

Feeding energy rich diets mostly decreases the intraruminal pH due to a higher production rate of SCFA combined with a lower inflow of saliva. Lowering the pH, in turn, increases the concentration of HSCFA, thereby promoting passive permeation of the lipid soluble form of SCFA. However, in most of the studies conducted so far [33; 49; 57; 68], the stimulatory effect of low pH did not reach the extent predictable by the Hendersson-Hasselbalch equilibrium of HSCFA and SCFA.
Part of the adaptive response can also be attributable to the elevated blood supply and the elevated motility inducible by SCFA [11; 32].

Unspecific adaptive changes of the ruminal epithelium

The key role in elevated transport capacity seems to be morphological transformations of the ruminal epithelium and adjacent tissues. Slowly increasing the concentrate intake leads to increases in the size of papillae, the number of epithelial cells and the number of capillaries [10; 21; 48; 59].
However, the increase of surface area resulting from the proliferative response mostly exceeds the increase of absorptive capacity [21; 59]. This might be attributable to down-regulation of transport proteins and/or to hyper-/ parakeratotic alterations of ruminal epithelium often seen when ruminants are fed high concentrate (pelleted) diets [30; 43].

Specific changes of the ruminal epithelium

Using the so called "SCFA-burst" feeding technique, Sehested et al. [55] revealed an up-regulation of SCFA transport in the absence of morphological changes. But specific changes of SCFA transport proteins are not characterized so far. This might be due to the fact that SCFA transport proteins are not fully elucidated. On the other hand, the high baseline absorption of SCFA as described above might complicate detection of specific up-regulation of SCFA transport proteins.
Like the changes in transport processes, the metabolic processes within the epithelium show signs of adaptive responses to energy rich diets. However, as for the absorptive processes, the specific metabolic changes of the single cell have to be distinguished from changes due to the increased cell mass.
On the basis of grams of tissue, grams of protein nitrogen or number of cells, both up- and down-regulation and also unaffected cell metabolism are reported [4; 28; 68]. Taking the of total cell mass into account, up-regulation of enzymes involved in SCFA degradation was detected [67].

Regulatory chains of specific and unspecific changes

Various factors induced by energy rich diets (viscosity and other physical factors, polyamines, nucleotides, specific nutrients, hormones) are discussed to trigger proliferative alterations of gastrointestinal epithelia [13; 24; 25; 26; 41; 63; 65]. Among these factors, n-butyrate probably plays a central role in ruminal epithelium [52]. However, both pro- and antiproliferative effects of n-butyrate are described.
The antiproliferative effect is probably due to a direct inhibition of DNA-synthesis by the n-butyrate taken up by the ruminal cell [22; 23]. The pro-proliferative effect of n-butyrate might be indirect. N-butyrate has been shown to induce the release of trophic hormones like insulin and growth factors [29; 59], which, in turn, are able to induce proliferation of the rumen epithelium [22; 23; 52].
Regarding the specific up-regulation of SCFA-transport proteins, evidence is lacking about the role of nutrients, hormones, growth factors and intracellular messengers that may regulate SCFA-transport of the single cell. Cyclic adenosine monophosphate (cAMP) could not be demonstrated to influence ruminal SCFA-transport, although sodium absorption was decreased [20].

SCFA transport in the colon

Basal transport processes for colonic SCFA-transport resemble those in the rumen [50]. However, role of MCT1 (see Fig. 1) in the colon is probably different, since it seems to be involved in the luminal uptake of SCFA itself [51]. Unlike for the forestomach, information about effects of energy rich diets on both morphology and colonic function is lacking.
A lot of studies have been conducted on the correlation between fiber, SCFA and epithelial morphology. However most of these studies provide no definitive clue for high yielding cows since

1. effects are not uniform,
2. effects depend on type of fiber and duration of diet,
3. effects are mostly seen when the animals and/or the tissues are in a deficient state [15; 39; 40; 53; 70].

Glucose absorption in the rumen

As pointed out below, glucose is absorbed from the small intestine of ruminants. Recent studies, however, also point to glucose absorption by the rumen epithelium. The expression of a secondary active glucose transporter (SGLT1) was detected in the ruminal epithelium of sheep [2]. Subsequent in-vivo studies underlined the ability of the rumen to absorb glucose [1]. In these studies, relative capacity of the rumen wall to absorb glucose increase with increasing glucose contractions in the ruminal fluid suggesting adaptive and/or regulatory influences.

Absorption from the small intestine

Like in monogastric animals, the small intestine is also the major site of nutrient absorption in ruminant species. Absorption from the reticulorumen is essentially limited to short-chain fatty acids, electrolytes (mainly sodium and chloride), minerals (magnesium, calcium) and ammonia. In contrast,

the majority of nutrients including glucose, di- and tripeptides, amino acids, nucleotides and nucleosides, fatty acids and monoglycerides, minerals and trace elements are absorbed by the small intestine. Fully differentiated enterocytes of the small intestinal mucosa are therefore equipped with a multitude of nutrient carriers, whereby the occurrence of specific mechanisms at the brush-border membrane and the basolateral membrane of the absorbing enterocytes enables vectorial transport across the epithelium. In monogastric species, ample evidence exists that most of the transport processes differentially respond to various stimuli, including various hormones, physiological state of the animal, and starvation or feeding; information about equivalent processes directly obtained in ruminant species is largely missing although it may be assumed that this also holds true for ruminants. Regarding adaptive changes of nutrient transporters in the small intestine, the expression and transport activity of the Na^+-dependent glucose carrier SGLT1 have been directly accessed in ruminant species. This will be further outlined below.

Glucose absorption by the gastrointestinal tract of ruminants

It has long been accepted by animal nutritionists, that in ruminants most dietary carbohydrates are extensively metabolised to SCFA in the reticulorumen. Thus, the amount of un-degraded glucose or glucose polymers, e. g. starch, delivered to the duodenum is generally low in ruminants. This would imply little or no need for the transport of free glucose in the gastrointestinal tract of ruminants after weaning [5; 45; 62]. However, in high-yielding dairy cows as well as in intensively fed growing animals, rations usually contain high amounts of starchy ingredients, which may partially pass the forestomach and subsequently are subjected to digestive and absorptive processes in the small and large intestine [27]. Even though the extent to which starch is digested in the small intestine is still under debate, there is no doubt, that starch is hydrolysed in the small intestine with subsequent absorption of free glucose [6; 8; 34; 35; 42].

Mechanism of glucose transport

Glucose is mainly absorbed from the small intestine by the transcellular route including carrier-mediated transport steps at the brush-border membrane as well as the basolateral membrane. Entry of glucose is mediated by a secondary active Na^+-dependent mechanism (SGLT1) whereas facilitated diffusion via the Na^+-independent carrier GLUT2 is responsible for glucose transport at the basolateral membrane [16]. Recently, however, a substantial role of GLUT2 in uptake of glucose across the brush-border membrane has been postulated whereby integration of GLUT2 into the brush-border membrane appears to be triggered by Na^+-dependent uptake of glucose [31].

Transcripts of SGLT1 mRNA have been detected in several tissues of the gastrointestinal tract of lactating cows including rumen, omasum, abomasum, duodenum, jejunum, ileum, and cecum, whereby translation to the functional SGLT1 carrier protein was restricted to the small intestine [71].

Adaptation of SGLT1 to luminal glucose

Most of the pertinent work clearly points to an adaptation of the glucose transport capacity to luminal glucose concentration in ruminants although experimental evidence has been presented that glucose transport capacity might limit overall capacity of starch digestion in the small intestine [6].

In pre-ruminant calves and lambs, glucose derived from the hydrolysis of lactose is absorbed across the brush-border membrane by SGLT1. In ruminants with a fully developed reticulorumen fed mainly roughage, the availability of starch/glucose at the small intestine is low. Associated with the decline of luminal sugars, there is a corresponding decrease in SGLT1 protein and mRNA abundance [17; 54; 61]. During early (ontogenetic) development, expression of the intestinal SGLT1 appears to be controlled post-transcriptionally [38] and may be governed by developmental factors [69]. In adult ruminants, however, the cellular regulation of SGLT1 is controlled transcriptionally. Postruminal infusion of glucose or glucose analogues into the duodenum in adult sheep enhances the level of functional SGLT1 protein as well as the mRNA abundance to those observed in the pre-ruminant state [12; 60]. A similar up-regulation of SGLT1 by the presence of glucose in the small intestinal contents occurs in ruminant animals fed high amounts of starch. Thus, Bauer et al. [7] demonstrated in steers and sheep that a 4 to 5 day abomasal infusion of a starch hydrolysate increased the capacity

for glucose absorption by the small intestine. Interestingly, differences appear to exist between cattle and sheep with respect to the fate of surplus glucose absorbed from the small intestine. Adaptation of steers to starch intake resulted in a clear increase in glucose delivery to the liver whereas in sheep absorbed glucose appears to be mainly consumed by portal drained viscera [7].

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W3. The molecular basis of lactation - metabolism in liver cells (Molekulare Basis der Laktation - der Leberzellstoffwechsel). M. Stangassinger* and H.P. Sallmann - München/Hannover

Lactation requires that nutrient uptake adapt and the body´s metabolic functions coordinate to enable the animal to remain in physiological equilibrium despite increased nutritional and metabolic demands.
In such a coordination of the whole body, the gut and the liver are particularly significant and are considered to be a functionally integrated organ, termed the splanchnic bed, having a profound impact on the supply of nutrients for maintenance and productivity (Stangassinger and Giesecke, 1986).

Some basic data on liver function: The liver, positioned at the anatomic and metabolic crossroads of the body and involved in a host of metabolic processes central to the maintenance of homeostasis and productive function (Stangassinger 1989), removes a high proportion of most nutrients or metabolites and subjects them to further metabolism. As a consequence, the pattern of nutrient uptake by, and metabolism in the liver, as well as the final availability of nutrients for the whole body are largely dependent on products of digestion and absorption and on the metabolic capacity of the liver. The latter is expected to be strongly influenced by diet and by hormones.

The type of diet fed has a profound influence, not only on carcass weight, but also on organ weights (Martin et al. 1973). Thus, compared to hay-fed animals, liver weight was 20 % higher in groups fed concentrates or 30 % higher in groups that had been re-fed either ration after fasting. According to further studies (Fell and Campbell 1974), this weight gain was due to cell proliferation and enlargement.
In lactating sheep fed to appetite maximum weights of liver were observed in week 6 of lactation and were maintained until weaning. The basal mechanism of these alterations may not simply be wholly attributable to hyperphagia. Mediation by hormones like prolactin that regulate mammary tissue during lactogenesis also play a role in the homeorhetic control of the other maternal tissues necessary to support lactation (Fell and Weekes 1975).
Costs to this increase in organ weights are considerably higher maintenance requirements per unit weight than is average for the body. Smith and Baldwin (1974) estimated that changes in digestive tract and liver size caused 25 and 56 % of the total increase in energy expenditure (10 – 15 %) of lactating cows for maintenance.

The liver is the most richly vascularized organ in the body, with blood comprising approximately 25 % of its mass. Despite constituting less than 2 % of body weight in adult ruminants, the liver also receives approximately 25 % of cardiac output. Total blood flow through the liver is sensitive to nutrition, plus other regulatory factors, and can reach values of 3 l min^{-1} kg^{-1} liver weight (or 60 ml min^{-1} kg^{-1} body weight). Blood supply to the liver arises from two sources, the hepatic portal vein and the hepatic artery, the latter making only a small contribution (8- 12 %) to total hepatic blood flow (Isserty et al., 1998).
Regulation of hepatic blood flow is unique. The main blood supply through the hepatic portal vein is not controlled by the liver. Furthermore, the well-known autoregulation of arterial blood flow through most other organs (driven by the level of tissue oxygenation) is absent from the liver. Instead, the arterial flow appears to be inversely linked to the portal venous flow, providing the liver with a constant total flow (Lautt 1996).

The energy requirements of the splanchnic bed, which constitute about 10 - 13 % of total body mass account for 38 - 50 % of whole body oxygen consumption. They are attributable to energy costs of

ion transport, protein synthesis and degradation and to other processes such as substrate cycling, free radical scavenging, glucose and urea syntheses, and detoxification of phenols and other compounds. Obviously, 16 – 55 % of total energy expenditure in ruminant liver is allocated to the Na^+/K^+-pump at cell membranes, and 20 - 30 % is used in protein turnover (Mc Bride and Kelly, 1990), underlining the very high activity of nutrient transport and metabolism in the liver. However, the exact costs of specific processes are difficult to ascertain as there is considerable interplay and exchange of metabolic intermediates.

Hepatic nutrient balances: Experiments with multicatheterized dairy cows enabling estimation of blood flow rates and nutrient balances across the liver show, there is net uptake of propionate, butyrate, valeriate, acetoacetate, long-chain fatty acids, lactate, amino acids, NH_4^+ and O_2, while there is net release of acetate, ketone bodies, glucose, urea and CO_2.

Of the VFA, excluding acetate, the liver in lactating as in non lactating cows removes 85 - 100 %, contributing carbon (C) either for glucose - or 3-hydroxybutyrate (3 HOB)- synthesis and precursors of acetyl-CoA provide C which can enter a number of metabolic pathways.
Liver **acetate** utilization is limited by a low activity of acetyl-CoA-synthetase (Bergman, 1990). In most studies, on a net basis, there was even acetate production by the liver. Therefore, acetate is the only VFA present in peripheral blood in substantial quantities.

Net liver removal of **n-butyrate** and acetoacetate accounts for 56 - 62 % of net liver 3HOB (Reynolds, 1995). Other sources for liver 3HOB include long chain fatty acids, branched chain VFA, n-valeriate, aminoacids and acetate. Both acetate and 3HOB provide oxidizable substrate for peripheral tissues.

There is a strong, positive relationship between ME intake and net liver removal of **propionat**. However, net liver removal of propionate is also affected by the carbohydrate and protein composition of diets fed at equal ME intake (Reynolds, 1995). In the liver, propionate is converted to cytosolic oxaloacetate (OXAC) via succinyl-CoA and mitochondrial malate, much of which (90 % in lactating cows) appears to be channelled towards glucose synthesis, but this does usually not cover more than 60 % of hepatic glucose outpout (Seal and Reynolds, 1993).
Mitochrondria of bovine liver contain acyl-CoA synthetases, but only propionyl-CoA-synthetase is highly specific (Reynolds, 1995). Its activity depends on portal propionate level and is inhibited or stimulated by the presence of other fatty acids (n-butyrate, palmitate, oleate).

The rate of arrival of **lactate** at the liver is expected to increase in productive animals. As for the VFA, a positive relationship between ME intake and net portal appearance of lactate has been established (Lomax & Baird, 1983). Interestingly, in contrast to 3-carbon and longer fatty acids, liver lactate removal is not determined solely by its supply to the liver, but appears determined to a large extent by liver carbon balance and redox state.
Experimental work with lactating cows has demonstrated that a decrease in liver carbohydrate content is associated with an increase in the rate of net lactate uptake by the liver and an increase in the rate of net uptake of endogenously derived lactate by the splanchnic bed (Stangassinger and Giesecke, 1986). While the latter is indicative of an increase in lactate formation in peripheral tissues, the elevated rate of hepatic lactate uptake increases the possibility of lactate acting as a glucose and glycogen precursor. Both these changes are likely to be associated with an increase in glucose-lactate interconversions. It is somewhat surprising, therefore, that the percentage of glucose converted to, or derived from lactate is substantially lower during lactation than during pregnancy in cows and ewes (Stangassinger and Giesecke, 1986). According to Baird et al. (1980), the rate of hepatic lactate uptake during lactation should be capable of providing 20-25 % of hepatic glucose output. Presumably, a substantial portion of that lactate assimilated in the liver is not used for glucose production, but for glycogenesis. Indeed, transorgan measurements on ewes (Baird et al. 1983) revealed that up to 80 % of hepatic lactate uptake is used for purposes other than glucose output.

As part of the homeorhetic adaptation to support lactation **NEFA** are mobilized from adipose tissue and enter the liver via hepatic artery (from peripheral tissues) as well as via portal vein (from mesenteric fat). This process of internalization of the lipid soluble long chain fatty acids occurs via diffusion and/or specific transport proteins (Vernon, 2002).
The ruminant liver has very low activity of (lipoprotein) lipases and, therefore, has little capacity for the uptake of triacylglycerol (TAG) from the blood (Emery et al. 1992).
In the liver NEFA, are either oxidized or reesterified and exported as very low density lipoprotein (s. Figure 1). The latter beeing low in ruminants can be increased (60 %) by lactation, but even then it accounts for less than 2 % of NEFA uptake (Emmison et al. 1991).
Therefore, accumulation, of TAG within the hepatocyte (fatty liver) and incomplete oxidation of NEFA (ketogenesis) are common metabolic changes for dairy cows during early lactation. Obese dairy cows at calving would develop more severe fatty liver and would be more susceptible to the induction of ketosis.

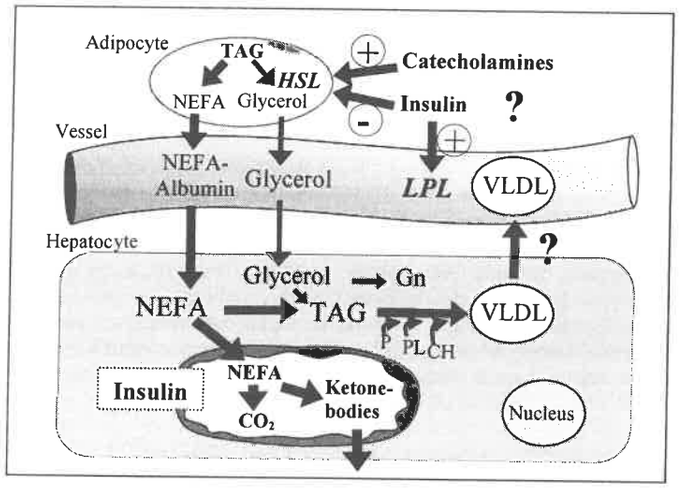


Figure 1: Lipid metabolism in cows during milking phase

TAG: triglycerides
NEFA: non esterified fatty acids
VLDL: very low density lipoprotein
Gn: gluconeogenesis
P: protein
PL: phospholipids
CH: cholesterol
LPL: lipoproteinlipase

Notwithstanding analytic uncertainties, hepatic fractional extractions of specific **amino acids (AA)** do vary widely. Although, there are some consistencies. For example, the branch-chain AA have the lowest rates of fractional removal, under a wide range of conditions, and glutamate is usually released by the liver (Lobley et al. 2000).
The varying removal of individual AA across the liver may relate, in part, to the physiological role of the liver as the side of synthesis of vital metabolites, e.g. glucose, nucleosides, porphyrins, neurotransmitters, xenobiotic conjugates etc., all of which involve the use of carbon skeletons derived from AA. The pattern of AA extracted by the liver to fulfil these various functions does not match that absorbed from the portal drained viscera (PDV) or required by peripheral tissues. But possibly, plasma export proteins of the liver can provide AA for peripheral tissue needs in ruminants as it has been shown in rodents, where as much as 20 % of albumin is degraded in each of skeletal muscle and skin (Maxwell et al., 1990).
For a number of interorgan AA cycles the liver is a central player. These include glutamate and glutamine, glycine and alanine and also arginine, ormithine and citrulline. These cycles shuttle C and N between the liver – as a site of glucose, protein and urea synthesis – and peripheral tissues – as sites of glucose utilization and protein synthesis and turnover. Thus, N from peripheral tissues is carried to the liver as alanine, glycine or glutamine for urea synthesis. Alanine, glycine and urea cycle intermediates serve the same function for N arising from PDV tissues. Most AA are removed on a net basis by the liver reducing the availability to other body tissues of AA absorbed across PDV.

Exceptions include the branched chain AA and glutamate, which are usually released by the liver (Lobley et al. 2000).

The amount of **ammonia-N** removed by the liver is dependent on dietary intake of fermentable protein and carbohydrates. There is a finite capacity for the rate of ureagenesis of 2 mmol urea-N/kg liver weight. This amounts to approximately 435 g urea-N/d for a 600 kg cow on high levels of nutrition (Sarraseca et al. 1998).
At higher intakes, unless there is no enzymatic adaptation, AA and ammonia will escape beyond the liver. This raises the important, but still unanswered question: When the capacity of ureagenesis is reached does the liver prioritize removal of NH_4^+ or of AA?

As in rodents, **glutamine** may have also in ruminants a key role in maintaining ureagenic flux and ammonia detoxification and may also impact on hepatic mechanisms related to acid-base balance. Production of urea involves utilization of both NH_4^+ and $HC0_3^-$, whereas synthesis of glutamine spares bicarbonate, which is then available to neutralize protons.
However, the activity of the ovine hepatic glutamine synthetase is only 15 % that of the rat (Häussinger, 1990). Thus, the maximal rate of urea production in ruminants exceeds hepatic glutamine synthesis by 5 – 10 fold (Lobley et al., 2000). This suggests that the ruminant liver is probably less import in regulation of acid-base balance.

With regard to **glucose**, the ruminant liver is a net producer, with 80 - 90 % of total glucose entry and unlike in non-ruminants rate of gluconeogenesis increases with feed intake. Thus, net hepatic glucose output has been described as a function of ME intake (Lomax & Baird, 1983) and of DE intake as well: y (mol/d) = 1.584 + 0.040 · DE (MJ/d), (Wieghart et al. 1986)
When diets contain high levels of cereal grain, and in particular ground maize, there is evidence - also from these equations – that significant quantities of starch may escape rumen fermentation. A net portal uptake may be observed at DE intakes of > 96 MJ/d. With a DE intake of 250 MJ/d about 16 % of total glucose entry will be delivered by portal exchange.
How are precursors for glucose synthesis provided in the ruminant? This question has been addressed by rather few experiments on lactating cows. The precursors for glucose synthesis are of both exogenous (dietary) and endogenous origin. There is general agreement that, in well fed cows propionate, exclusively exogenous, accounts for 50 – 60 % of liver glucose release. The supply of glycerol from adipose tissue lipolysis is low, unless cows are in severely negative energy balance. If used completely for gluconeogenesis, it could account for a further 15 - 20 %.
Lactate of dietary and endogenous origins would make a further small, but less predictable, contribution. Of the glucose precursors lactate appears to have the lowest priority in the well fed ruminant (Lomax & Baird, 1983). This fits to the finding that a marked decrease in the use of lactate for gluconeogenesis happens during transition from preruminating to the ruminating state (Reynolds, 1995).
Glycerol and endogenous lactate are both derived from glucose, and hence their contribution to gluconeogenesis via recycling represents no net synthesis of glucose. This means that in situations with limited feed intake as in early lactation the only possibility for the cow to increase the net production of glucose is to use mobilized AA from body protein. However, the quantitative significance of AA contributions to glucose synthesis in lactating ruminants is not well established. Data from literature (Danfaer et al. 1995) suggest that the proportion of glucose derived from AA can very considerably (from 2 - 40 %).
According to the potential significance of metabolized tissue protein as a source of AA for hepatic gluconeogenesis one has to conclude that over the period up to peak lactation its contribution to total needs is small. However, it may be critical during early lactation, when the cow is in substantial negative N balance. Indeed, net protein loss from skeletal muscle, the most likely tissue source of mobilized AA, is indicated by a 25 % reduction in muscle fiber diameter, in dairy cows immediately after calving (Bell, 1995). It seems likely, that hereby the net release of AA from muscle is achieved

entirely by suppression of protein synthesis rather than enhancement of protein degradation (Boisclair et al. 1993).
This peripheral mobilization of AA seems to be accompanied by enhanced synthetic activity and more efficient use of AA in the liver, already starting in preparturient cows and being a necessary prelude to the substantial hypertrophic and hyperplastic growth of the liver during early lactation (Campbell and Fell, 1970).

Metabolic adaptations in the liver and its regulation during the periparturient period: The periparturient period from 3 wk before calving to 3 wk after calving is marked by significant changes in endocrine status to accommodate parturition and lactogenesis. These changes, which are much more dramatic than at any other time during the gestation-lactation cycle, influence tissue metabolism and nutrient utilization. A reduction in feed intake by 30 - 35 %, maybe endocrine related, is initiated during the prepartum transition period, yet nutrient demands for support of conceptus growth and initiation of milk synthesis are increasing, but negative energy and protein balances are not as severe as during the week following parturition.
Circulating levels of NEFA and ketones tend to be elevated during late pregnancy, even in animals carefully fed to predicted energy requirements for conceptus growth and maintenance of nonuterine tissues. This trend becomes more evident close to term and is sharply exaggerated at calving or shortly thereafter. The massive mobilization of NEFA from adipose tissue during and after parturition is the metabolic hallmark of the transition from pregnancy to lactation. Thus, plasma NEFA concentration is highly correlated with the entry rate of NEFA, which in turn is representative of body fat loss.
Because ruminants do not efficiently export fatty acids as VLDL-TAG, significant amounts of the NEFA taken up and esterified by the liver are stored already prepartum.
Interestingly, the liver TAG to glycogen ratio somewhat greater than 2:1 at parturition may be an indicator of cow's susceptibility to ketosis (Smith et al., 1997). Thus, development of prepartum fatty liver appears to precede ketosis and to contribute to the onset of ketosis.
Plasma glucose concentrations remain stable or decrease slightly during the transition period, increase dramatically at calving by depletion of hepatic glycogen stores, and then decrease immediately postpartum. Although the demands for glucose by mammary tissue for lactose synthesis continues after calving, hepatic glycogen stores begin to replete and are increased by 2 wk postpartum (Grummer, 1995). This probably reflects an sufficiently increased gluconeogenic capacity to support lactation.

To summarize, metabolic patterns in maternal tissues of late-pregnant cows are characterized by increased hepatic glucose release but reduced glucose utilization in peripheral tissues, unchanged or decreased peripheral utilization of acetate, moderately increased mobilization of NEFA from adipose tissue, associated with similar increases in peripheral utilization of NEFA and their hepatic metabolite 3HOB, and by increased protein synthesis and reduced AA catabolism in the liver. All of these observed metabolic adaptations are consistent with promotion of availability of glucose and AA for conceptus metabolism and increasing reliance of maternal tissues on NEFA and ketones for oxidative metabolism.
However, within a few days of parturition, mammary demands for glucose, AA, NEFA and energy are 2.7, 2.0, 4.5 and 3.0 times those of the pregnant uterus before term (Bell, 1995; s. table 1).
The immediacy and magnitude of this increased demand is illustrated by the doubling of glucose production rate in dairy cows on the day of calving, compared with that observed a few days prepartum (Paterson and Linzell, 1974). Even post parturient increase in voluntary intake cannot satisfy this increased nutrient demand.
To sustain the impressive early performance of dairy cows given in table 1, they underlie the mobilization of tissue energy and protein reserves with substantial negative balance for net energy and metabolizable protein.

The challenge posed for the liver is apparent when mammary glucose uptake is compared with the estimated supply of dietary glucose precursors (propionate and AA). Even with the unlikely assumption that all absorbed propionate and AA (minus those required for milk protein) are available for hepatic gluconeogenesis, glucose synthesized from these substrates could account for only approximately 65 % of mammary glucose uptake.
The supply of glycerol from adipose tissue lipolysis and of lactate from dietary and endogenous origins would make a further small, but less predictable, contribution.

Table 1: Uterine as well as mammary uptake in relation to the nutrient supply (g/d) in Holstein cows during late pregnancy (250 d) and early lactation (4 d pp.) (Bell, 1995)

	Pregnant			Lactating		
	Supply	Uterine uptake		Supply	Mammary uptake	
Glucose	1476	666	(46 %)	2089	1775	(85 %)
Propionate	614	-		878	-	
Amino acids	998	718	(72 %)	1650	1374	(83 %)
Acetate	2196	270	(12 %)	3249	-	
NEFA	-	235		-	1224	

DMI (kg/d): 11.3 vs 14.6; Lactation performance: milk yield (kg/d) 29.8 ± 8; milk fat (g/kg) 46.7 ± 1.9; milk protein (g/kg) 42.3 ± 1.6;

In spite of these uncertainties, there is based on literature data a straight - line relationship between glucose production rates from 3 to 15 moles/d and milk yield (y, kg/d) in dairy cows: $y = (x - 1.64) / 0.396$; $R^2 = 0.95$ (Danfaer et al. 1995).
Thus, using this equation, the glucose need for a cow of 650 kg LW and producing 25 kg milk/d can be calculated as 11,5 moles/d with a requirement for milk production of 0,4 moles/kg leaving a basal requirements of only 0.5 $mmol \cdot h^{-1} \cdot kg^{-0.75}$.
The latter means, the early shortfall in the availability of glucogenic nutrients must be made up mainly, by major adaptations in the glucose utilization of extra hepatic, non mammary tisuess (e.g. limited irreversible loss of glucose via oxidation together with an increase in glucose-C-recycling) and by mobilization of stored glucogenic AA from "labile protein reserves", although both has not been directly documented in dairy cows.

