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- *Abstracts (Kurzfassungen der Originalmitteilungen)*
- *Workshop contribution (Workshop-Beitrag)*

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J. Hummel
Chairman

Citation recommendation

1. Abstracts / Review Lecture / Workshop-Contributions:

Sciascia QL, De Leonardis D, Görs S, Metges CC, 2025. The effect of glutamine supplementation on low birthweight suckling pig growth, organ mass and glutamine metabolism. Proc. Soc. Nutr. Physiol. 34:71.

Content

Conference program	7
Review lecture	21
Abstracts	25
Workshop	143
Index	151

Conference program

12:00		Opening and Welcome Prof. Dr. Jürgen Hummel, 1st Chairman of the Society of Nutrition Physiology e. V. Prof. Dr. Bernhard Brümmer, Vice President for Research at Georg-August University of Göttingen (requested)	
Feeding concepts			page
12:15		1 Development, digestive anatomy and physiology of calves weaned on hay or concentrate diets Zhao X., Jamrogiewicz M., Przybyło M., Flaga J., Kański J., Wojtysiak D., Mitko R., Ortmann S., Niu M., Górka P., Clauss M. — Zurich	27
12:30		2 Effect of a complementary feed containing pro- and prebiotics in combination with electrolytes and herbal components on the diarrhoea incidence and development of neonatal dairy calves. Altemeier M., Becklönne M.S., Middendorf L. — Essen / Göttingen	28
12:45		3 Body weight development in <i>ad libitum</i> milk-fed calves using different weaning protocols – preliminary results Stoschus A., Diesing L.E., Liermann W., Nieschulze T., Braun K., Hammon H.M. — Dummerstorf / Westerrönfeld	29
13:00		4 Effects of different weaning protocols after <i>ad libitum</i> milk feeding on metabolic traits in blood of dairy calves – preliminary results Stoschus A., Diesing L.E., Liermann W., Nieschulze T., Braun K., Hammon H.M. — Dummerstorf / Westerrönfeld	30
13:05		5 Exploratory investigation of animal- and environment-related factors influencing colostrum intake patterns within the first 24 h postpartum in dam-bound rearing systems Kurek J., Kurek A., Harms J., Zeiler E., Steinhoff-Wagner J. — Freising-Weihenstephan / Poing-Grub	31
13:10		6 Effects of individual colostrum composition on postnatal immune and metabolic adaptation in calves von Riedheim M. A., Kessler E.C., Gross J.J. — Bern	32
13:15		7 Associations of growth rates during the first two months of life with short- and long-term behavior and performance in Holstein heifers Hemmert K.J., Ostendorf C.S., Cohrs I., Koch C., Sauerwein H., Ghaffari M.H. — Bonn / Münchweiler an der Alsenz / Dummerstorf / Engen / Giessen	33
13:20		8 Evaluation of local protein feed crops as an alternative to soya protein in the diet of fattening Holstein bulls, which were fed milk replacer with either skimmed milk or whole milk during the rearing phase Scholz P., Buhrow F., Kersten S., Meyer U., Zeyner A., Dänicke S., von Soosten D., Frahm J. — Brunswick / Halle (Saale)	34
13:25		9 Feed choice strategies and lysine self-regulation in fattening pigs Schedle K. — Vienna	35
13:30		10 Nutrient booster diets and choice feeding effects on growth and efficiency in fattening pigs Schedle K., Razenberger D., Gierus M. — Vienna	36
13:35		11 Moulting of dual-purpose chickens – comparison of two feeding methods Kaiser A.M.G., Böttcher F., Lange F., Rettig A.M., Saliu E.-M. — Eberswalde	37
13:40		Session change	
Feed Processing			
13:45		12 Ensilability of leaves from common deciduous trees Sarfo T.S., Hummel J., Kuhla B., Hünerberg M. — Göttingen / Dummerstorf	38
14:00		13 Fermentation of alfalfa with various lactic acid bacteria strains and additives and their effect on fermentation characteristics Schmidle L., Klein N., Rodehutschord M. — Stuttgart-Hohenheim	39
14:15		14 Comparison of lactic acid bacteria for the fermentation of field peas Quinger F., Klein N., Rodehutschord M., Camarinha-Silva A., Seifert J. — Stuttgart-Hohenheim	40
14:20		Coffee break	
Digestion and absorption			
14:45		25 Effect of fermentation of field pea grains on the digestibility of nutrients in growing pigs Quinger F., Klein N., Camarinha-Silva A., Seifert J., Rodehutschord M. — Stuttgart-Hohenheim	51
15:00		26 Phytate degradation in growing pigs fed diets with unfermented or fermented field pea grains Klein N., Quinger F., Camarinha-Silva A., Seifert J., Rodehutschord M. — Stuttgart-Hohenheim	52
15:05		27 <i>In vitro</i> phytate degradation by laying hen phosphatases and their genetic background Hanauska A., Sommerfeld V., Schmid M., P. Haas V., Huber K., Bennewitz J., Rodehutschord M. — Stuttgart-Hohenheim	53
15:20		28 Accuracy and precision of measuring apparent dry matter digestibility in cattle: Comparison of the quantitative collection technique with the acid-insoluble ash and TiO ₂ indicator method Peschel M., Steinhoff-Wagner J., Windisch W. — Göttingen / Freising-Weihenstephan	54
15:35		29 Impact of timing of a second colostrum feeding on serum IgG dynamics in calves during the first week of life von Riedheim M.A., Erkiner H., Gross J.J. — Bern	55

12:00		See Lecture hall 008	
		Amino acids and nitrogen	page
12:15		15 Factors influencing fecal N excretion and it's relation to DMI based on digestion studies with wethers <i>Dreyer C., Losand B., Priepke A., Hummel J. — Dummerstorf / Sanitz / Göttingen</i>	41
12:30		16 Effects of <i>Hermetia Illucens</i> larvae meal on laying performance, feed efficiency, and nutrient composition of egg yolk and liver in Lohmann Sandy hens <i>Wang J., Geßner D., Most E., Schäfer L., Engel P., König S., Eder K. — Giessen</i>	42
12:45		17 Protein Reduction in Diets for fattening chickens: Effects on Growth, Health, and Carcass Traits in Male Dual-Purpose Chickens <i>Rettig A.M., Kaiser A., Lange F., Böttcher F., Trei G., Saliu E.-M. — Eberswalde</i>	43
13:00		18 Is the estimated optimal Tryptophan to Lysine ratio in finishing broilers influenced by the Leucine to Lysine ratio in the feed? <i>Siebert D.-C., Scharch C. — Frankfurt am Main / Wettin-Löbejun</i>	44
13:15		19 Influence of dietary glycine, serine, and betaine on plasma amino acid concentrations in broiler chickens, fed a low crude protein diet <i>Philippi H., Siegert W., van Milgen J., Mercier Y., Rodehutsord M. — Stuttgart-Hohenheim / Göttingen / Saint-Gilles / Lyon</i>	45
13:30		20 A meta-analysis on the influence of age on nitrogen accretion per body weight gain in broiler chickens <i>Dou W., Siegert W. — Göttingen</i>	46
13:35		21 Plane of nutrition and milk composition influences urinary constituents in neonatal calves during the first week of life <i>Meyer A., Vorndran A.M., Steinhoff-Wagner J. — Freising-Weihenstephan</i>	47
13:40 Session change			
		Fatty acids and lipids	
13:45		22 Dose-dependent effects of a dietary fish oil supplementation on the plasma lipidome of healthy adult cats <i>Paßlack N., Veit H., Funk H., Zentek J., Schuchardt S. — Oberschleißheim / Giessen / Munich / Berlin / Hanover</i>	48
14:00		23 Impact of different fat content in colostrum and transition milk on metabolic parameters in newborn calves <i>Erkinger H., von Riedheim M.A., Calisici O., Gross J.J. — Bern / Eltville am Rhein</i>	49
14:15		24 Longitudinal characterization of fecal lipids in dairy heifers from birth to first calving <i>Hosseini Ghaffari M., Sadri H., Hemmert K., Ostendorf C., Schuchardt S., Koch C., Sauerwein H. — Dummerstorf / Tabriz / Bonn / Hanover / Münchweiler</i>	50
14:20 Coffee break			
		Minerals	
14:45		37 Total and soluble phosphate content in wet cat food from the US market <i>Löffelmann S.F., Dobenecker B. — Oberschleißheim</i>	63
15:00		38 Effect of adding phosphate binders Ipakitine® and Renvela® to a high phosphate diet for cats on faecal and renal phosphate excretion <i>Sebold E., Pankratz C., Dobenecker B. — Munich</i>	64
15:15		39 Phosphate fractionation in complementary feed for dental hygiene in dogs <i>Pankratz C., Lewan L., Dobenecker B. — Munich</i>	65
15:30		40 Comparison of nutrient deposition and mineralization patterns in captive frugivorous bat (<i>C. perspicillata</i>) carcasses across developmental stages <i>Moscoso F.F., Moscoso D.R., Rüegg-van den Broek P., Liesegang A. — Zurich / Bogota / Kerzers</i>	66
15:45		41 Strain-specific ileal microbiome responses to low-phosphorus diets and their link to phosphorus utilization in laying hens <i>Chien Y.-H., Yergaliev T., Seifert J., Camarinha-Silva A. — Stuttgart-Hohenheim</i>	67

Digestion and absorption		page
15:50	 30 Development of amylase activity in pancreatic tissue and small intestinal content of C57Bl/6J mice before and after weaning <i>Ernst A., Steinhoff-Wagner J., Böswald L. — Planegg-Martinsried / Freising-Weihenstephan</i>	56
16:05	 31 Comparative <i>In Vivo</i> and <i>In Vitro</i> Digestibility of Wheat–Triticale Protein Concentrate and Poultry Meal in Dog Diets <i>Saengiem S., Van der Lee A., Heiden T., Grzeskowiak L., Zentek J. — Berlin / Zörbig</i>	57
16:10	 32 Stomach and intestinal <i>in vitro</i> starch digestion of hay and oat in horses: a pilot study <i>Schulz A., Nieradt A., Bernert S., Gemmeke P., Hauschild J., Klähn J., Watzke L., Trei G., Saliu E.-M. — Eberswalde</i>	58
16:15	 33 Comparison of methane emission in landrace and production breed sheep: A systematic review <i>Brandes-Samak J., Hummel J. — Göttingen</i>	59
16:20	Session change	
16:25	 34 Influence of reduced nitrogen and/or phosphorus supply on hepatic amino acid transporters in small ruminants <i>Wittenberg K.L., Hustedt K., Schnepel N., Muscher-Banse A. — Hanover</i>	60
16:40	 35 Effects of mineral phosphorus supply and <i>myo</i> -inositol feeding on insulin signaling in adipose tissue of two high-performing laying hen strains at different production stages <i>Ruda L.F., Szentgyörgyi A., Sommerfeld V., Huber K., Rodehutscord M. — Stuttgart-Hohenheim</i>	61
16:55	 36 The bovine hepatocyte cell line BFH12 as a potential <i>in vitro</i> model for liver metabolism in dairy cows <i>Dietz L.I., Cermak R., Grünberg W., Wilkens M.R. — Leipzig / Giessen</i>	62
17:00	Poster discussion – Feeding concepts / Feed Processing / Digestion and absorption / Intermediary metabolism	
18:00	Henneberg-Lehmann Award Ceremony (Aula am Wilhelmsplatz, Göttingen) followed by a reception (starting at 20:00) (separate registration required)	
20:00	END Day 1	

Minerals		page
16:00	 42 Effects of phosphorus deprivation during the periparturient period on the phospholipid composition of erythrocyte membranes in dairy cows <i>Eder K., Grundmann S.M., F. Brouwers J., van den Brink L.M., Cohrs I., Grünberg W. — Giessen / Breda / Utrecht</i>	68
16:15	 43 The effect of feeding poplar and willow leaves on blood plasma mineral concentrations in goats <i>Helffenstein K., Durkalec M., Kowalczyk J., Wilkens M.R., Kuhla B. — Dummerstorf / Leipzig</i>	69
16:20	Session change	
Gut health / Transport and epithelial physiology		
16:25	 44 Separate and synergistic short-term effects of ruminal and hindgut acidosis on fermentation and microbiome of dairy cows <i>Biber P., Atif R.M., Hartinger T., Reisinger N., Gressley T., Zebeli Q., Castillo-Lopez E. — Vienna / Tulln / Newark / Bonn</i>	70
16:40	 45 Differences in bile acid metabolism between neonatal calves with or without <i>Cryptosporidium parvum</i> infection <i>Liermann W., Dengler F., Dellling C., Bachmann L., Ulrich R., Sauerwein H., Henning A.-K., Hammon H.M. — Dummerstorf / Stuttgart-Hohenheim / Leipzig / Neubrandenburg / Bonn / Greifswald</i>	71
16:55	 46 Comparative effect of β -glucans vs β -glucans and mannan-oligosaccharides on fermentation characteristics of the equine hindgut in a batch culture system <i>Lange I., Richardt W., Zeyner A. — Halle (Saale) / Lichtenwalde</i>	72
17:00	 47 Bacterial and eukaryotic diversity in the gastrointestinal tract of horses fed concentrate containing vs. forage only diets <i>Santo M.M., Bachmann M., Schusser G.F., Wensch-Dorendorf M., Kleinstaub S., Pisch C., Bochnia M., Witow G., Heinichen K., Zeyner A. — Halle (Saale) / Leipzig</i>	73
17:05	 48 Comparison of different tannin supplements, tannin extracts and ways of application in Ussing chamber experiments <i>Krüselmann P., Ebersbach L., Saliu E.-M., Schulze Holthausen J., Vlasova I., Kostenko J., Rownicki M., Piwowarski J., Zentek J. — Berlin / Warsaw / Eberswalde</i>	74
17:20	 49 Impact of a reduced nitrogen and/or calcium diet on expression of genes involved in epithelial transport in young goats <i>Egler C.-Y., Heidelberg M., Burmester M., Hustedt K., Schnepel N., Muscher-Banse A.S. — Hanover</i>	75
18:00	See Lecture hall 008	

09:00	Review lecture Nutritional immunology in relation to farm animals <i>Lauridsen C. — Aarhus</i>	
10:00	Coffee break	
	Feedstuff evaluation and feeding	page
10:30	50 Effects of dietary dried <i>Laminaria japonica</i> on growth performance, blood metabolites, and <i>ex vivo</i> rumen microbial gas production in dairy sheep lambs during the late growth stage <i>Diepold D., Liesegang A., Foggi G., Brugger D. — Zurich / Helsinki</i>	76
10:45	51 Does the inclusion of duckweed in the diet of common carp (<i>Cyprinus carpio</i>) affect growth performance and product quality? <i>Gregersen E., Krone B., Siebenmorgen C., Mörlein J., Sünder A., Strack M., Mateus Vargas R., Wilkens M.R., Tetens J., Mörlein D., Hummel J., Rosenau S. — Göttingen / Leipzig</i>	77
11:00	52 Comparison of horse faeces as an alternative inoculum with rumen fluid for measuring gas production in the Hohenheim gas test <i>Kehraus S., Welter H., Jung C., Hilgers B., Hartinger T., Rippstein L., Rodehutscord M. — Bonn / Hohenheim</i>	78
11:15	53 Meta-analysis of the effects of feeding winery byproducts on production performance, carcass yield, and meat quality in ruminants and non-ruminants <i>Khiaosa-ard R., Gierus M. — Vienna</i>	79
11:30	54 <i>In vitro</i> fermentation characteristics of multi-species hay and silages with ryegrass, alfalfa, and plantain using the RUSITEC <i>Zhang X., Klevenhusen F. — Witzenhausen</i>	80
11:45	55 A minimalist machine learning approach to model <i>in vitro</i> gas production profiles obtained with different inocula <i>Ahmadi H., Rippstein L., Rodehutscord M. — Stuttgart-Hohenheim</i>	81
12:00	56 Functional analysis of faecal samples from cows fed different diets <i>Mewes S., Heinichen K., Wilkens M.R. — Leipzig / Großpösna</i>	82
12:15	57 Estimation of ruminal crude protein degradation parameters in concentrates based on literature data <i>Muche S., Richardt W., Herz R., Zeyner A. — Niederwiesa / Halle (Saale)</i>	83
12:30	58 Change in prediction of milk protein synthesis from the utilizable crude protein at the duodenum to the small intestinal digestible protein evaluation system for dairy cows <i>Peschel M., Seemann L., Padberg K., Mertens P., Unruh C., Bannert E., Meyer U., Dänicke S., von Soosten D. — Brunswick / Göttingen</i>	84
12:45	59 Nutrient composition and feeding value of regional organic by-products in Eastern Germany for livestock nutrition <i>Rühl E., Zentek J. — Berlin</i>	85
12:50	60 Utilization of lignocellulosic agricultural wastes as sustainable feed for <i>Zophobas atratus</i> larvae <i>Fazel Dehkordi N., Abd El-Wahab A., Rath A., Lingens J.B., Plötz M., Grabowski N., Rehman K.U., Visscher C., Schwennen C. — Hanover / Quakenbrück</i>	86
12:55	61 Characterizing nutrient composition and <i>in vitro</i> methane production of available feed resources used for dairy cattle in Bangladesh <i>Ismila J., Uddin M.M., Hünerberg M., Hummel J. — Göttingen / Mymensingh</i>	87
13:00	62 Effect of feed treats on gastrointestinal parameters in C57Bl/6J mice <i>Böswald L. — Planegg-Martinsried</i>	88
13:05	Lunch and Conversation break	
	Feedstuff evaluation and feeding	
14:20	63 Effects of <i>Hermetia illucens</i> larvae meal as a substitute for soybean meal on performance, egg quality, and yolk lipids in laying hens <i>Müller R., Hornfeck F., Schäfer L., Grundmann S., Most E., Eder K., Geßner D. — Giessen / Remptendorf</i>	89
14:35	64 Effects of <i>Hermetia illucens</i> fat as a substitute for soybean oil on performance, egg quality, and yolk lipids in laying hens <i>Geßner D., Hornfeck F., Müller R., Schäfer L., Grundmann S., Most E., Eder K. — Giessen / Remptendorf</i>	90
14:50	65 Utilization of by-products from certified organic farming and organic food processing as feed substrates for <i>Hermetia illucens</i> larvae <i>Schemmann K., Eder K., Geßner D. — Giessen / Remptendorf</i>	91