From in vivo measurements of glucose metabolism via i.v. tracer infusions in pygmy goats it is obvious, that a significant potential for metabolic adaptation in both, glucose irreversible loss rate (IRL) and in glucose-C-recycling to the metabolic state does exist in ruminants (Stangassinger, 1997). Thus, in severely catabolic animals, with a plasma glucose decrease of about 33 % and a plasma NEFA increase by a factor of 9.5 IRL of glucose decreased to 0.2 $mmol \cdot h^{-1} \cdot kg^{-0.75}$, corresponding to 20 % of the values of the control animals. At the same time, glucose-C-recycling via lactate and/or alanine increased by a factor of 3.3, making about 15 % of glucose entry rate (GER). Similar levels of recycling have been reported (Stangassinger and Giesecke, 1986) in pregnant and lactating ewes (21 and 15 % of GER) and in lactating cows deprived of food (13.5 % of GER). Remarkably lower levels of recycling have been measured (Stangassinger, 1997) in fed (4 % GER) as well as in fed + glucose loaded goats (0 % GER).
Blood glucose is one of the major factors involved in the regulation of glucose production as well as glycogen formation and breakdown in the liver. Therefore, one possible reason for the increase in

glucose recycling during glucose insufficiency may be found in the low blood glucose levels which prevent glucose-6-phosphate synthesized from lactate and/or alanine being diverted to glycogen.

In general, the glyconeogenic pathway is controlled by substrate availability – probably the major determinant in ruminants -, by quantities of the four key regulatory enzymes pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase) and glucose -6-phosphatase, in ruminant liver, and by the degree of its activation; all of which are influenced by hormones.

Mechanisms for hormonal control of key enzymes for hepatic gluconeogenesis have not been studied intensively in ruminants; however glucagon, insulin, growth hormone, glucocorticoids, and catecholamines play important roles in regulating glucose metabolism (Mc Dowell, 1983). Consequently, glucose availability is increased in early lactation, when insulin to glucagon ratio is low.
In the nonruminant, nutritional and hormonal regulation at the molecular level (e.g. gene expression for enzymes of glucose metabolism) have been extensively studied in vivo and in vitro, but few analogous studies have been done with ruminants (She et al. 1999).
For hepatic gluconeogenesis from precursors that enter the gluconeogenic path prior to the triose phosphates, PC and PEPCK are potential rate limiting enzymes.
Propionate-C is metabolized to OXAC through the TCA-cycle, whereas lactate-C is first metabolized to pyruvate and then directly to OXAC by PC. PEPCK catalyses the conversion of OXAC to phosphoenolphyruvate.
Pyruvate is a common entry point in the gluconeogenic pathway also for the carbons of glycine, serine, cysteine, tryptophan, alanine and threonine but it can also be converted to acetyl-CoA by pyruvate dehydrogenase and further metabolized through the TCA-cycle. Likewise, the abundance of NEFA at calving may be oxidized to acetyl-CoA and further metabolized in the TCA cycle or alternatively, can be partially oxidized to ketones.

There is increased PC mRNA-abundance and activity during the periparturient period (Greensfield et al., 2000) which appears to coincide with the period of maximal feed intake depression at calving and may reflect the necessity for hepatic adaptation to the use of lactate, glycerol and AA as gluconeogenic substrates when she supply of propionate in limiting. This adaptive mechanism allows pyruvate-C to be channelled through OXAC and simultaneously minimizes ketogenesis from nonlipid precursors. The direct allosteric activation of PC by acetyl-CoA from hepatic NEFA oxidation (Zhang et al., 1995) further augments the impact of increased PC mRNA expression in the periparturient dairy cow.

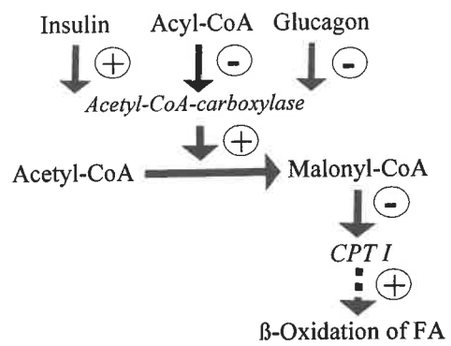
Interestingly, the period of transition to lactation is not accompanied by similar changes in PEPCK activity, suggesting a role for activated PC in OXAC formation also for other purposes (to maximize the anaplerotic metabolism of pyruvate and the mitochondrial oxidation of fatty acids by the liver).
The lack of change in PEPCK mRNA abundance suggests specific regulation of the PC enzyme rather than a general response to induce gluconeogenic potential in liver, and supports earlier findings (Filsell et al., 1969) that the ratio of PC: PEPCK enzyme activities in the liver from fasted sheep was elevated compared to the fed state. Obviously, during both, fasting and transition to lactation, this lack of change in PEPCK-activity may be of little consequence for the availability of glucose, if its total activity (cytosolic and mitochondrial) in liver is not limiting for gluconeogenesis.

The discordant timing of the activity of PC and PEPCK during the transition to lactation may reflect a response to the supply of endogenously derived lactate, glycerol and AA or to the release of fatty acids from adipose tissue shortly before and after calving. However, these changes are synchronous with expected glucose demand, gluconeogenic precursor availability, and availability of substrate for oxidation by liver (Greenfield et al. 2000), and they are in accordance with the suggestions (Danfaer et al. 1995) that increasing the recycling of carbon between glucose and lactate may support whole

body glucose economy of dairy cows during the transition period. During pregnancy over 80 % of lactate originates from glucose, whereas 36 % of lactate comes from glucose during lactation (Van der Walt et al. 1983). Therefore, an increase in gluconeogenesis from lactate per se may have some impact on the total glucose entry rate only around parturition. Alternatively, recycling of glucose -C occurs together with alpha-amino N via alanine providing a reserve supply of gluconeogenic carbon to meet glucose requirements during this period. Indeed, alanine accounts for approximately 25 % of the net portal appearance of α-Amino-N, and gluconeogenesis from alanine at calving is almost twice precalving rates (Overton et al. 1998).

Interestingly, postpartum during ongoing lactation there is a synchronous increase in mRNA-abundance for PEPCK and PC (Greenfield et al. 2000), reflecting the adaptation of liver metabolism to the increased glucose requirements and the coincident decrease in the insulin to glucagons ratio as milk production increases (Stangassinger et al. 1989).
Inspite insulin and glucagons are considered two of the primary regulators of gluconeogenesis in ruminants, only from nonruminants it is well established that insulin decreases the mRNA and activity of the PEPCK enzyme by directly regulating expression of the gene (O'Brien and Granner, 1990).
The ratio of insulin to glucagons decreasing through the days in milk after parturition, favours fatty acid mobilization and hepatic uptake, decreases malonyl-CoA concentration (Figure 2) and thereby the inhibitory effect on fatty acid mitochondrial transport and subsequent β-oxidation and ketogenesis (Zammit, 1990). Ongoing β-oxidation of fatty acids in early lactating cows produces NADH and acetyl-CoA, and both these metabolites indirectly stimulate gluconeogenesis: Acetyl-CoA activates PC; mitochondrial NADH can be used in the reduction of oxaloacetate to malate which in turn is transported to the cytosol and again via OXAC metabolized in the gluconeogenic pathway.

Figure 2: Regulation of acetyl-CoA-carboxylase



The immediate product of hepatic ketogenesis from NEFA via acetyl-CoA is acetoacetate, which is not reduced to 3HOB in liver mitochondria due to low dehydrogenase activity in ruminants. Instead, acetoacetate is transported to the cytosol in exchange for pyruvate which in turn is converted by activated mitochrondrial PC to oxaloacetate. Obviously, around parturition several mechanism do exist to ensure that according to rapidly increasing milk yield a high rate of gluconeogenesis can be maintained in the ruminant liver even during conditions of inadequate energy intake, lipid mobilization and hepatic fatty acid oxidation.

As lactation progresses, feed intake and propionate availability for gluconeogenesis increase. This causes higher hepatic concentrations of methylmalonyl-CoA and succinyl-CoA which are inhibitors

of β-oxidation and ketogenesis (Brindle et al. 1985; Lowe and Tubbs, 1985). Gluconeogenesis from most AA does not yield methylmalonyl-CoA, potentially allowing for simultaneous gluconeogenesis and ketogenesis.

Conclusions: Measurements of net nutrient flux across the splanchnic bed have provided important insights into the integration of nutrient supply from the gut with nutrient availability to peripheral tissues and how liver metabolism responds to changes in diet intake and composition as well as to the metabolic state.
Evidence is accumulating that productivity in ruminants depends on the coordinated dynamism of metabolism. Particular substrates can be diverted and interchanged to allow the most favourable balance to be achieved between available nutrients and the prevailing demands on the animal, e.g. pregnancy, lactation. Central to this adaptability in the liver with its dual role as an important organ for most energy-yielding oxidative processes and as the provider of key intermediates in the interconversion of carbon skeletons of fat, protein, and the glycogenic metabolites. The pathways traced at any one time by particular molecules derived from food or mobilized body tissue are neither stereotyped nor random. Substrates are routed, directed, and interconverted within the liver to achieve optimum metabolism for the whole animal. The extent of any metabolic interactions depends on both the amount and type of substrate available.
Thus, in situations of nutritional stress, such as the transition period, in dairy cows when energy output exceeds input, obviously, the amount of "available protein" determines the overall efficiency of the starting lactation. Catabolism may not always be merely a passive response to a need for removal of surplus material. It is well known that AA-carbon act as a precursor for the synthesis of many specific and very important metabolites especially glucose and glycolytic intermediates. Thus, AA provide an important supplemental source of these intermediates and may then have an influence on the efficiency and utilization of other substrates.

Another important example of metabolic interactions in the liver to achieve optimum metabolism is the process of gluconeogenesis. Reliance on hepatic oxidation to remove excess NEFA during the transition period may support gluconeogenesis to fulfil increasing lactational demands; However, in case of the preferential use of glycogenic AA this metabolic situation also may result in a deleterious increase in the flux of ketones.

The magnitude of this dynamism in metabolism and its coordination by the liver probably has a major impact not only on milk production but also on the amount of stress a cow encounters during the transition period. Many of the undesirable metabolic outcomes among early lactating cows that experience turbulent transition have either taken place by that time or may be programmed to take place in the following weeks.

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W4. Metabolic Performances of the Mammary Gland (Stoffwechselleistungen der Milchdrüse).

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Introduction

The function and accordingly the metabolism of the mammary gland markedly change depending on the physiological states (mammogenesis, lactogenesis, galactopoiesis). Milk ejection is associated with enhanced blood flow through the mammary gland, but metabolic and energy requirements are likely small relative to those needed for the synthesis of organic milk components during established lactation. This review will focus on metabolism of the mammary gland, on associated whole-body metabolism, and on the coordination of mammary gland and whole-body metabolism during the periparturient period and especially during established lactation in dairy cows.

Metabolic changes during the periparturient period

Besides mammogenesis, an optimal preparation for lactation is essential for high milk production. Lactogenesis is a complex phenomenon because alveolar epithelial cells (lactocytes) proliferate and differentiate to be ready for the later synthesis and lactocytes start with the secretion of specific milk components. After phase I of lactogenesis (differentiation of lactocytes), the mammary gland during phase II of lactogenesis starts to take up substrates (glucose, acetate and amino acids [AA]) for the synthesis of specific milk components. This is associated with enhanced blood flow as an indication of enhanced metabolic activity. Synthesized specific milk components, such as citrate ("a harbinger of lactation") and triglycerides, lactose, casein, α-lactalbumin and β-lactoglobulin are then secreted into the alveolar lumen. In addition, immunoglobulins (especially IgG_1) and various peptides (such as lactoferrin, insulin, prolactin, growth hormone [GH], insulin-like growth factors [IGFs] and enzymes) are transported from blood into colostrum by transcytosis or paracellular routes. The concentrations of many of these peptides in (pre-)colostrum during the last days before parturition exceeds concentrations in blood, i.e. these peptides are transported against a concentration gradient. Some of these peptides, once secreted into the alveolar lumen, exert paracrine effects on lactocytes.

The functional and metabolic changes of lactocytes during the periparturient period are regulated by hormones. Cortisol and prolactin are essential for phase I and II of lactogenesis, respectively. The glucocorticoid-induced differentiation of lactocytes is important to allow prolactin to later induce the synthesis of specific milk components. The rise of cortisol at parturition is associated with enhanced glucose uptake of the mammary gland and thus for enhanced lactose production. The blood plasma status of other hormones, such as GH, insulin, IGF-1, IGF binding proteins (IGFBPs) and 3.5.3'-triiodothyronine (T3) needs to be normal to allow successful lactogenesis, but these hormones are not necessarily directly lactogenic. Furthermore, the decrease of plasma progesterone in particular and in addition of 17β-estradiol concentrations are preconditions for the successful induction of lactation. It has recently been shown that lactoferrin, retinol (all-trans retinoic acid) and IGFBP-3 depend on each other with respect to their effects on bovine lactocytes especially in the involution phase of the lactation cycle (BAUMRUCKER & ERONDU, 2000) and in this respect it is worth to note plasma concentrations of lactoferrin, retinol and IGFBP-3 decrease at parturition. Enhanced release of oxytocin at first milking or suckling is essential for milk let-down of secreted milk components. Failure to release oxytocin especially in first calving heifers seems to be an increasing problem.

Metabolic changes during lactation and endocrine control of mammary gland and whole-body metabolism

During galactopoiesis the production and secretion of protein, fat and lactose and handling of other milk components depend on mammary gland metabolism. Up to peak of lactation (during the "starting phase of lactation") the synthesis of fatty acids (FA), fats, AA, peptides, proteins and lactose absolutely increases (although protein concentrations in milk transiently decrease), followed by a slow decline during the "production phase" up to an abrupt decrease during drying-off. In high-yielding cows at peak of lactation several kg of triglycerides, proteins and lactose are produced per day.

Specific milk proteins are synthesized *de novo* in lactocytes from blood plasma AA and in part from small peptides that are taken up by specific carrier mechanisms. Some of the AA used for protein synthesis are delivered by erythrocytes. Importantly, arterio-venous (A-V) differences at the level of the bovine mammary gland are not linear for all essential AA, indicating that there are marked differences in their metabolic handling. The AA primarily originate from ingested proteins and from microbial protein synthesized in the rumen and only in limited amounts (during the first weeks of lactation) from AA that are mobilized from tissues (muscle, involuting uterus > others). During the first 2-3 days of lactation (colostral period) many peptides and proteins are transported in relatively great amounts from blood between or through lactocytes into the alveolar lumen (BLUM & BAUMRUCKER, 2002). The transcellular transports of some of these peptides and of IgG_1 are receptor-mediated and thus are selective. As a result there are marked differences in the composition of milk compared with blood. Concentrations of milk urea, on the other hand, closely mirror concentrations in blood plasma because urea diffuses (passive transport) through mammary epithelia.

Milk FA in triglycerides up to C_{14}/C_{16} are the result of *de novo* synthesis, starting mainly from acetate that is produced in the rumen and to a smaller extent from β-hydroxybutyrate (BHBA) that is produced in the rumen wall or in the liver. There are linear correlations for acetate and β-hydroxybutyrate between A-V differences and arterial concentrations (as long as arterial concentrations are in the physiological range) at the level of bovine the mammary gland (RULQUIN, 1986), indicating efficient utilization by the mammary gland, but if BHBA concentrations are markedly elevated (as in cows with ketosis), its uptake is limited (BALDWIN et al.., 1980). Enhanced availability of glucogenic substrates (propionic acid administered into the rumen or glucose infused into the duodenum) decreased milk fat content and fat yields, but while propionic acid decreased short-, medium- and long-chain FA in a homogenous manner, glucose administration reduced only short- and long-chain FA, while medium-chain FA increased (RIGOUT et al.., 2003). The decrease of milk fat content was mainly due to a decreased production of C18 FA, probably due to decreased lipolysis (HURTAUD et al., 2002). About 40% of triglycerides derive from preformed, mainly long-chain FA that originate from depot fat, from liver or from food. The uptake of long-chain FA by the mammary glands depends on the activity of lipoprotein lipase, whose activity in the mammary gland is inversely related to the activity in depot fat and hormonally regulated. Glycerol for triglyceride formation needs to be present as glycerophosphate and the NADPH serves as a hydrogen donor. The mostly saturated long-chain FA that are absorbed in the small intestine can in part be secondarily desaturated, thus explaining the relatively high amounts of palmitoleic and oleic acid that derive from palmitic and stearic acid, respectively. Although linoleic acid (cis-9, cis 12 C18:2) is in part transformed into conjugated linoleic acid (CLA; cis-9, trans-11 C18:2), to vaccenic acid (trans-11 C18:1) and finally to stearic acid (C18:0), part of the vaccenic acid can be re-transformed into CLA by Δ9-desaturase, a process that is responsible for relatively high amounts of CLA in bovine milk. Marked reductions of milk fat concentrations are often due to a combination of rations that contain low amounts of physical structure and roughage, but high amounts of starch and especially of unsaturated FA that are transformed into trans-FA, of which the trans-10, cis-12 isomer has an especially marked milk-fat depressing effect. *De novo* synthesis of short- and medium chain FA is reduced if high amounts of long-chain FA are fed and this is associated with reduced activity of rate-limiting enzyme for fat synthesis (acetyl-Co-carboxylase).

Lactose (= glucose + galactose) is synthesized from glucose in the mammary gland. Epimerisation of glucose to galactose requires glucose binding to UDP and transfer of UDP-galactose to glucose by lactose synthetase, an enzyme consisting of galactosyltransferase (protein A) and α-lactalbumin (protein B). Thus, α-lactalbumin synthesis is associated with glucose metabolism and thus with energy metabolism. Lactose is the most osmotically active substance in milk. As a consequence, it attracts water from blood plasma into the alveolar lumen and because water is the quantitatively most important component of milk, water requirements and turnover are markedly enhanced during lactation. Thus, from a physiological standpoint, milk yield primarily depends on lactose synthesis and thus on sufficient availability of glucose to and uptake by lactocytes for lactose synthesis. A

sufficient availability of blood glucose therefore largely determines milk yield. However, it is well known that induction of hyperglycemia in euglycemic cows does not enhance milk yields, likely because hyperglycemia, through enhanced insulin release, reduces the availability of other substrates (such as AA) needed for milk synthesis. Thus, parenteral glucose infusions enhanced milk yield only if AA were simultaneously administered. Infusions of high amounts of glucose into the duodenum even decreased milk yields of dairy cows, possibly due to reduced glucose-1-phosphate to glucose-6-phosphate ratios in the mammary gland (RIGOUT et al., 2002a). Sufficient gluconeogenesis in dairy cows is a precondition for high milk yields because only part of ingested carbohydrates escape metabolisation to volatile FA in the rumen, because amylase activity in the small intestine is insufficient to digest high amounts of starch that have possibly escaped rumen degradation, because absorptive capacity of the small intestine for glucose is limited and because eventually absorbed glucose is primarily used to cover energy needs of the gastrointestinal tract. Gluconeogenesis is enhanced by pyruvate kinase (PC) and by phosphoenolpyruvate kinase (PEPCK). Our recent studies in high-yielding dairy cows demonstrate that mRNA levels of PC, mitochondrial and cytosolic PEPCK (PEPCK-M and PEPCK-C, respectively) are lowest in week 2 a.p., highest in week 4 p.p. and decrease almost to a.p. levels in week 20 p.p. (M. Reist, H. Hammon, C. Morel and J. Blum, unpublished observations). There were significant negative relationships of the energy balance with PC, of plasma leptin with PC and with PEPCK-C, and of plasma T3 with PEPCK-M. Gluconeogenesis primarily depends on sufficient availability of substrates (propionate, glucoplastic AA, lactate and glycerol), i.e. depends mainly on carbohydrate (starch) and protein intake. Enhanced availability of glucogenic precursors (such as propionate) increase milk yield and protein yields, but decrease fat yields (RIGOUT et al., 2002b, 2003). For limited times gluconeogenesis can be maintained based on mobilisation of glycerol from depot fat (triglycerides) and of AA from body proteins. If gluconeogenesis is reduced because of insufficient amounts of glucose precursors, hypoglycaemia occurs and if plasma glucose concentration falls below about 0.3 g/L, milk production decreases drastically.

The mammary gland uses energy for its basal metabolism, for synthetic processes and for the transport/exchange of substances (for example of ions). The synthesis of milk und especially of proteins is an energy-consuming process. Substrates for the formation of ATP are glucose, acetate, ketone bodies, FA and AA. Glucose and acetate are important energy yielding substrates of the mammary glands in ruminants. As a consequence of the enhanced metabolic rate and energy requirements of the mammary gland, if yields are increased (for example after the administration of GH), blood flow to the mammary gland and thus the transport of energy-yielding substrates is increased.

A sufficient availability and uptake by lactocytes of substrates used for the synthesis of specific milk components and for energy requirements of synthetic processes is essential for high milk production. Because intakes of energy and of absorbable protein in the small intestine in high yielding dairy cows in early lactation are insufficient, metabolic adjustments are needed to maintain high milk production. Metabolic adjustments involve enhanced mobilisation and decreased synthesis of depot fat, enhanced mobilisation of AA from and decreased synthesis of protein in skeletal muscles and in part in other organs, enhanced gluconeogenesis and reduced whole-body glucose oxidation, and adjustments in mineral metabolism (for example increased mobilisation of calcium from the skeleton). Non-esterified FA, that derive from depot fat, become increasingly important to cover energy requirements. Decreased glucose oxidation helps to spare glucose that can be used for lactose synthesis – an important adjustment for the maintenance of high milk yields. During the first weeks (up to the peak) of lactation, glucose, FA, AA (as well as of other components, such as some minerals, that are essential parts of milk) are partitioned in favour of the mammary gland. Thus, anabolic processes in the mammary gland are paralleled by extramammary catabolic processes, especially of protein and fat metabolism. Metabolic diseases, followed by reduced milk production and reproduction, occur, if these catabolic changes are too marked and last too long.

Mammary gland synthetic capacities during the first weeks of lactation can only be maintained by adjustments and coordination of whole-body metabolism. Hormones are of primary importance for the regulation of the metabolism of the mammary gland and of the other organs of the body. Further

adjustments at the level of the mammary gland are possible by changes in the activity of the sympathetic nervous system.

The GH – IGF-1 system is of primary importance for the coordination of mammary gland and whole-body metabolism in dairy cows (ETHERTON & BAUMAN, 1998). Plasma concentrations of GH during early lactation, i.e. during energy deficiency, are typically increased. Effects of GH involve enhanced feed intake, enhanced blood flow to the mammary gland (and thus enhanced delivery of substances needed for milk synthesis), enhanced lipolysis and FA oxidation, enhanced gluconeogenesis (from propionate and glycerol) and reduced glucose oxidation, and enhanced uptake of AA by the mammary gland. These adjustments result in enhanced availability of glucose, AA and FA for mammary lactose, protein and fat synthesis. Although adipocytes have GH receptors, effects of GH on whole-body metabolism are in part indirect. Thus, stimulation of lipolysis by GH is in part indirect through enhanced sensitivity of adipocytes to catecholamines. Furthermore, GH typically reduces insulin-dependent glucose utilisation and FA uptakes by skeletal muscles (through a decrease of glucose-4 transporters) favour the partitioning of nutrients to the mammary gland by the insulin-independent glucose-transporter-1. Thus, stimulating effects of GH are primarily mediated by modifications of whole-body metabolism and by partitioning of nutrients in favour of the mammary gland. Direct effects of GH on the mammary gland are small because there are, if at all, only small numbers of GH receptors present on lactocytes. However, GH stimulates through GH receptors present on hepatocytes and fibroblasts of the mammary gland (besides other organs) the production of IGF-1. IGF-1 can exert direct effects mainly through type-1 receptors that are present in high amounts during lactation on lactocytes (PLATH-GABLER et al., 2001) and it has been shown that IGF-1 may also enhance mammary gland blood flow and thus contribute to increased delivery of substrates for milk synthesis. Despite elevated plasma GH concentrations during energy deficiency in early lactation, plasma concentrations of IGF-1 are, however, typically low because of GH resistance in the liver and possibly also in other organs (RONGE et al., 1988). If GH is administered in amounts that result in plasma concentrations that exceed physiological concentrations and if GH is administered some weeks after peak lactation and thus during a phase during which energy and protein intakes meet requirements, plasma IGF-1 concentrations increase (BLUM, 1992). Under these conditions there is some evidence that IGF-1 enhances the number of lactocytes (mitotic effect) and (or) reduces apoptosis of lactocytes. Although IGF-1 is not directly galactopoietic, milk yield and the persistency of lactation increase because more lactocytes are in a differentiated state and thus are able to synthesize milk. Plasma glucose increased linearly with increasing duodenal glucose infusions, plasma glucose levels were linearly associated with increased plasma IGF-1 levels, and plasma IGF-1 was associated with glucose uptake by the mammary gland, but arterial plasma IGF-1 levels were not linearly correlated with milk yields, i.e. milk yields were decreased at highest IGF-1 levels (RIGOUT et al., 2002a; LEMOSQUET et al., 2004). In addition, GH enhances the conversion of thyroxine (T4) into T3 within the mammary gland. As a consequence, the metabolic rate and synthetic rates of lactocytes are expectedly enhanced while the peripheral T4 to T3 conversion in extramammary tissues is at the same time reduced. Low plasma T4 and T3 levels during early lactation are thought to contribute to reduced heat production of extramammary tissues and thus to spare energy in favour of the mammary gland, thus allowing an enhanced metabolic priority of the mammary gland. Additional hormones, such as insulin, glucagon, cortisol and leptin contribute to metabolic adjustments especially during early lactation in favour of the mammary gland. Concentrations of plasma insulin, IGF-1, T4, T3, and leptin behave very similarly, i.e. decrease during energy deficiency in early lactation and then continuously increase when energy intakes increase and energy balances become positive (REIST et al., 2003a,b). Thus, in early lactation the ratios of GH relative to IGF-1, insulin, glucagon, leptin, T4 and T3 increase when energy balances decrease. Low plasma insulin levels relative to high GH levels in early lactation are a prerequisite for enhanced breakdown of glycogen, fat and proteins and thus are of particular importance for the partitioning of nutrients toward the mammary gland, although in the dairy cow. However, insulin barely or not at all directly stimulate the uptake of substrates for milk synthesis by the mammary gland because insulin receptors are lacking. Nevertheless, insulin is important for protein yields, as shown in euglycemic-hyperinsulinemic glucose clamps, as reviewed by PETITCLERC et al. (2000).

Increases in milk protein by insulin may be due to stimulation of AA uptake by lactocytes or increased plasma IGF-1 and changes in IGFBPs. Low leptin levels in early lactation may moderate excessive fat mobilisation and stimulate feed intake. Other endocrine systems help to maintain calcium and phosphorus homeostasis (parathyroid hormone and $1.25(OH)_2$-vitamin D), sodium and potassium (aldosterone) and blood pressure and thus create an environment that allows the mammary gland to produce high amounts of milk at least for time and despite of insufficient intakes of energy and protein. Our studies in very high yielding dairy cows compared with lower yielding cows in the same farm (mean 46 and 36 kg energy-corrected milk/day during the first 100 days of lactation, respectively) demonstrate, that high yielders are basically able to maintain metabolic homeostasis (AEBERHARD et al., 2001a,b). The studies are in accordance with other investigations (KNIGHT et al., 1999) and do not support the often held opinion, that high yielders - although optimally fed and hold under optimal management conditions - are characterized by a particularly high frequency of metabolic diseases. However, if optimal feeding and management conditions are not maintained, metabolic diseases in the periparturient period and in early lactation are frequent.

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W5. Interactions between milk yield and production diseases in dairy cows (Hohe Milchleistung und Tiergesundheit – ein Widerspruch?) J. Rehage* and M. Kaske – Hannover

Milk yield of dairy cows in Northern Germany increased within the past three decades nearly linearily by about 2.4 % per year. While on herd level an average of about 8,000 kg FCM per year and herdbook cow has been reached in 2000, the top 10 % of the dairy enterprises obtain already an annual milk yield of more than 10,000 kg FCM per cow. Individual cows in such herds produce far more than 12,000 kg FCM (16).

This development was caused preliminary by the economic basic conditions in the EU favouring a high individual milk yield to reduce the costs per unit of milk produced. As these economic conditions will not change on the short run the trend towards a further increase will proceed. There is general concern that the enhancement of milk yield jeopardizes the health of the dairy cows raising an animal welfare issue (5). Increasing incidences of specific production diseases conditional on a variety of abiotic, biotic and operational factors were brought about by the drive for higher yields in the last years. Accordingly, it has to be asked whether a further increase of milk yield is physiologically possible, economically desirable, ethically accountable, and politically meaningful in respect to consumer´s product image of milk.

Already 25 years ago, higher health costs for groups of selected cows with superior genetics for milk production compared to average cows were reported (15, 27). However, as the income from increased milk yield was found to raise superproportionally, the selection for high milk yield appeared to be still economically favourable. This conclusion seems to be valid until today (9).

A genetic selection to achieve a reduction of health risks in dairy cows is possible, but any attempts are hampered by the low heritabilities for disease traits (Table 1) while those for milk yield and percentage of fat are moderate to high (h^2 0.25 – 0.46). Genetic correlations between milk yield and health traits have been investigated but the results are quite controversial. There is agreement that selection for high genetic merit leads to a deterioration in fertility (13), but the magnitude of effects of milk yield on health traits varies considerably (Table 1). An unambiguous influence has only been shown for mastitis.

Table 1: Heritability and genetic correlations between individual traits and milk yield (compilation of literature data; 17, 18, 20, 29)

Trait	h^2	Genetic correlation
Retained placenta	0.02	-0.43
Endometritis	0.01	-0.17
Cystic ovaries	0.05 - 0.12	-0.01 - -0.06
Mastitis	0.00 - 0.14	0.18 - 0.37
Displaced abomasum	0.09 - 0.28	-0.04 - -0.15
Milk fever	0.00 - 0.30	-0.67 - -0.33
Ketosis	0.01 - 0.09	0.17 - 0.77
Foot problems	0.26	0.27 - 0.31

Epidemiological attempts to investigate the relation between risks of diseases and milk yield have been undertaken frequently, but due to methodological threats it is difficult to obtain valid data describing quantitative influences. On the one hand, a broad data basis comprising data from commercial dairies is required, on the other hand, the risk of systematic lapses fudging the evaluation is thereby increased (14, 22, 23), e. g.

- substandard cows are less likely to be investigated by a veterinarian than high-yielding animals which may pretend a higher disease frequency in the latter group,
- diseased cows with a low milk yield are more likely to be culled than those with a higher breeding value,
- various diseases cause a decrease of milk yield in the present lactation; accordingly, for epidemiological purposes the milk yield in the previous lactation has to be considered,
- lactation number has a significant effect on the incidence of several diseases (e.g., periparturient paresis, mastitis). As also milk yield is positively related to the number of parities, the age-dependency may feign an influence of milk yield.

Based on the evaluation of 15 epidemiological papers, Erb (1987) could not verify evidence for a relation between milk yield and the risk of retained placenta, metritis, cystic ovary, ketosis, mastitis and LDA while high milk yield was found to be a risk factor for milk fever. By contrast, when 2197 lactations of 1074 German HF cows from 10 farms in Lower Saxony were analysed, significant correlations between milk yield and typical production diseases were demonstrated (12, Table 2). Especially the risk for mastitis and ovary cysts appeared closely related to milk yield while the relations between milk yield and the risks for claw problems and retained placenta were significant, but less pronounced.

Table 2: Estimated probability of appearance (EPA) demonstrating the influence of 305 d milk yield on the incidence risk of certain diseases; DIM – day in milk (12)

Disorder complex	EPA (%) 6000 kg	EPA [%] 12,000 kg	kg of milk per 1 % EPA increase	Median DIM at day of diagnosis
Retained placenta	6.4	17.0	570	1
Metritis	16.9	25.6	not significant	24
Ovarian cysts	9.0	27.5	323	61
Mastitis	17.9	44.2	228	54
Claw diseases	16.2	32.2	375	76
Milk fever	4.0	13.2	650	1

Obviously, neither studies looking at genetics nor those investigating epidemiologic findings provide unambiguous evidence to support or refute the relation between risk of most production diseases and milk yield. Firstly, this conclusion is remarkable by itself: if a straight and grave relation would exist this would foil in practise any surpluses achieved by the additional milk yield. Accordingly, the efforts for increased yield in the field would shrivel. Another aspect may explain the surprising lack of evidence, i.e., most production diseases are found in high-yielding dairy cows only within a short time frame and their incidence is influenced to a considerable extent by environmental and management factors.

Roughly 70 % of all diseases affecting dairy cows emerge only during six weeks of the reproductive cycle of a cow, i.e. the transition period defined as the period from 3 weeks ante partum until 3 weeks post partum. The majority of the remaining health problems are observed within early lactation (until 100 days in milk). This accumulation of various diseases within a short period is attributed predominantly to the negative energy balance (NEB) during the fresh cow period caused by rapidly increasing milk production in face of delayed increasing feed intake. Thus, most diseases reflect the direct or indirect consequence of failing homeostatic and homeorrhetic adaptation processes within

the first weeks of lactation leading to a drastic NEB (Bauman and Currie 1980). The consequence is a dissonance of the endocrinologic orchestra stimulating excessive lipomobilisation and favouring the accumulation of triglycerides in the liver (7). Massive fat infiltration represents an increased risk for liver failure (Table 3) and may affect physiologic hepatic functions as, e. g., detoxification of ammonia and endotoxins. Accordingly, the liver plays a pivotal role in the transition period: on the one hand, the liver provides the basis for general health (e.g., an efficient immune system [26, 35]), moreover, the liver makes available by gluconeogenesis the vast amount of glucose which is indispensable for milk production in the udder (7, 8).

Table 3: Relative risk for the development of liver failure in cows depending on the extent of hepatic fat infiltration; TGL – triglyceride; FW – fresh weight (24)

Liver-TGL [mg/g FW]	OR	p
< 35	1.0	
35 – 65	1.8	0.16
66 – 100	3.7	0.03
> 100	5.5	0.006

As a health problem affects the general behaviour and, in particular, the appetite of the cow, the worsening NEB aggrevates the per se existing metabolic stress for the cow. Thus, not surprisingly, most diseases increase the risk for the development of a further additional health problem (Table 2); e. g., the risk to develop an abomasal dislocation is 39 times higher in cows suffering from ketosis as compared to metabolically inconspicuous cows (25). A vicious circle may be initiated ending with the loss of the cow.

Table 4: Association between parturient paresis, periparturient disorders and fertility; OR – odds ratio

Disorder	OR	p	N	Source
Dystocia	6.5	< 0.0001	2190 (33 herds)	5
Retained placenta	3.2	< 0.0001		5
Ketosis	8.9	< 0.0001		5
Mastitis	8.1	< 0.0001		5
Abomasal displacement	3.4	0.06		5
	4.8		510	19
Calving to first service [days]	88.3 (controls: 75.5)		804	4

Accordingly, it has to be emphasized that the NEB represents the most threatening factor for an increased risk for production diseases in the transition period. The magnitude of the NEB is influenced by feed intake and energy output due to milk production. Obviously, the NEB tends to be higher in high-yielding cows compared to standard cows. However, high milk yield is not necessarily combined with a drastic NEB. It represents the art of successful dairy farmers to to minimize the NEB during the fresh-cow period in face of a high inset yield after parturition (7). As long as this attempt succeeds a high milk yield will not be accompanied by an increased risk of production diseases. A variety of sophisticated management factors has been established which have only one superordinate

target: to maximize dry matter intake after parturition and to maintain at the same time an appropriate ruminal fermentation (i.e., to avoid a subclinical acidosis) (8). In older textbooks, a maximal intake of 20 kg dry matter per day is depicted while nowadays 30 kg and even more can be achieved by many cows. Tools to maximize feed intake post partum have to be adopted fairly before the start of the next lactation; the importance of the dry period for the health status post partum has been acknowledged and emphasized especially in the last years (1, 7). The major aims are:

- to avoid overconditioning during the far-off period,
- to adapt the ruminal flora to the concentrate-based diet after parturition by increasing the amount of concentrate in the close-up period gradually,
- to prevent milk fever (e.g., by inducing a mild metabolic acidosis due to the supplementation of the close-up diet with anionic salts),
- to provide a clean and spacious calving pen.

After parturition, to achieve a high intake it is necessary:

- to afford an optimal climate with a high ventilation rate,
- to offer superior, tasty feedstuffs with a high energy density on the one hand and a sufficient amount of structure on the other,
- to organize a sophisticated bunk management allowing each cow of the group an unimpeded access to feed at each time of the day,
- to provide clean water in sufficient quantity,
- to avoid the exposure of the cows to social stress,
- to maximize cow comfort in respect to stall design, floor surface, bedding, waste management and regular claw trimming,
- to allocate a separate housing area for the fresh cows enabling an intensive monitoring of the health status during this extremely sensible period and promoting an early treatment of any health problems.

This list points up that the endeavours to increase both the level of milk yield and dry matter intake over the last decades went ahead with a considerable improvement of husbandry conditions for the majority of the dairy cows. However, the consideration of each of these factors requires a high awareness and expertise of the farmer, constructional prerequisites and adjusted work routines adopted to the unique situation of each specific farm. The ability to implement these tools into everyday life appears to be the predominant difference between farmers running high-producing or low-producing dairy enterprises.

Not only the conditions listed above favour a high intake; concomitant with breeding efforts to increase milk yield the size and body weight of the cows increased and this development still proceeds (Figure 1). Thereby, also feed intake capacity increased. This desirable effect has to be taken into account by those planning new stalls – today's cows are not comparable to those from yesterday!

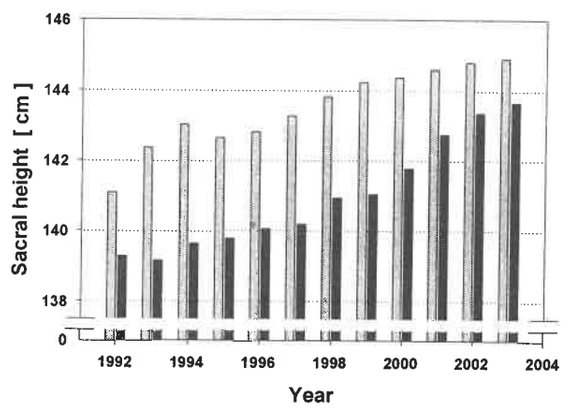

Figure 1:

Development of the sacral height of heifers during the past ten years in Schleswig-Holstein (federal state of Germany); grey bars: German Holstein Friesian (N = 48,420 totally); dark bars – German Red Holstein (N = 26,170 totally); (32)

Perspectives

At present, even a high level of milk yield seems to be conformable with a proper herd health status as long as the environment and management of the herd is appropriate. According to statements of animal breeders, a selection plateau in respect to milk yield is not yet reached even in Holstein Friesians (28). The question remains whether a further increase of milk yield far above the level reached at present is a striking goal. Doubts are admissible. The mean expected useful life of a dairy cow decreased within the last decades considerably reaching now only 2.7 lactations (21). As the majority of cows are culled due to unsatisfactory fertility or health problems (and not because of low milk yield), the low mean number of lactations reflects considerable problems even with young animals. Nevertheless, a strict correlation between average milk yield on a farm and culling rate has not been found (2, 34). In contrast, looking at individual cows, the highest producers have the lowest risk and the lowest producers have the highest risk of being culled (33).

Having in mind the outstanding importance of the NEB for the risk of peripartal diseases, any raise in milk yield necessitates a concomitant increase in feed intake capacity to avoid further health risks. However, based on a compilation of literature data, the genetic correlation between milk yield and feed intake is only 0.64 (30, 31). Accordingly, the gap between energy requirement and energy input increases with further selection towards extremely high-producing cows, i.e., a breakeven point exists for a justifiable enhancement of milk production. Individual cows will always be able to exceed considerably the average level of milk production. One reason is the enormous variability between individual cows within a herd to cope with a period of severe NEB and to manage successfully the endocrinological and metabolic rearrangements during the fresh cow period. However, the health status of the majority of the cows is put on a higher risk with increasing milk yields. Finally, also from the economic point of view an unreflected further selection of cows for increased milk yield seems not to be prudent as the financial surplus per unit increase in milk yield decreases with each further increment (11).

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W6. Milchviehfütterung unter systemorientierten Gesichtspunkten in der Ökologischen Landwirtschaft (Feeding dairy cows in organic agriculture from a system-oriented point of view) A. Sundrum und U. Schumacher - Kassel/Bielefeld

Ausgangsbedingungen: Die Ökologische Tierhaltung ist durch die EG-Verordnung (EWG Nr. 1804/1999) eindeutig definiert. Sie verfolgt das Ziel einer tiergerechten und umweltverträglichen Erzeugung qualitativ hochwertiger Produkte in einem weitgehend in sich geschlossenen Nährstoffkreislauf. Die Umsetzung dieses Organisationsprinzips basiert auf einem ausgewogenen Verhältnis zwischen den verschiedenen Betriebszweigen innerhalb der Grenzen des Betriebssystems. Die Fütterung dient nicht primär der Leistungsmaximierung, sondern der effizienten Verwertung der betrieblich verfügbaren Nährstoffressourcen mit dem Ziel einer gesamtbetrieblichen Wertschöpfung. Entsprechend sind die Leistungen der tierischen Erzeugung als Teilleistung eines Gesamtsystems zu bewerten. Unterschiede zur herkömmlichen Tierernährung bestehen vorrangig auf der Betriebsebene hinsichtlich der Zielsetzungen und der daraus abzuleitenden Optimierungsstrategien.
Potenziale: Durch die Beschränkungen beim Futterzukauf werden solche gesundheitliche Risiken für die Nutztiere minimiert, die mit einem ungemäßigten Einsatz von Produktionsmitteln (z.B. Kraftfutter) einhergehen. Die deutliche Reduzierung des Nährstoffimports in den Betrieb und die Nutzung pflanzenbaulicher Quellen für die N-Versorgung (Leguminosenanbau) hat bei guter Betriebsorganisation eine erhebliche Reduzierung von Nährstoffausträgen zur Folge (1). Auch besteht ein relevanter ökonomischer Anreiz zur Umsetzung emissionsmindernder Maßnahmen, da aufgrund der begrenzten Verfügbarkeit von Düngemitteln jede Verringerung von Nährstoffverlusten einer Erhöhung der ertrags- und einkommenswirksamen Düngemittelverfügbarkeit gleichkommt.
Problembereiche: Die Fütterung basiert auf dem betriebseigenen, leguminosenbetonten Futterbau. Dies setzt eine ausgeglichene, auf den Tierbestand und dessen Leistungsniveau abgestimmte Fruchtfolge voraus und bedingt eine hohe Abhängigkeit von den spezifischen Gegebenheiten des Standorts sowie der Betriebsstruktur. Zu den Kernproblemen der Fütterung in der Ökologischen Landwirtschaft gehört daher die begrenzte Verfügbarkeit von qualitativ hochwertigen Nährstoffen. Eine ausbalancierte und leistungsorientierte Nährstoffversorgung der Tiere kann nicht zu jeder Zeit auf allen Betrieben gewährleistet werden. Infolge muss auf ökologisch wirtschaftenden Betrieben das herkömmliche Primat der Leistungsmaximierung deutlich relativiert werden.
Wissenschaftliche Fragestellungen: Ausgehend von den unterschiedlichen Rahmenbedingungen und Zielsetzungen können wissenschaftliche Ergebnisse, die im konventionellen Betriebskontext ermittelt wurden, nur bedingt auf die betriebliche Situation in der Ökologischen Landwirtschaft übertragen werden. Auch sind Systemvergleiche zwischen ökologischer und konventioneller Wirtschaftsweise aufgrund der großen Variation innerhalb der Produktionsweisen nur von geringer Aussagekraft. Unterschiede gegenüber der herkömmlichen Milchviehfütterung betreffen insbesondere Strategien zur Förderung der Tiergesundheit und zur Reduzierung von Umweltbelastungen.
Fütterungsstrategien, die primär auf eine Minimierung fütterungsbedingter Erkrankungsrisiken abzielen, unterscheiden sich erheblich von solchen, die auf eine maximale Ausschöpfung des genetischen Leistungspotentials setzen und das Erkrankungsrisiko erst in zweiter Linie berücksichtigen. Die Fütterungsstrategien in der Ökologischen Tierhaltung sind kontextvariant, d.h. was im einzelbetrieblichen Kontext sinnvoll ist, kann für andere Betriebe kontraproduktiv sein.
Abgesehen von der Vermeidung von Nährstoffimbalancen, welche die Anpassungsfähigkeit der Tiere übersteigern, sind herkömmliche Fütterungsstrategien, die auf eine Minimierung von Nährstoffüberschüssen in der Futterration abzielen, in der Ökologischen Tierhaltung nicht zielführend. Aus ökologischer Sicht ist nicht maßgeblich, wie der Anteil von Nährstoffen in den Wirtschaftsdüngern reduziert werden kann, sondern inwieweit die Nährstoffe im Betriebskreislauf gehalten und effizient genutzt werden können. Das Emissionsminderungspotenzial resultiert aus dem Zusammenspiel diverser Einzelmaßnahmen auf der Betriebsebene und im vorgelagerten Bereich. Die Effizienz und Plausibilität umweltrelevanter Maßnahmen erschließt sich nicht aus einer sektoralen

Betrachtung, sondern erst im betrieblichen Kontext, in der wechselseitigen Bedingtheit mit der pflanzlichen Erzeugung und aus der Effizienz bei der Nutzung wirtschaftseigener Düngemittel.
Analog zum tierischen Organismus lässt sich aus der alleinigen Kenntnis von Inputgrößen keine hinreichende Vorhersage der Outputgrößen des *Betriebsorganismus* ableiten. So wie die Stoffumsätze im Verdauungstrakt können die Stoffumsätze auf der Betriebsebene nicht ohne Kenntnis der spezifischen Prozesse in den einzelnen Bereichen des landwirtschaftlichen Betriebes erfasst und beurteilt werden. Jedes Teilkompartiment unterliegt eigenen Gesetzmäßigkeiten und Einflussgrößen. Aufgrund der vielfältigen interagierenden Wechselwirkungen sind daher verallgemeinernde Aussagen zur Nährstoffdynamik wissenschaftlich nur bedingt belastbar. Das Hauptaugenmerk der Forschung fokussiert daher in erster Linie auf Optimierungsstrategien innerhalb von Betriebssystemen.
Forschungsbedarf: Die Ökologische Tierhaltung erfordert eine Neuorientierung nicht nur in der praktischen Abläufen, sondern auch in der wissenschaftlichen Bearbeitung von Fragestellungen. Aus ökologischer Sicht besteht ein erheblicher Forschungsbedarf in der Entwicklung von Instrumentarien zur Systemanalyse, d.h. zur Erfassung des betriebsspezifischen Ist-Zustandes, dessen Analyse und der Erarbeitung optimaler systembezogener, den Eigenheiten der Rahmenbedingungen entsprechenden Abläufen innerhalb der jeweiligen Betriebssysteme. Zu den spezifische Fragen der Fütterung gehören:

- Verallgemeinerungsfähigkeit von Ergebnissen aus standardisierten konventionellen und ökologischen Versuchsanstellungen auf die jeweilige betriebliche Situation,
- Toleranz der Tiere gegenüber Nährstoffimbalancen unter besonderer Berücksichtigung der Tiergesundheit sowie hinsichtlich der tierischen Leistungen und der Produktqualität,
- Risikominimierung bezüglich fütterungsbedingter Erkrankungen,
- Möglichkeiten der Verbesserung der Produktqualität,
- Potenziale bei der Bereitstellung qualitativ hochwertiger Futtermittel,
- Verbesserung der Versorgung mit Spurenelementen und Vitaminen,
- Genotyp/Umweltinteraktionen im Hinblick auf die Futteraufnahme und -verwertung,
- Aufbereitung von Futtermitteln zur Steigerung der Futteraufnahme und Nährstoffverfügbarkeit,
- Ökonomische Bewertung der fütterungsrelevanten Mehraufwendungen.

Schlussfolgerungen: Die Entwicklung der Tierhaltung innerhalb der Ökologischen Landwirtschaft steht erst in den Anfängen. Aus den aktuellen Erhebungsdaten können folglich keine weitreichenden Schlussfolgerungen sondern nur Einschätzungen hinsichtlich der Produktions- und Qualitätspotenziale abgeleitet werden. Die bewusste Herbeiführung einer Ressourcenknappheit erfordert eine erhebliche Umstrukturierung des landwirtschaftlichen Betriebes, um mit den betriebsspezifischen Ressourcen eine möglichst effiziente Nährstoffnutzung zu erzielen. Als systemorientierter Ansatz kann die Ökologische Tierhaltung nicht allein aufgrund einzelner verfahrenstechnischer Maßnahmen, sondern nur im betrieblichen Kontext beurteilt werden. Die Nutztierhaltung unterliegt dabei anderen Steuerungs- und Regelgrößen als in der herkömmlichen Tierproduktion. Zielkonflikte bei der Nutzung begrenzt verfügbarer Ressourcen müssen betriebsintern geregelt werden, d.h. Folge- und Umweltwirkungen werden internalisiert und nicht - wie vielfach üblich - externalisiert. Der systemorientierte Ansatz der Ökologischen Tierhaltung ist allerdings nur dann wirtschaftlich tragfähig, wenn die Wertschöpfung nicht nur über die Produktmengen sondern auch über eine gesamtbetriebliche Wertschöpfung durch Produkte von hoher Qualität und in Verbindung mit Tier- und Umweltschutzleistungen erfolgt und diese Leistungen angemessen honoriert werden.

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W7. Fütterungscontrolling in ökologisch wirtschaftenden Milchviehbetrieben in Nordrhein-Westfalen (Feedingcontrolling in organic dairy farms in North-Rhine-Westfalen) C. Drerup*, K. Kempkens – Wiedenbrück-Bonn

Ausgangssituation: Bio-Milchviehbetriebe in NRW liefern bis auf wenige Ausnahmen ihre Milch an Molkereien mit ausschließlicher oder gesonderter Bio-Milchabholung. Entsprechend muss sich die Fütterung an den Richtlinien der EU-Bio-Verordnung oder bei Mitgliedschaft auch an den meist strengeren Fütterungsrichtlinien der Bio-Verbände (Bioland, Demeter, Naturland) orientieren. Kennzeichnend für die Winterrationen ist der hohe Grassilageanteil von Grünland- oder Acker-Kleegrasflächen mit 60 bis 100% der Grundfutter-TS. Wo ackerbaulich möglich wird mit Maissilage und/oder Ganzpflanzensilage die Energiekonzentration angehoben. Als Saftfuttermitteln stehen begrenzte Mengen an Bio-Preßschnitzeln und Bio-Sojapülpe zur Verfügung. Konventioneller Biertreber wird nach einer Übergangszeit bis 2004 auf den verbandsgebundenen Milchviehbetrieben nicht mehr eingesetzt werden. Bei den Kraftfuttermitteln herrschen Eigenmischungen mit Getreide, Körnerleguminosen und Eiweißkomponenten als Kuchen von Bio-Ölmühlen vor. Thermisch oder druck-thermisch aufgeschlossene Körnerleguminosen sind aktuell noch in der Erprobungsphase. Im Sommer sind alle Weidevarianten von der Ganztagsweide bis zur stundenweisen Siesta-Beweidung vorzufinden. Mit Zunahme der Milchleistung in den vergangenen Jahren wurde der Weidegang zurückgefahren zugunsten einer stärkeren Stallfütterung. Aktuell erreichen spezialisierte Milchviehbetriebe eine Milchleistung von durchschnittlich 7.200 kg/Kuh (Spanne von 4.500 kg/Kuh bis 9.400 kg/Kuh).
Mit der Zielsetzung, Anhaltspunkte für eine Verringerung der Bestandsergänzungsrate zu finden, wurden vor 10 Jahren die Gründe für den Abgang jeder Milchkuh im Zeitraum „Wirtschaftsjahr 1993/94“ auf 15 Bio-Milchviehbetrieben ausgewertet. Zur besseren Orientierung lagen Vergleichswerte von 170 konventionell wirtschaftenden Betrieben vor, die im Jahr davor in gleicher Weise die Abgangsgründe untersucht hatten. Im Ergebnis kristallisierten sich als Hauptabgangsgründe die Störung der Fruchtbarkeit und eine mangelhafte Eutergesundheit heraus. Erst an sechster Stelle waren Stoffwechselerkrankungen Hauptabgangsgrund, wobei dieser im Vergleich zu den konventionellen Betrieben wesentlich seltener genannt wurde. Mit dem Wissen von heute muss rückblickend gefragt werden, ob die Abgangsgründe Fruchtbarkeitsstörungen, Eutererkrankungen und Stoffwechselerkrankungen auf Unzulänglichkeiten in der Fütterung als eigentlichen Grund zurückzuführen waren. So wird heute das vermehrte Auftreten von Zysten, Euterentzündungen mit dem bakteriellen Befund staph.uberis und Milchfieber ganz wesentlich mit der Fütterung in Verbindung gebracht. Es gibt viele Anhaltspunkte, auch aktuell von dieser Reihenfolge an Abgangsgründen auf Bio-Milchviehbetrieben auszugehen, wobei die gestiegene Milchleistung noch ein häufigeres Auftreten von Stoffwechselproblemen nahe legt. Um hier Besserung zu erzielen, ist der Fütterungsoptimierung und dem rechtzeitigen Erkennen von Stoffwechselproblemen mehr Beachtung zu schenken.
Situationsbeschreibung: Aktuell ist die Fütterung auf Bio-Milchviehbetrieben – in NRW überwiegend verbandsgebunden – wie folgt zu charakterisieren: Es überwiegen Boxenlaufstallsysteme mit einem Fressplatz-Tier-Verhältnis von 1:1. Seit Ende der 90er Jahre hat der Futtermischwagen zunehmend Verbreitung gefunden, mit dem in der Regel getreide- oder körnerleguminosenaufgewertete Grundrationen am Trog angeboten werden. Die Zuteilung von Milchleistungsfutter erfolgt aus Kostengründen leistungsgerecht über Transponder oder manuell am Trog. Die Grundration wird überwiegend auf die aktuelle, durchschnittliche, tägliche Milchleistung plus 2-3 kg Milch/Tag eingestellt. Besonderes Augenmerk liegt bei der Grundration auf das ausreichende Angebot von pansenverfügbarer Energie (pansenverfügbare Kohlenhydrate, pKH) und pansenverfügbarem Eiweiß (RNB), damit die berechnete, maximale Grundfutteraufnahme auch möglich wird. Als untere Grenze für eine ausreichende Versorgung werden mind. 130 g pKH/kg TS und ein RNB in der Gesamtration von + 20 g N angenommen. Eine lt. Rationsberechnung ausgeglichene Mineralstoffbilanz

(Mengenelemente) wird auch aus Gründen der Grundfutteraufnahme als wesentlich angesehen. Hinsichtlich der Notwendigkeit einer über das Jahr durchgängigen, mineralfuttergebundenen Versorgung mit Spurenelementen und Vitaminen bestehen dagegen auf Seiten der Praxis Zweifel. Die Kraftfuttermischungen erreichen aufgrund des hohen Getreideanteils leicht die Energiestufe 4, bleiben aber wegen der teuren Eiweißkomponenten in der Regel unter 160 g nXP/kg bei positivem RNB. Der hohe Grundfutteranteil in der Gesamtration (in der Praxis mind. 60% der TS aus Grundfuttermitteln) stellt selbst bei hohen Kraftfuttermengen eine ausreichende Rohfaser- oder Strukturversorgung sicher. Als Probleme sind aus der Praxis dagegen die Erreichung einer ausreichenden Dichte an Energie (beständige Stärke) und Eiweiß (nXP, UDP) zu nennen. Die Vermutung einer eher knappen Eiweißversorgung wird durch die Hoftorbilanzen viehstarker Bio-Betriebe gestützt, die im Gegensatz zu konventionellen Milchviehbetrieben in der gleichen Region praktisch ausgeglichene N-Bilanzen aufweisen.