09:00 See Lecture hall 008

10:00		Coffee break	
Undesirable substances and antinutrients			page
10:30		69 ConTrans: A Tool to Predict the Transfer of Contaminants from Feed to Food of Animal Origin <i>Numata J., Salewski M., Moenning J.-L., Pieper R. — Berlin</i>	95
10:45		70 Effects of feeding Per- and polyfluoroalkyl substances (PFAS) contaminated hay on the liver of dairy goats <i>Bode L., Bühler S., Just H., Pieper R., Kowalczyk J., Fischer-Tenhagen C., Frahm J., Dänicke S., Kersten S. — Brunswick / Berlin</i>	96
11:00		71 Toxicokinetics of per- and polyfluoroalkyl substances (PFAS) in German Improved White dairy goats <i>Just H., Przygoda L., Kowalczyk J., Bode L., Kersten S., Jahnke A., Fischer-Tenhagen C., Moenning J.-L., Numata J., Kuerbis C., Pieper R. — Berlin / Brunswick</i>	97
11:15		72 A systematic review and meta-analysis on the use of lupins and fattening pig diets <i>Buchallik-Schregel J., Wagner B., Ghoreishi N., Pieper R., Kowalczyk J. — Berlin</i>	98
11:30		73 Effect of caustic soda treatment of lupin seeds on quinolizidine alkaloid concentrations – preliminary results <i>Bachmann M., Buchallik-Schregel J., Mitterhuber M., Hoppe A., Pieper R. — Berlin / Nossen</i>	99
11:35		74 Transfer of per- and polyfluoroalkyl substances (PFAS) from feed into edible tissues of chicken for fattening (ROSS308) <i>Just H., Jahnke A., Wagner B., Bode L., Kowalczyk J., Kersten S., Pieper R. — Berlin / Brunswick</i>	100
11:40		75 Effects of ensiling jimsonweed (<i>Datura stramonium</i> L.) in maize on tropane alkaloid concentrations <i>Bachmann M., Wagner B., Kowalczyk J., Pieper R. — Berlin</i>	101
11:45		Session change	
11:50		Energy / Carbohydrates and fibre	
11:50		76 Impact of replacing starchy feeds by industrial by-products on performance and net food production of high-yielding dairy cows <i>Ped S., Scheu T., Koch C., Hayer J., Dusel G., Sauerwein H., Hartinger T. — Münchweiler an der Alsenz / Bonn / Bingen am Rhein</i>	102
12:05		78 Effects of algae β -glucan supplementation on the performance and intestinal immune system of weaned piglets <i>Ringseis R., Gutöhrlein F., Zeitz J.O., Henrich M., Most E., Whelan R., Htoo J.K., Eder K. — Giessen / Hanau</i>	103
12:10		79 Effects of different carbohydrates substituting dietary lactose on metabolism in formula fed calves <i>Bierlein G.F., Bruckmaier R. M., Gross J.J. — Bern</i>	104
12:15		80 The effect of inulin on faecal bacterial metabolites and blood amino acids in healthy sled dogs <i>Schulze Holthausen J., Neumer F., Zentek J. — Berlin / Obrigheim</i>	105
12:20		81 Fat and protein mass in the body of growing bulls grouped by dairy or dual-purpose and beef breeds <i>Honig A.C., Rodehutschord M., Windisch W., von Soosten D., Gruber L., Eittle T. — Hohenheim / Poing-Grub / Freising-Weihenstephan / Brunswick / Vienna</i>	106
12:35		82 Prediction of the energy value in colostrum and transition milk of Brown Swiss cows <i>Ostendorf C.S., Vorndran A.M., Meyer A., Steinhoff-Wagner J. — Freising-Weihenstephan</i>	107
12:50		Lunch and Conversation break	
Transport and epithelial physiology / Others			
14:20		83 Investigation of free water intake of dairy calves via water buckets or bowls within the first two weeks of life <i>Hayer J., Fenrich L., Dusel G. — Münchweiler an der Alsenz / Bingen am Rhein</i>	108
14:35		84 Nutrient accretion of fattening boars <i>Krieg J., van Asten A.L., Becker S., Schollenberger M., Rodehutschord M., Maak S. — Bad Sassendorf / Stuttgart-Hohenheim / Dummerstorf</i>	109
14:50		85 On-farm assessment of external traits to monitor body condition and nutritional status of milk-deprived and milk-fed calves in the first four weeks after birth <i>Kurek J., Liesalz T.M., Vorndran A.M., Kurek A., Huber V., Hammerl G., Spiekers H., Steinhoff-Wagner J. — Freising-Weihenstephan / Achselschwang / Poing-Grub</i>	110

Feedstuff evaluation and feeding		page
15:05	 66 Feed intake of separately offered insects (<i>Acheta domesticus</i> , <i>Hermetia illucens</i> flies) and macroalgae (<i>Palmaria palmata</i> , <i>Ulva lactuca</i>) by dual-purpose pullets <i>Spieß F., Godglück A., Hankel J. — Hanover</i>	92
15:20	 67 Ensiling instead of drying – an alternative to preserve wet-separated <i>Hermetia illucens</i> larval protein <i>Heidemann C.K., Hartinger K., Khiaosa-ard R., Resch R., Zebeli Q., Schedle K., Hartinger T. — Bonn / Vienna / Raumberg-Gumpenstein</i>	93
15:35	 68 Effects of <i>Hermetia illucens</i> larvae meal on growth performance, feed efficiency, and nutritional quality indicators in Lohmann Sandy brother roosters <i>Wang J., Geßner D., Most E., Schäfer L., Engel P., König S., Eder K. — Giessen</i>	94
15:40	Poster discussion – Feedstuff evaluation and feeding	
16:10	Coffee break	
16:30	Networking Event · Social Media Posts Moderation: <i>W. Windisch</i>	
16:30	Impulse: Social media posts about science (separate registration required) <i>Fiack S. — Berlin</i>	
17:15	Impulse: Dos and don'ts in social media communication: How do I build a network? (separate registration required) <i>Richter T. — Göttingen</i>	
18:00	END Technical Programme Day 2	
18:00	Conversation break	
18:30	GfE Member Assembly (separate invitation required)	
20:00	END Day 2	

Transport and epithelial physiology / Others		page
15:05	 86 Preliminary data on the validation of the RumiWatch System for automated detection of short-term feeding behavior and estimation of feed intake in pastured dairy goats <i>Haus A., Hummel J. — Göttingen</i>	111
15:20	 87 Skeletal Muscle Myokines, Myogenic Regulators, and Satellite Cell Markers in Dairy Cows from Late Pregnancy to Early Lactation <i>Sadri H., Bliese L., Meyer I., Mueller U., Schuh-von Graevenitz K., Hofs I., Hosseini Ghaffari M., Sauerwein H. — Tabriz / Bonn / Bingen am Rhein / Dummerstorf</i>	112
15:25	Poster discussion – Undesirable substances and antinutrients / Energy / Carbohydrates and fibre / Others Amino acids and nitrogen / Fatty acids and lipids / Minerals / Gut health / Transport and epithelial physiology	
16:10	Coffee break	
16:30	See Lecture hall 008	
18:00	END Technical Programme Day 2	
18:00	Conversation break	
18:30	See Lecture hall 008	

... News from the Committee for Requirement Standards (AfBN)

09:00 Rodehutschord M. — University of Hohenheim, Institute of Animal Science, Stuttgart-Hohenheim

09:20 Session change

Environmental effects

page

09:25  88 ProBioHuhn: Temporal variation in the organic chicken gut microbiota and influencing factors
Cheng Y.-C., Camarinha-Silva A. — Stuttgart-Hohenheim 11309:40  89 Heat stress in rats: Antioxidant impact of a wood based complementary feed
Schwarz C., Baidlowi A., Kraus V., Gierus M. — Vienna / Yogyakarta 11409:55  90 Impact of 3-Nitrooxypropanol on methane production, blood biochemical parameters and immune response in dairy cows throughout an entire lactation
Reiche A.-M., Sizzano F., Siegenthaler R., Eggenschwiler L., Silacci P., Dohme-Meier F. — Posieux / Nyon 11510:10  91 Seasonal variation in methane production of lactating cows in pasture-based feeding systems
Lizardo R.C., Mathaiyan G., Dickhoefer U. — Kiel 11610:25  92 Food Conversion Efficiency of different diets in Simmental steers: Implications for sustainable beef production
Noh L., Weindl P.A., Saliu E.-M., Hautzinger T., Zeiler E. — Eberswalde / Freising-Weihenstephan 11710:40  93 Impact of the temperature-humidity-index on dairy cow performance at two contrasting German regions (Baltic Sea and Central Region) during Summer 2025
Koch F., Schulz J., Mosler A.M., Melzer N., Wilkens M.R., Kuhla B. — Rostock / Leipzig / Witzenhausen 11810:55  94 Epigenetic and transcriptional responses in the liver of primiparous Holstein dairy cows to short-term heat stress or feed restriction
Sarkar S.M., Voelker-Albert M., Kuhla B., Koch F. — Rostock / Munich 11911:00  95 Decreasing feed-food competition and environmental impact: By-products in diets of fattening pigs
Puntigam R., Preißinger W., Schneeberger E., Hörtenhuber S. — Soest / Poing-Grub / Pöchlarn / Vienna 12011:05  96 The relationship between Carcass Yield in Fattening Pigs and Performance and Carbon Footprint
Rosengart S., Timme C., Büngener-Schröder S., Sehlen K., Visscher C. — Hanover / Schneiderkrug 121

11:10 Session change

Nutrigenomics and metabolomics / Omics in animal physiology

11:15  97 Effects of stress of the endoplasmic reticulum on genome-wide gene expression in a bovine liver cell model
Bajrami E., Wen G., Ringseis R., Geßner D., Eder K. — Giessen 12211:30  98 In ovo administration of myo-inositol and its implications for metabolic programming in broilers
Shomina N., Sommerfeld V., Rodehutschord M., Huber K. — Stuttgart-Hohenheim 12311:45  99 MicroRNA profiling reveals distinct immune and metabolic adaptations in sows and piglets around parturition
Kreuzer-Redmer S., Koger S., Verhovsek D., Metzler-Zebeli B. — Vienna 12411:50  100 Towards standardization in pig microbiome research: Biases introduced by DNA extraction kits are more prevalent in 16S rRNA gene datasets compared to shotgun metagenomic profiles
Enokela S., Yergaliyev T., Camarinha-Silva A. — Stuttgart-Hohenheim 12512:05  101 The kidney proteome of Holstein dairy cows with divergent milk urea concentrations
Koch F., Albrecht D., Müller-Kiedrowski C.B., Görs S., Oster M., Wimmers K., Kuhla B. — Dummerstorf / Greifswald / Innsbruck 126

12:20 Poster discussion – Environmental effects / Nutrigenomics and metabolomics / Omics in animal physiology

12:45 Conversation break

09:00	See Lecture hall 008	
09:20	Session change	
Feed additives		page
09:25	102 Comparison of the efficacy of a phytogetic feed additive composed of pomegranate polyphenols, aromatic essential oils and quillaja saponin compared with zinc oxide on growth performance and postweaning diarrhea indices in weaned piglets <i>Mueller A.S., Syriopoulos K., Tona R., Boehlen R., Wandel R., Maenner K. — Buetzberg / Berlin</i>	127
09:40	103 Prececal disappearance of phytate and digestibility of phosphorus and amino acids in nursery piglets: investigations on their relationship <i>Jlali M., Loibl P., Grimm A., Consuegra J. — Saint-Fons / Frankfurt am Main</i>	128
09:55	104 Effects of dietary microalgae species on growth performance, health, and physiological responses in broilers <i>Abd El-Wahab A., Schubert D.C., Lingens J.B., Krschek C., Starke S., Visscher C. — Hanover / Melle</i>	129
10:10	105 Investigations on the effects of 3-nitrooxypropanol on performance and methane emission of dairy cows <i>Huber V., Armstrong R., Müller I., Faas J., Schomaker T., Blümel F., Ettle T. — Poing-Grub / Tulln / Bramsche / Utting</i>	130
10:25	106 Effects of gallic acid on rumen fermentation, nutrient digestibility and rumen bacterial community in beef steers at low and high dietary crude protein levels <i>Bao Y., Zhou K., Zhao G. — Beijing</i>	131
10:40	107 Use of 2'-Fucosyllactose, a Human Milk Oligosaccharide, in Calf Rearing <i>Scherneck S., Schönleben M., Wemhoff S., Hauling F., Dusel G., Koch C. — Bingen am Rhein / Grafenwald / Bönen</i>	132
10:55	108 Individual promotion of calf health using specific colostrum additives and phytogetic substances <i>Groß C., Arnold C., Calisici O., Voltenauer H., Visscher C. — Hanover / Eltville am Rhein</i>	133
11:00	109 Supplementation of rumen-protected choline during warm summer days reduces body surface temperatures and improves production traits of dairy cows <i>Abousoliman I., Schwerdtfeger J., Koch F., Schneider M.R., Kuhla B. — Dummerstorf / Leipzig</i>	134
11:05	110 Impact of the green algae <i>Ulva intestinalis</i> on ruminal methanogenesis and fermentation parameters <i>in vitro</i> using the Rumen Simulation technique (RUSITEC) <i>Brunnbauer S., Meyer U., Visscher C., Reich M., Schweder T., Schulz C., Paschen M., Dänicke S., von Soosten D. — Brunswick / Hanover / Greifswald / Rostock</i>	135
11:10	111 Impact of increasing dosages of a phytase in broiler feed on nutrient digestibility and performance parameters. <i>Ader P., Amerah A.M., Heidysz M., Kaczmarek S. — Ludwigshafen / Aman / Poznan</i>	136
11:15	Session change	
Ruminal transformations		
11:20	112 Impact of pastures composition on calves microbiota in saliva, rumen and faeces <i>Lauer-Weich C., Werner J., Mesbahi G., Leiber F., Seifert J. — Stuttgart-Hohenheim / Frick</i>	137
11:35	113 WIMiQ: Metabolic adaptation patterns in dairy cows following changes in dietary energy supply: a multivariate approach to analyzing classical feeding trials <i>Fingerhut A., Sauerwein H., Malik A., Meyer U., Dänicke S., Huber K., Seifert J., Bakiasi M., Kersten S., Frahm J. — Brunswick / Bonn / Stuttgart-Hohenheim</i>	138
11:50	114 WIMiQ: Influence of different dietary energy levels on the rumen microbiota of dairy cows <i>Bakiasi M., Billenkamp F., Fingerhut A., Malik A., Sauerwein H., Huber K., Dänicke S., Frahm J., Seifert J. — Stuttgart-Hohenheim / Brunswick / Bonn</i>	139
12:05	115 WIMiQ: Milk-Derived Extracellular Vesicles: Characterization and Proteomic Profiling of their Response to Dietary Energy Transitions in Dairy Cows <i>Malik A., Fingerhut A., Hosseini Ghaffari M., Frahm J., Dänicke S., Huber K., Bakiasi M., Seifert J., Sauerwein H. — Bonn / Brunswick / Dummerstorf / Stuttgart-Hohenheim</i>	140
12:20	116 WIMiQ: Rumen fermentation patterns and ruminal pH values reflect host-microbiome interactions after energy-related dietary shifts in dairy cows <i>Fingerhut A., Sauerwein H., von Soosten D., Meyer U., Kersten S., Dänicke S., Frahm J. — Brunswick / Bonn</i>	141
12:25	Poster discussion – Feed additives / Ruminal transformations	
12:45	Conversation break	

Workshop	
13:00	Successfully implementing recommendations for energy and nutrient supply in dairy cows!
13:00	Moderation / Introduction <i>Schneider S., Krieg J. — Nürtingen, Bad Sassendorf</i>
13:05	WS1 Implementation of GfE (2023) in DLG (2025) – Concept for science, industry and practice <i>Spiekers H., Kampf — Freising-Weihenstephan, Frankfurt am Main</i>
13:20	WS2 DLG guidelines for accurate energy calculation and protein supply <i>Böttger C., Meißner L., Gerster E. — Bad Sassendorf, Aulendorf</i>
13:35	WS3 Ration optimisation and feeding control realigned! <i>Losand B. — Sanitz</i>
13:50	WS4 Application in analytics <i>Richardt W. — Lichtenwalde</i>
14:05	WS5 Digital implementation in ration planning and control using the example of Zifo2 <i>Rauch P., Schuster H. — Poing-Grub</i>
14:20	WS6 Agenda for further implementation <i>Kampf D., Schuster H. — Frankfurt am Main, Poing-Grub</i>
14:30	Discussion
14:55	Closing remarks <i>Prof. Dr. Jürgen Hummel, 1st Chairman of the Society of Nutrition Physiology</i>
15:00	End of conference

Review Lecture

■ Nutritional Immunology in Farm Animals

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Nutritional immunology in farm animals studies how diet modulates immune function, growth, and disease resistance. Proper nutrition—specifically balancing energy, protein, vitamins (A, D, E), and minerals (zinc, selenium)—is critical to support immune cell proliferation, reduce oxidative stress, and minimize health issues in intensive farming, such as immune stress or metabolic disorders. In the context of One Health, nutritional interventions may be an opportunity by which alternatives to the use of antibiotics can be applied in livestock production. Antimicrobial resistance (AMR) is a natural phenomenon that is forced by the overuse of antimicrobials threatening human and animal health and welfare. Livestock production has been a primary user of antibiotics for growth promotion, and although this was banned in the EU in 2006, conventional pork production remains to be a major consumer of antibiotics relative to other animal species (DANMAP, 2024, <https://www.danmap.org/reports/2024>). Pigs are especially exposed to antibiotic treatments during the weaning transition when diarrhea is frequent, the so-called post weaning diarrhea (PWD). During the weaning transition, the intestinal microbiome is highly affected by dietary changes, i.e., the change from suckling to solid feed, and by other external factors such as mixing of pigs, changing of environment and antibiotic use. Populated by trillions of microorganisms, the intestine is not only vulnerable to disruption by antibiotics, but because of its high-density, the intestinal microbiome is also an important source of AMR genes spreading among bacterial species, including animal and human pathogens. Diet and nutrition can be used as a strategic tool to manipulate the host intestinal microbiome and its immune system. In the present lecture, research will be presented showing how lipids and micronutrients can influence mechanisms of oxidative stress and inflammation, and how these bioactive nutrients can affect humoral immunity of the suckling and post weaned piglet. Furthermore, results of the international project entitled PIG-PARADIGM (Preventing Infection in the Gut of developing Piglets -and thus Antimicrobial Resistance - by disentangling the interface of Diet, the host and the Gastrointestinal Microbiome) will be presented, with special emphasis on how nutrition can affect immunological mechanisms.

Abstracts

1.

Development, digestive anatomy and physiology of calves weaned on hay or concentrate diets

Entwicklung, Verdauungsanatomie und – physiologie von auf Heu oder Konzentrat entwöhnten Kälbern

*Zhao X., Jamrogiewicz M., Przybyło M., Flaga J., Kański J., Wojtysiak D., Miltko R., Ortmann S., Niu M., Górka P., Clauss M. — Zurich

Introduction: Although ruminants are evolutionarily adapted to utilize forages as their primary energy source, livestock in modern production systems are often fed diets high in starch, to enhance growth performance and gastrointestinal tract (GIT) development. However, in adult ruminants, a high starch diet elevates the risk of metabolic disorders, primarily by disrupting ruminal homeostasis. We hypothesized that a high-starch diet during the initial weeks of life would significantly influence feed intake, growth trajectories, rumen function, digesta passage kinetics, and macroanatomical development, thereby altering the overall developmental trajectory of the GIT.

Methods: We evaluated a high-starch versus a hay-only weaning diet on intake, body mass, average daily body mass gain (ADG) in 48 newborn male calves randomly allocated to two treatments and fed milk replacer (6L/d in week 1, 8L/d in week 2, 9L/d weeks 3-6; reduction in week 7) with *ad libitum* access to either chopped hay (HAY; crude protein 18.2% and NDF 41.9% dry matter) or a high-starch concentrate mixture with only 5% chopped hay included (STARCH; crude protein 18.0% and NDF 23.1% dry matter). *Ad libitum* access to the solid diet was continued for two weeks after weaning (at 9 weeks of age). In the last week of the study, feed intake, digestibility and retention time were measured on 7 randomly selected animals per treatment. Afterwards, those 14 animals were slaughtered 2h after feeding for gross measurements (tissue and digesta weights of different part of GIT) and digesta and tissue samples collection. Rumen fluid pH was measured using hand-held pH meter and SCFA concentration was determined by gas chromatography. Groups were compared using mixed models that accounted for individuals and calf batch for repeated measures, and only for batch as random effect in single measures.