Verfahren des Fütterungscontrollings: Seit Mitte 2000 werden gegen Gebühr in einigen Bio-Milchviehbetrieben in NRW zur Kontrolle und Feineinstellung berechneter Futterrationen die monatlichen Milchleistungsprüfungen mit dem Herdenmanagementprogramm ZMS32-Version 4.0 der DSP-Agrosoft GmbH in Paetz ausgewertet und beratend begleitet. Die Vorteile dieser Auswertungsroutine liegen in einer chronologischen Übersicht aller MLP-Ergebnisse, dem schnellen Zugriff auf umfangreiche Einzeltierdaten und der automatischen Ergebnisprüfung anhand veränderbarer Grenzwerte. Nachteilig aus Sicht der Praxis ist sicherlich die irritierende Fülle an Auswertungsmöglichkeiten und Ergebnissen, die von Seiten der Beratung durch eine einzelbetriebliche Zielformulierung und Kommentierung zu begrenzen ist. Die Anwendung dieses Programms wird von der Praxis aber fast durchgängig begrüßt, da das Erkennen von Mängeln unter anderem in der Fütterung erleichtert und beschleunigt wird. Spezialisierte Berater setzen es mit zwar hohem Aufwand, aber auch nachhaltigem Erfolg ein. Sie sind dabei aber auf eine intensive Kooperation der Betriebsleiter angewiesen. Aus der Erfahrung sind folgende Aussagen möglich:

- Bio-Milchviehbetriebe können bei gutem Management, gutem Tiermaterial und guten Fütterungsvoraussetzungen konstant Leistungen zwischen 8.000 und 9.000 kg Milch pro Kuh und Jahr erreichen.
- Häufig abgekoppelt vom Leistungsstand der Herde wird der konventionelle Zielwert beim Milcheiweißgehalt in der Hochlaktation (mind. 3,2%) von (zu) vielen Kühen nicht erreicht.
- Der Verdacht auf Ketose aufgrund eines zu weiten Fett-Eiweiß-Verhältnisses ist wesentlich häufiger als der Verdacht auf azidotische Verhältnisse.
- Die niedrigen Milchharnstoffwerte lassen trotz kleehaltiger Grundfuttermittel keinen Raum für die Vermutung einer Stoffwechselbelastung durch Eiweißüberfütterung im Pansen. Stoffwechselprobleme, wie sie sich auch in Problemen mit der Eutergesundheit und der Fruchtbarkeit ausdrücken, sind eher im Bereich der Energieversorgung incl. der Versorgung mit darmverfügbarem Eiweiß zu sehen.

Folgende Wünsche und Fragen bleiben offen:

- Wie können die Abläufe im Pansen im Hinblick auf die Erreichung des ernährungsphysiologischen Optimums vorherbestimmt und später kontrolliert werden?
- Welche Bedeutung für die Stoffwechselstabilität haben besondere, pansenstabile Energie- und Eiweißkomponenten, die nur mit energetisch fragwürdigem Aufwand hergestellt werden können?
- Wie sehen Fütterungsstrategien aus, die die Milchleistung in der Hochleistungsphase gezielt begrenzen zugunsten einer besseren Persistenz und Langlebigkeit?

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W8. Spezielle Einflüsse der Fütterung auf die Eutergesundheit im ökologischen Landbau

(Dairy nutrition and bovine mastitis on organic farms). R. Andersson* - Osnabrück

Mastitis ist auch in der Milchviehhaltung auf ökologisch wirtschaftenden Betrieben eines der Hauptprobleme. Als typische *Faktorenkrankheit* tritt sie immer dann auf wenn mehrere ungünstige Faktoren zusammentreffen. Mikroorganismen spielen dabei eine wesentliche Rolle. Euterpathogene Mikroorganismen sind ständig in sehr großer Zahl im Melk- und Haltungsbereich präsent und stellen daher ein kontinuierliches Risiko für die Eutergesundheit dar. Funktionieren die Abwehrkräfte, bleibt das Euter gesund; immunschwächende Situationen begünstigen die Entstehung der Mastitis und bestimmen die Dynamik des Krankheitsverlaufes nach einer Infektion.

Die Förderung sowie Sicherung der Gesundheit und nicht die Bekämpfung der Krankheit ist ein erklärter Ansatz der ökologischen Nutztierhaltung. In diesem Zusammenhang spielt auch die Fütterung eine maßgebliche Rolle.

Infolge eines nicht ausgeglichenen Energiestoffwechsels **(subklinische Ketose)** und Proteinstoffwechsels **(hohe Harnstoffgehalte)** treten häufig Zellzahlanstiege auf. Es wird diskutiert, dass aufgrund einer Energieunterversorgung u.a. die Phagozytoseleistung des unspezifischen Immunsystems gemindert wird.

Dieses kann einen Teil der gehäuften Probleme mit der Eutergesundheit am Beginn und Ende der Laktation erklären; Mastitis wird als Teil des *Partus Syndroms* gesehen. Verfetten die Tiere während der Trockenstehzeit, so erhöht sich die Gefahr von Geburts- und Stoffwechselproblemen nach der Kalbung. Ein Anstieg von Ketokörpern bei verfetteten und energetisch unterversorgten Tieren nach der Geburt senkt die ohnehin reduzierte Futteraufnahme zu Beginn der Laktation noch weiter. Das Problem der Energieunterversorgung wächst, da erhöhte Acetongehalte im Blut negativ auf die Futteraufnahme wirken.

Gleichzeitig wirken Ketokörper und besonders Harnstoff störend auf die gewünschte, symbiotische milchsäurebildende Zitzenflora.

Die auch im ökologischen Landbau steigenden Milchleistungen fordern entsprechende Rationsgestaltungen. Infolge **mangelnder Struktur** sowie hoher Anteile leicht verdaulicher Kohlenhydrate in der Ration können sehr leicht azidotische Verhältnisse eintreten.

Neben Mängeln in der bedarfsgerechten Energie-, Protein- und Strukturversorgung, die indirekt über die Tiergesundheit auf das Euter wirken, sind einige Rationskomponenten hervorzuheben, die in direktem Zusammenhang mit der Eutergesundheit diskutiert werden.

Natriummangel bzw. ein **K:Na – Verhältnis**, welches > 10 : 1 ist, beeinträchtigt die Funktionsfähigkeit der Zitzenschleimhaut. Hohe Kaliumgehalte oder Mängel in der **Kalziumversorgung** können die Ausbildung von Euterödemen fördern, die wiederum aufgrund der Strichkanalweitung im Zusammenhang mit Mastitis stehen.

Bei den Spurenelementen liegen sehr viele gesicherte Erkenntnisse bezüglich der **Selenversorgung** vor. Selen, ß-Carotin und **Vitamin E** spielen eine sehr große Rolle bei der Kontrolle freier Radikale und dienen somit letztlich dem Zellschutz.

Eine besondere Aufgabe kommt der **Zinkversorgung** im Rahmen der Keratinneubildung im Strichkanal nach dem Melken zu. Keratin kleidet den Strichkanal aus, wird aber durch den Melkvorgang in erheblichem Maße entfernt und muss ständig ersetzt werden. Im ökologischen Landbau, bei Leistungen unter 8.000 kg Milch, dürfte es keine Mangelzustände für Zink geben. Allerdings ist die Verfügbarkeit von Zink aus dem Futter von anderen Futterkomponenten abhängig. Hier ist vor allem Eisen zu nennen, welches in Graskonserven, teils durch hohe Anteile von Erde, sehr hohe Gehalte erreichen kann. D.h., bei hohen Eisengehalten kommt es trotz der eigentlich ausreichenden Zinkkonzentration im Futter zu einer nicht optimalen Zinkversorgung der Kuh.

Provitamin A (ß-Carotin) und **Vitamin A** sind wesentliche Faktoren des Zellschutzes und der Leistungsfähigkeit der Leukozyten. Im Spätwinter treten regelmäßig Probleme mit der Versorgung

der Kühe auf, weil die Konserven (Silage, Heu) aufgrund der Lagerdauer erhebliche Verluste an ß-Carotin aufweisen.
Vitamin E ist immer in Verbindung mit Selen zu sehen, hat aber auch eine eigenständige Bedeutung im Entzündungsgeschehen. Eine optimale Versorgung gestaltet sich aufgrund der Lagerungsverluste, besonders im Heu, ggfs. im Winterhalbjahr schwierig.
Phytoöstrogene kommen vor allem in Leguminosen oder in Schimmelpilzen vor. Hier handelt es sich um eine recht große Gruppe an Wirkstoffen, die je nach Konzentration im Futter eine positive, aber i.d.R. negative Wirkung auf das Immunsystem haben.
Mykotoxine werden in der Wiederkäuerfütterung bislang relativ wenig beachtet. Es ist aber auch bei Rindern davon auszugehen, dass Mykotoxine auf die Mikroorganismen im Pansen wirken, Zellen schädigen und dass sie bei entsprechender Konzentration das Immunsystem beeinträchtigen.

Das **theoretische Fütterungsmanagement** darf nicht überschätzt werden. Nicht die errechnete, optimal zusammengesetzte Ration ist entscheidend, sondern die wirkliche, den Tieren vorgelegte Ration, muss artgerecht und bedarfsgerecht sein. Häufig weicht aber die errechnete Ration von der vorgelegten Ration ab, diese ist wiederum nicht mit der gefressenen identisch.

Die **Wasserversorgung** muss insbesondere bei hohen Temperaturen ausreichend und artgerecht sein. D. h. selbst ein Wasserbedarf > 150 l/ Tag muss an heißen Tagen gedeckt werden können. Da die Kuh ein Saugtrinker ist, der große Mengen Wasser in einem Zuge aufnehmen möchte, kommen Schalentränken für Milchkühe nicht in Frage - zu empfehlen sind ausreichend dimensionierte Trogtränken mit guter Reinigungsmöglichkeit, z.B. Kipptränken. Kühe reduzieren ihre Wasseraufnahme, wenn sie das Wasser nur in kleinen Portionen aufnehmen können. Bei der Weidehaltung ist daran zu denken, dass die Wege zur Tränke nicht zu weit sind. Weiter als 50 – 100 m laufen Kühe ungern zur nächsten Tränke, dieses gilt auch an heißen Tagen, an denen sie es besonders nötig hätten.
Eine reduzierte Wasseraufnahme führt zu einer reduzierten Futteraufnahme und somit zu Defiziten in der Nährstoffversorgung.

Bei mittleren Milchleistungen und Weidegang auf kräuterreichen Weiden können viele der genannten Risiken sehr gut minimiert werden. Steigen die Leistungen und werden Konserven eingesetzt, gewinnt die Fütterung hinsichtlich der Eutergesundheit an Bedeutung. Eigene Erhebungen werden durch aktuelle Ergebnisse aus Futteranalysen des Ökoringes Niedersachsen sowie der LWK-Hannover dahingehend bestätigt, dass die Qualität der Grassilagen in vielen Fällen für Milchkühe nur bedingt geeignet ist, 9 von 35 Proben sind im Energiegehalt beim ersten Schnitt unter 5,8 MJ NEL, davon liegen 5 Proben im Energiegehalt unter 5,6 MJ NEL.
Aufgrund der Schranken des Ökologischen Landbaus können die Rationen nicht unbegrenzt durch Import von Nährstoffen oder Zusätzen optimiert werden. Folglich muss jeder Betrieb die Milchviehherde, deren Leistung auf seine individuellen Futtergrundlagen abstimmen. Das Optimum ist nicht das Maximum an Leistung, sondern eine stabile, belastbare Gesundheit.

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W9. Survey for feeding and metabolic health of dairy cows in ecological producing farms

(Erhebung zur Fütterung und Stoffwechselgesundheit von Milchkuhherden in ökologisch produzierenden Betrieben). Katrin Mahlkow-Nerge* - Kiel

Numerous hints from practice show, that the protein supply according to common recommendations may represent a problem in ecological producing farms. Furthermore there are rarely data in literature about metabolic health of dairy cows in ecological producing farms although metabolic profiles in diagnostics of the herds are used increasingly.
With this survey production data have been collected in connection with metabolic parameters from fresh calved cows and heifers from ecological producing farms. These data are compared with the results of conventionally managed high yielding dairy cows of a survey from the year 2000.

Method:

The agricultural chamber of Schleswig - Holstein and an advisory service for ecological production (Versuchs- und Beratungsring ökologischer Landbau Schleswig-Holstein e. V.) have analyzed five ecological producing farms with an average milk production of 7.900 kg (6.590-9.120 kg). Within the project parameters of ration composition, nutrient content, mineral content and metabolic parameters were analysed.

Results:

The data show very large differences in feeding management as well as in the nutrient and mineral supply between the ecological producing farms.
Especially the protein supply according to recommendations is a problem. This can be seen e.g.in the lower urea values in milk and blood.
The relative high starch contents in the total ration are a result of using mainly leguminosae like pea and bean with a high starch content as protein concentrate.
On the basis of the milk yield there were no metabolism problems expected, but the milk- and blood parameter show metabolic disturbances.
The supply with minerals was very different between the farms. Blood parameters usually showed the deficiencies in the supply of trace elements. On the other hand a surplus or the correct quantity in the ration was not always reflected in the blood values.

Conclusion:

In the first weeks of lactation there can frequently be a lack of energy and protein for cows and heifers in ecological producing farms. This danger should be met by consistent feeding strategies for dry cows (splitting the dry period in two parts with more emphasis on milk fever- and ketosis prevention) and better ration optimisation, during the first part of lactation (high digestible fibre/structure, limiting starch content).
In agreement with other literature(1,2) is to assume that metabolic regulations and their consequences take place on similar levels even if there are great differences in milking capacity.
Very high milk yields can be a problem for ecological producing farms because in their feed is not enough protein.

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W10. Aspects of nutrient supply in calves kept under organic farming conditions (Problematik der Nährstoffversorgung von Kälbern in extensiv bzw. ökologisch wirtschaftenden Betrieben).

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In organic farming as well as in suckler herds milk is the main nutrient source during the first three month of life. It is insinuated, that milk and the progressive intake of pasture, hay or silages will be optimal and sufficient in this period. Supplementations with minerals and vitamins are not obsolete (suckler herds) or partly forbidden (organic farming). The aim of the study was therefore, to estimate exemplary the mineral and vitamin supply in calves of suckler herds during the first 3 month of life.

Methods: The first part of the trial was focussed on estimating basal data for milk and feed intake of 142 calves (53%♀; 47%♂) during the winter feeding period (December to March) between the 7th and 111th day post partum under farming conditions. In each yard unit an average of 35 cows (Fleckvieh; 525 ± 48 kg body weight) and their crossbred calves were housed in a ventilated stable with a total of 4 units. Lying, water supply and supplementary feed for calves and cows was separately. Dry cows and cows until 6 days after calving were fed individually and from the 7th day post partum onwards per unit at 6:30 and 14:30 h. The daily requirement was met by a total mixed ration (20 kg grass-silage, 1 kg creep feed, 1 kg hay, 100 g mineral feed). Calves were offered milk and hay ad libitum. The milk yield was estimated by supervising the body weight of 15 cows in 14 day intervals with regard to the daily energy intake. Body loss or gain of 1 kg was adjusted to 5.8 litre milk more or less. The average hay intake of calves was documented daily per unit. The nutrient supply of calves was calculated by the analysed composition of milk and hay with regard to the estimated daily intake and the blood plasma concentrations (day 5, 30 and 80 post partum). The second part of the trial was focussed on improving the mineral and vitamin supply (ß-carotene, vitamin E) in calves (51%♀; 49%♂) during the first 3 month of life. The following feeding strategies were studied during the winter period: Improving the mineral and vitamin foetus store or the milk concentration by a modified mineral feed for dry cows 8 weeks ante partum (n= 480) and cows from the 7th day post partum (n=418) onwards or supplementing calves from the 7th day of life onwards with a mineral or mineralised creep feed, respectively. Each treatment consisted of 3 replicates with an average of 34 mother cows. During the pasture-period (April to October) calves (51%♂; 49%♀) of 2 herds (125 cows per herd) without or with supplement were studied, respectively. The dry matter intake of calves during the pasture period was calculated by using the date given in table 1. Mineral and vitamin analyses in pastures, hay, creep and mineral feed, milk and blood plasma were in accordance to the VDLUFA recommendations.

Results: The results of the calculated dry matter intake of calves are summarized in table 1.

Table 1 Estimated daily dry matter intake of calves during the first 3 month of life under housing conditions

	Average body weight (kg)	Dry matter intake (kg/d)		
Month of life		total	milk	hay
1st	63.8 ± 5.9	1.8	1.7	0.1
2nd	82.5 ± 9.0	2.0	1.8	0.3
3rd	103.3 ± 23.7	2.3	1.7	0.5

Based on these data and on the composition of the nutrient sources the average daily intake was calculated (table 2). Official recommendations (1) were documented in parenthesis. With the exception of energy, crude protein and sodium milk and hay are not sufficient sources for nutrient supply. Corresponding results (except vitamin E and ß-carotene) were found under pasture conditions. In order to overcome the mineral and vitamin deficiency dry and lactating cows were fed daily with 100 g of a modified mineral feed (per kg premix: 1400 mg Zn-, 500 mg Cu-, 25 mg Se-methionate, 22820 mg α-thocopherol, 300 mg ß-carotene). The blood-plasma concentrations of Zn, Cu, Se, ß-carotene and α-tocopherol in neonatal calves were higher than in cows of the control group fed with

Table 2: Nutrient daily intake of calves during the first 3 month of life

	Month of life		
	1st	2nd	3rd
Body weight (kg)	63.8 ± 5.9	82.5 ± 9.0	103.3 ± 23.7
Body weight gain (g)	750 ± 180	623 ± 230	690 ± 250
Crude Protein (g)	473 ± 25 (247)	501 ± 20 (274)	437 ± 19 (315)
ME (MJ)	33.0 ± 1.3	34.6 ± 1.1	31.2 ± 1.5
Ca (g)	16.2 ± 1.2 (15.4)	13.9 ± 1.9 (17.1)	9.54 ± 1.4 (19.7)
P (g)	10.6 ± 1.3 (7.2)	11.9 ± 1.9 (8.0)	12.1 ± 1.5 (9.2)
Mg (g)	1.54 ± 0.4 (2.6)	1.43 ± 0.4 (2.9)	1.61 ± 0.5 (3.3)
Na	11.4 ± 0.7 (2.1)	11.5 ± 0.6 (2.3)	9.7 ± 0.4 (2.7)
Fe (mg)	13.8 ± 3.1 (36)	22.8 ± 2.9 (100)	50.6 ± 3.5 (126)
Cu (mg)	7.75 ± 0.8 (18)	8.67 ± 0.7 (20)	8.70 ± 0.8 (23)
Mn (mg)	2.74 ± 0.72 (90)	5.92 ± 0.79 (100)	32.4 ± 0.67 (115)
Zn (mg)	44.9 ± 3.2 (90)	51.2 ± 2.9 (100)	29.5 ± 3.4 (115)
Se (mg)	0.25 ± 0.07 (0.27)	0.24 ± 0.08 (0.30)	0.20 ± 0.07 (0.35)
Vitamin E (mg)	9.6 ± 1.0 (27)	10.2 ± 1.1 (30)	11.5 ± 1.2 (34.5)
ß-carotene (mg)	0.96 ± 0.06 (27)	0.96 ± 0.07 (30)	0.80 ± 0.07 (34.5)

the unmodified mineral feed (per kg premix: inorganic Zn (1400 mg), Cu (1500 mg), Se (40 mg), 350 mg α-thocopherol). However, after 14 days the values in plasma of calves decreased and were within the concentrations of the control. Feeding the modified mineral feed to cows from the 7th day post partum onwards Zn, Cu, Se, vitamin E and ß-carotene in the milk were with 0.6, 2.0, 0.01, 0.7 and 0.2 mg per litre higher compared to the control. Because of the limited milk intake the demands (Cu excepted) were not compensated. Therefore a mineral feed was offered calves from the 7th day of life onwards (per kg: 165 g Ca; 60 g P; 55 g Na; 20 g Mg; 1400 mg Fe; 500 mg Mn; 750 mg Cu; 50 mg J; 16 mg Co; 50 mg Se). The daily intake during the first three month was with an average of 25 g per calf lower than expected and therefore still not sufficient to meet the requirement. However, the intake of hay was significantly higher compared to calves without mineral supplementation. In order to overcome the low intake, the premix was mixed with a creep feed. The intake was now sufficient to meet the recommended levels. The daily weight gain was not significantly improved by 8.3%. Corresponding results were found during the pasture period. The rate of calf-losses and the cases of sickness were significantly reduced by 38% during the winter period compared to calves without supplementation.

Conclusion: The results show, that the feeding conditions for calves during the first 3 month of life are inducing a severe mineral and vitamin (ß-carotene, vitamin E) deficiency. The only way to overcome the demands is offering a mineral and vitamin supplementation with a high acceptance from the 7th day of life onwards modified for the winter- and pasture- feeding period..

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W11. Health state in organic dairy farming – metabolic disorders, mastitis, lameness (Status quo der Tiergesundheitssituation in der ökologischen Milchviehhaltung – Stoffwechselstörungen, Mastitis, Lahmheiten). C. Winckler* and J. Brinkmann – Vienna/Goettingen

Production diseases are generally regarded as a serious problem in dairy farming due to their welfare relevance and economic impact (1). In organic production systems, the housing, feeding and management conditions according to the EU legislation and guidelines set by organic farming associations are supposed to be welfare friendly and beneficial to health. However, there is evidence that the adaptability of farm animals is often overstressed in organic farming (2). There is only little information available about the situation in german organic dairy farms. Whereas studies in other european countries revealed similar incidences like in conventional farming for the most important diseases, farm structures and housing systems differ from the german farming conditions. It was the aim of the present study to assess the health state in german organic dairy farming with regard to metabolic disorders, mastitis and lameness, and to investigate possible relationships with the specific housing conditions and the use of preventive measures.

Methods: A questionnaire on housing, herd health management and disease incidences during the last 12 months was sent to 1000 randomly chosen organic dairy farms with a herd size greater than 20 cows. A cross-sectional study was then carried out in a representative sample of 50 loose housed herds (mean herd size 53 cows), which had been selected out of the farms which had responded (n=283) and agreed to be included in further studies (n=158). The housing systems were either cubicle (n=39) or straw yard systems (n=11). Holstein-Friesian was the prevalent breed (n=30), other breeds were Simmental (n=11) and Brown Swiss (n=9). Milk yield did not differ significantly between cubicle housed herds (6434 kg/a, sd 1195) and herds housed in straw yard systems (5720 kg/a, sd 1419).

In each farm, herd health records from 2002 were copied and subsequently evaluated with regard to metabolic disorders, mastitis and lameness treatments. Data from milk performance testing were analysed as well. During the farm visit, lameness prevalence was recorded using a 5-point locomotion scoring system (3) and leg injuries were assessed taking size and severity of the lesions into account.

Results: The incidence of metabolic disorders showed a high level of variation. Milk fever incidence ranged between 0 and 20 % (mean 5,4 %, median 3,5 %). However, almost two thirds of the farms did not apply any specific measures to prevent milk fever (e.g. anion-rich diets, oral administration of calcium). Treatments of ketosis (0 – 13 %), acidosis (0 – 9 %) and displacement of the abomasum (0 – 3 %) were less frequent and seemed to be only of relevance on single farms.

Mastitis incidence ranged between 0 and 202 % (mean 38 %). Interestingly, there was no obvious effect of several preventive measures such as teat disinfection on mastitis incidence. The average lameness prevalence was 18 % (2 – 53 %) with herds housed in cubicles housed showing significantly more lameness (20 %, sd 11,7) than herds on straw yard systems (10 %, sd 5,2; $p<0.01$, Mann-Whitney).

Conclusions: These preliminary results demonstrate that production diseases such as metabolic disorders (milk fever), mastitis and lameness play a considerable role in organic dairy farming in Germany.

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Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology

Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

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Prediction of Metabolisable Energy (ME) in total mixed rations (TMR) for ruminants
Schätzung des Gehaltes an Umsetzbarer Energie in Mischrationen (TMR) für Wiederkäuer

Introduction
While different equations are recommended for the prediction of ME in grass, grass products, and maize silage (GfE, 1998) as well as in compound concentrates (GfE, 1996) for ruminants no equations have been recommended so far for routine evaluation of total mixed rations (TMR). The ME content in TMR cannot be satisfactorily estimated with any of the already published equations for single feeds and compound concentrates (Boguhn et al. 2003). Therefore, separate equations were developed and validated. TMR in the sense of this communication are mixed diets that contain at least two feedstuffs of which at least one is a forage silage.

Data pool for calculating the equations (Pool A)
Thirty TMR were investigated for crude nutrient digestibilities according to standard procedures (GfE 1991) using wether sheep (Boguhn et al. 2003), and the content of ME was calculated from digestible crude nutrients according to GfE (1995). Ingredients had been selected in a way that TMR were representative for the practice, and contents of crude nutrients, NDF and ADF in these diets covered the relevant range (Table 1). Gas production (GP) and enzymatically degradable organic matter (EDOM) were analysed as well. Regression equations were predicted on the basis of multiple regression analysis (Boguhn et al. 2003).

Data pool for validation (Pool B)
The number of data sets which could be used for independent validation of the equations was limited. A total of 28 TMR were investigated for nutrient digestibility based on the standard method in different institutions in recent years. Only such data were considered that were within the range of nutrient contents in datapool A. The committee gratefully acknowledges the contribution of data from the following institutions representing different regions in Germany: Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern, Landwirtschaftskammer Rheinland, Sächsische Landesanstalt für Landwirtschaft, Bayerische Landesanstalt für Landwirtschaft and Universität Halle-Wittenberg. Data for this pool are summarized in Table 1. Not all analyses relevant for validation of all equations were run with all rations. Therefore, the data basis for validation is weak, especially for equations considering NDF and EDOM. Furthermore, the variation in ME content of these diets was small. Dry matter content was given for 24 out of the 28 rations.

Table 1: Description of the rations used for the calculation (Pool A) and for the validation (Pool B) of prediction equations

		Pool A (n=30)				Pool B		
		Min.	Max.	Av.	n	Min.	Max.	Av.
DM[1)]	%	27	57	42	24	30	52	43
OM	g/kg DM	884	946	920	28	888	946	920
XA	g/kg DM	54	116	80	28	54	112	80
XP	g/kg DM	118	234	169	28	125	215	159
XF	g/kg DM	131	250	180	28	127	234	185
XL	g/kg DM	26	48	34	28	26	45	33
EDOM	g/kg DM	652	800	744	15	653	762	705
GP	ml/200 mg DM	41	54	49	10	46	54	52
NDF	g/kg DM	281	488	364	10	304	482	351
ADF	g/kg DM	173	304	222	10	185	249	214
ME[2)]	MJ/ kg DM	9.6	11.9	11.2	28	9.6	12.1	11.1
NEL[3)]	MJ/ kg DM	5.7	7.4	6.8	28	5.7	7.5	6.7

[1)] Abbreviations: DM dry matter, OM organic matter, XA crude ash, XP crude protein, XF crude fibre, XL crude fat, EDOM enzymatically degradable organic matter, GP gas production, NDF neutral detergent fibre, ADF acid detergent fibre

[2)] Calculated on the basis of digestible crude nutrients according to GfE (1995)

[3)] Calculated from ME and gross energy according to equations 4 and 5

Recommended predictive equations and their validations

The following equation is recommended for routine application when crude nutrients were analysed:

$$\text{ME (MJ/kg DM)} = 6.0756 + 0.19123\times XL + 0.02459\times XP - 0.000038\times XF\times XF - 0.002139\times XL\times XL - 0.000060\times XP\times XP \quad [\text{equ. } 1]$$

r^2: 0.83, $s_{y.x}$: 0.25 MJ/kg DM

with XP (crude protein), XL (crude fat) and XF (crude fibre) in g/kg DM.

The validation of this equation showed an overall good agreement between measured and predicted ME (Fig. 1 and Table 2).

When NDF is used as a measure of fibre content instead of crude fibre, equation 2 can be alternatively used to predict ME with a similar level of accuracy. Using ADF, however, resulted in a poorer accuracy of prediction and is not recommended. Independent validation of equation 2 indicates the suitability of the equation, but it is based on 10 data sets only (Fig. 1).

$$\text{ME (MJ/kg DM)} = 11.9955 - 0.00753\times NDF + 0.00566\times XP + 0.03987\times XL - 0.00513\times XA \quad [\text{equ. } 2]$$

r^2: 0.81, $s_{y.x}$: 0.26 MJ/kg DM

with XP (crude protein), XL (crude fat), XA (crude ash) and NDF (neutral detergent fibre) in g/kg DM.