Results: Body mass and solid feed intake increased significantly over time but did not differ between groups. STARCH showed, at a similar dry matter intake, a higher dry matter digestibility ($P < 0.001$) and longer mean retention times for a fluid (in the lower GIT; $P = 0.039$), a small hay particle (whole GIT and reticulorumen [RR]; $P = 0.002$ and $P = 0.014$) and a large hay particle marker (whole GIT; $P = 0.003$), whereas the ratio of large to small particle retention in the GIT and RR was higher in HAY ($P = 0.010$ and $P = 0.008$). At slaughter, there was no body mass difference between the groups, but a trend emerged for heavier carcass mass in STARCH (68.4 ± 9.1 kg vs. 59.0 ± 5.9 kg in HAY; $P = 0.063$). HAY had more digesta in the RR and the caecum ($P = 0.001$ and $P = 0.041$) but not in any other GIT sections. GIT tissue mass was higher in STARCH for the caecum ($P = 0.002$), but not different for other GIT sections – importantly, not for the rumen. Although total SCFA concentration did not differ, the rumen propionate, butyrate and valerate proportions in STARCH were higher (33.9 ± 4.4 %, 14.6 ± 6.7 and 3.2 ± 1.0 vs. 13.8 ± 2.4 , 8.2 ± 2.3 and 1.2 ± 0.2 in HAY, respectively; $P < 0.001$, $P = 0.020$ and $P < 0.001$) at the expenses of acetate and isobutyrate, which were higher in the rumen liquid of HAY. The pH of rumen and caecum contents were higher in HAY (6.79 ± 0.23 and 7.26 ± 0.14 vs. 5.62 ± 0.22 and 6.43 ± 0.20 in STARCH, respectively; $P < 0.001$ and $P < 0.001$). The spleens of STARCH were larger (379 ± 75 g or 0.29 ± 0.06 % of body mass vs. 276 ± 27 g or 0.23 ± 0.04 % in HAY; $P = 0.011$).

Conclusions: These results indicate that early-life concentrate feeding need not be advantageous in terms of growth and GIT development if high-quality forage is available, and that concentrate feeding has the expected effects on digestibility (with consecutive slow passage of digesta), SCFA and pH. Whether the low pH is detrimental for animal health, especially later in life, will be assessed in the older animals from this experiment. The absolutely and relatively larger spleens of the STARCH group might indicate a higher immunological challenge in this group.

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Effect of a complementary feed containing pro- and prebiotics in combination with electrolytes and herbal components on the diarrhea incidence and body weight gain of neonatal dairy calves

Effekt eines Ergänzungsfuttermittels mit Pro- und Präbiotika in Kombination mit Elektrolyten und pflanzlichen Bestandteilen auf das Durchfallgeschehen und Gewichtsentwicklung bei neugeborenen Milchkälbern

*Altemeier M., Becklönne M.S., Middendorf L. — Essen / Göttingen

Introduction: First days of young animals' life set the basis for their healthy and productive adulthood: insufficient nutrient supply or occurrence of diseases during rearing phase both lower performance of the adult animals and increase the risk of health problems. Diarrhea problems are the most common complications in early calfhood. This multifactorial problem directly reduces the well-being and growth performance of animals. The latter often results in poorer physical development of young animals, which can also reduce their lifetime performance.^[1] Strengthening immune system, improving hygiene, and directly reducing pathogens are essential components in prevention of diarrhea. ^[2] Prevention of diarrhea symptoms can be supported by feeding methods. This includes application of trace elements and vitamins for an increased immune function as well as the usage of feed additives (like phytochemicals, pro- and prebiotics)^[3] Aim of this study was to investigate if a synergistic complementary feed can reduce the incidence and severity of diarrhea symptoms as well as development of newborn calves when used in preventive way.

Methods: 50 newborn calves were divided into two groups immediately and randomly after birth and cared for according to standard procedures of the commercial farm. During first hours after birth, calves were kept directly next to the cow that had given birth and then relocated to individual calf houses followed by a controlled administration of colostrum. A quality check of the colostrum was not done. From day 2 p.p., calves were switched to whole milk feeding based on a fixed drinking protocol, which did not differ between the groups. Calves assigned to control group (CON, N=25) received no supplements. Calves in the trial group (TRL, N=25) received a powdered complementary feed at a dosage of 2 x 25 g per animal per day, mixed into their milk feed, from day 2 p.p. until day 11 p.p.. It contains mainly a combination of *Enterococcus faecium*, yeast cell walls, electrolytes (sodium, potassium, chloride), and herbal components like chestnut extract and carob flour. For all calves, a diarrhea score (1=no diarrhea, 2=mild diarrhea, 3=severe diarrhea) was recorded daily from day 2 p.p. until day 28 p.p. based on visual stool consistency monitoring. In addition, calf weights were measured on the day of birth, day 12 p.p., and day 28 p.p.. Occurrence of umbilical inflammation, pneumonia and need for medical intervention were noted. Quantitative data were analyzed using analysis of variance (ANOVA) for normally distributed variables with group, birth weight, occurrence of other diseases and sex as fixed effects. Non-normally distributed qualitative data were analyzed using the Mann-Whitney U test. For all statistical analyses a p-value <0.05 was determined as statistical difference.

Results: No statistical differences were found in birth weights, calving progress, or the incidence of other diseases besides diarrhea like umbilical inflammation and pneumonia, as well as the consumption of milk or need for medical intervention. Average daily weight gain from day 1 p.p. until day 12 p.p. differed significantly between the groups, with 0.555 kg for CON and 0.695 kg for TRL (p=0.015) but also between the sexes (p=0.014). There was no statistical effect from birth weight or occurrence of other diseases. Total number of days with diarrhea (d) differed between the groups, with an average of 4.16 d for CON and an average of 2.48 d for TRL (p=0.009). Furthermore, CON showed more severe diarrhea with an average diarrhea score >2 of 4.96 d than TRL with an average diarrhea score >2 of 3.12 d (p=0.05).

Conclusions: This study proves that the preventive use of this complementary feed reduces the severity and duration of diarrhea symptoms in neonatal calves. The promotion of daily weight gain in the first few days must be investigated through further studies with a larger number of animals, a balanced sex ratio and also including more detailed management factors.

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- [2] Foster D. M. and G. W. Smith 2009. Pathophysiology of diarrhea in calves. *Vet. Clin. North Am. Food Anim. Pract.* 25:13–36.
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3.

Body weight development in *ad libitum* milk-fed calves using different weaning protocols – preliminary results

Gewichtsentwicklung bei ad libitum mit Milch gefütterten Kälbern unter Verwendung verschiedener Fütterungsprotokolle während des Absetzens - erste Ergebnisse

Stoschus A., Diesing L.E., Liermann W., Nieschulze T., Braun K., *Hammon H.M. — Dummerstorf / Westerrönfeld

Introduction: Calves with a high milk feeding level, e.g., *ad libitum* milk intake, develop much better than calves fed restrictively. However, if these calves are weaned too fast, there is a decline in body weight (BW) development during weaning [1]. The hypothesis was tested that during weaning from high milk intake a decline in body growth can be avoided by adjusting the feeding protocol. The objective was to identify feeding protocols after *ad libitum* milk feeding that ensure consistently high nutrient intake during weaning.

Methods: Fifty Holstein calves (n=21 female and n=29 male) were supplied with milk replacer (MR; 14 % dry matter) *ad libitum* by an automatic feeding system until wk 7 of life. Calves were randomly assigned to one of 4 different weaning protocols starting in wk 8. Group 1 (n=12) and 2 (n=13) were weaned linearly either within 3 or 6 weeks. Calves in group 3 (n=12) were weaned based on concentrate intake. MR intake was limited to 12 L/d in wk 8. Further reduction of MR occurred when calves consumed corresponding amounts of concentrates. An increase of concentrate intake by 100 g/d led to a reduction of 0.5 L MR/d. Calves in group 4 (n=13) were weaned according to average daily gain (ADG) whereas weekly ADG should remain at > 900 g. BW of the calves was determined at each visit to the automatic feeder using a front foot scale, and the MR access was reduced by 200 mL/d if the ADG was > 900 g over 3 days. If calves in groups 3 and 4 had not been weaned by the end of their 15th week of life, they were weaned using a linear programme of the automatic feeder until week 18. Calves were weighed weekly and ADG was calculated up to wk 20. Calves had free access to water and hay from first week on and total mixed ration (TMR) based on grass and corn silage from wk 5 on. MR and concentrate consumption were documented daily by the automatic feeding system for each calf and daily means were calculated for each week from wk 7 to wk 14. In wk 20, daily TMR intake was measured for each calf. Data were analysed by the Mixed Model of SAS with feeding group, time, and sex as fixed effects and least squares means were tested using the Tukey-Kramer procedure.

Results: MR intake was reduced ($P < 0.05$) in group 1 and 2 as planned. MR intake was lowest ($P < 0.05$) in group 1 from wk 9 on, was lower ($P < 0.05$) in group 2 than in group 3 from wk 10 to wk 14 and in group 4 from wk 11 to wk 13, and was lower ($P < 0.05$) in group 4 than in group 3 from wk 12 to wk 14. In wk 14, MR intake was 6.1 ± 0.5 L/d in group 3 and 2.5 ± 0.5 L/d in group 4. MR intake was higher ($P < 0.05$) in male than female calves before start of the study in wk 7 but not during the study. Concentrate intake increased ($P < 0.05$) during weaning in all groups. Intake was highest ($P < 0.05$) in group 1 in wk 10 and 11 and was lower ($P < 0.05$) in group 3 than in groups 2 (wk 13 and 14) and group 4 (wk 13). Before start of weaning, male calves were ca. 10 kg heavier ($P < 0.05$) than female calves (BW: 90.5 ± 2.0 kg for male; 80.9 ± 2.5 kg for female). The effect of gender on BW remained consistent ($P < 0.05$) across treatment groups until the end of the study (BW in wk 20: 201.9 ± 2.6 kg for male; 175.9 ± 3.2 kg for female). There was a trend for lower BW in group 1 than group 4 from wk 17 to wk 19 ($P < 0.1$). ADG was higher ($P < 0.05$) in male than female calves (ADG: 1.22 ± 0.02 kg/d for male; 1.05 ± 0.02 kg/d for female). TMR intake in wk 20 was higher ($P < 0.05$) in male than female calves (TMR intake: 12.7 ± 0.4 kg for male; 11.0 ± 0.5 kg for female), but did not show any group effects.

Conclusions: No decline in BW development was observed in any of the treatment groups during the critical weaning period. Weaning by concentrate intake (group 3) resulted in highest MR and lowest concentrate intake during the weaning process. *Ad libitum* MR intake had already led to a significant difference in BW between male and female calves before start of weaning and the difference persisted postweaning.

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Effects of different weaning protocols after *ad libitum* milk feeding on metabolic traits in blood of dairy calves - preliminary results

Auswirkungen verschiedener Absetzprotokolle nach ad libitum-Milchfütterung auf Stoffwechselparameter im Blut von Milchkälbern – vorläufige Ergebnisse

Stoschus A., Diesing L.E., Liermann W., Nieschulze T., Braun K., *Hammon H.M. — Dummerstorf / Westerrönfeld

Introduction: Elevated milk supply, e.g., by *ad libitum* milk feeding, enhances anabolic processes and body growth in calves. Metabolic parameters such as glucose, urea, free fatty acids (FFS) and triglycerides reflect the stimulation of anabolic processes and can serve as indicators for identifying insufficient nutrient intake during weaning [1]. The objective of the present study was to investigate metabolic changes in *ad libitum* milk-fed calves during weaning from milk replacer (MR) using four different weaning protocols and to assess parameters that mirror the different weaning processes.

Methods: Fifty Holstein calves (n=21 female and n=29 male) were supplied with milk replacer (MR; 14 % dry matter) by an automatic feeding system. Calves were randomly assigned to one of 4 weaning protocols starting at wk 8. Calves of group 1 (n=12) and 2 (n=13) were weaned linearly either within 3 or 6 weeks. Calves in group 3 (n=12) were weaned based on concentrate intake. MR intake in 8th week was limited to 12 L/d. Further reduction of MR only occurred when calves consumed corresponding amounts of concentrates to maintain nutrient supply, e.g., with an increase of concentrate intake by 100 g/d MR was reduced by 0.5 L/d. Calves were weaned when proportion of metabolizable energy of MR was less than 10 percent. Calves in group 4 (n=13) were weaned according to average daily gain (ADG) whereas weekly ADG should remain at > 900 g. For this purpose, the BW of the calves was determined using a front foot scale at the automate, and the MR quantity was reduced by 200 mL/d if the ADG was > 900 g over 3 days. If calves in groups 3 and 4 had not been weaned by the end of their 15th week of life, they were weaned using a linear programme of the automate until week 18. Plasma concentrations of total protein, albumin, urea, glucose, lactate, beta-hydroxybutyrate (BHB), FFS and triglycerides were measured in blood taken before (wk 7), during (wk 8-16 of life), and post-weaning in wk 20 by biochemistry. Data were analysed by the Mixed Model of SAS with feeding group, time, sex as fixed effects.

Results: Data on feed intake and growth performance are reported in a companion abstract [2]. Plasma concentrations of total protein and albumin increased ($P < 0.05$) during the weaning process and plasma albumin in group 1 was higher ($P < 0.05$) than in group 2 (wk 11 to 13) and group 4 (wk 11 and 12) and tended to be higher ($P < 0.1$) than in group 3 in wk 11. Plasma lactate concentrations were lower ($P < 0.05$) in group 1 than in group 3 in wk 10. Plasma glucose concentrations declined ($P < 0.05$) during the weaning process and in wk 10 were lower ($P < 0.05$) in group 1 than in group 3. Plasma BHB concentrations increased ($P < 0.05$) during weaning, tended to be higher ($P < 0.1$) in group 1 than in groups 3 and 4 in wk 10, was highest ($P < 0.05$) in group 1 from wk 11 to wk 14, and was higher ($P < 0.05$) in group 1 than in group 3 (wk 15 and 16) and group 4 (wk 15). Plasma BHB was higher ($P < 0.05$) in group 2 than in group 3 (wk 14 - wk 16) and higher ($P < 0.05$) than in group 4 (wk 15). Plasma BHB was lower ($P < 0.05$) in group 3 than in group 4 in wk 16. Plasma FFS concentrations were changed ($P < 0.05$) by time. Plasma FFS in group 1 were highest ($P < 0.05$) in wk 10 and were lowest ($P < 0.05$) in wk 13. Plasma triglyceride concentrations decreased ($P < 0.05$) with time and were lower ($P < 0.05$) in group 1 than in group 4 in wk 12.

Conclusions: Changes in metabolic traits (BHB, glucose) mirrored the weaning process and indicated a greater rumen papilla activity especially in group 1 with the shortest weaning protocol [3]. Lowest plasma BHB concentrations in group 3 may point at reduced rumen papilla activity when calves were weaned based on concentrate intake. Higher plasma FFS concentrations in group 1 may point at temporary fat tissue mobilisation to compensate for lower nutrient availability due to lower MR intake during weaning [1].

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Exploratory investigation of animal- and environment-related factors influencing colostrum intake patterns within the first 24 h postpartum in dam-bound rearing systems

Explorative Untersuchung tier- und umweltbezogener Faktoren, die die Kolostrumaufnahme innerhalb der ersten 24 Stunden postpartum in kuhgebundenen Aufzuchtssystemen beeinflussen

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Introduction: An adequate intake of colostrum is essential for neonatal calves, as it plays a critical role in the development of the immune system and supports growth during the early stages of life [1]. In conventional rearing systems, colostrum is administered via bottle or bucket feeding to monitor the amount consumed. In contrast, dam-rearing systems present challenges in monitoring colostrum consumption at the udder, as intake is influenced by a variety of factors dependent on both the calf and the dam [2; 3]. This study examined the influence of various animal- and environmental factors on the colostrum intake patterns within the first 24 h postpartum (pp) in dam-bound rearing systems. The objective was to identify key determinants that contribute to the success of the suckling process.

Methods: The study was conducted on the TUM experimental farm Veitshof and included 13 Brown Swiss cows (primiparous $n = 4$; multiparous $n = 9$) and their calves. After birth, all calves remained with their dams. Cow-related data included an evaluation of the birth process using a dystocia scoring system ranging from normal to difficult births, as well as the duration of parturition and parity. Additionally, udder anatomy was assessed using 15 quantitative measurement points and 3 qualitative scorings, based on breed-specific exterior traits for Brown Swiss cows. Colostrum was sampled after birth and Brix was measured using a refractometer. Maternal and calf behavior (16 behavioral aspects) related to the colostrum intake pattern were analyzed using video recordings and a structured observation protocol, focusing on interactions that influence the calf's ability to access and consume colostrum. Searching time reflected the time from birth until the calf successfully located the udder, while suckling duration indicated the time span of apparent milk intake. Human assistance served as an indicator of management intervention, and calf activity summarized locomotion (standing and walking). Maternal licking events were considered a proxy for dam-calf care by the cow. Calf-related data comprised birth weight and 20 anatomical body measurements, such as withers height, sacrum height, etc., which were recorded immediately after birth. Environmental data included continuous monitoring of ambient temperature and relative humidity. Statistical analysis was performed using RStudio 4.4.2 (R Core Team, Vienna, Austria). Descriptive statistics and Spearman correlation coefficients were calculated to identify key factors influencing the colostrum intake patterns within the first 24 h pp.

Results: Within the first 24 h pp, calves spent on average 40.12 ± 4.98 min searching for the udder and 30.36 ± 7.32 min suckling. The average number of suckling events was 19 ± 3 in the first 24 h pp. Three calves required human assistance to successfully access milk from the udder within the first 24 h pp. Correlation analysis revealed that udder searching time was associated with calf activity and lying ($r = 0.53$ vs. -0.40 , $p \leq 0.05$), the number of maternal licking events ($r = 0.36$, $p \leq 0.05$), and udder depth ($r = 0.24$, $p \leq 0.05$). Regarding total suckling duration, correlations were found with calf activity ($r = 0.37$, $p \leq 0.05$), parity of the dam ($r = -0.29$, $p \leq 0.05$), and teat length ($r = -0.23$, $p \leq 0.05$). Suckling duration and events were positively ($r = 0.98$, $p \leq 0.05$) correlated. Udder searching time showed no or only weak ($r \leq 0.16$) correlations with environmental variables.

Conclusions: Multifactorial aspects, such as calf vitality, maternal behavior, and udder conformation influenced the success of colostrum intake (searching time and suckling duration) in dam-bound rearing systems. Calves require substantial time to locate and access the udder. These results highlight the importance of monitoring and supporting early calf-dam interactions, and warrant confirmation through further research.

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Effects of individual colostrum composition on early postnatal metabolic adaptation and serum IgG concentrations in calves

Einfluss der individuellen Kolostrumzusammensetzung auf die frühe postnatale Stoffwechselanpassung und Serum-IgG-Konzentrationen bei Kälbern

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Introduction: The first 24 h of life represent a critical transition in the neonatal calf metabolism. Nutritive factors in colostrum cover macronutrients such as protein, fat, and lactose, which provide energy and substrates for the rapidly developing calf. Non-nutritive, bioactive factors include hormones and growth factors such as insulin, IGF-1, and adiponectin, which influence intestinal development, nutrient absorption, and early metabolic regulation. This study aimed to examine associations between individual colostrum components and metabolic as well as immunological blood parameters in neonatal calves during the first 24 h postpartum (p.p.).

Methods: Forty-six Holstein calves received 2 L of their dam's colostrum at 4 h and 12 h p.p. Blood samples were taken immediately before feeding at 4 h, 12 h and 24 h. Colostrum was analyzed for fat, protein, lactose, IgG, insulin, and adiponectin. Blood samples were analyzed for IgG, total protein (TP), glucose, non-esterified fatty acids (NEFA), phospholipids (PL), triglycerides (TG), and total cholesterol (TC). Changes of blood parameters over time were analyzed using mixed models with time as fixed effect measure and individual calf as random subject. To assess the influence of colostrum composition on neonatal blood parameters, mixed models were used including colostrum variables (fat, protein, lactose, insulin, adiponectin, colostrum IgG) as fixed effects. Pearson correlation coefficients between colostrum variables and changes in blood parameters relative to baseline at 4 h p.p. (Δ 4–12 h and Δ 4–24 h) were calculated. Statistical significance was assumed at $P < 0.05$.

Results: Plasma total protein (TP), IgG, and glucose concentrations increased markedly during the first 24 h postpartum ($P < 0.001$), whereas non-esterified fatty acids (NEFA) declined after the first colostrum feeding at 4 h p.p. ($P < 0.001$). Colostrum fat content correlated positively with plasma phospholipids (12 h: $r = 0.65$; 24 h: $r = 0.76$), triglycerides (12 h: $r = 0.51$; 24 h: $r = 0.54$), and total cholesterol (12 h: $r = 0.42$; 24 h: $r = 0.67$; all $P < 0.01$), highlighting the importance of colostrum fat as an early energy source. Significant positive correlations were observed between colostrum protein and serum TP (12 h: $r = 0.45$, $P < 0.01$; 24 h: $r = 0.49$, $P < 0.05$) as well as serum IgG (24 h: $r = 0.56$, $P < 0.01$). In contrast, colostrum lactose tended to correlate negatively with serum IgG (12 h: $r = -0.26$, $P = 0.08$; 24 h: $r = -0.46$, $P = 0.06$). Positive associations were also detected between colostrum insulin and plasma IgG (12 h: $r = 0.52$, $P < 0.001$; 24 h: $r = 0.40$, $P = 0.07$), and between colostrum adiponectin and plasma TP (12 h: $r = 0.37$, $P = 0.01$).

Conclusions: The results suggest that variability in colostrum composition contributes to differences in early metabolic and immune adaptation. The correlations suggest that lactose, insulin, and adiponectin may influence IgG uptake, potentially through effects on intestinal development and closure, although these interpretations should be viewed as preliminary and require further confirmation.

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Associations of growth rates during the first two months of life with feeding behavior, long-term activity, and performance in Holstein heifers

Zusammenhänge zwischen den Wachstumsraten in den ersten beiden Lebensmonaten und dem Fressverhalten, der langfristigen Aktivität und der Leistung von Holstein-Färsen

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Introduction: Early nutrition and management shape growth, health, and lifetime productivity of dairy heifers [1]. Higher early-life gain is associated with improved later-life performance outcomes [2,3]. This study examined the natural variation in growth performance of dairy calves receiving up to 12 L/d milk replacer (MR) for eight weeks. The association with feeding behavior and subsequent performance during rearing and first lactation was tested. We hypothesized that calves with enhanced preweaning growth rate will differ in feeding behavior, earlier reproductive maturity, younger age at calving, and higher milk production in the first lactation.

Methods: Forty-two female Holstein calves were monitored from birth until their first lactation. Calves received up to 12 L/d of MR (140 g/L) until d 56 (preweaning) and were then gradually weaned over 6 wk. MR intake was recorded manually during individual housing (first 2 wk) and automatically during subsequent group housing using automatic calf feeders (VARIO smart, Förster-Technik GmbH). The activity levels of the heifers were continuously recorded starting with group housing and using a triaxial accelerometer attached to the collar (Smart Neckband, Förster-Technik). Activity peaks indicating estrus were detected in addition to visual observation. Body weight, body condition score (BCS) and backfat thickness (BFT) were measured regularly to monitor postweaning development. Daily milk yield and milk components were analyzed using repeated measures mixed-model ANOVA. Clustering was performed retrospectively based on preweaning (birth until d 56 of life) average daily gain (ADG) using K-Means.

Results: Preweaning ADG across all calves ranged between 0.73 and 1.16 kg/d. Two well-separated clusters with a silhouette score of 0.70 (Hedges' $g = 3.87$) were identified: maximum gain (MAX, $n = 21$, 1.06 ± 0.01 kg/d) and moderate gain (MOD, $n = 21$, 0.84 ± 0.05 kg/d; $P < 0.001$; means \pm SE). MAX calves were heavier than MOD calves throughout the entire milk feeding period ($P < 0.001$) and weighed 15 kg more than MOD at weaning ($P < 0.001$). MAX calves consumed 1.52 L/d more MR than MOD calves when housed individually; during preweaning group housing (until d 56) the difference was 1.1 L/d ($P = 0.001$). Total daily concentrate intake was consistent across clusters; however, disparities in visiting behavior of feeding stations were observed. MAX calves had a greater feed efficiency (i.e. higher gain:feed ratio, $P = 0.005$) during individual housing. MAX calves were more active during the first 2 mo, but MOD heifers had higher activity levels from mo 7 of life until mo 2 of lactation ($P < 0.05$). MAX heifers were heavier until month 25 ($P < 0.001$), with high postweaning variability in ADG. MAX heifers reached the requirement for insemination maturity (400 kg BW) 42 days earlier than the MOD cluster ($P < 0.001$); however, early life ADG had no influence on age at first calving (mean age 25.9 ± 0.40 months). MAX cows yielded 1,269 kg more milk than MOD cows ($P = 0.001$) during their 305-d lactation without differences in milk composition resulting in greater yields of protein, fat, and lactose. Milk urea concentration (MUN) was higher in MOD cows during 7 out of 9 months of lactation ($P = 0.01$). During the first month of lactation, MAX cows were heavier than MOD cows ($P = 0.02$) and had higher BCS ($P = 0.02$), but no further differences in body condition were evident between the two groups for the rest of the lactation period.

Conclusions: Monitoring feed intake and ADG during the initial weeks of life for dairy calves is important for early identification of appropriate replacement heifers. Automated recording of feeding-associated behavior has the potential to serve as a proxy for development. Higher milk yields and lesser MUN levels in MAX heifers support the notion that high growth rates in early life foster milk yield in later life and indicate improved N efficiency.

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Evaluation of local protein feed crops as an alternative to soya protein in the diet of fattening Holstein bulls, which were fed milk replacer with either skimmed milk or whole milk during the rearing phase

Bewertung von einheimischen Proteinpflanzen als Alternative zu Soja-Protein in der Fütterung von Holstein-Bullen in der Mast, die in der Aufzuchtphase mit einem Magermilch- oder Vollmilch-Milchaustauscher gefüttert wurden

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Introduction: Adequate livestock protein supply often relies on imported soya, associated with deforestation and high carbon footprints. Growing demands for sustainable, climate and environmentally friendly, GMO-free agriculture considering animal welfare have led to national strategies promoting regional protein crops. This study evaluates the long-term effects of replacing soya with the local protein feed crops peas and rapeseed meal (PR) in concentrate feed (CF) on the growth, feed intake, and metabolism of Holstein bulls, as well as the impact of different milk replacer (MR) qualities. It is hypothesised that protein source variation has no significant effect on these parameters.

Methods: Forty-nine male newborn calves were randomly assigned to one of four feeding groups, receiving either skimmed (SM) or whole milk (WM)-based MR during the 7-week rearing phase. Furthermore, the calves were fed either a soya- (S) or pea and rapeseed meal (PR)-based CF diet, which was fed continuously until the end of the fattening trial. At 291±28 d of age there was a zero sample (T0) 4 weeks before the start of a 11-week fattening trial. The bulls were fed a total mixed ration (TMR) consisting of 31.4% maize silage, 30.2% grass silage, 35% CF and 3.5% straw on dry matter (DM) basis *ad libitum* before and 70% maize silage and 30% CF *ad libitum* after feed change 3 weeks after T0. Individual feed and water intake was recorded daily using weighing troughs (Insentec B.V., Holland). Ultrasonographic rib fat thickness (RFT) [1] was measured at three time points (T0, T5, T11), and backfat thickness (BFT) [2] once at T11. Live weight (LW) was recorded weekly and the average daily live weight gain (ADG) was calculated. Blood samples were collected at T0, T5 and T11 and were analysed for cholesterol, triglyceride, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) using an automated analyser (IndikoPlus, ThermoScientific). Data were analysed using Proc MIXED of SAS (V9.4., SAS Institute Inc., USA) with Time (T), MR and CF as fixed factors, and interactions between T, MR and CF and an adjusted Tukey-test as a post-hoc procedure.

Results: Bulls fed a ration with PR-CF had a higher average DM intake (DMI, $p_{CF}=0.042$) of 9.3 ± 0.26 kg/d during fattening period, compared to those fed S-CF of 8.5 ± 0.27 kg/d. Bulls started the fattening trial with an average LW of 410 ± 69.7 kg in T0 and showed a consistent increase over time ($p_T < 0.001$). LW of bulls that received SM MR during rearing was higher at the end of the trial compared to the bulls that received WM MR as calves ($p_{MR*T}=0.005$). The ADG of bulls fed a PR-CF ration was 1.52 ± 0.047 kg/d, compared to 1.38 ± 0.048 kg/d in the S-CF group ($p_{CF}=0.053$). Mean RFT of bulls fed S-CF was significantly thicker than that of PR-CF fed bulls (17.6 mm vs. 15.9 mm, $p_{CF}=0.042$). MR fed during the rearing period resulted in higher RFT in the SM MR group during the trial ($p_{MR*T}=0.008$), compared to the WM MR group. BFT was unaffected by CF and MR. Serum cholesterol and triglyceride varied over time ($p_T < 0.05$), unaffected by CF and MR. NEFA levels were higher in bulls fed SM MR (133 μmol/L) as calves than in bulls received WM MR (115 μmol/L, $p_{MR}=0.033$). The PR-CF group showed higher mean BHB levels (0.56 mmol/L) compared to the S-CF group (0.49 mmol/L, $p_{CF}=0.023$).

Conclusions: These results indicate that local protein sources can effectively replace soya in bull fattening diets without impairing performance, while early-life nutrition may exert lasting effects on energy and fat metabolism.

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Feed choice strategies and lysine self-regulation in fattening pigs

Wahlfütterungsstrategien und Lysin-Selbstregulation bei Mastschweinen

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Introduction: Feeding strategies play a crucial role in optimizing nutrient intake and promoting efficient growth in pigs. Traditional fixed phase diets often lead to systematic over- or undersupply of amino acids (AAs), which can limit growth performance or result in inefficient nutrient utilization (1). Choice feeding, on the other hand, offers pigs the autonomy to regulate their own nutrient intake, potentially allowing them to fine-tune their AA consumption closely around their specific physiological requirements. Focusing exclusively on feed choice, we quantified (i) how closely pigs met sex-specific GfE targets for *pev* Lys (2), (ii) dose–response of performance and Lys efficiency to the proportion of the +10% AA diet (+ diet), and (iii) self-selection phenotypes from multivariate structure.

Methods: Thirty pigs (Large White × Landrace × Piétrain; 15 barrows, 15 gilts) were allocated to pens according to litter, sex and initial body weight (10 pigs per pen, three replicates). Pens were equipped with two identical dry feeders, each offering one diet. Feeder positions were swapped weekly to prevent habituation. With the help of an individual transponder chip, it was possible to record each feeding session and the amount of feed consumed per session for each feeder. Pigs had *ad libitum* access to feed and water. Two isoenergetic diets were formulated: a control diet (C) meeting nutrient recommendations (SID lysine: 9.5 g/kg starter, 8.5 g/kg grower, 7.5 g/kg finisher) and a nutrient booster diet (+) containing +10% SID lysine with identical AA ratios; Ca, digestible P, Na and the mineral premix were increased accordingly. Feeding phases were starter (30.8 kg ± 0.47 - 51.8 kg ± 0.64), grower (51.8 kg ± 0.64 - 94.4 ± kg 1.05 kg) and finisher (94.4 kg ± 1.05 - 116.0 kg ± 0.44). Phase-specific proportions of the + diet were used to calculate effective SID-lysine intake. GfE requirements were estimated by sex-specific bilinear interpolation (body-weight midpoint × ADG). Analyses focused on $\Delta\% = (\text{intake} - \text{requirement})/\text{requirement}$, lysine efficiency (g ADG per g SID-Lys), dose–response relationships (OLS with heteroscedasticity-robust SE), null-point estimation ($\Delta\% = 0$), and PCA/k-means clustering within choice-fed pigs.

Results: Choice-fed pigs clustered closely around $\Delta\% \approx 0$ during the starter and grower phases. In the finisher phase, positive $\Delta\%$ values occurred frequently without corresponding ADG responses, indicating lysine non-limitation. Most pigs selected diets close to GfE targets, particularly gilts. During starter and grower phases, ADG increased with rising + diet proportions before plateauing, while lysine efficiency peaked at moderate inclusion levels (≈ 35 – 55% + diet). In the finisher phase, ADG was independent of + diet proportion and lysine efficiency declined at higher inclusion levels. Barrows required higher + diet proportions to reach $\Delta\% \approx 0$ and tended toward oversupply, whereas gilts achieved $\Delta\% \approx 0$ at lower + diet proportions with higher efficiency. Multivariate analysis identified a balanced, efficient phenotype (moderate + diet, $\Delta\% \approx 0$; predominantly gilts) and an oversupply phenotype (high + diet, positive $\Delta\%$; predominantly barrows). Higher initial BW slightly increased + diet selection in the starter phase only.

Conclusions: Under choice feeding, pigs especially gilts self-selected SID-lysine intakes close to GfE recommendations in starter and grower phases, while additional lysine supply in the finisher phase provided no performance benefit and reduced efficiency.

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Nutrient booster diets and choice feeding effects on growth and efficiency in fattening pigs

Auswirkung einer erhöhten Nährstoffkonzentration und Wahlfütterungssystemen auf Leistung und Effizienz bei Mastschweinen

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Introduction: Precision feeding aims to match the pig's amino acid (AA) intake to its changing requirements for lean tissue deposition and nitrogen efficiency. Allowing pigs to choose between a standard and an AA-enriched diet could allow them to fine-tune their intake to pcv-Lys/the ideal protein pattern. Therefore, a choice feeding trial was initiated to determine if pigs could meet the pcv Lys requirement of the German Society for Nutritional Physiology (GfE) (1) when offered AA-enriched diets. The objectives were to verify (i) how closely pigs meet the GfE pcv-Lys target, (ii) growth and feed efficiency responses by sex and diet, and (iii) whether choice feeding avoids AA oversupply and undersupply.

Methods: A total of 60 pigs were used in the study (a mixture of barrows and gilts in each pen; (Large White x Landrace) x Piétrain)). Pigs were allocated to experimental units according to litter, sex and body weight in three treatments. Two no-choice treatments (control (C) and plus (+) diet) with 5 animals per pen and 3 pens per treatment. The feed choice (FC) group contained 10 animals per pen and had 3 replicates. To avoid habituation of diet type (C/+) in the dry-feeding station, the diets in the two feeders were changed weekly. Pigs had free access to the diets and drinking water. Both diets were calculated isoenergetically, but differed in a standard diet (C) according to the (1) nutrient recommendation (pcv Lys content was in starter 9.5 g/kg; 8.5 g/kg in grower; 7.5 g/kg in finisher diet) and a „nutrient booster diet“ (+). The + diet contained +10% pcv Lys and maintained the same AA ratio as the C-diet. Ca, dig. P, and Na, as well as the mineral and trace element premix, were also increased by 10% in these treatment. The study was divided into three feeding periods (starter: 30.7 kg ± 0.47 - 51.4 ± 0.64; grower: 51.4 ± 0.64 - 93.6 ± 1.05; finisher: 93.6 ± 1.05 - 115.9 kg ± 0.44). Theoretical GfE pcv Lys requirements were interpolated to each 5-kg body-weight class and multiplied by the group-specific mean feed intake to obtain a required daily AA intake curve for barrows and gilts. A two-factorial analysis of variance with diet and sex and their interaction was used to determine treatment differences in performance parameters. Requirements were interpolated from BW×lean growth grids. Outcomes included percentage deviation (Δ%), Lys efficiency, dose–response of average daily gain (ADG) to +share.

Results: Starter: Control supplied 18.0 ± 3.1 g pcv Lys d⁻¹, ~5 % below the GfE band (18.6–19.7 g). +diet provided 22.0 ± 3.5 g (+15 %) and FC delivered 20.5 g. Grower: C-, +diet and FC offered 23.0, 26.5 and 25.1 g, matching 95–110 % of GfE. Finisher: all diets exceeded the target by 12–20%, because feed intake rose while Lys concentration remained fixed. As the +diet raised all essential AAs and minerals in lock-step, no secondary AA became limiting. Whole fattening period: ADG did not differ statistically among diets (900 g d⁻¹). Feed conversion ratio (FCR) improved by 8 % in +diet (2.49 kg kg⁻¹) and FC (2.51 kg kg⁻¹) compared with C-diet (2.72 kg kg⁻¹). The coefficient of variation of ADG was 9% in C-diet and FC, but 14 % in +diet, indicating that self-selection homogenised performance. Barrows consumed 5–7% more AA than gilts and gained 5 % faster. No diet × sex interaction was detected. Nitrogen efficiency: Because finisher pigs received 2–4 g pcv Lys d⁻¹ above need, the incremental N conversion efficiency of +diet dropped from 49 % (starter) to 38 % (finisher), whereas FC remained at 42 %, illustrating a modest environmental benefit.

Conclusions: Raising all essential AAs and macro-minerals by 10 % lifts feed efficiency only when the basal diet is below the GfE band. Choice feeding enabled pigs to hit the GfE target for SID-Lys with ~5 % less SID-Lys than the fixed +diet, delivering the same ADG and better homogeneity.

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11.

Moulting of dual-purpose chickens – comparison of two feeding methods

Mauser von Zweinutzungshennen- Vergleich von zwei Fütterungsvarianten

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Introduction: In managed poultry systems, moulting can occur naturally or be induced to synchronize production cycles and rejuvenate the reproductive tract. This study examined two different moulting regimes for laying hens after their first laying period. Data on hen health during and after moulting, as well as performance parameters, were collected and evaluated.

Methods: In total, 244 Coffee dual-purpose hens (breed Coffee) were housed in two mobile houses, each divided into four equally sized compartments, under organic farming conditions. After the first laying period (71 weeks of age), two compartments per house received a moderately restricted oat-based diet (ROD; 50 g oats per hen per day), while free-choice feeding (FCF; layer feed [10.9 MJ AMEN, 15.1% CP] and oats) was applied in the remaining two compartments for two weeks. During this period, light duration was reduced to nine hours per day in all compartments. All hens were weighed on days zero, seven, 14, 21, 28, 56, and 103 after moult induction. Feed intake, laying performance, and egg size distribution were recorded continuously until 96 weeks of age. Whole-body analyses were conducted after the second laying period. Data were statistically analysed using Tukey's HSD and Kruskal-Wallis test ($p < 0.05$).

Results: During the reduction phase, daily feed intake per hen in the FCF group was 149.6 ± 3.96 g, consisting of 95.9 ± 9.34 g layer feed and 53.7 ± 6.18 g oats. Hens in the ROD group lost 12.3% of their body weight during the reduction phase but exhibited a 9.41% higher body weight 56 days after moult induction compared to the start of the study ($p < 0.001$). From day seven to day 21 after moult induction, body weight in the ROD group was lower than baseline, whereas from day 56 onwards it was higher ($p < 0.001$). The FCF group maintained a relatively constant body weight during moulting; 56 days after moult induction, body weight was 5.66% above baseline ($p < 0.001$). Up to day 28, body weight was lower in the ROD group than in the FCF group ($p < 0.001$), whereas no significant differences were observed from day 56 onwards ($p = 0.285$). Daily laying performance declined to 0% in the ROD group but only to 16.9–30.9% in the FCF group. After the recovery phase, laying performance increased to 81.6% in the ROD group and to 62.9% in the FCF group ($p < 0.001$). From six weeks after moult induction onwards, no differences in weekly laying performance were observed between the groups ($p > 0.556$).