In contrast to findings with individual forages (GfE, 1998), no improvement in accuracy is to be expected for ME prediction in TMR from the inclusion of GP. However, EDOM can significantly improve the accuracy when measured in addition to crude nutrients (equ. 3). Due to the lack of independent data the validation is weak and still needs more data (Fig. 1). The present validation indicates an average underestimate of ME by 2.9%. However, for specific questions that may need a higher level of accuracy the additional analysis of EDOM is recommended.

$$\text{ME (MJ/kg DM)} = 1.5473 + 0.00764\times EDOM + 0.23292\times XL - 0.002760\times XL\times XL - 0.000021\times XF\times XF \quad [\text{equ. } 3]$$

r^2: 0.90, $s_{y.x}$: 0.19 MJ/kg DM

with EDOM (enzymatically degradable organic matter), XL (crude fat), and XF (crude fibre) in g/kg DM.

Calculation of net energy for lactation (NEL)

Content of NEL can be calculated from ME on the basis of analysed crude nutrients according to the established equations (GfE, 2001) as follows:

$$\text{GE (MJ/kg DM)} = 0.0239\ XP + 0.0398\ XL + 0.0201\ XF + 0.0175\ XX \quad [\text{equ. } 4]$$

with XP, XL, XF and XX (nitrogen-free extract) in g/kg DM

$$\text{NEL (MJ)} = 0.6\ [1 + 0.004\ (q-57)]\ \text{ME (MJ)} \quad \text{with } q = ME/GE\times 100 \quad [\text{equ. } 5]$$

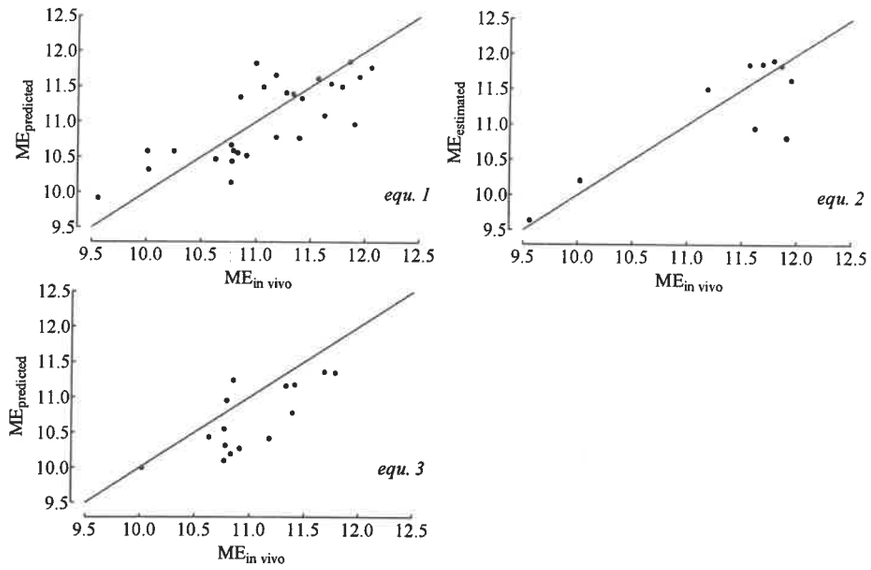

Figure 1: Relation between ME determined on the basis of digestible crude nutrients ($ME_{in\ vivo}$) and ME predicted by independent validation on the basis of crude nutrients (equ. 1), NDF (equ. 2) and EDOM (equ. 3). All data in MJ/kg dry matter. The line is the line of equality. For details see Table 2.

Table 2: Results of independent validation

Equation	No. of rations	Deviation between $ME_{predicted}$ and $ME_{in\ vivo}$ (%) Average	from	to
1	28	- 0.4	- 7.8	+ 7.6
2	10	- 0.7	- 9.1	+ 2.8
3	15	- 2.9	- 6.9	+ 3.5

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Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

Empfehlungen zur Energie- und Nährstoffversorgung der Mastputen

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1 Einleitung

Die Putenfleischproduktion hat in den letzten Jahren in der Bundesrepublik Deutschland beachtlich zugenommen, gleichfalls der Verbrauch an Putenfleisch und -verarbeitungsprodukten. Derzeit beträgt die Bruttoeigenerzeugung in Deutschland 325 kt/Jahr (ZMP, 2002a), die 61% des Gesamtverbrauchs abdeckt. Vom Pro-Kopf-Verbrauch an Geflügelfleisch (19 kg) entfallen etwa ein Drittel auf Putenfleisch (ZMP, 2002a). Bezogen auf den Gesamtfleischkonsum (92 kg pro Kopf der Bevölkerung) beträgt der Putenfleischanteil etwa 7%.

Für die Mast werden in Deutschland derzeit fast ausschließlich schwere Herkünfte verwendet; es dominiert der Genotyp B.U.T. Big 6 (*British United Turkeys Ltd.)* (MEYER, 2000). Diese kommerzielle Mastherkunft ist das Endprodukt einer 3-Linienkreuzung. Außer *British United Turkeys Ltd.* existieren weltweit nur noch zwei weitere Zuchtunternehmen (*Nicholas Turkey Breeding Farm, USA, Schottland*; *Hybrid, Kanada*) für schwere Mastputen. Die Vermarktung dieser schweren Schlachtkörper erfolgt in Form von Teilstücken und Verarbeitungsprodukten. Als Haltungsform kommt die Bodenhaltung auf Tiefstreu zur Anwendung. Die Mastdauer beträgt in der Regel bei den männlichen Tieren 20 bis 23 Wochen und bei den weiblichen Tieren 15 bis 17 Wochen. Die Fütterung basiert auf Alleinfuttermitteln bei Anwendung von Phasenfütterungsprogrammen (weibliche Tiere 5 Phasen, männliche Tiere 6 Phasen). In den letzten Jahrzehnten wurde die Mastleistung der Mastputen ständig verbessert. Entsprechende Daten für die Herkunft B.U.T Big 6 vermittelt Tabelle 1.1. Mit diesem enormen Zuchtfortschritt ist gleichzeitig eine deutliche Erhöhung des Schlachtertrages verbunden.

Neben Herkünften des schweren Masttyps gibt es außerdem Herkünfte, die dem mittelschweren bzw. leichten Typ zuzuordnen sind. Lebendmassedaten der drei Typen bei gleichem Alter sind in Tabelle 1.2 ausgewiesen. Die Lebendmassedifferenz zwischen mittelschweren und schweren Herkünften ist in den letzten Jahren geringer geworden. Für die Mast nach den Rahmenbedingungen des ökologischen Landbaus werden langsamer wachsende Putenherkünfte verwendet (FELDHAUS und SIEVERDING, 2001). Das Produktionsvolumen dieser Puten liegt derzeit unter 1% der Gesamterzeugung an Putenfleisch (ZMP, 2002b).

Tabelle 1.1: Kenndaten zur Entwicklung der Lebendmasse (LM) und des Futteraufwandes von Puten der Herkunft B.U.T. Big 6 im Zeitraum von 1984-1999 (MOORGUT KARTZFEHN 1993, 1999)

	Hahn (22 Wochen)		Henne (16 Wochen)	
	LM (kg)	kg Futter/kg Zuwachs	LM (kg)	kg Futter/kg Zuwachs
1984	15,70	3,20	7,87	2,71
1988	17,79	3,07	8,76	2,75
1993	19,45	2,77	9,56	2,50
1999	20,72	2,73	9,88	2,49

Tabelle 1.2: Lebendmassen (kg) leichter, mittelschwerer und schwerer Mastputen im Alter von 16 (weibliche Tiere) bzw. 22 Wochen (männliche Tiere)

Typ	Quelle	Herkunft	Weiblich	Männlich
Leicht	KELLY TURKEYS (2001)	SUPER MINI White und Bronze	5,0	10,1
Mittelschwer	B.U.T. (1999)[1)]	B.U.T. T 9	8,8	18,7
		B.U.T. T 8	8,2	17,7
	MEYER (1999)	B.U.T. T 8		18,7
Schwer	B.U.T. (1999)[1)]	B.U.T. Big 6	9,9	20,7
	GRAMZOW (2001),	B.U.T. Big 6		23,0
	JEROCH, pers. Mitt.	B.U.T. Big 6	10,4	
	MEYER (1999)	B.U.T. Big 6		20,6
	HYBRID	Converter	9,9	20,4
	(1999)[1)]	Euro FP	9,8	20,6
	NICHOLAS	N 300	8,8	19,5
	(1999)[1)]	N 700	9,5	20,5

[1)] Quelle: MEYER (2000)

Es fehlt derzeit eine wissenschaftlich fundierte Abhandlung über den Energie- und Nährstoffbedarf von Mastputen, die dem heutigen Leistungsniveau gerecht wird. Die letzten offiziellen Empfehlungen zur Energie und Nährstoffversorgung von Mastputen wurden 1994 vom National Research Council publiziert (NRC, 1994). Bereits kurze Zeit nach dem Erscheinen dieser Monografie zeigte sich, dass die Empfehlungen für essentielle Aminosäuren eine optimale Versorgung nicht gewährleisten (s. Literaturauswertung bei GRAMZOW, 2001). Die Grundlage für die NRC-Empfehlungen bildeten ältere Versuchsergebnisse, die mit einem Tiermaterial ermittelt wurden, das deutlich unter dem heutigen Leistungsniveau bezüglich der Mast- und Schlachtdaten liegt. Nachdem 1999 erstmalig Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler) publiziert wurden (GfE, 1999), werden nachfolgend Empfehlungen für schwere Mastputen vorgestellt. Die Beschränkung auf den schweren Masttyp erfolgt deshalb, weil die anderen Mastputentypen in Deutschland kaum gemästet werden und zu diesen außerdem wenig experimentelle Untersuchungen neueren Datums vorliegen. Die Zuchtputen (Elterntiere zur Bruteierproduktion) und deren Nachzucht wurden in diese Abhandlung nicht aufgenommen. Lediglich der Einfluss der maternalen Ernährung auf die postnatale Entwicklung der Küken findet Berücksichtigung.

Die Ableitung der Versorgungsempfehlungen für Energie und Nährstoffe erfolgte in dieser Abhandlung auf zwei verschiedenen Wegen. Bei ausreichender Datenbasis wurde faktoriell vorgegangen (Rohprotein und Aminosäuren). Auch bei der Energie sollte zunächst dieses Verfahren angewandt werden. Es zeigte sich jedoch, dass keine Mastputen-spezifischen Daten zur partiellen Verwertung der ME für den Ansatz von Protein- und Fett sowie zum Erhaltungsbedarf vorliegen und die Anwendung Masthuhn-spezifischer Daten keine Alternative ist. Deshalb beschränkt sich der

Ausschuss hier, ähnlich wie bei den Spurenelementen und Vitaminen, auf die Empfehlung von Konzentrationen. Die Basis für die Ableitung der Empfehlungen zur Versorgung mit essentiellen Fettsäuren, Spurenelementen und Vitaminen bildeten vorrangig die Ergebnisse von Leistungsversuchen, die nach dem Dosis-Wirkungs-Prinzip durchgeführt wurden.

2 Energie

2.1 Einleitung

Die Ableitung des Energiebedarfes und die energetische Futterbewertung erfolgen für das Geflügel und somit auch für Mastputen auf der Ebene der umsetzbaren Energie. Es handelt sich hierbei um die N-korrigierte scheinbare Umsetzbare Energie (AME_N). Eine ausführliche Begründung der Wahl dieses energetischen Bewertungssystems für das Geflügel ist vom Ausschuss für Bedarfsnormen der GfE (1999) gegeben worden. Dort finden sich auch die Formeln zur Berechnung des Gehaltes an umsetzbarer Energie von Mischfutter und Einzelfuttermitteln.

Im Gegensatz zu Masthühnern und Legehennen ist eine faktorielle Ableitung des Energiebedarfs für Mastputen infolge einer unzureichenden Datenlage derzeit nicht möglich (Abschn. 2.4). Daher beschränken sich die Empfehlungen zur Energieversorgung für Mastputen –abweichend von Legehennen und Masthühnern– auf die Angaben von AME_N-Konzentrationen im Futter (Abschn. 2.6).

2.2 Energieansatz

Die Berechnung des Energieansatzes in Form von Protein (N×6,25) (RE_p) und Fett (RE_f) erfolgte auf der Grundlage des mittels Gompertz-Funktion beschriebenen Protein- und Fettansatzes männlicher und weiblicher Puten der Herkunft BUT Big 6 (GRAMZOW, 2001; JEROCH et al., 2004; JEROCH, pers. Mitt.) sowie eines Bruttoenergiegehaltes von 23,86 kJ/g Körperprotein sowie von 39,77 kJ/g Körperfett (HOFFMANN und SCHIEMANN, 1980) (Abb. 2.1). Der Proteinenergieansatz ist für männliche und weibliche Tiere bis etwa zur 5. Lebenswoche von gleicher Größenordnung. Danach kommt der Geschlechtsdimorphismus immer deutlicher zum Tragen. Das Maximum des Proteinenergieansatzes wird bei weiblichen Tieren im Zeitraum von der 9. bis 11. Lebenswoche erreicht, während bei männlichen Tieren dieses Maximum erst zwischen der 13. und 15. Lebenswoche realisiert wird.

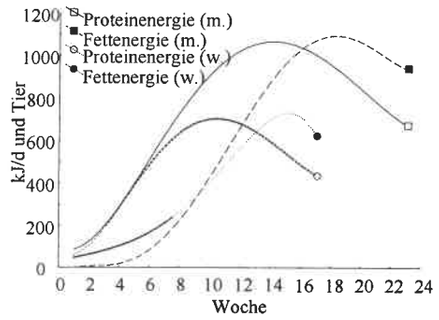

Abbildung 2.1: Altersabhängiger Verlauf des täglichen Energieansatzes in Protein und Fett männlicher (m.) und weiblicher (w.) Puten der Herkunft BUT Big 6

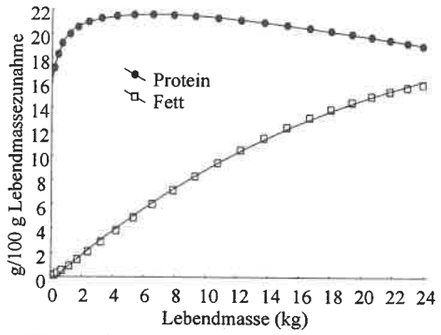

Abbildung 2.2: Gehalte an Protein und Fett im Lebendmassezuwachs männlicher Puten der Herkunft BUT Big 6 in Abhängigkeit von der Lebendmasse

Das Maximum des Fettenergieansatzes erreichen Hennen zwischen der 14. und 16. und Hähne zwischen der 17. und 19. Lebenswoche. Infolge des höheren Energiegehaltes des Fettansatzes sind die jeweiligen Maxima trotz geringeren stofflichen Ansatzes von gleicher Höhe wie die des Proteinenergieansatzes.
Diese altersabhängige Beschreibung des Energieansatzes besitzt streng genommen nur Gültigkeit für die in den zugrunde liegenden Versuchen erzielten Lebendmassen und Lebendmassezunahmen.

2.3 ME-Bedarf für Erhaltung (ME_m) sowie Zuwachs

Ansätze zur faktoriellen Aufteilung der aufgenommenen ME auf die Lebendmasse (Indikator für Erhaltungsbedarf) sowie auf den Zuwachs (Indikator für Energieretention) sind lediglich in den Arbeiten von HURWITZ et al. (1980), HURWITZ und BENGAL (1982) sowie HURWITZ et al. (1983a, b) zu finden. Diese Vorgehensweise orientiert sich nur an einfach zu erfassenden Messgrößen, wie Lebendmasse und Lebendmassezuwachs und berücksichtigt daher nicht die Unterschiede in der chemischen Zusammensetzung bzw. im Energiegehalt des Zuwachses. Diese Vereinfachung erscheint insbesondere im Hinblick auf die lange Mastdauer von schweren Mastputen und der damit einhergehenden Veränderung in der chemischen Zusammensetzung des Tierkörpers sowie des Zuwachses (siehe Abb. 2.2 und 2.3) als problematisch. Im Ergebnis ihrer umfangreichen Untersuchungen kommen oben genannte Autoren zu dem Schluss, dass der Erhaltungsbedarf für Broiler und Mastputen mit 8,4-9,2 kJ/g Lebendmasse$^{2/3}$ von gleicher Größenordnung ist. Aus dem deutlich niedrigeren Energieaufwand für den Lebendmassezuwachs von Mastputen im Vergleich zum Broiler schlussfolgerten HURWITZ et al. (1980), dass hierfür die Unterschiede in der chemischen Zusammensetzung des Lebendmassezuwachses verantwortlich sind. Dass Broiler stärker als Mastputen zur Verfettung neigen, geht aus vergleichenden Untersuchungen von KOUBA et al. (1992) hervor. Danach führte eine vergleichbare Fütterung hinsichtlich des Proteingehaltes im Futter ("broilergerecht" und "putengerecht" an Broiler und Puten bzw. umgekehrt) generell zu einem höheren Fettgehalt und niedrigeren Proteingehalt des Broilerkörpers. Diese tierartspezifischen Unterschiede erfordern zunächst die Überprüfung der Anwendbarkeit der Broiler-spezifischen Verwertungsfaktoren (GfE, 1999) bei der Mastpute.

2.4 Überprüfung der Eignung der faktoriellen Ableitung des ME-Bedarfes von Mastputen auf der Grundlage der für das Masthuhn abgeleiteten Faktoren

Untersuchungen zur partiellen Verwertung der ME für RE_p (k_p) und RE_f (k_f) sowie zum ME_m liegen für die Mastpute des schweren Typs nicht vor, so dass zur Überprüfung der Eignung der faktoriellen Ableitung des ME-Bedarfs auf die vom Ausschuss für Bedarfsnormen der GfE abgeleiteten entsprechenden Faktoren für das Masthuhn zurückgegriffen werden musste (k_p=0,52, k_f=0,84, ME_m= 480 kJ/d/$W^{0,75}$; GfE, 1999).
Auf der Grundlage dieser Masthuhn-spezifischen Daten sowie unter Verwendung des gemessenen Energieansatzes (Abschnitt 2.2) für die Mastpute wurde versucht, den ME-Bedarf faktoriell abzuleiten. Zur Validierung wurden publizierte Wachstumsversuche (LEHMANN, 1998; GRAMZOW, 2001; BOGUHN et al., 2002; RODEHUTSCORD et al., 2003) der entsprechenden genetischen Herkunft verwandt, wobei nur Informationen zur gemessenen Lebendmassezunahme und zur Lebendmasse zur Verfügung standen. Zur Abschätzung des Stoffansatzes wurden multiple nichtlineare Regressionsmodelle verwendet, welche den Protein- und Fettansatz in der Lebendmassezunahme (y) in Abhängigkeit von der Lebendmasse beschreiben. Die Anwendung dieser Regressionsgleichungen auf die Daten, die zur Validierung herangezogen wurden, ermöglichte somit eine Vorhersage des Stoff- und damit auch des Energieansatzes (Abb. 2.2 und 2.3), der dann zur Ableitung des ME-Bedarfes unter Verwendung der Masthuhn-spezifischen Verwertungsfaktoren herangezogen wurde.
Der so ermittelte ME-Bedarf ist dann der ME-Aufnahme gegenübergestellt worden, die tatsächlich in den zur Validierung herangezogenen Versuchen zugrunde gelegt wurde (Abb. 2.4). Als Einschränkung ist hierbei zu berücksichtigen, dass die ME-Aufnahme in allen Versuchen aus der Multiplikation der realisierten Futteraufnahme mit *kalkulierten* ME-Gehalten der in den Versuchen

verwendeten Futtermischungen abgeschätzt wurde. Darüber hinaus wurde die Validierung nur für männliche Tiere vorgenommen, da für weibliche keine genügende Datengrundlage für eine Validierung vorliegt.

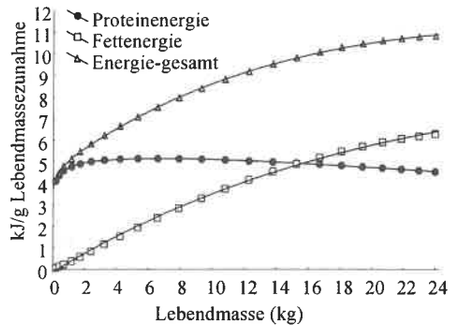

Abbildung 2.3: Energiegehalt im Zuwachs männlicher Mastputen der Herkunft BUT Big 6 in Abhängigkeit von der Lebendmasse

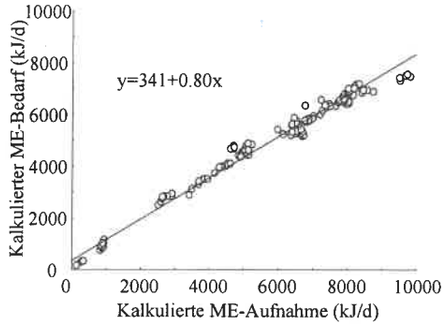

Abbildung 2.4: Vergleich der in den zur Validierung verwendeten Wachstumsversuchen kalkulierten ME-Aufnahme mit dem nach der faktoriellen Methode abgeleiteten ME-Bedarf

Es wird deutlich, dass die Verwendung der Masthuhn-spezifischen Faktoren zu einer systematischen Unterschätzung des ME-Bedarfes führt, wobei der vorhergesagte ME-Bedarf um ca. 20% unterschätzt wird, wenn die ME-Aufnahme als Bezugsbasis gewählt wird (Abb. 2.4). Wenngleich dieser Vergleich mit verschiedenen Fehlermöglichkeiten behaftet ist, muss dennoch die Schlussfolgerung gezogen werden, dass eine faktorielle Bedarfsableitung erst dann vorgenommen werden kann, wenn Messwerte für k_p, k_f und ME_m vorliegen.

2.5 Energiebedarfsdeckung und Energiekonzentration im Mastputenfutter

Untersuchungen zur Energiebedarfsdeckung von Mastputen konzentrierten sich hauptsächlich auf die Prüfung variierender Energiegehalte im Putenfutter auf die Parameter der Mast- und Schlachtleistung. Älteren Arbeiten ist übereinstimmend zu entnehmen, dass die Anforderung der Pute an die Energiekonzentration des Futters im Verlauf der Mast ansteigt (SCHOLTYSSEK et al., 1973; HERZ et al., 1975a, b; RICHTER und PRINZ, 1980; RICHTER et al., 1980). Auch neuere Untersuchungen an Hähnen der Herkunft BUT Big 6 bestätigen diese Relationen (MEYER, 1999). Schließlich sind auch praktische Fütterungsprogramme in der Regel durch steigende Energiekonzentrationen im Futter charakterisiert (Tab. 2.1). Modifikationen im Energiegehalt innerhalb der Mastphasen können sich dabei aus unterschiedlichen Produktionsbedingungen (insbesondere Klima) ergeben.

2.6 Empfehlungen zur Versorgung mit umsetzbarer Energie

Aufgrund der ungenügenden experimentellen Datenlage für eine faktorielle Bedarfsableitung sowie der dargestellten Befunde zum Einfluss der Energiekonzentration auf die Leistungsdaten werden zunächst AME_N-Konzentrationen für die einzelnen Altersabschnitte empfohlen, wobei die angegebenen Spannweiten unterschiedliche Fütterungs- und Haltungsprogramme berücksichtigen sollen (Tabelle 2.2).

Tabelle 2.1: AME_N-Konzentrationen im Futter für schwere männliche (m) und weibliche (w) Mastputen nach verschiedenen Angaben

	Mastphase						
	1	2	3	4	5	6	7
LARBIER und LECLERCQ (1994)							
Wochen (m/w)	1-4	4-8	8-12	12-16			
AME_N (MJ/kg) (m/w)	13,0	13,0	13,0	13,0			
NRC (1994)							
Wochen (m)	1-4	4-8	8-12	12-16	16-20	20-24	
Wochen (w)	1-4	4-8	8-11	11-14	14-17	17-20	
AME_N (MJ/kg) (m/w)	11,7	12,2	12,6	13,0	13,4	13,8	
CUNNINGHAM und FARMS (1996) - Vergleich von Fütterungsprogrammen							
Kanada, Ontario							
Wochen (m)	1-3	4-6	7-9	10-12	13-15	16-18	
Wochen (w)	1-3	4-6	7-9	10-12	13-15	16-17	
AME_N (MJ/kg) (m/w)	11,8	12,1	12,5	13,0	13,4	13,8	
USA, Südost							
Wochen (m)	1-3	4-6	7-9	10-12	13-15	16-18	
Wochen (w)	1-3	4-6	7-9	10-12	13-15	16-17	
AME_N (MJ/kg) (m/w)	12,0	12,7	13,4	13,7	14,1	14,6	
Europa							
Wochen (m/w)	1-4	5-8	9-12	13-16	ab 17		
AME_N (MJ/kg) (m/w)	12,1	12,6	13,4	13,9	14,1		
POLSKA AKADEMIA NAUK (1996)							
Wochen (m/w)	0-3	4-6	7-11	12-15	ab 16		
AME_N (MJ/kg) (m/w)	11,3-12,1	12,1-13,4	12,1-13,4	12,1-13,4	12,1-13,8		
LEESON und SUMMERS (1997)							
Wochen (m)	1-2	2-4	4-8	8-12	12-15	15-20	
Wochen (w)	1-2	2-4	4-8	8-12	12-16		
AME_N (MJ/kg) (m/w)	11,9	12,3	12,8	13,2	13,5/13,8	13,6	
FERKET (2000)							
USA, Südost							
Wochen (m)	1-3	4-6	7-9	10-12	13-15	16-18	18-21
AME_N (MJ/kg) (m)	12,1	12,8	13,2	13,6	14,2	14,6	14,9
Europa							
Wochen (m)	1-4	5-8	9-12	13-16	ab 17		
AME_N (MJ/kg) (m)	11,9	12,6	13,4	13,8	14,0		
VELDKAMP (2002)							
Wochen (m)	1-4	5-8	9-12	13-16	17-20		
AME_N (MJ/kg) (m)	11,1	12,1	12,6	14,1	14,1-14,6		
JEROCH und DÄNICKE (2003)							
Wochen (m/w)	1-2	3-4	5-8	9-12	13-16	17-20[1]	>20[1]
AME_N (MJ/kg) (m/w)	11,5	11,5	12,0	12,5	12,5	13,0[1]	13,5[1]

[1] männliche Tiere

Tabelle 2.2: Empfehlungen zum Gehalt an umsetzbarer Energie in Futtermischungen für Mastputen (bei 88 % Trockenmasse)

	Phase					
	1	2	3	4	5	6
männlich						
Wochen	1-2	3-4	5-8	9-12	13-16	ab 17
AME_N (MJ/kg)	11,2-11,7	11,5-12,0	11,8-12,3	12,3-12,8	12,8-13,2	13,2-13,6
weiblich						
Wochen	1-2	3-4	5-8	9-12	ab 13	
AME_N (MJ/kg)	11,2-11,7	11,5-12,0	12,3-12,8	12,8-13,2	13,2-13,6	

3 Protein und Aminosäuren

3.1 Einleitung

Die Ableitungen erfolgen analog zur Vorgehensweise beim Hühnergeflügel (GfE, 1999) nach der faktoriellen Methode. Ausgangsdaten sind dabei für Protein (N×6,25) und einzelne Aminosäuren jeweils der Erhaltungsbedarf, der Ansatz und die Verwertung. Beim Ansatz werden der federfreie Tierkörper und die Federn getrennt quantifiziert, da diese beiden Fraktionen hinsichtlich des Aminosäurenmusters sehr unterschiedlich sind (GRAMZOW, 2001). Bei der Verwertung handelt es sich um die Gesamtverwertung, die sowohl die Verdaulichkeit als auch die postabsorptive Verwertung beinhaltet. Der Ausschuss ist der Meinung, dass Empfehlungen auf der Basis von verdaulichen Aminosäuren derzeit wegen des Fehlens von Daten noch nicht gegeben werden können. Auf den hier gemachten Angaben zum Nettobedarf aufbauend, kann aber zu einem späteren Zeitpunkt eine Modifikation der Empfehlungen vorgenommen werden.

3.2 Proteingehalt und Aminosäurenzusammensetzung

Es liegen neuere Daten zur Entwicklung des Proteingehaltes und zur Aminosäurenzusammensetzung (g essentielle AS/16 g N) im Körper männlicher und weiblicher Puten für einen schweren Masttyp vor (GRAMZOW, 2001; GRAMZOW et al., 1998, 1999; JEROCH et al., 2004, JEROCH, pers. Mitt.). Dieses Datenmaterial weist alters- und geschlechtsbedingte Unterschiede in der AS-Zusammensetzung des Ganzkörpers und des federfreien Körper auf, die vermutlich aus den wachstumsbedingten Veränderungen in den Relationen der einzelnen Körperfraktionen und Organe im Körper resultieren. Insbesondere der Anteil des Lysins im Protein des Ganzkörpers steigt mit zunehmender LM kontinuierlich an, wobei der Anstieg bei den männlichen Tieren (1. Tag 5,4, 23. Woche 7,2 g/16 g N) wegen des höheren Brustmuskelanteils etwas stärker ausfällt als bei den weiblichen. In Tabelle 3.1 sind zur Übersicht Mittelwerte für männliche Tiere dargestellt.