Conclusions: Based on the results of this study, both methods appear suitable for inducing moulting. The restrictive feeding regime was associated with complete moulting of all hens and a temporary reduction in body weight. However, this body weight loss was regained in the weeks following moulting. Thus, dual-purpose chickens showed relative tolerance to a temporary restrictive diet.

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Ensilability of leaves from common deciduous trees

Silierbarkeit von Blättern häufiger Laubbäume

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Introduction: Utilizing sustainable feed resources is a key step toward more resilient livestock systems. Tree and shrub foliage is an abundant but largely overlooked feed source that can reduce reliance on conventional forages and support circular agricultural practices. In temperate regions, its seasonal availability necessitates effective conservation to ensure year-round use. However, successful conservation of foliage as silage requires effective ensiling strategies. This study evaluated ensiling methods for foliage from seven deciduous tree species that are very common in central Europe, aiming to identify effective preservation methods and to assess silage quality and nutritional characteristics.

Methods: Foliage of common beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), field maple (*Acer campestre*), norway maple (*Acer platanoides*), black alder (*Alnus glutinosa*), linden (*Tilia cordata*), and aspen (*Populus tremula*) were harvested between August 28th and September 7th 2023 from stands in and around Goettingen (max. 4 km radius). Immediately after harvest, the foliage was transported to the University of Goettingen. Upon arrival, the leaves were separated by hand from the branches for a laboratory silo experiment. Four ensiling treatments were applied to 3-kg batches of leaves from each species: (1) control (no additive), (2) sodium benzoate/sodium propionate mixture (Bergo®Silostabil; 4 ml/kg), (3) sugar beet molasses (30 g/kg), and (4) sugar beet molasses with a mix of homofermentative lactic acid bacteria (LAB; 3×10^8 CFU/kg; all fresh matter [FM]). The treated leaves (500 g FM) were vacuum-sealed in PA/PE bags (n=4; [1]) and stored at 15.8 ± 2.3 °C for 91 d. Fresh foliage (d 0) and silages (d 91) were analyzed for nutrient composition, true protein, and NH₃. Water-soluble carbohydrates and buffering capacity were only analyzed on d 0. Fermentation products and pH were only determined after 91 d. Metabolizable energy (ME) was calculated according to Menke and Steingass (1988; [2]). Data were analyzed as 7×4 factorial design, using the MIXED procedure of SAS (Version 9.4). The model included the fixed effects of species, treatment and their interaction, and mini silo as random effects. Treatment means were compared by Tukey-Kramer test. Effects were declared significant at $P \leq 0.05$.

Results: Silage DM content ranged from 30% (linden) to 38% (field maple). The crude protein content of the silages varied from 106 g/kg (ash-chemical) to 202 g/kg DM (black alder-control), and was strongly impacted by species \times ensiling treatment ($P < 0.001$). Similarly, true protein (range: 67 to 90% CP across all treatments) was affected by species \times ensiling treatment ($P < 0.05$). Metabolizable energy was between 7.3 MJ/kg (beech-control) and 11.3 MJ/kg DM (field maple-control) and also impacted by species \times ensiling treatment ($P < 0.001$). Silage pH ranged from 4.1 (beech-molasses+LAB) to 6.1 (linden-control), and was strongly impacted by treatment and species \times ensiling treatment (both $P < 0.001$). Supplementation of molasses or molasses+LAB were most effective to lower silage pH. Adding molasses+LAB resulted in a reduction in pH in all species, compared to the respective control ($P < 0.05$); while only in two species (alder and ash) adding only molasses did not reduce pH, compared to the control (within all other species: control vs. molasses = $P < 0.05$).

Conclusions: Most of the evaluated foliage types have attractive nutrient profiles and can be effectively conserved through ensiling, with relatively good nutrient retention. Adding only molasses, or molasses+LAB, seems to improve fermentation quality, supporting the use of tree-leaf silages as sustainable forage alternative. The feasibility of using ensiled foliage as alternative feed resource will, among other factors, highly depend on the practicality of mechanical harvest.

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Fermentation of alfalfa with various lactic acid bacteria strains and additives and their effect on fermentation characteristics

Fermentierung von Luzerne mit verschiedenen Milchsäurebakterienstämmen und Zusätzen und deren Auswirkung auf Fermentationsparameter

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Introduction: Studies indicated that higher inclusion rates of alfalfa (*Medicago sativa L.*) in non-ruminant diets adversely affect weight gain and feed conversion [1]. The primary factors responsible for these limitations include low protein digestibility, high fibre content, and antinutritional factors like saponins [2]. Feed processing methods such as lactic acid bacteria fermentation have been shown to increase protein digestibility and reduce antinutritional factors [3]. However, the effect and quality of the fermentation may largely depend on the fermentation conditions including the microorganisms used as starter culture, the addition of exogenous carbohydrases, or the addition of readily fermentable energy sources. This study compared four inoculations, with and without adding sugar cane molasses and exogenous carbohydrases.

Methods: Three homofermentative lactic acid bacteria strain inoculants were used at a dosage of 3.42×10^7 cfu/g DM. Fresh Alfalfa (*Medicago sativa L.*) at the bud stage was finely chopped and mixed with distilled water in a ratio of 1:2.53 to a DM content of 7.6 %. Each alfalfa–water mixture was either inoculated with a single strain (*Pediococcus pentosaceus*, *Lactiplantibacillus plantarum*, and *Companilactobacillus formosensis*) or a combination of *P. pentosaceus* and *C. formosensis*. Each of these treatments was tested without and with the addition of carbohydrases or sugar cane molasses. After inoculation, the fermentation vessels were placed in an incubator for 72 h at 30 °C. The pH was measured after 0, 8, 12, 16, 20, 24, 36, 48, 60, and 72 h. The concentrations of lactate, acetate, $\text{NH}_4\text{-N}$, and crude protein were analyzed after 72 h. Data were analysed in a 4×3 -factorial analysis of variance in R to test for the main effects of starter culture (*P. pentosaceus*, *L. plantarum*, *C. formosensis*, or a combination of *P. pentosaceus* and *C. formosensis*) and treatment (no additive, addition of carbohydrases, or addition of sugar cane molasses), as well as their interaction.

Results: The starter culture affected the pH from 8 h onwards ($P < 0.05$). From 24 h onwards, sugar cane molasses reduced pH to similar levels across all starter cultures (starter culture \times treatment: $P < 0.05$), reaching ≤ 4.0 , whereas starter cultures alone or combined with carbohydrases maintained pH values of approximately 4.7. The addition of carbohydrases did not affect pH within each starter culture ($P > 0.05$). After 72 h, the effect of sugar cane molasses on $\text{NH}_4\text{-N}$ concentrations was dependent on the starter culture, as indicated by a significant interaction ($P < 0.05$). The $\text{NH}_4\text{-N}$ concentrations ranged from 2.4 to 2.9 g/kg DM with or without carbohydrases, while sugar cane molasses reduced $\text{NH}_4\text{-N}$ concentration to 0.8 g/kg DM for *L. plantarum*, and to around 1.4 g/kg DM for all other starter cultures. The effect of sugar cane molasses on lactate and acetate concentrations was also dependent on the starter culture (starter culture \times treatment: $P < 0.05$). Without and with the addition of carbohydrases the lactate concentrations were around 50 g/kg DM. Lactate concentrations increased with sugar cane molasses from 63 g/kg DM for *L. plantarum* and to 155 g/kg DM for *C. formosensis*. Without and with the addition of carbohydrases the acetate concentrations were around 18 to 30 g/kg DM. The addition of sugarcane molasses decreased acetate concentration by approximately 18 g/kg DM in *C. formosensis*, whereas in all other starter cultures, the decrease was around 10 g/kg DM.

Conclusions: Stable alfalfa ferments can be produced without sugar cane molasses or enzyme addition. However, in the absence of additional energy sources, pH remains relatively high, increasing the risk of nitrogen loss through proteolysis. The addition of carbohydrases does not appear to increase the energy availability for the starter culture tested on alfalfa. Thus, to improve fermentation quality, it is necessary to provide an external energy source for the tested strains.

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Comparison of lactic acid bacteria for the fermentation of field peas

Vergleich von Milchsäurebakterien zur Fermentation von Körnererbsen

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Introduction: Field peas (*Pisum sativum L.*) are used as a protein and energy source in pig diets. However, peas contain considerable amounts of protein, starch, and fibre that are precaecally indigestible [1] but may be reduced by feed processing techniques such as fermentation. Feed fermentation can reduce the concentration of antinutritional factors, including α -galactosides or phytate, and increase the precaecal digestibility of nutrient fractions, such as crude protein and starch, in feedstuffs [2]. The effect of the fermentation may largely depend on the fermentation conditions, including the microorganisms used as starter cultures. Different starter cultures possess distinct genetic potential that enables specific metabolic conversions, thereby determining their suitability for fermenting specific feedstuffs [3]. This study compared nine bacterial strains for their ability to ferment field pea grains.

Methods: Nine strains of different genera, including homofermentative and heterofermentative lactic acid bacteria and *Bacillus*, were selected as starter cultures based on their genetic potential to degrade pea carbohydrates. Ground pea grains (2 mm) were mixed with double-distilled water prewarmed to 37 °C in a ratio of 1:1.5 (w/w). The pea-water mixture was inoculated with 100 μ l/100 g of an overnight culture adjusted to an OD₆₀₀ of 1 of either one of the starter cultures or left uninoculated as a control. After inoculation, the ferments were incubated for 24 h at 37 °C in an incubator. The pH of the ferments was measured after 0, 2, 4, 6, 8, 12, and 24 h (n = 3) and concentrations of lactate, acetate, and NH₄⁺-N were analysed after 24 h (n = 1). Based on these results, three of the nine strains were selected to form combinations of two or three strains as the starter culture in the second trial. The fermentation was carried out as described above and pH (n = 6), lactate, acetate, and NH₄⁺-N (n = 5) were measured. Data were analysed with a linear mixed-effects model using the lmer package in R. Statistical significance was set at P < 0.05.

Results: The starter culture affected the pH value from 2 h onwards (P < 0.05). After 8 h, the lowest pH value was observed for strains of *Pediococcus pentosaceus*, *Companilactobacillus kimchii*, *Weissella confusa*, and *Lactiplantibacillus plantarum* (pH 4.7–5.2), whereas the pH for *Bacillus licheniformis* and *Bacillus subtilis* strains did not differ compared to the uninoculated control (P > 0.05). After 24 h, homofermentative *L. plantarum* and *C. kimchii* strains showed a pH value below 4.0 on average, while *Leuconostoc suionicum*, *W. confusa*, and *Bacillus* strains had a pH value not different from the control (P > 0.05) at around pH 4.5. The highest concentration of lactate was measured for *C. kimchii* (138 mmol/kg) followed by other homofermentative lactic acid bacteria strains and *Bacillus* strains (77–100 mmol/kg), whereas heterofermentative strains had lower concentrations (58–77 mmol/kg). The concentration of acetate was higher for heterofermentative strains (29–40 mmol/kg) than homofermentative strains (7–27 mmol/kg). The NH₄⁺-N concentration was lower for *L. plantarum* strains (4–6 mg/100 g), followed by other homofermentative lactic acid bacteria strains (15–16 mg/100 g), and higher for heterofermentative lactic acid bacteria and *Bacillus* strains (19–23 mg/100 g). For the second trial, heterofermentative *Levilactobacillus spicheri* and homofermentative *C. kimchii* and *L. plantarum* were selected. Any combination of the three strains showed values for pH, lactate, acetate, and NH₄⁺-N-concentration between the values observed for the respective single strains.

Conclusions: The results suggest that among the tested strains, primarily homofermentative lactic acid bacteria as starter cultures for pea grains produce sufficient lactic acid and reduce the pH to 4.0 or below within 24 h of fermentation. Combinations of the three selected strains did not show any additive effects on the measured fermentation parameters.

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Factors influencing fecal N excretion and its relation to DMI based on digestion studies with wethers

Untersuchungen zu Einflussfaktoren auf die Kot-N-Ausscheidung und deren Verhältnis zur Trockenmasseaufnahme anhand von Verdauungsversuchen mit Hammeln

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Introduction: Following the modelling of a new estimation equation for nitrogen (N) excretions of dairy cows [1], the distribution of nitrogen in feces and urine has become a matter of concern, given that only nitrogen from urine contributes to ammonia emissions. Compared to N excretion with urine, the N content in feces is more constant [2]. In addition, a close correlation with dry matter intake (DMI) is considered. In order to test this hypothesis and to investigate the influence of crude protein (CP) content, organic matter digestibility (OMD) and feedstuff on the relation of fecal nitrogen excretion to DMI, data from digestion studies with wethers from the Institute of Livestock Farming of the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries (LFA) in Dummerstorf were analysed.

Methods: A total of 201 digestion trials conducted at the LFA in Dummerstorf between 2011 and 2023 were evaluated. The examined test rations were both complete feeds including TMR (direct test) and mixtures of test feed and previously tested supplementary feed (difference test). The tested feedstuffs were categorised in the roughage feed groups whole crop silage, grass, clover/alfalfa, and corn, and all mixed rations (TMR) were treated separately as one group. The roughage used as supplementary feed in the difference tests was also included in the grouping according to roughage type. The following test parameters were available: DMI (g DMI/kg BW), CP (g/kg DM), OMD (%), and fecal N excretion (g N/kg DMI). For statistical evaluation, a linear generalized model (PROC GLM) was created for the dependent variable g fecal N/ kg DMI using SAS. The fixed factors CP group (<120, 120 - <150, 150 - < 180, 180 - < 210, >210 [g/kg DM]), OMD group (< 65, 65 – 75, >75 [%]) and feed group (whole crop silage, grass, clover/alfalfa, corn, TMR) were considered in the model. To estimate the adjusted mean values, least square means (LSM) were calculated and then compared using the Tukey post-hoc test with a significance level of $\alpha = 0.05$.

Results: The average DMI was 14.2 g/kg BW and varied between 8.2 and 23.4 g/kg BW. The mean CP content was 165 g/kg DM and ranged between 59 and 299 g/kg DM, while the OMD ranged from 50.3 to 84%, with an average value of 71.7%. The mean fecal N excretion per kg DMI was 57.2 g, with a range from 27 to 95 g N/kg DMI. The present study has revealed that there is no influence of DMI on the fecal N excretion per kg DMI in either the linear regression or the analysis of variance. Furthermore, statistical analysis demonstrated a significant decrease in g N/kg DMI when the CP content was below 120 g/kg DM and a significant increase when the CP content was above 210 g/kg DM. Within the range of 120 to 210 g CP/kg DM the results showed no significant differences between the values of g N/kg DMI. In relation to OMD, a significant negative correlation with g fecal N/kg DMI was identified. The results also demonstrated that grass-based feeds had significantly higher fecal N amounts/kg DMI than the feed groups whole crop silage, clover/alfalfa, and TMR, while all other feedstuffs did not differ significantly.

Conclusions: The investigations showed a significant negative impact of OMD on the quantity of nitrogen in feces per kg DMI. The CP content demonstrated a significant influence solely at extreme values (< 120 or > 210 g/kg DM). Only the grass feed group showed higher values for fecal N content/kg DMI; otherwise, the feed group had no influence. The amount of fecal N excretion per kg DMI appeared to be independent of the level of DMI. This is a precondition for using a constant relationship between fecal N excretion and DMI to predict the total fecal N excretion and ammonia emissions, respectively. However, due to the small range of DMI in wethers, the significance of these results is limited and should be verified in the next step with values from N balance studies with dairy cows.

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Effects of *Hermetia Illucens* larvae meal on laying performance and nutrient composition of egg yolk and liver in Lohmann Sandy hens

Auswirkungen von Hermetia illucens-Larvenmehl auf Legeleistung und Nährstoffzusammensetzung von Eidotter und Leber bei Lohmann Sandy Legehennen

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Introduction: Sustainable alternatives to conventional protein sources are increasingly important in poultry nutrition. Among them, meal black soldier fly (*Hermetia illucens*, HI) larvae has received growing attention due to its favorable amino acid profile, efficient feed conversion of organic residues, and low environmental footprint. This study investigated the effects of partially replacing conventional protein sources with HI larvae meal on laying performance and nutritional quality of egg yolks in Lohmann Sandy hens, a genotype suitable for organic farming systems.

Methods: The trial was conducted at the Oberer Hardthof experimental farm of Justus-Liebig University Giessen (Germany) under organic farming standards. Chicks were fed a standard commercial starter diet (CP 22.50%, ME 12.00 MJ/kg) until 6 weeks of age, then randomly assigned to two groups (n = 69 each). Each group was kept in one cage. From 7 to 52 weeks, hens were divided into three phases: young hens, pre-layers, and laying hens. The control diet (HI0) was formulated with peas and mixed protein supplements (soybean cake, sunflower cake, rapeseed cake) as primary protein sources, with CP and ME ranging 16.89-18.34% and 11.05-12.06 MJ/kg in the three phases, respectively. In the experimental diet (HI15), a part of the protein sources was replaced by HI larvae meal (15% of the diet), maintaining comparable energy and nutrient levels. Number of eggs and egg weight were recorded weekly to calculate hen-day egg production (HDEP) and total egg output. Eggs were sampled in weeks 31, 41, and 51 for yolk analyses. Fifteen hens per group were slaughtered at week 52 for liver and plasma collection. Parameters analyzed included growth performance, carcass traits, vitamins (retinol, α - and γ -tocopherol), fatty acid composition, triglycerides, cholesterol, and plasma metabolites. Statistical analysis was performed using one-way ANOVA with $P < 0.05$ considered significant.

Results: Egg production rate (81.2% in HI15 vs. 77.6% in HI0), egg weight (61.9 g vs. 61.0 g) and daily egg mass (52.7 g vs. 49.4 g) were similar in both groups. At the end of the experiment, HI15 hens had a higher carcass weight, and a lower relative liver weight than HI0 hens. At week 41, yolks from HI0 hens showed higher levels of retinol and α -tocopherol compared to those from HI15 hens. Across the study period, HI0 hens also exhibited higher α - and γ -tocopherol concentrations in yolks at weeks 31 and 51 ($P < 0.05$). Hepatic α - and γ -tocopherol concentrations were greater in HI0 hens than in HI15 hens. Fatty acid profiles indicated increased saturated fatty acids (SFA) and decreased monounsaturated (MUFA), polyunsaturated fatty acids (PUFA) levels as well as MUFA/SFA, and PUFA/SFA ratios in HI15 yolks ($P < 0.05$), while HI0 yolks had higher levels of n-6 PUFA. Yolk triglyceride and cholesterol concentrations did not differ, but liver cholesterol was higher in HI15 than in HI0 hens ($P < 0.05$). Plasma metabolomics analysis via principal component analysis (PCA) revealed a clear separation between groups, with the first two components explaining 76.5% of the total variance.

Conclusions: Replacing conventional protein with HI meal (15% of the diet) had no adverse effects on laying performance in Lohmann Sandy hens, while altering vitamin A and E concentrations and yolk fatty acid composition. These findings contribute to the understanding of the potential of HI meal as a sustainable protein source in organic egg production.

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17.