3.3 Ansatz von Protein und Aminosäuren

Auf der Grundlage quantitativer experimenteller Daten modellierte GRAMZOW (2001) den täglichen Ansatz von Rohprotein und Aminosäuren eines schweren Masttyps. Eine Zusammenfassung ist in Tabelle 3.2 gegeben. Tryptophan ist bei dieser Betrachtung ausgenommen, weil keine Messungen vorgenommen wurden. Die Maxima des Ansatzes werden bei dieser Herkunft in der 13.-16. (männlich) bzw. in der 9.-12. Lebenswoche (weiblich) erreicht.

Tabelle 3.1: Aminosäurenzusammensetzung (g/16 g N) männlicher Puten

	FISCHER u. SCOUGALL (1982)	HURWITZ et al. (1983b)		GRAMZOW (2001)		
	Ganzkörper	federloser Tierkörper	Federn	Ganzkörper	federloser Tierkörper	Federn
Arg	6,55	6,9	7,2	6,43	6,33	6,73
Cys	1,54	-	-	1,35	1,16	7,41
His	2,17	3,0	0,7	2,47	2,52	0,86
Ile	3,93	4,4	5,0	4,06	4,03	4,71
Leu	7,16	9,0	8,1	7,37	7,36	8,15
Lys	5,49	8,0	1,9	6,99	7,15	1,75
Met	1,94	2,7	0,7	2,38	2,44	0,45
Met+Cys	3,48	3,0	9,1	3,73	3,60	7,86
Phe	3,71	4,6	5,1	3,92	3,85	5,36
Thr	3,92	4,6	4,6	3,83	3,81	4,66
Trp	0,99	-	0,7	-	-	-
Val	4,91	5,4	7,7	4,56	4,45	7,39

Tabelle 3.2: Mittlerer Ansatz von Protein und essentiellen Aminosäuren männlicher und weiblicher schwerer Mastputen (g/d) (GRAMZOW, 2001)

Alter Wochen	N× 6,25	Cys	Met	Met+ Cys	Thr	Val	Ile	Leu	Phe	His	Lys	Arg
						männlich						
1-2	3,7	0,05	0,11	0,15	0,16	0,20	0,17	0,32	0,18	0,10	0,31	0,27
3-4	9,5	0,10	0,24	0,34	0,36	0,43	0,37	0,70	0,39	0,24	0,67	0,60
5-8	23,6	0,22	0,53	0,75	0,78	0,92	0,82	1,51	0,83	0,53	1,47	1,32
9-12	40,8	0,38	0,87	1,25	1,30	1,53	1,38	2,51	1,34	0,89	2,46	2,20
13-16	45,1	0,46	0,97	1,43	1,50	1,75	1,60	2,88	1,50	1,01	2,82	2,50
17-20	38,2	0,43	0,85	1,28	1,36	1,57	1,46	2,62	1,33	0,90	2,55	2,24
>20	29,0	0,36	0,66	1,03	1,11	1,27	1,18	2,14	1,06	0,72	2,07	1,80
						weiblich						
1-2	3,6	0,04	0,08	0,12	0,14	0,16	0,15	0,24	0,13	0,10	0,22	0,23
3-4	9,8	0,13	0,22	0,34	0,39	0,44	0,40	0,68	0,36	0,29	0,62	0,62
5-8	21,7	0,29	0,49	0,78	0,88	1,00	0,90	1,54	0,81	0,66	1,42	1,38
9-12	28,9	0,39	0,65	1,04	1,19	1,36	1,24	2,09	1,08	0,93	1,95	1,82
13-16	23,8	0,31	0,54	0,85	0,99	1,15	1,05	1,73	0,89	0,81	1,65	1,48
17	20,5	0,23	0,41	0,64	0,77	0,89	0,82	1,34	0,69	0,64	1,29	1,14

3.4 Erhaltungsbedarf und Verwertung von Protein und Aminosäuren

Zum Erhaltungsbedarf an Stickstoff (NEB) liegen für Mastputen nur wenige Daten vor (GRAMZOW, 2001; HURWITZ et al., 1983b). Als Grundlage für die weiteren Berechnungen wird hieraus ein Wert von 280 mg N je kg $LM^{0,67}$ für den täglichen Erhaltungsbedarf geschätzt. Aus Gründen der Praktikabilität wird der Erhaltungsbedarf bei Aminosäuren jedoch, wie allgemein üblich, auf die Lebendmasse bezogen angegeben.

Für Lysin ermittelte GRAMZOW (2001) einen täglichen Erhaltungsbedarf von 40 mg/kg LM, während SAUNDERSON et al. (1997) 65 mg/kg LM und Tag mitteilen. Aus Literaturdaten wurden für Broiler 41 mg/kg LM und Tag abgeleitet (GfE, 1999). Der Erhaltungsbedarf für alle weiteren

essentiellen Aminosäuren wurde nach der von der GfE (1999) ausführlich beschriebenen Vorgehensweise ermittelt (siehe auch Fußnoten zu Tabelle 3.3).

Die Verwertung wurde nur in wenigen Studien mit Mastputen gemessen. SUMMERS et al. (1985) verglichen die Verwertung zwischen 35 Tage alten Broilern und Puten. Puten verwerteten das Rohprotein aus den verabreichten Rationen im Mittel mit 54% und lagen damit um 7 Prozentpunkte über dem Broiler. Solche Vergleiche sind allerdings schwer zu bewerten, wenn die Relation zwischen der Rohproteinversorgung und dem jeweiligen Aminosäurenbedarf der Tiere nicht bekannt ist. Die aus Bilanzversuchen von GRAMZOW (2001) abgeleiteten Werte für die N-Verwertung liegen zwischen 59 und 66%. Mit zunehmendem Alter der Tiere nahm die Verwertung ab. Angesichts der Fülle von Faktoren, die die Verwertung des Rohproteins beeinflussen können, wird für die weitere Ableitung ein Verwertungskoeffizient von 60% unterstellt.

Für Lysin schwanken die Angaben zur Verwertung in der Literatur zwischen 52 (SAUNDERSON et al., 1997) und 64% (GRAMZOW, 2001). Ein Teil dieser Differenz mag durch Unterschiede in der Absorbierbarkeit des Lysins bedingt sein. Im Sinne eines Bewertungssystems lassen sich Verwertungsgrößen aber immer nur dann interpretieren, wenn sie im Bereich einer knappen Versorgung mit der jeweiligen Aminosäure gemessen wurden. HURWITZ et al. (1983b) unterstellen bei einer faktoriellen Ableitung eine Verwertung aller Aminosäuren von 85% und gehen davon aus, dass die absorbierten Aminosäuren vollständig für Ansatz und Erhaltung zur Verfügung stehen. Da allerdings selbst bei einem Mangel an einer Aminosäure ein Teil unvermeidlich oxidiert wird (WILKE et al., 1987; SIMON et al., 1995), führt diese Annahme zu einer Überschätzung der Verwertung.

In Auswertung der Literatur für Broiler hat die GfE (1999) eine Lysinverwertung von 68% abgeleitet. Für Mastputen unterstellt der Ausschuss in Anlehnung an GRAMZOW (2001) einen Wert von 64%. Für alle übrigen essentiellen Aminosäuren konnten die Verwertungsgrößen nur geschätzt werden. Hierbei wurde auf Literaturdaten zur praecaecalen Verdaulichkeit (FIRMAN und REMUS, 1992; FIRMAN, 1992) und zur intermediären Verwertung zurückgegriffen (GfE, 1999). In Anlehnung an BAKER (1991) wird für alle Aminosäuren (außer Lysin) eine postabsorptive Verwertung von 73% angenommen. In der Tabelle 3.3 sind die Ausgangsdaten zum Erhaltungsbedarf und zur Verwertung für die faktorielle Bedarfsableitung zusammengefasst. Es wird unterstellt, dass es bei diesen Faktoren keinen Unterschied zwischen den Geschlechtern gibt. Tryptophan konnte wegen des Fehlens von Daten nicht berücksichtigt werden. Hier wird auf die Empfehlungen des NRC (1994) verwiesen.

Tabelle 3.4 vermittelt die Ergebnisse der faktoriellen Bedarfsableitung für schwere männliche und weibliche Mastputen. In der Tabelle 3.5 erfolgt eine Transformation der Daten auf die Bezugsebene g/kg Alleinfutter. Für diese Berechnungen wurden die regressiv verrechneten Daten zur Futteraufnahme aus den Arbeiten von JEROCH et al. (2004) und von JEROCH (pers. Mitt.) unter Berücksichtigung der empfohlenen ME-Gehalte (Tabelle 2.2) verwendet. Als eine Konsequenz der hier vorgenommenen faktoriellen Ableitung und des unterschiedlichen Verlaufes von Feder- und Restkörperwachstum sind die Unterschiede zwischen den Geschlechtern im Bedarf an einzelnen Aminosäuren nicht zwangsläufig in den Unterschieden im Proteinbedarf widergespiegelt.

In Tabelle 3.6 sind neben den NRC-Empfehlungen neuere experimentelle Daten zum optimalen Gehalt an bedeutsamen essentiellen Aminosäuren, die vorrangig nach Dosis-Wirkungsstudien ermittelt wurden, zusammengestellt.

Die faktoriell abgeleiteten Versorgungsempfehlungen überschreiten in der Mehrheit und zum Teil beachtlich (z. B. Lysin) die NRC-Empfehlungen (1994). Der relativ hohe Lysinbedarf in den letzten Mastwochen lässt sich durch das noch starke Brustfleischwachstum in der Endmastphase begründen. Insgesamt muss festgestellt werden, dass die NRC-Empfehlungen die Anforderungen der in Deutschland gemästeten schweren Puten für einige essentielle Aminosäuren unterschätzen. Werden die NRC-Empfehlungen hinsichtlich ihrer Literaturquellen analysiert, so ist festzustellen, dass 21 Publikationen in die Empfehlungen eingeflossen sind, deren Veröffentlichung fast vollständig vor 1983 liegt. In neueren Arbeiten wurde untersucht, ob die Empfehlungen des NRC (1994) zur Aminosäurenversorgung schwerer Mastputen ausreichend sind (KIDD et al., 1997; WALDROUP et al., 1998; JANKOWSKI et al., 1998; VELTKAMP et al., 2003). Diese Arbeiten führten u.a. zu dem

Resultat, dass eine Anhebung des gesamten Aminosäurenniveaus in den Futtermischungen über die Empfehlungen des NRC (1994) hinaus die Leistung der Mastputen verbesserte.

Tabelle 3.3: Ausgangsdaten für Erhaltungsbedarf und Verwertung

	AS-Erhaltungsbedarf (mg/kg LM)[1]	Gesamtverwertung[2] (%)
Arginin	51	70
Histidin	6	60
Isoleucin	35	65
Leucin	61	65
Lysin	40[3]	64[3]
Met+Cys	56	60
Phenylalanin	40	65
Threonin	35	60
Valin	56	60

[1] Abgeleitet aus dem N-Erhaltungsbedarf und der durchschnittlichen Aminosäuren-Zusammensetzung der Federn

[2] gerundet unter Einbeziehung einer postabsorptiven Verwertung von 73% in Anlehnung an BAKER, 1991

[3] GRAMZOW (2001)

Die hier faktoriell abgeleiteten Empfehlungen liegen für viele Aminosäuren und Altersabschnitte höher als die aus einzelnen Dosis-Wirkungsstudien abgeleiteten Werte (LEHMANN et al., 1996; WESTERMEIER et al., 2000, LEMME et al., 2002) (Tab. 3.6). Dabei dürfte von Bedeutung sein, dass die Verdaulichkeit der zur Steigerung der Versorgung verwendeten freien Aminosäuren vollständig ist und somit geringere Gesamtgehalte in solchen Untersuchungen resultieren werden als bei ausschließlichem Einsatz proteingebundener Aminosäuren.

Tabelle 3.4: Bedarf männlicher und weiblicher schwerer Mastputen an Rohprotein und essentiellen Aminosäuren (g/Tier und Tag)[1]

Alter Wochen	FV[2] g/Tier/d		Rohprotein		Lys		Met		Met+Cys		Thr		Arg		Val		Ile		Leu		Phe		His	
	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w
1-2	22	21	5,6	5,3	0,38	0,36	0,12	0,12	0,19	0,18	0,21	0,20	0,33	0,31	0,24	0,23	0,21	0,20	0,40	0,38	0,23	0,22	0,12	0,12
3-4	62	61	15,0	14,5	0,94	0,99	0,33	0,34	0,50	0,52	0,52	0,55	0,85	0,89	0,63	0,66	0,53	0,56	1,01	1,05	0,58	0,60	0,32	0,34
5-8	130	130	30,5	30,8	1,82	2,01	0,64	0,71	0,99	1,10	1,01	1,12	1,67	1,84	1,22	1,35	1,04	1,16	1,94	2,14	1,09	1,21	0,65	0,72
9-12	305	254	63,9	53,4	3,89	3,71	1,38	1,31	2,19	2,09	2,17	2,07	3,60	3,44	2,61	2,49	2,29	2,18	4,14	3,95	2,26	2,15	1,43	1,36
13-16	455	333	80,5	51,3	5,28	3,78	1,83	1,31	2,96	2,12	2,94	2,10	4,84	3,47	3,52	2,52	3,14	2,25	5,61	4,02	2,97	2,13	1,93	1,38
17-20	556		84,6		5,57		1,87		3,07		3,09		5,03		3,66		3,33		5,92		3,05		2,01	
>20	627		71,2		5,16		1,69		2,79		2,84		4,58		3,35		3,08		5,48		2,76		1,83	

[1] Bedarf jeweils zu Beginn des Abschnittes [2] FV: Futterverbrauch

Tabelle 3.5: Erforderliche Gehalte an Rohprotein und essentiellen Aminosäuren in den Putenmastmischungen (g/kg Alleinfutter bei 88 % Trockenmasse)[1]

Alter Wochen	Rohprotein		Lys		Met		Met+Cys		Thr		Arg		Val		Ile		Leu		Phe		His	
	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m	w
1-2	255	253	17,1	17,0	5,7	5,7	8,4	8,4	9,4	9,3	14,9	14,9	11,1	11,1	9,4	9,4	18,2	18,2	10,5	10,5	5,5	5,5
3-4	242	238	15,2	16,2	5,3	5,6	8,0	8,5	8,4	9,0	13,7	14,6	10,1	10,8	8,6	9,1	16,2	17,3	9,3	9,9	5,2	5,6
5-8	235	237	14,0	15,5	4,9	5,5	7,6	8,4	7,8	8,6	12,8	14,2	9,4	10,4	8,0	8,9	14,9	16,5	8,4	9,3	5,0	5,5
9-12	209	210	12,8	14,6	4,5	5,2	7,2	8,2	7,1	8,2	11,8	13,5	8,6	9,8	7,5	8,6	13,6	15,6	7,4	8,5	4,7	5,4
13-16	177	154	11,6	11,4	4,0	3,9	6,5	6,4	6,5	6,3	10,6	10,4	7,7	7,6	6,9	6,8	12,3	12,1	6,5	6,4	4,2	4,2
17-20	152		10,0		3,4		5,5		5,6		9,1		6,6		6,0		10,7		5,5		3,6	
>20	114		8,2		2,7		4,5		4,5		7,3		5,4		4,9		8,8		4,4		2,9	

[1] für Futtermischungen bei Gehalten an umsetzbarer Energie (mittlere Werte) entsprechend Versorgungsempfehlungen in Abschnitt 2.6, Tabelle 2.2

Tabelle 3.6: Optimale Aminosäurengehalte in den Futtermischungen für männliche Mastputen nach neueren Literaturquellen (g/kg Alleinfutter)

			Lys					**Met+Cys**			
Altersabschnitt (Wochen)	0-4	5-8	9-12	13-16	17-20	21-24	0-4	5-8	9-12	13-16	17-20	21-24
LEHMANN et al. (1996)				9,2	7,0-7,6				5,2-5,8	4,4-5,0	4,0-4,2	
LEMME et al. (2002)		12,4		9,9			9,0	7,8	5,1	5,0	4,1	3,3
NRC (1994)	13,6	12,3	8,3	7,7	6,0	4,7						
WESTERMEIER et al. (2000)					8,1-8,5	6,0-6,2						
VELTKAMP et al. (2003)	16,8	16,7	11,9	10,8	9,4							
GRAMZOW (2001)	15,1	11,9	10,4	9,2	7,6	6,4	8,1	7,8	7,4	6,5	5,6	4,9

			Thr					**Trp**			
Altersabschnitt (Wochen)	0-4	5-8	9-12	13-16	17-20	21-24	0-4	5-8	9-12	13-16	17-20	21-24
LEHMANN et al. (1997)			5,2-5,6									
KIDD et al. (1998)	8,0-7,7	6,7	5,5									
MOORE et al. (2001)		7,4-6,7										
KAMYAB und FIRMAN (1999)	6,5-5,9											
NRC (1994)	8,5	7,8	5,1	5,8	4,5	3,6	2,2	2,0	1,3	1,4	1,1	0,9
GRAMZOW (2001)	8,5	7,8	7,0	6,1	5,6	4,6	2,5	2,0	1,8	1,6	1,4	1,1

			Arg					**Val**			
Altersabschnitt (Wochen)	0-4	5-8	9-12	13-16	17-20	21-24	0-4	5-8	9-12	13-16	17-20	21-24
KAMYAB und FIRMAN (1999)							10,6					
NRC (1994)	13,6	11,5	7,1	6,9	5,6	4,3	10,2	9,9	7,2	6,2	5,2	4,3
GRAMZOW (2001)	12,4	10,7	9,8	8,7	6,9	5,8	10,6	9,3	8,5	7,4	6,3	5,4

4 Essentielle Fettsäuren

Die essentiellen Fettsäuren werden nach ihrer Struktur in ω-6- und ω-3-Fettsäuren eingeteilt. Zu den essentiellen Fettsäuren gehören als ω-6-Fettsäuren die Linolsäure und die Arachidonsäure und zu den ω-3-Fettsäuren die α-Linolensäure, die Eicosapentaensäure und die Docosahexaensäure. Auf Grund ihrer chemischen Struktur sind Umwandlungen innerhalb der verschiedenen ω-6- oder ω-3-Fettsäuren möglich, aber ω-6- und ω-3-Fettsäuren können nicht ineinander überführt werden und haben außerdem unterschiedliche Funktionen im Organismus. Deshalb sind die Mangelsymptome auch unterschiedlich. Die essentiellen Fettsäuren bestimmen als Bausteine der Zellmembranen wesentlich deren Funktionen mit und sind die Ausgangssubstanzen für verschiedene Gewebshormone (BURR und BURR, 1929; NEURINGER et al., 1988; SIMOPOULOS, 1989; SIMOPOULOS et al., 1990).

Die häufigste ω-6 Fettsäure, die Linolsäure (C 18:2 ω-6), kommt in vielen Pflanzenölen vor. Arachidonsäure (C 20:4 ω-6) ist Bestandteil von Land- und Seetierfetten. Von den Pflanzenölen, die α-Linolensäure (C 18:3 ω-3) enthalten, sind Lein-, Soja- und Rapsöl zu nennen. Die höher ungesättigten ω-3 Fettsäuren (Eicosapentaen- (C 20:5 ω-3) und Docosahexaensäure (C 22:6 ω-3) kommen besonders hoch in marinen Fetten vor. In der Geflügelfütterung werden sowohl Pflanzenöle in reiner oder gemischter Form sowie mechanisch bzw. hydrothermisch bearbeitete Vollfettsamen (Sojabohnen, Sonnenblumensamen, Rapssaat) eingesetzt.

In der Literatur existieren lediglich 2 experimentelle Arbeiten, die die Versorgung von Putenküken mit essentiellen Fettsäuren untersucht haben. KETOLA et al. (1973) staffelten den Linolsäuregehalt im Futter (1. bis 21. Lebenstag) zwischen 0 und 42,5 g/kg und leiten anhand der Parameter Zunahme und Futteraufwand einen Mindestgehalt von 10 g Linolsäure/kg Futter ab. FRIEDMAN und SKLAN (1997) ermittelten den Einfluss des Fettsäuremusters der Ration auf das Wachstum sowie auf die Immunantwort bei männlichen Putenküken (BUT) in den ersten 6 Lebenswochen. Die Gruppe, deren Futtermischung 12,4 g Linolsäure und 1,35 g α-Linolensäure je kg enthielt, wies ein schlechteres Wachstum im Vergleich zur Gruppe mit den nächst höheren Gehalten an Linol- und α-Linolensäure (22,3 und 2,8 g/kg) auf. Im Alter von 28 Tagen war der Unterschied in der Lebendmasse statistisch gesichert. Weiterhin wurde in dieser Arbeit ein Zusammenhang zwischen der Antikörperproduktion und der Konzentration an Linolsäure sowie an allen ω-6 Fettsäuren im Serum der Puten ermittelt. Eine Beziehung zum Gehalt an α-Linolensäure war nicht nachzuweisen. Da die Abstufung zwischen den genannten Gehalten relativ groß war, kann aus dieser Arbeit keine konkrete Versorgungsempfehlung abgeleitet werden.

Vom NRC (1994) werden folgende Gehalte an Linolsäure je kg Futter (bei 90% Trockensubstanz) empfohlen: vom Schlupf bis zur 8. Lebenswoche 10 g und danach bis zum Mastende (24. Woche) 8 g. Eine Versorgungsempfehlung für α-Linolensäure wurde nicht gegeben.

Untersuchungen von DING und LILBURN (1997) zu Veränderungen des Fettsäureprofils im Putenei während der Embryonalentwicklung zeigten eine Verringerung der Konzentration an Docosahexaensäure in den Phospholipiden von 6% im unbebrüteten Ei auf 1,6% nach einer 28-tägigen Brutdauer. Dieser Rückgang im Ei war verbunden mit dem Einbau der genannten Fettsäure in das embryonale Gewebe und weist damit auf die Bedeutung dieser Fettsäure für die embryonale Entwicklung hin.

Während von WHITEHEAD und HERRON (1988) der Bedarf an der ω-6 Fettsäure Linolsäure für Putenzuchthennen nach Reproduktionsversuchen ermittelt und mit einer Größenordnung von 11 g pro kg Futter angegeben wurde, fehlen Untersuchungen zum Bedarf von Zuchthennen an ω-3 Fettsäuren, insbesondere mit dem Ziel, ein für die Embryonalenwicklung ausreichendes Depot im Brutei anzulegen.

NOBLE (1991) ermittelte im Eifett kommerziell gefütterter Putenhennen folgende Konzentrationen: Linolsäure 14%, Arachidonsäure 2%, α-Linolensäure 1% und Docosahexaensäure 1%. Entsprechend der Menge an Dotterfett, die zwischen 7,7 und 10,4 g bezogen auf den Legeabschnitt der Henne liegen kann (REIDY et al., 1994), wären 150-210 mg ω-3 Fettsäuren (α-Linolen- plus Docosahexaensäure) im Ei enthalten und ein Verhältnis von ω-3 : ω-6 von etwa 1:8 gegeben.

Aus den wenigen Untersuchungen und aufgrund des enormen Leistungsanstiegs gegenüber den den Normen des NRC (1994) zu Grunde liegenden Mastdaten wird für Mastputen von der 1. bis zur 8. Lebenswoche ein Gehalt von 12 g Linolsäure und danach bis zum Mastende von 10 g Linolsäure je kg Futter (bei 88% Trockensubstanz) empfohlen. Für eine vorläufige Versorgungsempfehlung mit α-Linolensäure bildet das errechnete Verhältnis der ω-3 zu ω-6 Fettsäuren im Ei die Basis (s. oben). Danach ergeben sich 1,5 g (1. bis 8. Woche) bzw. 1,25 g (ab 9. Woche) α-Linolensäure je kg Futter.

5 Mengenelemente

5.1 Einleitung

Unter den Mengenelementen werden die Elemente Calcium (Ca), Phosphor (P), Magnesium (Mg), Natrium (Na), Kalium (K), Chlor (Cl) und Schwefel (S) verstanden. In den in der Geflügelernährung eingesetzten Futtermitteln ist der Gehalt an Kalium so hoch, dass nicht mit einer unzureichenden Versorgung zu rechnen ist. Nachfolgend werden dennoch Empfehlungen zur Versorgung mit Kalium gegeben, um den Betrieben für die Nährstoffbilanzierung entsprechende Daten mitzuteilen. Da bei angemessener Versorgung mit schwefelhaltigen Aminosäuren die Versorgung mit Schwefel ausreichend ist, wird auf die Angabe besonderer Empfehlungen für dieses Element verzichtet.
Empfehlungen zur Versorgung von Mastputen mit Mengenelementen sind von der WPSA (1985) sowie vom NRC (1994) publiziert worden. Während die Daten der WPSA (1985) faktoriell abgeleitet wurden, bildeten für die Angaben des NRC (1994) die Ergebnisse von Wachstumsversuchen die Basis, in denen neben der Wachstumsleistung der Gehalt an Knochen- oder Zehenasche bzw. das Auftreten bzw. Ausbleiben von Mangelsymptomen als Kriterien dienten. Die hier vorgelegten Empfehlungen zur Versorgung mit Mengenelementen beziehen die wenigen neueren Daten zum Ansatz, zu den unvermeidlichen Verlusten und zur Verwertung ein. Für eine faktorielle Ableitung war die Datengrundlage aber auch hier nicht ausreichend.

5.2 Stand des Wissens zu den Faktoren des Bedarfes

5.2.1 Gehalte an Mengenelementen im Tierkörper und im Lebendmassezuwachs

In der Literatur sind nur wenige Daten zur Konzentration an Mengenelementen im Mastputenganzkörper zu finden (Tabelle 5.1). Die Daten der WPSA (1985) weisen für 91 Tage alte Puten höhere Gehalte an Ca und P im Körper auf als für 42 Tage alte. Dieser Unterschied erscheint angesichts des mit dem Wachstum abnehmenden Anteils des Skelettes für moderne Putenherkünfte nicht plausibel. In einer neueren Untersuchung mit einer schweren Herkunft zeigte sich das Gegenteil (Dänner, pers. Mitt.). Aus diesen Daten ließ sich berechnen, dass die Gehalte im LM-Zuwachs männlicher Puten zwischen der 3. und 21. Lebenswoche kontinuierlich zurückgingen (in g/kg LM-Zuwachs): Ca 10,8 bis 3,3; P 8,5 bis 4,2; Mg 0,56 bis 0,22; Na 1,6 bis 1,2 und K 4,3 bis 2,4. Bei den weiblichen Tieren, die bis zum Alter von 17 Wochen untersucht wurden, zeigte sich, mit Ausnahme des Ca, ein ähnlicher Trend. Die Gehalte waren bei weiblichen Tieren auf einem grundsätzlich niedrigeren Niveau als bei den männlichen. Für Ca und P lassen sich weitere Werte aus den Bilanzuntersuchungen von SANDERS et al. (1992) und QIAN et al. (1996) kalkulieren. In diesen Untersuchungen wurden bei männlichen Tieren im Alter zwischen 14 und 21 Tagen Konzentrationen an Ca und P im LM-Zuwachs von 8,4 und 6,0 g/kg Lebendmassezuwachs nicht überschritten.

Tabelle 5.1: Gehalte an Mengenelementen im Körper von Mastputen (g/kg Lebendmasse)

	Ca	P	Mg	Na	K	Cl	Anmerkungen[1)]
CASTALDO et al. (1990)	7,94			0,98	2,52		91. Tag, Nicholas Large White, w
RANAWEERA und WISE (1981)				0,68	1,47		66 und 73 Tage, B.U.T. T6, m
WPSA (1985)	4,3	3,4	0,24	1,6	2,00	1,50	Schlupf
	8,9	6,4	0,37	1,5	2,10	1,18	42 Tage, w
	8,2	6,0	0,35	1,6	2,06	1,05	42 Tage, m
	10,1	6,4	0,39	1,2	2,10	1,40	91 Tage, w
	10,8	7,2	0,43	1,5	2,04	1,25	91 Tage, m

[1)] m: männlich; w: weiblich

5.2.2 Unvermeidliche Verluste

Die Höhe der unvermeidlichen Verluste lässt sich derzeit noch nicht für alle Elemente sicher quantifizieren. Sie machen allerdings bei schnell wachsenden Tieren nur einen geringen Anteil des Gesamtbedarfes aus. Bei Puten einer schweren Herkunft betrugen im Alter von 63 Tagen die unvermeidlichen Verluste 13 mg P/kg W je Tag (Dänner, pers. Mitt.). Da für die übrigen Mengenelemente keine Daten vorliegen, erfolgt die Schätzung der unvermeidlichen Verluste in Anlehnung an die Empfehlungen zur Versorgung der Legehennen und Broiler nach GfE (1999).

5.2.3 Verwertung

Die Gesamtverwertung stellt den Mengenanteil des mit dem Futter aufgenommenen Elementes dar, der im Tierkörper angesetzt wird. Die Gesamtverwertung gibt nur dann gleichzeitig auch die Verwertbarkeit eines Elementes als Eigenschaft eines Futtermittels wieder, wenn die Versorgung des Tieres mit dem entsprechenden Element im suboptimalen Bereich liegt. Literaturdaten zur Gesamtverwertung von Ca und P bei Mastputen liegen von BRADLEY und SAVAGE (1995), HOCKING et al. (2002a), LEESON und ATTEH (1995), SANDERS et al. (1992), SLAUGH et al. (1989), YI et al. (1996) und ZYLA et al. (1996) vor. Bei den zitierten Literaturstellen beträgt die Spanne der Gesamtverwertung für Ca 31-69% und für P 28-62%. Als Verwertbarkeit im Sinne einer charakterisierenden Eigenschaft eines Futtermittels lassen sich diese Werte nicht interpretieren. Neben der unklaren Herkunft der verwendeten Mineralstoffträger ist in vielen Fällen nicht nachvollziehbar, ob die Versorgung der Tiere mit dem entsprechenden Element im suboptimalen Bereich lag.
In praxisüblichen Putenmastrationen liegen in der Summe aus pflanzlichen und mineralischen Quellen etwa zwei Drittel des gesamten P als Nicht-Phytin-Phosphor (NPP) vor, wobei dieser Anteil mit zunehmendem Alter der Tiere in der Regel abnimmt. Unter Zugrundelegung einer Verwertung des NPP von 70% (GfE, 1999) kann eine mittlere Gesamtverwertung des in der Ration enthaltenen Phosphors von rund 50% kalkuliert werden. Auf Grund der deutlich längeren Mastdauer bei Puten im Vergleich zum Broiler und des abnehmenden Zusatzes von mineralischem Phosphor im Verlaufe der Mast ist bei Puten eine unterschiedliche Höhe der Gesamtverwertung des Phosphors in den einzelnen Mastphasen zu erwarten. Da die methodischen Grundlagen für eine standardisierte P-Bewertung noch nicht ausreichend sind (GfE, 1999), werden für Mastputen neben den Empfehlungen zur Versorgung mit verwertbarem P auch Empfehlungen zur Versorgung mit Gesamt-P gegeben. Diese sind als Näherungswerte zu verstehen. Bei Vorliegen von Messdaten zur Verwertbarkeit des Phosphors sind diese Empfehlungen zu überprüfen und gegebenenfalls anzupassen.
Für die Verwertbarkeit von Ca, Mg, Na, Cl und K liegen keine Ergebnisse für Mastputen vor. Deshalb wurde unterstellt, dass diese Mengenelemente in derselben Größenordnung wie von der GfE (1999) für Legehennen und Broilern angenommen verwertet werden.

5.3 Empfehlungen zur Versorgung mit Mengenelementen

Die in den vorangegangenen Abschnitten erläuterten Daten zu Teilfaktoren des Bedarfes erlauben die Ableitung von Konzentrationen im Alleinfutter (Tabelle 5.2). Für das Element P werden Angaben auf Basis von Gesamt-P und verwertbarem P gemacht. Hierdurch soll der Tatsache Rechnung getragen werden, dass speziell für P die Variation in der Verwertbarkeit zwischen verschiedenen Quellen sehr hoch sein kann und außerdem für dieses Element eine höhere ökologische Relevanz gegeben ist als für die übrigen Mengelemente. Sobald Messdaten zur Verwertbarkeit des P aus ausreichend vielen Einzelkomponenten vorliegen, wird eine differenziertere Berechnung der Gehalte an Gesamt-P möglich sein. Insofern sind die Angaben zum Gesamt-P in Tab. 5.2 als vorläufig anzusehen.

Tabelle 5.2: Empfohlene Gehalte an Mengenelementen im Futter für Mastputen (g/kg bei 88% Trockenmasse)[1,2)]

Alter,	Ca		P		verwertb. P		Mg		Na[3)]		K	
Wochen	m	w	m	w	m	w	m	w	m	w	m	w
1-2	13,0	13,0	10,0	10,0	5,0	5,0	0,60	0,60	1,3	1,3	3,5	3,5
3-4	13,0	13,0	10,0	10,0	5,0	5,0	0,60	0,60	1,3	1,3	3,5	3,5
5-8	12,0	11,0	9,0	9,0	4,5	4,5	0,55	0,55	1,2	1,2	3,2	3,2
9-12	10,0	9,0	7,5	6,0	3,8	3,0	0,50	0,45	1,1	1,0	3,0	2,5
13-16	9,0	7,0	6,0	5,0	3,0	2,5	0,45	0,35	1,1	0,8	2,5	2,0
17-20	7,0		5,0		2,5		0,40		1,0		2,0	
>20	5,0		4,0		2,0		0,35		0,9		1,8	

1) m: männlich; w: weiblich 2) es sind die Gehalte an AME gemäß Tabelle 2.2 unterstellt
3) für Chlor werden dieselben Werte wie für Na unterstellt

Für alle Elemente können die Gehalte im Futter im Verlaufe der Mast deutlich reduziert werden. Dies ist die Konsequenz aus den Veränderungen in der Zusammensetzung des Zuwachses und im Futteraufwand auf (Futter/Zuwachs). Neuere Versuche mit Variation in der P-Versorgung belegen, dass mit den hier empfohlenen P-Gehalten auch bei einem hohen Wachstumsniveau keine negativen Effekte eintreten (HOCKING et al., 2002a,b; RODEHUTSCORD et al., 2003). Speziell in den letzten beiden Phasen scheint das Potenzial zur Reduzierung der P-Gehalte im Futter mit diesen Empfehlungen nicht voll ausgeschöpft zu sein.
Insgesamt wird deutlich, dass zur experimentellen Absicherung der Angaben zu den Gehalten im LM-Zuwachs, zu den Einflussfaktoren auf die unvermeidlichen Verluste und zur Verwertbarkeit noch umfangreiche Forschungsarbeit geleistet werden muss.

6 Spurenelemente

6.1 Zusammenfassende Empfehlungen zur Versorgung

In der Fütterung von Mastputen sind die essentiellen Spurenelemente Eisen, Kupfer, Zink, Mangan, Jod und Selen von Bedeutung. Sie müssen in mehr oder weniger großem Umfang dem Futter zugelegt werden. Für weitere essentielle Spurenelemente liegen bislang keine ausreichenden Befunde vor, um Angaben über den erforderlichen Bedarf machen zu können. In Tabelle 6.1 sind für die genannten Spurenelemente Versorgungsempfehlungen für Mastputen zusammengestellt.

Tabelle 6.1: Empfohlene Konzentrationen an Spurenelementen in Rationen für Mastputen (mg je kg Futtertrockenmasse)

Element	Woche	
	1 – 4	> 4
Eisen	80	60
Kupfer	15	6
Zink	50	40
Mangan	60	50
Jod	0,5	0,5
Selen	0,2	0,2

Die Angaben stellen Versorgungsempfehlungen dar, die aus verschiedenen Dosis-Wirkungs-Versuchen abgeleitet wurden. Allerdings stützen sich die Daten teilweise auf ältere Experimente mit Tieren, deren Wachstumspotential das der heutigen Zuchtprodukte nicht erreichte. Eine faktorielle Ableitung des Bedarfs ist derzeitig nicht möglich, denn die Datenbasis über die Gehalte im Zuwachs ist unzureichend und es existieren keine Angaben über die endogenen Verluste und die Elementverwertung.
In der Literatur wird diskutiert, dass verschiedene Belastungen, wie Stress oder Infektionen (z.B. Kokzidiose) aufgrund verminderter Absorption und/oder eines veränderten Stoffwechselverhaltens eine höhere Elementaufnahme erfordern (NOY et al., 1994).

6.2 Eisen

Systematische Untersuchungen zum Eisenbedarf von Mastputen fehlen bislang weitgehend. Das INRA (1984) empfiehlt eine Zulage von 40 mg/kg Futter in den ersten acht Wochen sowie von 30 bzw. 20 mg/kg in den darauf folgenden Mastabschnitten. Nach den Angaben des NRC (1994) liegen die Versorgungsempfehlungen altersabhängig im Bereich von 80 bis 50 mg/kg Futter mit 90 % T. Im Hinblick auf diese Angaben werden für eine ausreichende Versorgung 80 bzw. 60 mg/kg T empfohlen.
Hohe Eisenzulagen von bis zu 500 mg/kg haben einen geringen Einfluss auf die oxidative Stabilität von Putenfleisch während der Lagerung (BARTOV und KANNER, 1996). Eisenzulagen von 50 mg/kg konnten bei Putenküken die Beeinträchtigung des Wachstums durch überhöhte Kupfergaben (0,05 % Kupfersulfat) nur teilweise kompensieren (HARMS und EBERT, 1974).

6.3 Kupfer

Systematische Dosis-Wirkungsuntersuchungen zur Ableitung des Kupferbedarfs für Mastputen sind in der Literatur nicht vorhanden. In jüngsten Untersuchungen (SCHENKEL et al., 2004) zum Kupfergehalt in Ganzkörpern wurden bei Putenküken deutlich höhere Kupfergehalte im Ganzkörper ermittelt als bei Tieren in der anschließenden Mast. Daher wird für die Aufzucht eine höhere Versorgungsempfehlung angesetzt (15 mg/kg T) als für die anschließende Mast (6 mg/kg T). Das INRA (1984) sieht für verschiedene Altersstufen bzw. Mastabschnitte Kupferzulagen von 2 bis 4 mg/kg vor. Das NRC (1994) empfiehlt Kupfergehalte in den ersten acht Wochen von 8 mg/kg, die in den folgenden Mastabschnitten auf 5 mg/kg reduziert werden.
Eine Reihe von Untersuchungen, die in der Literatur vorliegen, beschäftigt sich mit dem Einfluss erhöhter Kupfergaben über das Futter und Trinkwasser. Hierbei konnte bei Zulagen im Bereich 60 bis 250 mg/kg teilweise ein verbessertes Wachstum, verbunden mit einem erniedrigten Futteraufwand, ermittelt werden. Noch höhere Zulagen führten zu Wachstumsdepressionen, zum Teil verbunden mit einer Schädigung des Muskelmagens (GUENTHNER et al., 1978; KASHANI et al., 1986; HARMS und BURESH, 1987; LEESON et al., 1997). In mehreren Experimenten hatte die erhöhte Kupferergänzung eine vermehrte Retention in den untersuchten Organen, vor allem in der Leber (GUENTHNER et al., 1978; WARD et al., 1994, 1995) zur Folge. In den Untersuchungen von GUENTHNER et al. (1978) konnten durch hohe Kupfergaben (120 mg/kg) Ausfälle durch Aortenrupturen deutlich reduziert werden. Die Effekte der hohen Kupfersupplementation waren abhängig von der Rationszusammensetzung (v.a. Protein-, Methionin-, Sulfat- und Eisengehalt) (HARMS und EBERT, 1974; GUENTHNER et al., 1978; CHRISTMAS und HARMS, 1979; KASHANI et al., 1986; HARMS und BURESH, 1987). Auch eine gezielte Coccidioseinfektion beeinflusste die Kupferwirkungen (WARD et al., 1995). Hohe Kupfergaben können zu einer unerwünschten Steigerung der Viskosität des Caecuminhaltes und der Exkremente führen (LEESON et al., 1997).

6.4 Zink

Dosis-Wirkungsversuche mit Putenküken auf der Basis von halbsynthetischen Diäten führten DEWAR und DOWNIE (1984) durch. Je nach erfasstem Kriterium lag der Bedarf zwischen 25 (optimales Wachstum) und 41 mg/kg (Zn-Konzentration im Plasma). Unter Verwendung von

Sojaisolaten als Proteinquelle hatten andere Autoren in früheren Arbeiten (KRATZER et al., 1958; SULLIVAN, 1961) einen Zinkbedarf für Putenküken von 50 bis 60 mg/kg abgeleitet. Die Untersuchungen wurden allerdings an Tieren durchgeführt, deren Zuwachsleistungen deutlich unter denen heutiger schwerer Mastputen liegen. Unter Berücksichtigung der vorhandenen Literatur werden für eine ausreichende Versorgung 50 mg/kg T in den ersten 4 Wochen und während der anschließenden Mastperiode 40 mg/kg T festgelegt.

6.5 Mangan

Aus Untersuchungen von KEALY und SULLIVAN (1966) an bis zu vier Wochen alten Putenküken ergab sich im Hinblick auf das Wachstum und die Verhinderung von Beinschäden ein Bedarf im Bereich von 37 bis 72 mg/kg. Nach Übersichten von HILL (1988) und NOY et al. (1994) werden für eine ausreichende Manganversorgung bei Mastputen meist 50 bis 60 mg/kg T empfohlen. Einige Zuchtfirmen weisen Konzentrationen von bis zu 100 mg/kg als Versorgungsempfehlung aus (Noy et al., 1994).

6.6 Jod

Für Jod liegen in der Literatur keine Dosis-Wirkungs-Untersuchungen zur Bedarfsermittlung an Mastputen vor. Deshalb müssen gewisse Rückschlüsse aus Untersuchungen an Masthühnern gezogen werden.

CREEK et al. (1954) fanden bei Masthähnchen mit 0,3 mg Jod/kg eine normale Schilddrüsenstruktur vor. Bei Mastküken konnten GROPPEL et al. (1991) bei Jodzulagen zwischen 0,1 und 10 mg/kg keinen Einfluss auf die Lebendmasseentwicklung ermitteln. Die Jodeinlagerung in den untersuchten Organen stieg entsprechend der Jodsupplementierung an. Die Zulage von 10 mg/kg bewirkte eine Schilddrüsenhypertrophie. In Anlehnung an die Empfehlungen für Mastküken (GfE, 1999) dürften 0,5 mg/kg T auch bei intensiv wachsenden Mastputen eine ausreichende Versorgung sicherstellen.

Vom NRC (1994) wird der Jodbedarf von Mastküken mit 0,35 mg/kg und für Mastputen mit 0,4 mg/kg Futter mit 90 % T angegeben. Von Zuchtunternehmen werden insbesondere für Jungtiere in den ersten 4 bis 8 Lebenswochen z. T. wesentlich höhere Versorgungsempfehlungen ausgesprochen (NOY et al., 1994). Auch das INRA (1984) empfiehlt eine Jodzulage bei der Aufzucht und Mast zwischen 1,0 und 0,5 mg/kg. Für die Notwendigkeit derart hoher Gaben werden allerdings keine experimentellen Befunde genannt. Bei Legeputen führten bei bestimmten Herkünften 4,2 mg J/kg bereits zu einer Beeinträchtigung der Legeleistung und des Schlupfs (CHRISTENSEN et al., 1991).

6.7 Selen

Der Selenstoffwechsel steht in vielfältiger Wechselbeziehung zu anderen Nahrungsfaktoren. Häufig beschrieben ist die Wechselbeziehung zwischen Selen und Vitamin E und den schwefelhaltigen Aminosäuren (SCOTT et al., 1967; SELL et al., 1997a; UNDERWOOD und SUTTLE, 1999).

SCOTT et al. (1967) ermittelten, dass 0,18 mg Se/kg T erforderlich waren, um bei ausreichend mit Vitamin E versorgten Putenküken Myopathien an Muskelmagen und Herz zu vermeiden. Auch SELL et al. (1997a) verweisen auf die Bedeutung der Selenversorgung im Zusammenhang mit ihren Versuchen zur Steigerung der nutritiven Vitamin E-Zufuhr an Mastputen. Aus Untersuchungen von SCOTT und THOMPSON (1971) ergibt sich bei Putenküken ein Anstieg der Selengehalte in den untersuchten Organen bis zu einer Dosierung von 0,2 bis 0,3 mg/kg. Eine weitere Seleneinlagerung konnte bei Zulagen von deutlich über 0,5 mg/kg ermittelt werden. In kurzfristigen Untersuchungen von BARTOV (1983) an älteren, ausreichend mit Vitamin E versorgten Mastputen hatten Zulagen von Selen im Bereich von 0,1 bis 1,1 mg/kg keinen Einfluss auf die Lebendmasseentwicklung bzw. die Vitamin E-Konzentration im Blutplasma. HADLEY und SUNDE (1997) fanden bei weiblichen Putenküken, die halbsynthetische Diäten erhielten, dass mit steigenden Selenzulagen zum Futter bei 0,2 mg/kg T die Aktivität der Glutathionperoxidase in der Leber ein Plateau erreichte. Auch die Selenkonzentration in der Leber wies in diesem Versorgungsbereich ein Plateau auf, während die Aktivität der Glutathionperoxidase im Blutplasma ein Maximum bei 0,3 mg/kg erreichte.

Selenzulagen im Bereich von 0,05 und 0,5 mg/kg zu einer Basaldiät mit weniger als 0,007 mg/kg hatten keinen Einfluss auf die Zunahmen während des 23-tägigen Versuchs. Aufgrund dieser Befunde werden für eine ausreichende Versorgung 0,2 mg/kg T festgelegt.

BONOMI (2001) postuliert anhand seiner Versuchsergebnisse im Hinblick auf Zuwachs, Futteraufwand und Schlachtkörperzusammensetzung einen Bedarf von 0,3 mg/kg. Die Versorgungsempfehlungen des INRA (1984) liegen bei 0,15 mg/kg Zulage in den ersten 8 Wochen und 0,1 mg/kg während der übrigen Mast. Das NRC (1994) empfiehlt Selenkonzentrationen von 0,2 mg/kg während der gesamten Mast. Ähnliche Empfehlungen liegen von HILL (1988) bei optimaler Vitamin E-Versorgung vor.

7 Fettlösliche Vitamine

7.1 Zusammenfassende Empfehlungen zur Versorgung

Für die Herleitung von Versorgungsempfehlungen (Tab. 7.1) liegt bislang nur eine begrenzte Anzahl von experimentellen Studien vor. Im Falle von Vitamin E wurde eine Reihe von Untersuchungen durchgeführt, bei denen relativ hohe Dosierungen eingesetzt wurden, um die Immunabwehr der Tiere oder die oxidative Stabilität des Fleisches bzw. des Fettes zu verbessern. Die hierbei ermittelten Dosierungen überschreiten deutlich den ernährungsphysiologischen Bedarf. Hinsichtlich der Einschätzung der Versorgung mit Vitamin K ist es schwierig, den Anteil der intestinalen Eigensynthese abzuschätzen.

Tabelle 7.1: Empfehlungen zur Versorgung mit fettlöslichen Vitaminen (je kg Futtertrockenmasse)

	Woche		
	1–2	3–8	>8
Vitamin A, IE	5 000	5 000	5 000
Vitamin D_3, IE	1 500	1 100	1 100
Vitamin E, mg[1]	15	15	10
Vitamin K, mg	1,5	1,5	1,0

[1] plus 0,6 mg Vitamin E (DL-α-Tocopherolacetat) je g Polyenfettsäuren im Futter über den Bedarf

7.2 Vitamin A

Vitamin A wird vor allem in der Leber gespeichert. Ein Gehalt von 2750 IE/kg hatte in einer Untersuchung von AYE et al. (2000a) eine Depletion der Leberspeicher zur Folge. Nach Studien von PRINZ et al. (1981) findet eine ausreichende Einlagerung bei einem Zusatz von 5000 IE/kg Futter statt. Eine hohe Vitamin A-Zulage (20 000 IE/kg) führte zu einem Anstieg der Vitamin A-Konzentration in Leber und Muskulatur, wobei signifikante Unterschiede zwischen verschiedenen Muskeln (Brust, Keule) bestanden (SALLMANN et al., 1998).

Putenküken, deren Diät mit nur 100 IE/kg supplementiert war, wiesen eine verminderte Wachstumsrate auf (DORR und BALLOUN, 1976). PRINZ et al. (1981) stellten bei Putenküken, die Rationen mit weniger als 500 IE/kg erhielten, bereits nach wenigen Tagen deutliche Mangelsymptome (schwere Koordinationsstörungen) fest. Bei Dosierungen zwischen 500 und 1000 IE/kg wurden Verzehrsminderungen beobachtet. SKLAN et al. (1995) verfütterten an Putenküken Soja-Sorghum-Rationen. Erfolgte keine Vitaminergänzung, starben die Küken innerhalb von zwanzig Tagen bei stark reduzierten Vitamin A-Gehalten in Blut und Leber. AYE et al. (2000a) beobachteten bei Vitamin A-Mangelputen (2750 IE/kg Futter) markante epitheliale Schäden, vor allem im Bereich des Oropharynx und Oesophagus, begleitet von einer raschen Depletion der Leberspeicher. Nach Meinung der Autoren steht die erhöhte Mortalität in Zusammenhang mit einer Schädigung des Immunsystems.

JEROCH et al. (1978) ermittelten in Mastversuchen (112 Tage), dass Vitamin A-Zulagen von 5000 IE/kg in der Starterperiode und 2500 IE/kg in der anschließenden Mastperiode für optimale Mastergebnisse (Lebendmassezunahme, Futteraufwand, Verluste) ausreichend waren. Nach Auswertung des gesamten Datenmaterials halten sie eine Supplementierung mit 3000 IU/kg über die gesamte Mastperiode für ausreichend. PRINZ et al. (1981) leiten aus Mastversuchen unabhängig vom Geschlecht eine erforderliche Zulage an Vitamin A von 2000 IE/kg für optimalen Zuwachs und Futteraufwand ab. Soll eine ausreichende Leberspeicherung erreicht werden, halten die Autoren Zulagen von 5000 IE/kg für erforderlich. In einem zweifaktoriellen Fütterungsversuch (PRINZ et al., 1986) bestand eine Wechselwirkung zwischen der Rohprotein- und der Vitamin A-Versorgung. Bei geringerem Proteinversorgungsniveau ergab sich ein etwas höherer Vitamin A-Bedarf. Die Autoren kommen anhand ihrer Ergebnisse zu der Schlussfolgerung, dass ein Vitamin A-Zusatz von 5000 IE/kg, unabhängig von der Proteinversorgung, bezüglich einer ausreichenden Futteraufnahme und Lebendmasseentwicklung ausreichend ist. Dementsprechend wird eine Versorgung mit 5 000 IE Vitamin A/kg T für alle Mastabschnitte empfohlen.
Die Versorgungsempfehlungen des INRA (1984) liegen bei 10 000 IE/kg für Küken sowie bei 8 000 IE/kg für Masttiere. Das NRC (1994) empfiehlt für Mastputen 5 000 IE/kg.

Erhöhte Gaben von Vitamin A können bei Mastputen einen positiven Effekt auf die humorale Immunantwort haben. Allerdings wurde dieser Effekt nicht bei allen geprüften Antigenen bestätigt (PRINZ et al., 1983; 1986). SKLAN et al. (1995) wiesen bei Puten eine verbesserte zelluläre und humorale Immunantwort nach, wenn die Versorgung mit Vitamin A die NRC- Empfehlung um etwa das Doppelte überschritt. Hohe Gaben von Vitamin A hatten keinen Einfluss auf den Verlauf einer experimentellen Histomoniasis-Infektion bei Puten (SCHILDKNECHT und SQUIBB, 1979). Nach Befunden von AYE et al. (2000b) hatte eine unterschiedliche Versorgung mit Vitamin A keinen Einfluss auf die Erregerausbreitung nach einer experimentellen Infektion mit Pasteurella multicodia.
Der Einfluss hoher Vitamin A-Gaben (20 000 IE/kg) in Kombination mit hohen Vitamin E-Gaben auf die oxidative Stabilität in der Putenmuskulatur wurde von GÖTZKE (1993) untersucht. Hohe Vitamin A-Gaben führten bei niedrigen Vitamin E-Gaben zu einer zusätzlichen Reduktion der Vitamin E-Gehalte im Plasma.
Eine hohe Vitamin A-Zulage (16 000 IE/kg) beeinträchtigte die Lebendmassezunahme und die Femurentwicklung bei Putenküken (DORR und BALLOUN, 1976). METZ et al. (1985), VELTMAN und JENSEN (1985) sowie VELTMAN et al. (1987) konnten bei Putenküken antagonistische Effekte zwischen Vitamin A und Vitamin D nachweisen. Hohe Vitamin A-Gaben führten vor allem bei marginaler Vitamin D-Versorgung zu Störungen der Knochenmineralisierung und des Calciumstoffwechsels, die mit einer Beeinträchtigung des Vitamin D-Stoffwechsels in Zusammenhang stehen. Hohe Vitamin D-Gaben vermochten zum Teil die toxische Wirkung von Vitamin A auf Wachstum, Skelett- und Mineralstoffwechsel zu mildern oder zu beseitigen. STEVENS et al. (1983) ermittelten, dass der Grad der Interaktion zwischen beiden Vitaminen auch von der Art und Höhe der Fettsupplementierung abhängig war.

7.3 Vitamin D

In umfangreichen Fütterungsversuchen von JEROCH et al. (1978) an Mastputen waren nur in einem Versuchsdurchgang mit 500 IE/kg in der Starterphase Einbrüche in der Mastleistung festzustellen. STEVENS et al. (1983) ermittelten bei Putenküken bei einer Steigerung der Vitamin D-Zulage von 900 auf 2400 IE/kg einen verbesserten Zuwachs sowie positive Effekte auf die Knochenmineralisierung. VELTMAN und JENSEN (1985) fanden in dreiwöchigen Untersuchungen bei Steigerungen der Vitamin D_3-Zulagen eine Verbesserung der Zunahmen, einen günstigeren Futteraufwand und einen deutlichen Rückgang der Knochenschäden. Zwischen der Zulage von 900 und 1800 IE/kg Futter waren die Unterschiede allerdings statistisch nicht mehr abzusichern. In Untersuchungen von JIN et al. (2001) führten Rationen mit 275 IE Vitamin D_3/kg zu verminderter

Futteraufnahme, geringerem Wachstum und deutlichen Veränderungen im Mineralstoffwechsel einschließlich verminderter Knochenaschegehalte. Bei 550 IE Vitamin D_3/kg war im Vergleich zur Ration mit 1650 IE/kg das Wachstum vermindert (JIN et al., 2001). Für Putenküken wurde daher die Versorgungsempfehlung mit 1500 und für Mastputen mit 1100 IE/kg T festgelegt.
Das NRC (1994) empfiehlt 1100 IE/kg. Das INRA (1984) gibt mit 1500 (Küken) bzw. 1200 IE/kg (Masttiere) etwas höhere Zielwerte an.
HURWITZ et al. (1973) beschreiben eine Vitamin D-resistente Rachitiserkrankung bei Putenküken mit einer leichten Hypocalcämie und vermindertem Knochenaschegehalt. Die Konzentration an Calcium-bindendem Protein in der Darmmukosa war deutlich reduziert.
Eine Vitamin D-Hypervitaminose führt zu Mineralisierungen im Bereich der Nierentubuli sowie der Arterien. LANGENA et al. (1999) ermittelten bei Gaben von 990 µg 25-Hydroxyvitamin D_3/kg eine erhöhte Mortalität bei männlichen Mastputen. METZ et al. (1985) sowie VELTMAN und JENSEN (1985) konnten zeigen, dass stark erhöhte Vitamin A-Gaben zu einer Beeinträchtigung des Vitamin D-Stoffwechsels führten, die durch gleichzeitig hohe Vitamin D-Gaben vermieden wurden.