Protein Reduction in Diets for fattening chickens: Effects on Growth, Health, and Carcass Traits in Male Dual-Purpose Chickens

Proteinreduktion in Mastfuttermitteln: Auswirkungen auf Wachstum, Gesundheit und Fleischqualität männlicher Zweinutzungshühner

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Introduction: Nutrient requirements of slow-growing genotypes, such as dual-purpose (DP) chickens, remain insufficiently understood. Our previous investigations indicated that reducing dietary crude protein (CP) to 16% does not impair performance or health. The present study aimed to identify the optimal dietary CP level for male DP chickens.

Methods: A total of 213 male DP chickens (ÖTZ Coffee) were reared under organic conditions. At six weeks of age (woa), birds were randomly assigned to four dietary groups and fed isoenergetic diets (11.4 MJ AMEN) differing in CP content: CP16.0 (control; 16.0% CP, 0.74% lysine, 0.29% methionine), CP15.5 (15.5% CP, 0.74% lysine, 0.29% methionine), CP13.9 (13.9% CP, 0.66% lysine, 0.28% methionine), and CP13.7 (13.7% CP, 0.65% lysine, 0.28% methionine). Each group consisted of 52 - 54 birds in two replicates. Body weight (BW) and feed intake (FI) were recorded weekly, and feed conversion ratio (FCR) was calculated. At seven and eleven woa, comb injuries and plumage condition were scored. At 13 woa, birds were slaughtered. During slaughter, carcass characteristics, organ weights, breast blisters, keel bone deviations, and pododermatitis were assessed. After slaughter, meat quality traits were measured, and tibiae were collected and examined using a three-point bending test. Data were statistically analysed using Tukey's HSD and Kruskal-Wallis tests ($p < 0.05$).

Results: Body weight was higher in the CP16.0 and CP15.5 groups compared with the CP13.7 and CP13.9 groups ($p = 0.03$). Carcass characteristics; breast, thigh, wing, and organ yields; abdominal fat-to-body weight ratio; and breast fillet tenderness did not differ among dietary treatments ($p > 0.05$). The CP16.0 group showed the highest cut-up part weight. No differences were detected among groups with regard to footpad dermatitis, breast blisters, keel bone deviations, comb injuries, plumage condition, or liver abnormalities ($p > 0.05$). Tibiae were heavier, longer, and thicker in the CP16.0 group compared with CP13.9 and CP13.7 ($p < 0.001$). No differences in tibial fracture strength were observed among the groups.

Conclusions: A dietary CP level below 15.5% reduced growth performance but did not impair meat quality traits, organ development, health, or animal welfare. Thus, a moderate reduction in dietary protein appears ethically acceptable for male DP chickens (Coffee).

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Is the estimated optimal Tryptophan to Lysine ratio in finishing broilers influenced by the Leucine to Lysine ratio in the feed?

Wird das optimale Tryptophan zu Lysin-Verhältnis bei Endmastbroйлern durch das Leucin zu Lysin-Verhältnis des Futters beeinflusst?

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Introduction: Tryptophan (Trp) has an important role in the metabolism of broilers as it is not only required for protein synthesis but also serves as a precursor for the synthesis of serotonin, melatonin and niacin. Leucine (Leu) and other large neutral amino acids compete with Trp to cross the blood-brain barrier [1]. This competition can affect Trp availability in the brain, affecting serotonin synthesis, appetite regulation and thus growth in broilers. Aim of this study was to test whether the estimated optimal Trp to lysine (Lys) ratio for growth is depending on the Leu to Lys ratio.

Methods: A total of 684-day old male Ross 308 broiler chickens were reared with a commercial starter and grower feed. From day 19 until day 33, birds were allocated to one of eight dietary treatments in a 2 x 4 factorial design, with seven replicates each, holding 12 birds per pen. Animals received a diet with one of four standardized ileal digestible (SID) Trp to Lys ratios (0.137, 0.170, 0.200, 0.230), in combination with either 1.10 or 1.60 SID Leu to Lys. Basal diets (BD) mainly consisted of wheat, barley, soybean meal and peas. BDs were supplemented with free L-Trp and L-Leu to achieve the corresponding Trp and Leu levels. The SID Lys content of the finisher diets was reduced by about 11% as compared to breeder's recommendations (1.06%) [2] to evaluate broiler performance in relation to the Trp to Lys ratios. Feed intake (FI) and body weight (BW) were measured. Feed conversion ratio (FCR), daily weight gain (DWG) and European production efficiency factor (EPEF) were calculated. At day 33, eight birds per pen were used for carcass evaluation. Additionally, blood samples of two animals per pen were taken during the slaughter process to analyze serotonin and corticosterone. Data was analyzed using one-factorial ANOVA with the fixed effect of treatment. Means were compared using Tukey test to account for multiple comparisons ($p < 0.05$). Additionally, Trp to Lys recommendations to achieve 98% of maximum performance were determined using an exponential model.

Results: In general, no statistical difference in performance parameters between the two Leu to Lys levels were detectable, e.g. the average final BW was observed to be 2359 g (1.1 Leu to Lys) vs. 2353 g (1.6 Leu to Lys). The estimation of Trp to Lys ratio to achieve the 98% of maximum performance at 1.1 Leu to Lys was 0.170, 0.153, and 0.141 for DWG, FI, and EPEF, respectively. It was not possible to estimate the optimal Trp to Lys ratio for feed conversion, carcass parameters and blood parameters. The highest relative breast weight was observed at 0.20 Trp to Lys independently of Leu to Lys level. The serotonin and corticosterone levels remained stable among the treatment groups.

Conclusions: Higher Leu to Lys ratios did not seem to impact the optimal Trp to Lys ratio under the conditions of this trial. A recent dose-response study in finisher broilers found a lower value than 1.1 Leu to Lys for optimal performance [3], consequently the lack of interaction in this trial may be explained because the Leu to Lys ratio of 1.1 was already above the animals need. The optimal Trp to Lys ratio is most likely not higher than 0.170 given the optimal rearing conditions during this trial might have contributed to a lower optimal Trp to Lys compared with the literature. The lack of response of serotonin and corticosterone to different levels of Trp and Leu remained to be further elucidated.

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Influence of dietary glycine, serine, and betaine on plasma amino acid concentrations in broiler chickens, fed a low crude protein diet

Einfluss von Glycin, Serin und Betain in Niedrigproteinrationen auf Aminosäurenkonzentrationen im Blutplasma von Broilern

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Introduction: Glycine (Gly) may become a limiting amino acid in low crude protein (CP) broiler diets. Gly and serine (Ser) are interconvertible, and several studies showed an equal effect of Gly and Ser when considered on an equimolar level; thus, it is assumed that Gly and Ser are best evaluated together as Gly equivalent (Gly_{equi}) [1]. However, the Gly_{equi} does not consider other molecules involved in the metabolic interconversion, like one-carbon units (1C units). When Gly is converted to Ser, a 1C unit is required and, vice versa, a 1C unit is released when Ser is converted to Gly. Another 1C unit is released when Gly is degraded, leading to different molar quantities of 1C units that can be produced from Gly or Ser [2]. Because of the different roles of Ser and Gly in 1C synthesis and utilization, we hypothesized that equal utilization of Gly and Ser might be impaired under circumstances such as a high metabolic 1C unit load. We aimed to investigate whether the availability of 1C units from betaine supplementation affects the utilization of equimolar levels of dietary Gly and Ser by analyzing plasma amino acids and relevant plasma metabolites in broiler chickens.

Methods: Male broiler chickens (Ross 308) were housed in metabolism units and were distributed to test 10 diets with 7 replicate pens (10 birds per pen) following a randomized complete block design. Experimental diets were fed from d7 to d22 of age. The diets were based on corn and soybean meal, contained 15% CP, and were formulated to provide adequate concentrations of all proteinogenic amino acids except Gly and Ser by supplementing high proportions of free amino acids. Betaine was supplemented to the diets at 0 or 2 g/kg (Bet-, Bet+). The diets were formulated to provide 10.0 g Gly_{equi}/kg DM (no Gly or Ser supplementation (Basal)), 13.5 g Gly_{equi}/kg DM (from either Gly (Gly+) or Ser (Ser+) supplementation), or 17 g Gly_{equi}/kg DM (from either Gly (Gly++) or Ser (Ser++) supplementation). Plasma samples were collected on d22 from one bird per metabolism unit. Data were analyzed with a mixed model using the MIXED procedure of SAS. Contrasts were calculated to compare treatment groups (1: Bet- vs. Bet+, 2: Bet-Gly+(+) vs. Bet+Gly+(+), 3: Bet-Ser+(+) vs. Bet+Ser+(+), 4: Bet-Gly+(+) vs. Bet-Ser+(+), 5: Bet+Gly+(+) vs. Bet+Ser+(+)).

Results: Plasma Gly concentrations were affected by treatment ($P < 0.001$) and increased with Gly supplementation in a dose-dependent manner, with Bet+Gly++ showing the highest value. Ser supplementation numerically increased plasma Gly concentrations in Bet- treatments, whereas in Bet+ treatments, Ser supplementation significantly increased plasma Gly concentration compared to the basal diet (Bet-). Plasma Ser concentrations were affected by treatment ($P < 0.001$) and reacted in a dose-dependent manner to Ser supplementation. Gly supplementation numerically increased plasma Ser concentrations in Bet- treatments. Bet supplementation increased plasma Ser concentrations (contrast 1: $P < 0.001$). Threonine concentrations were higher in Bet+ than Bet- treatments (contrast 1: $P < 0.001$), this difference was particularly apparent for Ser supplemented treatments (contrast 3: $P = 0.011$). Within the Bet- treatments, concentrations of sarcosine (Sar), methionine (Met), and cysteine were on a similar level irrespective of the level of Gly or Ser supplementation. Concentrations increased with Bet supplementation (contrast 1: $P < 0.001$) and increased even further for the Gly++ (Sar) and Ser++ (Sar, Met) treatments.

Conclusions: Bet supplementation enhanced the effect of Ser supplementation on plasma Gly concentrations and increased the effects of Gly and Ser supplementation on plasma Ser concentrations. Whether betaine supplementation contributed to higher plasma concentrations of Ser and Gly through its role as 1C unit donor or via other mechanisms and whether the increased plasma levels coincide with a higher availability of Gly and Ser require further elucidation.

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A meta-analysis on the influence of age on nitrogen accretion per body weight gain in broiler chickens

Eine Meta-Analyse zum Einfluss des Alters auf den Stickstoffansatz im Gewichtszuwachs bei Masthühnern

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Introduction: Nitrogen accretion per body weight gain (NBWG) is a key figure relevant for determination of nitrogen utilisation efficiency under practical conditions and can be used to derive amino acid supply recommendations. The GfE (1999) recommendations described a nonlinearly increasing and asymptotic relationship of NBWG and age of up to 8-week-old broiler chickens, with no specific model reported [1]. Furthermore, assumptions of increasing NBWG over the years were made, which was explained by continuing breeding efforts for higher meat yields and leaner body composition [2]. Therefore, this contribution aimed to quantify the influence of age on NBWG in broiler chickens in a meta-analysis based on recent literature.

Methods: A dataset of 528 data lines was extracted from 90 studies published between 2016 and 2024, where nitrogen excretion of broiler chickens was determined based on quantitative excreta collection. Data lines were excluded if the growth performance was significantly reduced due to an intervention. The average age during the excreta collection phases ranged from 3.5 days to 42 days (median 21 days) while NBWG ranged from 152 g/kg to 527 g/kg (median 315 g/kg). In an attempt to identify strain effects, 3 subsets were further filtered by major strains, comprising Arbor Acres (94 lines from 12 studies; Ar), Cobb 500 (60 lines from 10 studies; Cb), and Ross 308 (309 lines from 55 studies; Rs). The remaining 65 data lines were from 6 other strains. The following models were used to identify the most-fitted regression: linear (Lm), logarithmic (Log) as a model without an asymptote, and the asymptotic models logistic (Logi), Michaelis-Menten with log-transformed independent variable (MiMe), and Mitscherlich (Mit). All models included a random trial effect on the intercept and were fitted using a Bayesian approach with R package “brms”. As goodness-of-fit criteria, root mean square error (RMSE), Leave-One-Out Information Criterion (LOOIC), and Widely Applicable Information Criterion (WAIC) were used. Paired t-tests were conducted for both observed and fitted NBWG to check for differences of model predictions. Differences in NBWG among the strains were investigated by separately fitting the subsets of the three strains to Lm, Log, and MiMe. Other models did not converge for all strains.

Results: For the whole dataset, goodness-of-fit criteria showed minor differences among models. The lowest and highest LOOIC and WAIC was observed for MiMe (2451 and 2442, respectively) and Logi (2461 and 2453, respectively). The lowest and highest RMSE was from Log (2.075) and Lm (2.082), respectively. All paired t-tests comparing observed NBWG and model predictions had a p-value greater than 0.5, indicating no significant differences among all model predictions in the observed age range of up to 42 days. The Lm described NBWG (g/kg) as 274 (standard error 8) + 2.1 (standard error 0.2) × age (days). Among the strains, Ar led to similar results as the whole dataset, while the age effect in Rs was minor. Cb showed about twice the increase in NBWG with age compared to Ar.

Conclusions: All nonlinear and linear models performed similarly in describing NBWG by age, as the predicted values within the observed age range of up to 42 days were close to each other. Deviations among the models only occurred for extrapolated age. This indicated that Lm as the simplest regression is sufficient in this age range. Differences among the strains appeared unreasonably pronounced and possibly were a consequence of the big variation in sample size of the strain subsets.

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Plane of nutrition and milk composition influences urinary constituents in neonatal calves during the first week of life

Das MilCHFütterungsniveau und die Milchzusammensetzung beeinflusst den Urin neugeborener Kälber in der ersten Lebenswoche

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Introduction: The composition of urine provides a wide range of information about metabolism, water and electrolyte balance. This preliminary study compared twice unlimited intake [1] with a commonly on commercial farms used restrictive milk feeding strategy [2] and investigated the influence of plane of nutrition on urine parameters in neonatal calves during the first week of life.

Methods: At the TUM research facility Veitshof (Freising, Germany) 24 Brown Swiss calves were separated from their mothers immediately after birth (BW 44,17 ± 1,14kg) and divided into three groups for their first week of life: the first group was fed whole milk (MM) twice daily in unlimited volume, the second was fed milk replacer (MR) twice daily in unlimited volume (n=10/ group, [3]) and the third (n=4) was fed whole milk twice daily restricted at a relative amounts of 10% body weight (RES). Before the group specific feeding, all calves received the colostrum and transition milk of their mothers at unlimited amounts until day 4. The amounts of fed colostrum, milk, MR and excreted urine were recorded. Milk and urine samples were taken before each feeding. In the laboratory, urine samples were analyzed for dry matter (DM), nitrogen (N), crude ash (CA), salinity, electrical conductivity, brix and density and the urine samples additionally for urea. The results were statistically analyzed using RStudio using a mixed model with feeding no and group as fixed effects.

Results: Colostrum and milk intake until day 4 was comparable between MM and MR groups and previously published [1,3]. The feed and DM intake, as well as the CA intake was lowest in RES, whereas N intake was highest in MM (p=0.001). RES calves excreted significantly less urine than the other two groups. However, urine of RES contained relatively more DM, N and urea resulting in comparable total N, urea and total CA excretion as MM calves (p≤0.001).

Conclusions: Plane of nutrition exerts a major influence on the urine composition and the amount of urine excreted in calves in their first week of life. A higher feed allowance, regardless of feedstuff seemed to positively influence the hydration status.

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Dose-dependent effects of a dietary fish oil supplementation on the plasma lipidome of healthy adult cats

Dosisabhängige Effekte einer Fischölsupplementierung zum Futter auf das Plasmalipidom gesunder, adulter Katzen

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Introduction: We could previously demonstrate that a dietary fish oil supplementation lowered the triglyceride concentrations and increased the total and low-density lipoprotein cholesterol concentrations in the plasma of healthy adult cats [1]. However, although triglycerides and cholesterol are important clinical markers, more in-depth analyses could provide further insight into the metabolic effects of the dietary fatty acid supply. Therefore, targeted lipidomics analyses were performed with the plasma samples of our previous study [1] to characterize the observed impact of a dietary fish oil supplementation on the lipid metabolism of cats in more detail.

Methods: Ten healthy adult cats received a complete low fat diet (9 % in dry matter) without (control) or with the supplementation of fish oil (0.5 g/kg body weight (BW)/day and 1 g/kg BW/day), using a randomized crossover design. Fasting blood samples were collected at the end of each 3-week feeding period. Lipidomic analyses were performed with the MxP® Quant 500 XL Metabolomics kit (biocrates life sciences ag, Austria) and triple quadrupole mass spectrometry. In the case of missing values for one variable, half the minimum value detected for the respective metabolite in the other cats was used to complete the data set. All values were log-transformed before statistically tested by one-way ANOVA. FDR-adjusted P-values < 0.05 were considered statistically different between groups. Those values were further analyzed with Tukey's post-hoc test. In addition, a principal component analysis (PCA) was conducted (MetaboAnalyst6.0).

Results: The PCA analysis showed a clear clustering between the lipidome of the control group and the supplementation groups. In addition, 259 plasma metabolites assessed by the lipidomics analyses differed depending on the dietary treatment ($P < 0.05$). These metabolites were assigned to the following lipid classes: triacylglycerols (n=75), phosphatidylcholines (n=59), phosphatidylethanolamines (n=58), phosphatidylglycerols (n=22), phosphatidylinositols (n=16), cholesterol esters (n=7), phosphatidylserines (n=7), diacylglycerols (n=4), sphingomyelins (n=4), fatty acids (n=3), phosphatidic acids (n=2), ceramides (n=1), and glycosylceramides (n=1). Most differences were observed when the two supplementation groups were compared with the control group, i.e., independently of the dietary fish oil dose. However, some plasma metabolites were only significantly different between the control treatment and the group receiving the highest supplementation dose. With regard to the previously observed triglyceride-lowering effect of a fish oil supplementation [1], the lipidomics analyses revealed a more precise picture, demonstrating both lowering and increasing effects on specific species of TGs.

Conclusions: A dietary fish oil supplementation significantly affected the plasma lipidome of cats. As the blood collection was performed in the fasting state of the animals, general modulations of the lipid metabolism of the animals can be assumed, even after a relatively short feeding period of 21 days.

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Impact of different fat content in colostrum and transition milk on metabolic parameters in newborn calves

Einfluss eines unterschiedlichen Fettgehaltes im Kolostrum und der Transitmilch auf Stoffwechselparameter neugeborener Kälber

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Introduction: Thermoregulation in neonatal calves is essential to maintain a constant body core temperature. Especially during the cold season, successful adaptation is required to avoid animal welfare, health and production losses. Colostrum and transition milk are characterized by an elevated fat content compared to mature milk. Calves born during the cold season may benefit from this increased fat content as energy source to support thermoregulation during the first days of life. Although colostrum fat content is known to vary considerably, its specific effect on postnatal metabolic adaptation in calves under cold conditions has not been well characterized. The objective of the present study was to examine the impact of a different fat content in colostrum and transition milk on energy and lipid metabolism in newborn calves.