7.4 Vitamin E

SCOTT (1953) ermittelte einen günstigen Effekt von Vitamin E-Zulagen bei der Verhinderung der Perosis, wobei der Vitamin E-Gehalt der Basisration nicht mitgeteilt wird. JORTNER et al. (1985) fanden bei Putenküken unter Vitamin E-Mangel sehr niedrige Vitamin E-Konzentrationen im Blut, verbunden mit klinischen Enzephalopathien, die durch Vitamin E-Supplemente (5 IE/kg) vermieden wurden. APPLEGATE und SELL (1996) fanden bei relativ niedrigen Basisgehalten von 5 IE α-Tocopherol/kg und erhöhten Gehalten an ungesättigten Fettsäuren bei jungen Mastputen keine Hinweise auf eine Erhöhung von Enzephalopathien. MELDRUM et al. (2000) fanden bei Putenküken mit dem Knock-Down Syndrom signifikant erniedrigte Vitamin E-Spiegel im Blut.
RICHTER et al. (1988) ermittelten in mehreren Fütterungsversuchen mit Mastputen, dass sowohl im Küken- als auch im Mastfutter, von einem Versuch abgesehen, die nativen Vitamin E-Gehalte (5-17 mg/kg) ausreichend waren. Vitamin E-Zulagen führten zu keiner Verbesserung der Futteraufnahme, des Futteraufwandes oder einer Reduktion der Ausfälle. Entsprechende Versuchsergebnisse liegen auch von weiteren Autoren vor (BARTOV, 1983; SOTO-SALANOVA et al., 1993; APPLEGATE und SELL, 1996; SOTO-SALANOVA und SELL, 1996). In mehreren Fütterungsversuchen mit männlichen Mastputen fanden SELL et al. (1997a), dass die nativen Vitamin E-Gehalte zwischen 6 und 20 IE α-Tocopherol ausreichend für ein normales Wachstum und Wohlbefinden der Tiere waren. Steigende Zulagen zwischen 12 und 300 IE DL-α-Tocopherylacetat hatten keinen Einfluss auf die Mastleistung. Ferner ergaben sich keine Beziehungen zu Sprunggelenksveränderungen oder Myopathien des Magens.
SELL et al. (1997a) diskutieren, ob nicht zusätzlich zu der Vermeidung von Mangelerscheinungen wie Sprunggelenksverdickungen, Muskeldystrophie sowie Wachstum und Futteraufwand noch weitere Kriterien wie oxidative Vorgänge im Gewebe und Hämolyseanfälligkeit der Erythrozyten zur Beurteilung der Versorgung herangezogen werden müssten. Aufgrund der vorliegenden Ergebnisse wird eine Versorgung mit 15 mg/kg T vorgeschlagen, die ab der 9. Woche auf 10 mg/kg T abgesenkt werden kann. Das INRA (1984) empfiehlt 20 mg/kg für Küken sowie 15 bis 10 mg/kg für Mastputen. Das NRC (1994) schlägt für Masttiere zwischen 12 und 10 mg/kg vor.
In der Literatur wird mehrmals darauf verwiesen, dass in verschiedenen Gewebeproben von Mastputen deutlich geringere Tocopherolkonzentrationen nachweisbar waren als bei vergleichbar gefütterten Hühnerküken. Dies wird mit als Grund für die erhöhte Oxidationsanfälligkeit von Putenfleisch und -fett angesehen (MECCHI et al., 1956a, b; WEN et al., 1997; SALLMANN et al., 1998). MECCHI et al. (1956a, b), MARUSICH et al. (1975), BARTOV (1983) sowie APPLEGATE und SELL (1996) begründen dies mit einer ineffizienten intestinalen Vitamin E-Absorption, vor allem bei jungen Putenküken. SKLAN et al. (1982) ermittelten eine schlechtere Vitamin E-Absorption als beim Hühnerküken, vermutlich bedingt durch eine verstärkte Bildung und

Ausscheidung von Tocopheryl-Glucuroniden im Darm. In einer Reihe von Untersuchungen (SOTO-SALANOVA et al. 1993; SOTO-SALANOVA und SELL, 1995, 1996; SELL, 1996) wurde bei jungen Putenküken in den ersten Lebenstagen ein deutlicher Abfall der α-Tocopherol-Konzentration im Plasma und Lebergewebe analysiert, der auch durch erhöhte Vitamin E-Gaben nicht vollkommen kompensiert werden konnte (APPLEGATE und SELL, 1996; SELL et al., 1997a).
Zwischen der Vitamin E-Aufnahme mit dem Futter und der Tocopherolkonzentration im Blut besteht eine enge Beziehung (BARTOV, 1983; SHELDON, 1984; SELL et al., 1997a, b; HEFFELS-REDMANN et al., 2001 a, b), wobei sich bei jungen Puten allerdings ein Sättigungsplateau einstellt (BARTOV, 1983; SOTO-SALANOVA et al., 1993). Ferner ergibt sich eine Beziehung zwischen der Vitamin E-Konzentration im Blut und im Schlachtkörper (MARUSICH et al., 1975).
Hohe Vitamin E-Zulagen führten zu einem Anstieg der α-Tocopherolgehalte in Blut und Muskulatur, wobei sich zwischen verschiedenen Muskeln eine Differenzierung ergab (SKLAN et al., 1983; SHELDON, 1984; HIGGINS et al., 1998a, b; SALLMANN et al., 1998; RENERRE et al., 1999). MERCIER et al. (2001) konnten einen Anstieg der Vitamin E-Gehalte in den Mikrosomenmembranen der Muskulator bei erhöhten α-Tocopherolgaben zeigen. SELL et al. (1997a) und WEN et al. (1997) verfolgten die α-Tocopherolgehalte in verschiedenen Organen und Geweben von Mastputen in Abhängigkeit von Mastdauer und Dosis, während von SURAI et al. (1999) entsprechende Studien an Embryonen vorliegen.
In der Literatur existieren zahlreiche Untersuchungen zum Einfluss erhöhter Vitamin E-Gaben auf die oxidative Stabilität von Putenfleisch unmittelbar nach dem Schlachten sowie nach längerem Lagern bzw. der Lagerung von Putenprodukten. Dabei wurde neben der Dosis auch die Art und Menge des gleichzeitig verfütterten Fettes sowie der Einfluss zusätzlicher Eisengaben untersucht (MARUSISCH et al., 1975; GOTZKE, 1993, BARTOV und KANNER, 1996; WEN et al., 1997; HIGGINS et al., 1998 a, b; RENERRE et al., 1999; MERCIER et al., 2001). Dabei ergab sich ein positiver Einfluss hoher Vitamin E-Zusätze im Dosierungsbereich von 200 bis 600 mg/kg auf die oxidative Stabilität von Lipiden wie auch Proteinen. Eine Vitamin E-Zulage in Abhängigkeit vom jeweiligen Gehalt an Polyensäuren im Futter, wie sie für das Hühnergeflügel hergeleitet wurde (GfE, 1999), bietet sich auch in der Putenfütterung an.
Mehrere Arbeiten befassten sich mit dem Einfluss von Vitamin E-Zulagen auf die Immunabwehr bei Puten (FRANCHINI et al., 1990; HEFFELS-REDMANN et al., 2001a, b). So konnte zum Teil eine erhöhte Antikörperproduktion bzw. eine vermehrte Lymphozytenproliferation gefunden werden (SELL et al., 1997a; GORE und QURESHI, 1997). Allerdings sind die Ergebnisse nicht einheitlich. Offensichtlich scheint die Versorgung mit Vitamin A bzw. die Aufnahme an verschiedenen Fettsäuren zusätzlich Einfluss zu nehmen (SOTO-SOLANOVA und SELL, 1996; SELL et al., 1997b.)

7.5 Vitamin K

Bei Putenküken mit Vitamin K-Mangel wurden eine verlängerte Prothrombinzeit im Blut sowie Schädigungen im Muskelmagen beobachtet (GRIMINGER, 1957). GRIMINGER (1957) kommt auf Grund seiner Versuche zur Schlussfolgerung, dass bei jungen Putenküken die enterale mikrobielle Vitamin K-Synthese kaum zur Versorgung der Tiere beiträgt. Orale Antibiotikagaben hatten keinen Einfluss auf den abgeleiteten Vitamin K-Bedarf. Dies zeigt, dass der Beitrag aus der intestinalen mikrobiellen Synthese bei den jungen Tieren relativ gering ist (JIN und SELL, 2001).
GRIMINGER (1957) hält für Putenküken in den ersten vier Wochen für eine ausreichende Prothrombinzeit einen Zusatz von 1,8 mg Menadion/kg für erforderlich. Er fand für den wasserlöslichen Menadion-Natrium-Bisulfit-Komplex eine etwa dreifach bessere Wirksamkeit als für Menadion. Diese Empfehlungen wurden auch vom NRC (1994) übernommen.
Neuere Untersuchungen mit Putenküken ergaben für die Parameter Prothrombin-Zeit im Plasma und Prothrombinkonzentration im Plasma einen Bedarf an Vitamin K_1 im Bereich von 0,079 und 0,13 mg/kg (JIN und SELL, 2001; JIN et al., 2001). Die Biopotenz hinsichtlich der Reduzierung der

Prothrombinzeit bzw. einer Konzentrationserhöhung an Prothrombin im Plasma war für Vitamin K_1 höher als für Menadion-Dimethylprimidinolbisulfit bzw. Menadion-Nikotinamidbisulfit (JIN und SELL, 2001) Eine unterschiedliche Vitamin K_1-Supplementierung hatte nur einen geringen Einfluss auf die Knochenentwicklung junger Puten (JIN et al. 2001). Die Versorgungsempfehlungen wurden auf 1,5 bzw. 1 mg Vitamin K/kg T festgelegt. Während in Bereich der Tierernährung eine Supplementierung sowohl mit Vitamin K_1 als auch Vitamin K_3 in Form verschiedener Menadion-Präparate möglich ist (Petersen und Kruse, 2002), ist der Einsatz von Menadionpräparaten in der Humanernährung nicht statthaft (ANONYMOUS, 2002).
Die Versorgungsempfehlungen des INRA (1984) liegen mit 2 bis 4 mg/kg relativ hoch. Das NRC (1994) empfiehlt 1,75 mg/kg für Küken und 1,5 bis 0,75 mg/kg für Masttiere (abnehmend mit steigendem Alter).
Hohe Vitamin K-Gaben hatten keinen positiven Effekt auf den Verlauf einer experimentell induzierten Histomoniasis-Infektion (SCHILDKNECHT und SQUIBB 1979).

8 Wasserlösliche Vitamine

8.1 Zusammenfassende Empfehlungen zur Versorgung

Die Kenntnisse über den Bedarf von Mastputen an verschiedenen wasserlöslichen Vitaminen sind zum Teil sehr lückenhaft. So liegen in den wenigsten Fällen systematische Steigerungsversuche über mehrere Dosierungen hinweg vor, die eine sichere Ableitung von Versorgungsempfehlungen zulassen. Auch hinsichtlich der Versuchsdauer ist die Aussagefähigkeit einiger Untersuchungen eingeschränkt, da sich die Experimente häufig nicht über eine ganze Mastperiode, sondern nur über einen relativ kurzen Zeitraum erstrecken. Hinsichtlich älterer Versuche ist auch der eingetretene deutliche Zuchtfortschritt in Rechnung zu stellen. In der Regel wurden nur relativ unspezifische Kriterien wie Wachstum, Futteraufwand oder die Vermeidung klinischer Mangelerscheinungen erfaßt. Teilweise ist dies auch in der Tatsache begründet, daß die nativen Gehalte der üblichen Futterkomponenten bereits ausreichende Gehalte aufweisen und Versuche im Vitaminmangel nur mit halbsynthetischen Diäten möglich sind.

Tabelle 8.1: Empfehlungen zur Versorgung mit wasserlöslichen Vitaminen (mg/kg Futtertrockenmasse)

	Woche		
	1–2	3–8	>8
Thiamin	2,0	2,0	2,0
Riboflavin	4,5	4,5	4,0
Vitamin B_6	4,0	4,0	3,5
Vitamin B_{12}	0,01	0,01	0,01
Pantothensäure	10	10	10
Niacin	50	50	40
Folsäure	1,0	1,0	0,8
Biotin	0,3	0,25	0,20
Cholin	1 600	1 400	1 200

8.2 Thiamin (Vitamin B_1, Aneurin)

Im Mangel kommt es zu Ataxie sowie zu Muskelschwäche und Muskeldegeneration. Das rückwärtige Verbiegen von Kopf und Hals sind typische nervöse Symptome. Thiaminmangel führt bei jungen Putenküken zu Veränderungen der Aminosäurengehalte in der Leber und in einigen Gehirnregionen. In verschiedenen Gehirnregionen war die Konzentration einzelner biogener Amine (Epinephrine und v.a. Serotonin) verändert. Die erniedrigten Serotoningehalte dürften in enger Beziehung zu der im Thiaminmangel auftretenden Anorexie stehen (REMUS und FIRMAN, 1988, 1989, 1991).

Aufgrund dieser Befunde und in Anlehnung an die Vorschläge des NRC (1994) wird eine Versorgungsempfehlung von 2 mg/kg T festgelegt.
Zur Ableitung des Thiaminbedarfs liegen keine neueren Untersuchungen vor. Das INRA (1984) geht bei Putenküken von einer ausreichenden Versorgung bei 2 mg/kg und im Verlauf der weiteren Mast bei 1 mg/kg aus. Das NRC (1994) schlägt für die Versorgung eine Konzentration von 2,0 mg/kg für Starter- und Mastfutter vor.

8.3 Riboflavin (Vitamin B_2, Lactoflavin)

Eine Vitamin B_2-Unterversorgung führte bei Putenküken zu einer erhöhten Mortalität, schlechtem Wachstum, schlechter Befiederung und ausgeprägter Paralyse der Beine (BIRD et al., 1946; RUIZ und HARMS, 1989a). Unklar ist, ob die in älteren Untersuchungen beschriebene Dermatitis evtl. auch durch Biotinmangel bedingt war (PATRICK et al., 1944; BIRD et al., 1946, RUIZ und HARMS, 1989a).
In älteren Fütterungsversuchen an Bronzeputen ermittelten BIRD et al. (1946) einen Bedarf von 3,25 bis 3,75 mg/kg für 4 Wochen alte Küken. PATRICK et al. (1944) hatten den Riboflavinbedarf von Putenküken für die ersten sechs Lebenswochen mit 2,7 mg/kg angegeben. In den Untersuchungen von JEROCH et al. (1978) war der native Gehalt der Ration ausreichend, um optimale Mastergebnisse zu erzielen. Die Autoren schlagen daher Gehalte von 3,2 mg/kg (1.-6. Woche), 2,3 mg/kg (7.-12. Woche) und 1,4 mg/kg (13.-16. Woche) zur ausreichenden Versorgung vor. RUIZ und HARMS (1989a) ermittelten im Altersabschnitt bis 3 Wochen mit Hilfe der „broken-line"-Technik einen Bedarf für maximales Wachstum von 3,8-4,4 mg/kg. Für die sichere Vermeidung von Beinschwächeproblemen sind nach Meinung der Autoren unter Umständen noch etwas höhere Zulagen notwendig. Daher werden für die ersten 8 Wochen 4,5 und für die verbleibende Mast 4,0 mg/kg T empfohlen.
Vom INRA (1984) wird eine Versorgung empfohlen, die sich von 6 mg/kg beim Küken auf 4 mg/kg für die weitere Mast vermindert. Das NRC (1994) empfiehlt im Starterfutter Gehalte von 4 mg/kg, die sich im weiteren Verlauf der Mast auf 2,5 mg/kg vermindern.

8.4 Vitamin B_6 (Pyridoxin)

In Mastversuchen von JEROCH et al. (1978) waren die nativen Vitamin B_6-Gehalte ausreichend für die Versorgung. Zulagen erbrachten keine Verbesserung der Mastleistungsparameter. Nach Auffassung der Autoren sind Gehalte von 5,7 mg/kg im Starterfutter sowie 4,0-4,2 mg/kg in den einzelnen Mastphasen ausreichend. In den Empfehlungen der Tabelle 8.1 ist eine Orientierung an den Angaben des NRC (1994) erfolgt, das 4,5 mg/kg in der Starterphase sowie 3,5-3,0 mg/kg in der anschließenden Mast für ausreichend hält. Das INRA (1984) weist mit 2 mg/kg eine deutlich geringere Versorgungsempfehlung aus.

8.5 Vitamin B_{12} (Cyanocobalamin)

In Mastversuchen von JEROCH et al. (1978) ergaben Vitamin B_{12}-Zulagen keine Verbesserung der Mastergebnisse. Die Gehalte lagen im Starterfutter bei 0,019 mg/kg und in den Mastfuttermischungen zwischen 0,009 und 0,002 mg/kg. Während das NRC (1994) für alle Lebensabschnitte der Mastputen eine einheitliche Versorgung mit 0,003 mg/kg empfiehlt, liegen die Angaben des INRA (1984) mit 0,015 mg/kg für Küken und 0,01 mg/kg für Masttiere deutlich höher. Der Ausschuss empfiehlt für die gesamte Mastdauer 0,01 mg/kg.

8.6 Pantothensäure

Zulagen von Ca-Pantothenat (50 mg/kg) konnten bei Putenküken die aufgetretenen Mangelerscheinungen (gebrochene Federn, Dermatitis, Perosis und Durchfälle) mildern. Erst eine kombinierte Gabe mit Folsäure und Biotin führte zu einer weitgehenden Vermeidung der Krankheitserscheinungen (ROBBLEE und CLANDININ, 1953).

In Untersuchungen von JEROCH et al. (1978) war der native Gehalt der Futtermischungen an Pantothensäure ausreichend für ein optimales Wachstum. Die Autoren halten daher eine Versorgung mit 13 mg/kg in den ersten 6 Wochen und von 10 bzw. 8 mg/kg in der darauf folgenden Mast für ausreichend. Bei Putenküken bewirkten Zulagen von 0,8 bis 3,8 mg Pantothensäure zu einer Basaldiät mit 9,8 mg/kg keinen Effekt auf Futteraufnahme und Wachstum. Tendenziell ergab sich ein günstigerer Futteraufwand (RUIZ und HARMS, 1989b). Auf der Grundlage der vorliegenden Literatur wird eine Versorgungsempfehlung von 10 mg/kg T gegeben.
Die Empfehlungen des NRC (1994) liegen für Putenküken (bis 4 Wochen) bei 10 mg/kg und für Mastputen bei 9 mg/kg. Das INRA (1984) empfiehlt für Putenküken eine Versorgung mit 10 mg/kg und für Mastputen mit 5 mg/kg.

8.7 Niacin (Nicotinsäure, Nicotinsäureamid)

In frühen Untersuchungen hatte SCOTT (1953) im Hinblick auf die Verhinderung der Perosis bei Verwendung halbsynthetischer Rationen einen Bedarf von ca. 70 mg/kg postuliert. In einem Fütterungsversuch von JEROCH et al. (1978) bewirkten Niacin-Zulagen keine Verbesserung der Mastleistung von Puten. Die Autoren halten daher eine Versorgung mit 43 mg/kg in den ersten sechs Wochen als ausreichend für ein optimales Wachstum. Für verschiedene Mastphasen schlagen sie Gehalte von 32 bzw. 24 mg/kg vor. Nach ZAVIEZO und McGINNIS (1980) benötigen Putenküken je nach Tryptophanversorgung zwischen 45 und 35 mg/kg für optimales Wachstum bei Verhinderung von Perosiserkrankungen. Bei Putenküken im Alter von bis zu drei Wochen ermittelten RUIZ und HARMS (1988) mit Hilfe der „broken line"-Auswertung einen Minimalbedarf von 44 mg/kg für die Kriterien maximales Wachstum, optimale Futteraufnahme und günstiger Futteraufwand sowie einer Vermeidung des Beinschwächesyndroms. CHRISTMAS et al. (1986) konnten bei Puten im Alter zwischen 4 und 12 Wochen keinen Einfluss von Niacinzulagen zwischen 0 und 50 mg/kg auf Lebendmasseentwicklung, Beinschwäche oder Mortalität finden. Die Basisdiät auf der Grundlage von Mais und Sojaschrot enthielt 21 mg/kg. Die Tiere waren in dieser Untersuchung den ersten 4 Lebenswochen mit einer Ration mit 80,6 mg/kg gefüttert worden.
Von einigen Autoren wird die erforderliche Niacinzufuhr von der Versorgung mit Tryptophan abhängig gemacht (ZAVIEZO und McGINNIS, 1980). Allerdings konnten RUIZ und HARMS (1990) an Putenküken mit gestaffelter Niacin- bzw. Tryptophanversorgung und ausreichender Pyridoxinversorgung nur eine sehr ineffiziente Synthese von Niacin aus Tryptophan ermitteln, deutlich ungünstiger als bei Hühnerküken.
Es wird für die ersten 8 Wochen eine Versorgung mit 50 mg/kg T und für die restliche Mast mit 40 mg/kg T empfohlen.
INRA (1984) und NRC (1994) empfehlen eine Versorgung mit 60 mg/kg im Starterfutter und mit 50 bzw. 40 mg/kg in der Mastphase.

8.8 Folsäure

Folsäuremangel führt zu einer Beeinträchtigung des Wachstums sowie der Schlupffähigkeit. Ferner führt eine Unterversorgung mit diesem Vitamin zu Perosis und Störungen in der Befiederung. Unzureichende Folsäureversorgung der Hennen führte zu einer Beeinträchtigung der Lebendmasseentwicklung der jungen Putenküken (MILLER und BALLOUN, 1967).
In Untersuchungen von JEROCH et al. (1978) bewirkten Folsäurezulagen in einem Putenmastversuch keine Verbesserung der Lebendmasseentwicklung und des Futteraufwandes. Die nativen Gehalte (1,3 mg/kg in der Starterphase; 1,8 bis 0,9 mg/kg in der anschließenden Mast) gewährleisteten eine optimale Entwicklung. FROEHLICH (1987) schlägt Folsäurezulagen im Starterfutter von 1-2 mg/kg und für Masttiere von 1-1,5 mg/kg vor.
In Übereinstimmung mit dem NRC (1994) wird eine Folsäurekonzentration von 1,0 mg/kg T in den ersten 8 Wochen empfohlen. Im weiteren Verlauf der Mast sind 0,8 mg/,g T ausreichend. Die Angaben des INRA (1984) unterscheiden sich davon nur unwesentlich.

8.9 Biotin

Biotinmangel führt unter anderem zu Hautschäden, Fissuren an den Fußballen und Verkrustungen im Schnabelwinkel. Bei mit Biotin unterversorgten Küken waren vermehrt Beinschäden (verdrehte Füße, verkürzte Knochen) und Wachstumsdepression festzustellen (JENSEN und MARTINSON, 1969, ARENDS et al., 1971). Dermatitis trat an den Beinen, insbesondere an Zehen, Fußballen, Schenkeln sowie um den Schnabel und die Augenlider herum auf. Die Augenlider waren zum Teil durch ein Exudat verklebt. Ein Teil der Hautschäden war durch Biotininjektionen heilbar (MARUSICH et al., 1970; ARENDS et al., 1971). ROBBLEE und CLANDININ (1953) konnten zeigen, dass sich durch kombinierte Gaben von Biotin und Ca-Pantothenat bei Putenküken Mangelsymptome, wie gebrochene Federn, Dermatitis, Perosis und Durchfälle weitgehend beseitigen lassen.

JENSEN und MARTINSON (1969) ermittelten bei Putenküken bis zum Alter von vier Wochen für maximales Wachstum einen Bedarf im Bereich von 0,24 bis 0,28 mg/kg bei Verwendung halbsynthetischer Diäten. WAIBEL et al. (1969) konnten bei Putenküken keinen Einfluss einer Biotinzulage von 0,3 mg/kg zu einer Basaldiät mit 0,105 mg/kg auf Zunahmen, Befiederung und Beindeformationen erkennen. MARUSICH et al. (1970) postulierten beim Einsatz halbsynthetischer Diäten aufgrund ihrer Untersuchungen an Putenküken in den ersten 3 Lebenswochen einen Bedarf von 0,325 mg/kg. Für übliche Praxisrationen empfehlen sie eine Versorgung mit mindestens 0,45 mg/kg zur Verhinderung von Mangelerscheinungen.

ARENDS et al. (1971) fanden bei Verwendung halbsynthetischer Diäten (Basalgehalt 0,07 mg/kg) mit steigenden Biotinzulagen (bis zu 0,3 mg/kg) signifikant steigende Biotingehalte in den Lebern der Küken. Für maximales Wachstum in den ersten 4 Lebenswochen wurde ein Bedarf von 0,12 bis 0,17 mg/kg abgeleitet. Nach den Ergebnissen von JEROCH et al. (1978) sind 0,24 mg/kg in den ersten 6 Lebenswochen und 0,18 bis 0,11 mg/kg in der anschließenden Mast für eine optimale Versorgung ausreichend.

MISIR und BLAIR (1988), die Untersuchungen zur Bioverfügbarkeit von Biotin aus verschiedenen Futtermitteln mit jungen Putenküken durchführten, fanden bei vordepletierten Tieren bis zu Zulagen von 0,2 mg/kg eine lineare Steigerung der Wachstumsrate.

Hinsichtlich der Biotinversorgung ist anzumerken, dass offensichtlich erhebliche Unterschiede in der Biotinverfügbarkeit aus verschiedenen Futtermitteln bestehen. Dies ist bei der Rationsgestaltung zu berücksichtigen (JENSEN und MARTINSON, 1969; KRUEGER et al., 1976, GfE, 1999). BUDA (2001) und PLATT et al. (2001) fanden bei einer Verdopplung der Biotinsupplementierung bei männlichen Mastputen eine leichte Verbesserung der Mastendgewichte. Eine mikroskopische Untersuchung der Fußballen ergab teilweise einen positiven Effekt der höheren Biotinzulagen auf Ballenläsionen.

Es werden Biotingehalte von 0,3 (<3 Wochen), 0,25 (Wochen 3-8) und 0,2 (>8 Wochen) mg/kg T empfohlen. Nach INRA (1984) kann der Biotingehalt von 0,3 mg/kg bei Putenküken auf 0,05 mg/kg in der Mast abgesenkt werden. Die Versorgungsempfehlungen des NRC (1994) bewegen sich mit 0,25 mg/kg für Küken und 0,1 mg/kg für Masttiere in einer ähnlichen Größenordnung.

8.10 Cholin

In den Untersuchungen von JEROCH et al. (1978) verbesserten Zulagen von 250 mg/kg in den ersten sechs Lebenswochen die Lebendmassezunahmen und verminderten die Verluste. Die Autoren schlagen für eine ausreichende Versorgung Gehalte in der Starterphase von 1590 mg/kg und in der Mast zwischen 990 und 675 mg/kg vor. BLAIR et al. (1986) konnten bei Methioninzulageversuchen mit jungen Putenküken keinen Einfluss einer zusätzlichen Cholingabe auf Wachstum, Futteraufnahme und Futteraufwand ermitteln. Der Cholingehalt der Basaldiät lag bei 1250 mg/kg. In Versuchen von PESTI et al. (1979) und MILES et al. (1983) war auf Grund der positiven Effekte von Cholinzulagen (1500 mg/kg) bei marginaler Methioninversorgung von einem Spareffekt (Methylgruppen) ausgegangen worden.

Es werden Cholingehalte von 1600 (<3 Wochen), 1 400 (Wochen 3-8) und 1 200 (>8 Wochen) mg/kg T empfohlen. Das INRA (1984) empfiehlt eine Zulage an Cholinchlorid von 800 bis 500 mg/kg für Mastputen. Die Versorgungsempfehlungen des NRC (1994) nehmen von 1600 mg/kg für Küken auf 800 mg/kg bis zum Mastende ab.

8.11 Vitamin C (Ascorbinsäure)

Vögel sind in der Regel in der Lage, ausreichend Ascorbinsäure aus Glucoronsäure im Stoffwechsel zu synthetisieren und sind damit unabhängig von einer exogenen Zufuhr. LECHOWSKY und NAGORNA-STASIAK (1995) fanden bei Puten eine relativ hohe Aktivität der L-Gulono-gamma-oxidase, einem Enzym, welches an der Biosynthese von Vitamin C beteiligt ist. In Abhängigkeit von Lebensalter und Haltungsbedingungen konnten sie unterschiedliche Syntheseraten in einzelnen Organen aufzeigen (LECHOWSKY und NAGORNA-STASIAK, 1995; NAGORNA-STASIAK et al., 1999). Puten können verschiedenen Stressfaktoren (Belegdichte, Temperatur etc.) ausgesetzt sein, die den Vitamin C-Bedarf erhöhen, so dass sich nach einer Supplementierung positive Effekte ergaben (NAGORNA-STASIAK et al., 1999). So berichten DORR und BALLOUN (1976) nach Ascorbinsäurezulage über positive Effekte auf die Lebendmassezunahme bedingt durch eine Steigerung der Futteraufnahme bei sehr jungen Putenküken. Eine unzureichende Vitamin A-Versorgung führte zu einer Beeinträchtigung des Vitamin C-Stoffwechsels. In Untersuchungen von SELL et al. (1997a) hatten Ascorbinsäurezulagen von 300 mg/kg bis zum 41. Lebenstag keinen Einfluss auf Zunahme, Futteraufwand und Lebensfähigkeit.

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