Methods: Twelve calves (n = 9 Holstein, n = 3 Holstein x Limousin crossbred; birth weight 41.3 ± 1.8 kg) born to cows without dystocia during cold season (February to March 2025) were enrolled in the study. Depending on sex, birth weight and breed, 6 calves were assigned to a group receiving full-fat colostrum (FF; 4.81% fat), and 6 calves to a low-fat colostrum group (LF; 0.58% fat). At 4 and 12 h postpartum (p.p.), the respective colostrum was fed (2.5 L each). On days 2 and 3 p.p., the colostrum was diluted with bulk tank milk to simulate transition milk feeding. FF and LF were fed twice daily 3 L of transition milk that differed only by the respective colostrum used (on average 5.17% fat for FF and 3.8% fat for LF). From day 4 onwards, all calves were fed identically with bulk tank milk (average 4.5% fat). In parallel, clinical vital parameters (rectal temperature, heart rate and respiratory rate) were determined. In blood, various metabolic and health parameters were measured: Glucose (GLU), gamma-glutamyl transferase (GGT), aspartate aminotransferase (ASAT), cholesterol (CHOL), high-density lipoprotein (HDL), triglycerides (TG), non-esterified fatty acids (NEFA) and total protein (TP). Data are presented as mean values \pm SEM. Statistical analysis was performed with SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). A mixed model with group (FF, LF), time and group \times time as fixed factors was used. Significant effects were assumed at $P < 0.05$.

Results: The average temperature and temperature-humidity-index (THI) during the experiment was 4.3°C and 42, respectively. In both LF and FF, glucose concentration in plasma increased during the first days after parturition. Glucose concentration was greater in FF compared to LF, especially during the first 4 days of age ($P < 0.05$). Activity of GGT was higher in LF compared to FF ($P < 0.05$). The GGT activity increased in both groups up to 24h of age (LF: from 11 ± 2 U/L to 1953 ± 306 U/L; FF: from 20 ± 7 U/L to 1148 ± 258 U/L) and decreased thereafter. Although ASAT activity showed a similar pattern to GGT, no differences were observed between LF and FF ($P = 0.31$). Throughout the observation period, plasma concentrations of CHOL and HDL concentrations were greater in FF compared to LF ($P < 0.05$). Concentrations of TG were higher in FF compared to LF ($P < 0.05$). No significant differences were observed in NEFA ($P = 0.25$) and TP concentration ($P = 0.27$) between LF and FF.

Conclusions: The present study showed that fat content of colostrum and transition milk affected metabolic adaptation in neonatal calves. In particular, glucose and lipid metabolism were affected, which reflected the overall lower energy availability in LF calves. Further experiments investigating the impact of elevated ambient temperatures (e.g., heat stress during warm season) on metabolic adaptation as well as immune competence are warranted.

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Longitudinal characterization of fecal lipids in dairy heifers from birth to first calving

Langzeitliche Charakterisierung fäkaler Lipide bei Milchrindfärsen von der Geburt bis zur ersten Kalbung

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Introduction: Dairy heifers undergo developmental transitions from birth to first calving that program growth, metabolic adaptation, and future productivity (1). Among these, gastrointestinal maturation, coordinated physiological and microbial changes enabling the shift from milk dependence to fermentative digestion, drives functional transformation (2). Fecal metabolomics offers a non-invasive assessment of this development (3); however, the longitudinal dynamics of fecal lipid classes remain poorly defined. Therefore, we quantified their temporal profiles at key developmental stages in Holstein heifers from birth to first calving.

Methods: Ten Holstein heifers were prospectively enrolled and sampled at 5 critical developmental stages: immediately after birth (12 h), preweaning (week 6), at weaning (week 14), postweaning (month 8) and at first calving (mean age 26 ± 2.3 months). Fecal samples were collected rectally at each stage and analyzed by targeted metabolomics (MxP Quant 500 kit) using flow injection analysis–tandem mass spectrometry with normalization to internal standards and strict quality control. Multivariate analyses, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and hierarchical clustering, were used to assess temporal changes in fecal lipid composition.

Results: The PCA model explained 69.3% of the total variance. A robust separation by developmental stage was observed (PCA $R^2 = 0.76$; PLS-DA $R^2 = 0.98$, $Q^2 = 0.87$), reflecting clear metabolic differentiation throughout the study period. PLS-DA-VIP scores highlighted key lipids, including FA, lyso-PC (e.g., 16:0, 18:0), and lyso-PS (e.g., 18:0, 16:0), which play a crucial role in metabolic changes, lipid metabolism, membrane remodeling, and cellular signaling in the gut. Fecal FA profiling revealed a consistent dominance of myristic acid (C14:0), suggesting microbial synthesis or host-microbe lipid metabolism, with potential implications for intestinal lipid metabolism and gut health. Longitudinal assessments on triglycerides (i.e., TG 16:0_32:1), diglycerides (i.e., DG 18:1_18:1), and monoglycerides revealed stage dependent changes in lipid metabolism in relation to the process of digestive lipolysis and dietary transition from birth to calving. Additionally, the data indicated significant developmental changes in lipid composition, with notable increases in phosphatidylcholine species (e.g., PC 36:5, PC 34:3), phosphatidylethanolamine species (e.g., PE 38:4, PE 36:1), phosphatidylserine species (e.g., PS 36:2, PS 40:5), and sphingomyelin species (e.g., SM 34:1, SM 43:1) after birth and colostrum feeding, highlighting the role of milk-derived phospholipids in the remodeling of gut membranes, cellular signaling, and protection against gastrointestinal infections. Additionally, elevated levels of sphingolipid metabolites, such as ceramides (e.g., Cer d18:1/16:0) and hexosylceramides (e.g., Hex-Cer d18:1/22:0), suggest their involvement in maintaining gut integrity and modulating host-microbe interactions. Our analysis of 39 acylcarnitine species revealed significant stage-dependent changes in 30 species, with two main temporal clusters: the first showing high concentrations after birth and in the preweaning period, indicating active fatty acid oxidation, and the second showing peaks at weaning, postweaning, and calving, suggesting metabolic adaptation as the calf's diet changes and the rumen becomes functional. Stage-dependent changes were also evident for fecal cholesteryl esters and acylcarnitines, with early elevations after birth and again during the preweaning stages. This may infer distinct lipid transport and energy metabolism and later fluctuations in signaling physiological changes in response to dietary transitions and rumen development.

Conclusions: The study highlights significant changes in the fecal lipid profiles of dairy heifers across critical developmental stages, offering valuable insights into the dynamics of lipid metabolism, gastrointestinal maturation, and host-microbiota interactions.

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Effect of fermentation of field pea grains on the digestibility of nutrients in growing pigs

Einfluss der Fermentation von Körnererbsen auf die Verdaulichkeit von Nährstoffen bei Mastschweinen

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Introduction: Field peas (*Pisum sativum L.*) serve as a source of protein and energy in pig nutrition. Nevertheless, a substantial proportion of protein, starch, and fibre in pea grains remains precaecally undigested [1]. Feed processing methods such as fermentation have been shown to increase the precaecal digestibility of nutrient fractions, including crude protein and starch [2]. During fermentation, bacteria produce lactic acid and acetic acid, which lower the pH of the feed and thereby support the proteolytic activity in the stomach [3]. Additionally, fermentation may reduce the concentration of antinutritional factors such as α -galactosides [2]. Antinutritional factors in peas can be further reduced by using exogenous carbohydrases, which may also increase the availability of nutrients for bacteria. Therefore, the objective of the experiment was to evaluate the effect of fermentation of pea grains with or without the addition of carbohydrases on the precaecal and total tract digestibility in pigs.

Methods: Four diets based on wheat (20 %), barley (13 %), rapeseed meal (10 %), and 50 % pea grains were formulated. The peas were either unfermented (control), unfermented with added lactic and acetic acid, fermented, or fermented with added carbohydrases. The fermented peas were produced by inoculating a mixture of ground pea grains and water (1:1.5 w/w) with an overnight culture of *Companilactobacillus kimchii* and incubating it at 37 °C for 24 h. Titanium dioxide was included as an indigestible marker. All diets were formulated to meet or exceed the nutrient supply recommendations of the GfE. Eight barrows (27.6 \pm 2.0 kg initial BW) were fitted with a T-cannula at the distal ileum and assigned to the four dietary treatments in a 4 \times 4 double Latin-square design. Daily feed allowance was 4 % of the mean BW. The experiment included 4 experimental periods of 11 days each with 7 days of adaptation to the diet, followed by 2 days of faeces collection and 2 days of ileal digesta collection. Data were analysed using a linear mixed-effects model with the lmer package in R. The model included the dietary treatment as fixed effect and the animal and the experimental period as random effects. Statistical significance was set at $P < 0.05$.

Results: Fermentation with and without carbohydrase addition reduced the concentration of total dietary fibre (TDF) in the pea grains (189 vs. 168 g/kg DM) but did not affect the concentrations of other nutrients. The precaecal digestibility of DM and gross energy was increased for the two fermented compared to the two unfermented treatments ($P < 0.05$) by around 4 %-units. The diet with fermented pea grains with addition of carbohydrases increased the precaecal disappearance of TDF compared to the control (22.1 vs. 13.2 %; $P < 0.05$) but not compared to the diet with fermented pea grains ($P > 0.05$). The precaecal digestibility of starch and CP for the two fermented treatments were increased compared to the diet with unfermented pea grains with added lactic and acetic acid ($P < 0.05$) but not compared to the control ($P < 0.05$). The total tract digestibility of DM and total tract disappearance of TDF were lower for the control than all other treatments ($P < 0.05$). Fermentation with or without addition of carbohydrases increased the total tract digestibility of gross energy by around 2.5 %-units compared to the control ($P < 0.05$). The highest hindgut disappearance of DM and TDF was calculated for the diet with unfermented pea grains with added lactic and acetic acid ($P < 0.05$).

Conclusions: The fermentation of pea grains with *C. kimchii* can increase the precaecal digestibility of DM and gross energy above the effect of acidification, which may be attributed to the breakdown of carbohydrates. The addition of carbohydrases to the fermentation process did not alter the degradation of pea carbohydrates but increased precaecal TDF disappearance, possibly by targeting carbohydrates from other feed ingredients in the precaecal tract.

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Phytate degradation in growing pigs fed diets with unfermented or fermented field pea grains

Untersuchungen zum Phytatabbau bei Mastschweinen und Einsatz von Rationen mit unfermentierten oder fermentierten Erbsen

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Introduction: Field peas (*Pisum sativum L.*) are widely used as a protein and energy source in pig diets. In unprocessed peas, a considerable proportion of phosphorus (P) is bound to inositol hexakisphosphate (InsP₆), making it poorly accessible to pigs due to low endogenous and plant intrinsic phytase activities. The InsP₆ content may be reduced by processing techniques such as feed fermentation, thereby increasing the availability of P and other nutrients [1]. This InsP₆ degradation may result from microbial production of phytase and activation of intrinsic pea phytase by fermentation in a moist environment at 37 °C with a reduced pH. Additionally, the reduced pH may enhance proteolytic activity, thereby facilitating the release of InsP₆ from protein complexes. Therefore, the objective of this study was to evaluate the effect of fermentation or the addition of lactic acid and acetic acid on the precaecal and total tract InsP₆ degradation in pigs.

Methods: Four diets based on wheat (20 %), barley (13 %), rapeseed meal (10 %), and 50 % pea grains were formulated. The peas were either unfermented (control), unfermented with added lactic and acetic acid, fermented, or fermented with added carbohydrases. The fermented peas were produced by inoculating a mixture of ground pea grains and water (1:1.5 w/w) with 1 ml/kg of an overnight culture of *Companilactobacillus kimchii* and incubating it at 37 °C for 24 h. Titanium dioxide (Ti) was included as an indigestible marker. All diets were formulated to meet or exceed the nutrient supply recommendations of the GfE. Eight barrows (27.6 ± 2.0 kg initial BW) were fitted with a T-cannula at the distal ileum and assigned to the four dietary treatments in a 4×4 double Latin-square design. Daily feed allowance was 4 % of the mean BW. The experiment included 4 experimental periods of 11 days each with 7 days of adaptation to the diet, followed by 2 days of semi-quantitative faeces collection and 2 days of ileal digesta collection. The flow of inositol phosphates (InsP_{3,6}) and *myo*-inositol per kg of diet at the end of the ileum was calculated using Ti: $\text{InsP}_{\text{flow}} = (\text{Ti}_{\text{diet}} / \text{Ti}_{\text{ileal digesta}}) \times \text{InsP}_{\text{ileal digesta}}$. Data were analysed with a linear mixed-effects model using the lmer package in R. The model included the dietary treatment as fixed effect and the animal and the experimental period as random effects. Statistical significance was set at $P < 0.05$.

Results: *C. kimchii* fermentation with and without carbohydrase addition reduced the concentration of InsP₆ in the pea grains (9.5 vs. 8.2 μmol/g DM). Intrinsic phytase activity was 475 U/kg of unfermented pea grains and not detectable after 24 h of fermentation. The precaecal disappearance of InsP₆ was higher ($P < 0.05$) for the diets containing fermented pea grains (36 %) compared to the diet with unfermented pea grains with added lactic and acetic acid (30 %). The diet with unfermented pea grains with added lactic and acetic acid showed higher precaecal InsP₆ disappearance ($P < 0.05$) compared to the control (26 %). The flow of InsP₃, InsP₄, and InsP₅ at the end of the ileum was increased for the diets containing fermented pea grains compared to the control ($P < 0.05$), while the flow of InsP₆ was decreased ($P < 0.05$). The calculated flow of P bound in InsP_{3,6} and of *myo*-inositol at the end of the ileum did not differ among diets. The total tract disappearance of InsP₆ was overall very high but lower ($P < 0.05$) for the control diet compared to the diet with unfermented pea grains with added lactic and acetic acid and diets with fermented pea grains (96 vs. 98 %).

Conclusions: Fermentation of pea grains or acidification of the diet increased the disappearance of InsP₆ but did not affect the total amount of P bound in InsP_{3,6} at the end of the ileum. This indicates that fermentation of pea grains or acidification enhanced InsP₆ hydrolysis yet led to greater accumulation of lower InsP isomers. In contrast, in the control diet, less InsP₆ disappeared, but hydrolysis of lower InsP isomers was more intense.

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***In vitro* phytate degradation by laying hen phosphatases and their genetic background**

In vitro-Phytatabbau durch Phosphatasen der Legehennen und deren genetischer Hintergrund

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Introduction: Chickens can degrade dietary phytate (InsP_6) to a variable extent through the activity of endogenous mucosal phosphatases. Laying hens, in particular, exhibit a relatively low level of phytate degradation, which is affected by dietary phosphorus (P) and calcium (Ca) concentrations, genetic strain, and age of the hens. Regarding strain differences, Lohman Brown-classic (LB) and Lohman LSL-classic (LSL) differed in terms of P utilization and genes related to intracellular InsP metabolism [1]. The present study aimed to investigate InsP_6 disappearance following *in vitro* incubations using duodenal mucosa of individual hens from two genetically distinct strains (LB, LSL) at two ages in the laying peak and receiving diets with or without mineral P supplementation.

Methods: The experiment followed a 2×2 -factorial design with the factors hen strain (LB, $n = 110$ and LSL, $n = 110$), hen age (30 and 42 weeks of age), and dietary mineral P supplementation (0 (P-) and 1 (P⁺) g P/kg feed). Diets were provided for 3 weeks, whereby 200 hens received the P- diet and 20 hens received the P⁺ diet as a control. In weeks 30 and 42 of age, hens were sacrificed, and duodenum mucosa was rinsed and collected. The mucosa samples were freeze-dried before use in a three-step *in vitro* assay [2], simulating crop, stomachs, and small intestine at 40 °C, incorporating digestive enzymes and pH conditions. A corn-soybean meal mixture, ensuring negligible plant intrinsic phytase activity, served as the phytate substrate, containing 10.8 $\mu\text{mol InsP}_6/\text{g}$. At the start of the small intestine step, 5 mg of freeze-dried mucosa protein were added to the system. The incubation was followed by InsP_6 extraction, chromatographic detection, and calculation of InsP_6 disappearance (%). Controls without mucosa addition were included, and each sample was run at least in duplicate. Data were analyzed using a 3-factorial analysis of variance using the MIXED procedure of SAS, and *in vitro* InsP_6 disappearance of the two strains fed P- was further assessed for variance component estimation using ASReml-R. Genetic parameters of the *in vitro* InsP_6 disappearance were estimated separately for each strain using mixed linear model approaches, with the aim of providing a basis for quantifying the genetic background of *in vitro* InsP_6 disappearance.

Results: Three-way interactions for *in vitro* disappearance of InsP_6 were not detected. The disappearance did not differ between both ages when fed P- (54.0% and 54.6%) but was reduced when fed P⁺ to 47.8% (42-wk-old hens) and 39.4% (30-wk-old hens) (age \times diet: $P = 0.016$). Moreover, the *in vitro* InsP_6 disappearance exhibited high heritability in LB hens (h^2 if gte mEquation 12] $\langle m:\text{oMath}\rangle\langle m:\text{sSup}\rangle\langle m:\text{sSupPr}\rangle\langle \text{span style="font-family:'Cambria Math', serif; mso-ascii-font-family:'Cambria Math'; mso-hansi-font-family:'Cambria Math'; mso-bidi-font-family:'Times New Roman'; mso-ansi-language:EN-US; font-style:italic; mso-bidi-font-style:normal; vertical-align:super}\rangle\langle m:\text{ctrlPr}\rangle\langle m:\text{ctrlPr}\rangle\langle \text{span}\rangle\langle m:\text{sSupPr}\rangle\langle m:\text{e}\rangle\langle i style="mso-bidi-font-style:normal'\rangle\langle \text{sup}\rangle\langle \text{span lang=EN-US style="font-size: 11.0pt; line-height:107%; font-family:'Cambria Math', serif; mso-fareast-font-family:Calibri; mso-fareast-theme-font:minor-latin; mso-bidi-font-family:'Times New Roman'; mso-ansi-language:EN-US; mso-fareast-language:EN-US; mso-bidi-language:AR-SA'\rangle\langle m:\text{r}\rangle\langle m:\text{r}\rangle\langle \text{span}\rangle\langle \text{sup}\rangle\langle i\rangle\langle m:\text{e}\rangle\langle m:\text{sup}\rangle\langle i style="mso-bidi-font-style:normal'\rangle\langle \text{sup}\rangle\langle \text{span lang=EN-US style="font-size: 11.0pt; line-height:107%; font-family:'Cambria Math', serif; mso-fareast-font-family:Calibri; mso-fareast-theme-font:minor-latin; mso-bidi-font-family:'Times New Roman'; mso-ansi-language:EN-US; mso-fareast-language:EN-US; mso-bidi-language:AR-SA'\rangle\langle m:\text{r}\rangle\langle 2/m:\text{r}\rangle\langle \text{span}\rangle\langle \text{sup}\rangle\langle i\rangle\langle m:\text{sup}\rangle\langle m:\text{sSup}\rangle\langle m:\text{oMath}\rangle\langle ![\text{endif}][[\text{if } \text{mEquation}][\text{endif}] = 0.59, P = 0.004$), and a polygenic nature of the InsP_6 disappearance was identified, with small genetic effects contributing additively to the overall phenotype. No significant genetic variation was observed for LSL.

Conclusions: Dietary P renunciation and hen aging in the laying peak enhanced phytate degradation, potentially by upregulation of expression of endogenous mucosal phosphatases. The high heritability observed in only one strain supports the strain differences found in other studies. The observed quantitative-genetic differences between laying hen strains in *in vitro* InsP_6 disappearance suggest that *in vitro* data can serve as refined phenotypic indicators for genetic analyses independent of environmental influences like feed or host-specific microbiota.

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Accuracy and precision of measuring apparent dry matter digestibility in cattle: Comparison of the quantitative collection technique with the acid-insoluble ash and TiO_2 indicator method

Richtigkeit und Präzision der Messung der scheinbaren Verdaulichkeit der Trockenmasse bei Rindern: Vergleich der quantitativen Sammelmethode mit der Säure-unlöslichen Asche und TiO_2 Indikatorermethode

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Introduction: Measurement of dry matter digestibility (DMD) through quantitative collection of individual faecal excretions along 7 days is considered as the experimental “gold standard” in ruminant nutrition research [1]. But this procedure implies severe welfare issues [2]. Shorter quantitative collection periods as well as application of alternative techniques such as the indicator method (acid-insoluble ash (AIA); titanium dioxide (TiO_2)) solve the initial welfare problem but might as well entail a loss in accuracy and precision. This investigation compared accuracy and precision of quantitative sampling with AIA and TiO_2 method.

Methods: Nine dry dairy cows were housed in a tie-stall for the duration of the 21-week trial, which consisted of 3 periods of each 7 weeks. Cows were fed restrictively a TMR (6.5 kg DM /day), consisting of 50% grass silage, 40% maize silage, 4.6% wheat, 4.6% soybean cake, 0.7% mineral premix and 0.1% TiO_2 on a DM basis. Feed was offered twice daily at 0700h and 1600h and consumed completely. Silage samples were pooled per period and dried at 60°C. Silage and concentrate samples were analysed for chemical composition, AIA and TiO_2 . During the last week of each period, daily faecal excretions were recorded quantitatively starting from 0700h to 0700h the subsequent day. From these 24h periods two sets of aliquots were sampled, of which one set served as daily samples and the other set was merged to a 7-day pool sample. Additionally, faecal grab samples were taken twice daily from the first defecation after morning and evening feeding. These quantities of faeces were added to daily excretions. Pool, daily and grab samples were analysed for DM, AIA and TiO_2 content. Marker concentration in corresponding pool, daily and grab samples were analyzed with a linear model using “lm” procedure of RStudio. Intercept and slope were tested to differ significantly from 0 and 1, respectively. In order to evaluate accuracy and precision independently of each other, a concentration of the marker was assumed in the feed that corresponded to an average recovery of 100% within the respective period. Different methods were analysed for the potential to detect differences in DM digestibility (least significant difference [LSD]) at $\alpha < 0.05$ and statistical power of $\beta < 0.20$.

Results: AIA concentration in TMR averaged 0.52% of DM with a range from 0.50% to 0.55%. Recovery of TiO_2 in pool samples was 97.2%, 97.8% and 93.3% and recovery of AIA was 192.6%, 220.2 and 130.9% for individual periods. The corresponding DMD was 74.3%, 72.9% and 75.8% for TiO_2 and 86.9%, 87.8% and 82.5% for AIA. DMD measured from 7-day quantitative sampling was 75.1%, 73.5% and 77.4%. Regression equation for the mean AIA concentration from all 7 daily samples and corresponding pool samples was: $0.95x$ ($p=0.17$) + 0.02 ($p=0.87$). Comparison of AIA concentration in combined morning and evening grab samples and corresponding daily samples resulted in: $1.10x$ ($p < 0.05$) – 0.28 ($p=0.07$). With 9 animals per group, the 7-day quantitative collection method resulted in an LSD of 2.2%. Application of the TiO_2 and AIA indicator method led to an LSD of 2.1% and 3.3%, respectively. Shortening the sampling period to 4 days increased LSD for quantitative collection to 2.7%. With 4 days of morning and evening grab sampling LSD increased to 3.8% for AIA indicator method but for TiO_2 indicator method LSD remained unchanged. In period 2 mean AIA concentration in daily samples on days 5, 6 and 7 was 24%, 37% and 18% higher compared to the average of days 1 to 4.

Conclusions: In this experiment, the precision of measuring apparent DMD using AIA as marker was lower in comparison to quantitative collection and TiO_2 indicator method. The accuracy of the AIA indicator method seems to be very susceptible to changes in AIA concentration from feed. For a more accurate measurement of DMD feed sampling should be as corresponding as possible to faecal sampling, in order to also detect short-term changes in AIA concentration from feed.

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Impact of timing of a second colostrum feeding on serum IgG concentrations and persistence in calves during the first week of life

Einfluss des Zeitpunkts einer zweiten Kolostrumgabe auf die Serum-IgG-Konzentrationen und Persistenz bei Kälbern in der ersten Lebenswoche

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Introduction: Efficient colostrum management within the first hours after birth is critical for achieving adequate serum immunoglobulin G (IgG) concentrations in calves, as intestinal IgG absorption rapidly declines after birth. While two colostrum feedings within the first 12 h postpartum (p.p.) are recommended, a timely second feeding is not always feasible under field conditions. However, previous studies suggest that intestinal closure may not be complete by 24–36 h p.p. and that higher IgG concentrations in colostrum may partially compensate for declining intestinal permeability, allowing some continued IgG absorption at later time points [1,2]. This study tested the hypothesis that administering a high-IgG colostrum replacer at 24 h p.p. can partially compensate for reduced intestinal absorptive capacity compared with a second colostrum feeding at 12 h p.p.

Methods: Twelve Holstein and crossbred calves (birth weight ~40 kg) were assigned to two groups. Calves in the early-colostrum-feeding group (CCT, n = 6) received colostrum replacer (2.5 L; 100.7 mg IgG/mL) at 4 and 12 h p.p., followed by reconstituted transition milk at 24 h p.p.; whereas calves in the delayed-colostrum-feeding group (CTC, n = 6) received colostrum replacer at 4 and 24 h p.p., with reconstituted transition milk at 12 h p.p. (reconstituted transition milk = bulk tank milk supplemented with colostrum replacer, 7.8 g IgG/L, 2.5 L per feeding). Colostrum replacer powder composition on a dry matter basis was 23.1% fat, 55.8% protein, 18.9% carbohydrates and 28.1% IgG. Blood samples were collected before the first feeding (4 h p.p.), 12, 24 h p.p., on day 2 (d2; 32–38 h p.p.), daily on d 3–5 and on d 7 prior to feeding. Serum IgG, total protein, γ -glutamyl transferase and apparent efficiency of absorption (AEA; calculated according to Quigley and Drewry, 1998 [3]) were measured. Differences between groups over time were evaluated using a mixed model (MIXED procedure, SAS). Fixed effects included group, day, and the group \times day interaction, while the individual calf was treated as a repeated measure. P-values < 0.05 were considered significant.

Results: Baseline serum IgG concentrations were close to 0 mg/mL in both groups before the first colostrum feeding (4 h p.p.). After the second colostrum feeding (12 h p.p. in CCT and 24 h p.p. in CTC), serum IgG concentrations were assessed at 24 h p.p. in CCT and on d 2 in CTC, revealing higher IgG concentrations in CCT compared with CTC (23.3 \pm 1.8 vs. 16.9 \pm 1.1 mg/mL; P < 0.05), accompanied by greater apparent efficiency of absorption (16.8% vs. 11.7%; P < 0.05). Serum total protein followed a similar pattern to IgG, whereas γ -glutamyl transferase (GGT) increased after the first feeding and then plateaued in both groups. During the first week, IgG concentrations declined more slowly in CTC compared to CCT (P < 0.05), indicating greater apparent persistence, although absolute IgG concentrations remained consistently higher in CCT calves.

Conclusions: Providing the second colostrum feeding at 12 h p.p. results in higher absolute serum IgG concentrations and superior passive transfer of immunity compared with a second feeding at 24 h p.p. Lower initial IgG levels were associated with apparently greater IgG persistence, but this does not compensate for the substantially higher absolute IgG concentrations achieved through early feeding.

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Development of amylase activity in pancreatic tissue and small intestinal content of C57Bl/6J mice before and after weaning

Entwicklung der Amylase-Aktivität in Pankreasgewebe und Dünndarminhalt von C57Bl/6J-Mäusen vor und nach dem Absetzen

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Introduction: Among digestive enzymes, α -amylase is central for carbohydrate digestion, hydrolysing dietary starch into absorbable oligosaccharides [1]. Comparative studies have shown large interspecies variation in pancreatic amylase activity: in omnivorous species, pancreatic amylase activity is higher than in herbivores and much higher than in carnivores [2]. Age and weaning also influence amylase activity. In neonates, milk provides lactose as the exclusive carbohydrate source, which is hydrolysed by lactase, whereas solid-food diets of juveniles and adults contain starch. While this developmental shift has been well documented in several species, a knowledge gap remains for mice, one of the most widely used research model. This study therefore aimed to characterize the age-dependent amylase activity in pancreatic tissue and small intestinal content of C57Bl/6J mice before and after weaning, providing insights into basic digestive physiology.

Methods: Forty C57Bl/6J inbred mice, fed a pelleted standard diet (Altromin rat & mouse breeding, based on cereals, *ad libitum*), were studied between the age of 12 days (d) and 10 weeks (wks). Animals were sampled at different time points before (12d, 3 wks) and after weaning (4, 5, 6, 8, and 10 wks). The entire pancreatic tissue and defined portions of the small intestinal content were collected, weighed, homogenized, and diluted in bovine serum albumin buffer (1:1000). Amylase activity was determined using a standardized enzymatic assay (Phadebas kit). In addition, organs were weighed and referenced to body weight (pancreas, liver, caecum). Small intestinal and colon length were determined. To compare the age groups, ANOVAs were performed (GraphPad Prism, $\alpha=0.05$).

Results: Pancreatic amylase activity increased significantly between 12 d and 5 wks of age, reaching a plateau thereafter. The most abrupt increase occurred between 12 d and 3 wks, from 1978 to 3398 U/g wet weight of pancreatic tissue, corresponding to the transition period prior to weaning. In contrast, amylase activity in the small intestinal content showed no systematic pattern. Most relative organ weights increased significantly until the age of 5 wks. The increase in caecum weight corresponded with the start of intake of solid feed. Small intestinal and colon length increased significantly and in a fixed ratio until the age of 5 wks.

Conclusions: The data confirm that pancreatic amylase activity in young mice increased significantly at weaning and shortly thereafter. This can likely be explained both by pancreas growth and induction of amylase production when dietary starch content increases rather abruptly at weaning. Amylase activity in the small intestinal content might be more sensitive to feeding status and digesta passage rate, which needs to be investigated in further experiments. To more precisely characterize the developmental phase, future studies could include ages between 12 d and 3 wks, different weaning ages, and specifically test earlier pre-weaning starch consumption. Moreover, possible strain differences cannot be excluded.

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Comparative *in vivo* and *in vitro* digestibility of wheat–triticale protein concentrate and poultry meal in dog diets

Vergleichende in vivo- und in vitro-Verdaulichkeit von Weizen–Triticale-Proteinkonzentrat und Geflügelmehl in Hundefutter

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Introduction: The growing demand for sustainable protein sources in pet food has increased interest in plant-based ingredients and by-products from industrial processing. A protein concentrate derived from wheat–triticale processing by-products is a sustainable alternative to animal proteins [1]. This study aimed to assess the *in vivo* and *in vitro* digestibility of a wheat–triticale protein concentrate compared with a poultry meal-based diet in adult dogs.

Methods: Forty adult, healthy dogs were divided into four groups and fed either a dry extrude control diet (G0) or a wheat–triticale protein concentrate at 5% (G5), 10% (G10), or 15% (G15) with TiO_2 as an indigestible marker [2] for 14 days. Each dog was offered 500 g of dry food daily. The study included a 14 day adaptation period on the G0 diet. Nutritional and proximate analyses of all diets were conducted using the Weende method. Faecal samples were taken from D12-D14 and analysed for protein and fat to determine the apparent total tract digestibility (ATTD). The INFOGEST *in vitro* digestibility method was performed based on the published assay with some modifications [3]. Data were analysed using a repeated-measures ANOVA general linear model in NCSS (version 12.0.11). Significant differences were considered at $p \leq 0.05$.

Results: The dogs were healthy throughout the trial and showed no signs of intolerance to the various food compositions. No statistically significant difference in food and energy intake between groups, with energy intake consistently ranging from 302 to 354 per kg BW. Furthermore, the ATTD of crude protein ranged from 74.8 % to 78.1 %, Crude fat digestibility was consistently high across all diets, ranging from 95.6 % and 95.9 % no statistically significant. *In vitro* crude protein digestibility of all diets was from 84.8 % to 85.8 %, with no significant differences between the control poultry meal and wheat-triticale protein diets. These findings suggest that wheat-triticale protein can be used as an alternative to poultry meal in dog diets, offering similar digestibility.

Conclusions: Overall, wheat-triticale protein appears to be a viable alternative to poultry meal in dog diets, providing comparable levels of crude protein and fat digestibility. Further research is needed to understand the observations related to long-term dietary use in dogs.

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Stomach and intestinal *in vitro* starch digestion of hay and oat in horses: a pilot study

Gastrale und intestinale in vitro Stärkeverdauung von Heu und Hafer beim Pferd: Pilotprojekt

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Introduction: Oats are commonly fed to horses as a source of energy. However, starch digestion in the intestinal tract of horses is limited. For better understanding of starch digestion and to avoid animal digestion trials, *in vitro* models can be helpful. Hence, the study aimed to modify the *in vitro* digestion protocol INFOGEST 2.0 [1] to mimic the digestion in horses.

Methods: The INFOGEST 2.0 model was adjusted for pH, enzyme activity, time and an addition of lactic acid bacteria in the stomach phase. Hence, the gastral phase was 2 h and started at pH 3 with the addition of a commercial product containing lactic acid bacteria. In the intestinal phase, the α -amylase activity was 80 U/mL, the pH was 7 and the digestion time 2 h. Hay and oat samples were evaluated for starch digestion: in the gastral and intestinal phase, the glucose concentration was measured with a glucometer and starch digestion was calculated.

Results: Glucose concentrations in the fluid was increased in the intestinal phase compared to the gastral phase for all feed samples. Glucose concentration in the gastral ($p \leq 0.006$) and intestinal phase ($p < 0.050$) differed between feed samples. The highest glucose concentrations were measured for oat samples at the end of the intestinal phase (oat: 25.9 ± 4.68 mmol/L vs. hay: 16.3 ± 2.70 mmol/L). As starch digestion was calculated by glucose concentration in the feed and fluid phase, starch digestion in hay samples was significantly higher (654%) than in oat samples (4.76%).

Conclusions: The *in vitro*-model showed differences in starch digestion between hay and oat samples. The model should be validated using animal trials.

[1] (Brodkorb et al, 2019)

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Comparison of methane emission in landrace and production breed sheep: A systematic review

Vergleich der Methanemissionen bei Landschafen und Schafrassen mit höherer Produktionsleistung: Ein systematischer Review

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Introduction: Sheep represent a domestic ruminant with a large variety of breeds, ranging from breeds for extensive conditions (Landrace; LR) to breeds with higher performance potentials (Production breed; BR). It is an open question to which degree such breed types differ in their digestive performance, e.g. methane (CH₄) production. Factors like ruminal volume, bodyweight (BW) and dry matter intake (DMI) are known to have the potential to influence CH₄ production (g/d) and CH₄ yield (g/kg DMI) in ruminants. A classic work on one LR (Heidschnucken) and one BR breed (Blackface) Weyrether et al. (1987) [1] suggests that the traditional breed had a better ability to increase ruminal volume, e.g. to maintain intake level at high fiber forage. This systematic review sets out to discover if there are general differences between sheep breed types with regards to CH₄ emission.

Methods: The research and evaluation of studies took place between August and October 2025. Studies were selected if they met the following criteria: a) inclusion of both breed types (LR plus BR), b) information on enteric CH₄ production measurements, c) information on dry matter intake and ration nutrient composition, d) at least two different treatments. Eight publications fulfilled all inclusion criteria, resulting in 20 data sets for CH₄ emission and DMI and 11 data sets for BW. Seven of the studies included European breeds, while one study worked with African breeds. For one publication, one of three BRs had to be chosen randomly to fit the statistical model. Feeding levels and number of treatments in the studies varied (32.3 - 78.0 g DM/kg BW^{0.75}; 2-16 treatments/study). While most treatments offered the diet *ad libitum*, one publication included a treatment of restricted hay feeding. Data was extracted and checked for plausibility in Excel for further analysis with R. Normality in the data was checked with the Shapiro-Wilk-Test. BW, DMI, CH₄ emission and CH₄ yield were checked for differences between the two types of breeds by using the mean difference as effect size. A random effects model was applied to account for within and between study variance. Level of significance was at $p \leq 0.05$, p -values of $0.1 \geq p > 0.05$ were considered as trend.

Results: Mean BW showed a trend of difference between the two groups of BR and LR. Mean BW for BR was 55.2 kg (± 13.4) and for LR 43.3 kg (± 10.1), showing LR group being less heavy in comparison ($p=0.087$). No significant differences between the groups were found for mean DMI in kg/d, DMI in g/kg metabolic bodyweight (BW^{0.75}), total CH₄ g/d and CH₄ g/kg DMI. DMI for BR was 1157.95 g/d (± 470.37) and for LR 977.71 g/d (± 200.65), ($p=0.127$). Mean DMI per BW^{0.75} for BR was 55.82 g/d (± 13.62) and for LR 56.60 g/d (± 10.92), ($p=0.844$). Mean absolute CH₄ production for BR was 21.14 g/d (± 7.82) and for LR 18.29 g/d (± 5.72) ($p=0.425$). Mean CH₄ yield for BR was 19.27 g/kg DM (± 4.29) and for LR 19.04 g/kg DM (± 3.66) ($p=0.871$). In meta-analytical tests absolute CH₄ emissions were on average 5.06 standard deviations lower than for BR compared to LR but not statistically significant at $p=0.218$. Between-study heterogeneity ($\tau^2 = 133.79$) is rather large, indicating differences in true effects between publications (Q-test=303.07, $p=0.0001$) while within-study heterogeneity ($\tau^2 = 0.996$) is moderate.

Conclusions: Despite the expected trend in BW, most variables did not differ significantly between the two sheep types. The included studies in this meta-analysis were characterized by substantial diversity in methodology. While all studies involved sheep breeds that met the characterization of BR and LR, the specific breeds used varied across publications, and differed in animal age, sex, environmental conditions, CH₄ measurement techniques, experimental design and statistical methodology further contributing to between-study heterogeneity. The meta-analytical tests highlighted this heterogeneity.

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Influence of reduced nitrogen and/or phosphorus supply on hepatic amino acid transporters in small ruminants

Einfluss einer reduzierten Stickstoff- und/oder Phosphorversorgung auf hepatische Aminosäuretransporter bei kleinen Wiederkäuern

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Introduction: Economic efficiency while ensuring climate protection and resource conservation, remains a key challenge in modern agriculture. One promising strategy to reconcile these aspects may be the optimisation of livestock nutrition by minimising the inclusion of limited or costly feed components. Ruminants, owing to their unique metabolic characteristics, offer promising opportunities for feed optimisation. In particular, their specialised nitrogen (N) metabolism and endogenous phosphate (Pi) recycling suggest that dietary N and phosphorus (P) levels could be reduced, thereby lowering both production costs and environmental impact. However, the metabolic consequences of such dietary restrictions are still largely unknown. A study by Weber et al. demonstrated that the amino acid (AA) profile in the blood of ruminants fed an N-restricted diet was altered. Specifically, the proportion of non-essential amino acids increased, while that of essential amino acids (EAA) decreased compared with the control group. The resulting deficiency in EAAs, caused by the limited supply of the key element N, activates the AA signalling pathway (GCN2-ATF4), which upregulates the expression of genes involved in AA transport and metabolism [1]. P, as an essential constituent of ATP, may also modulate the signalling pathways that regulate cellular metabolism when its dietary supply is reduced, potentially affecting the expression of genes related to AA transport. Based on this rationale, we hypothesised that restrictive feeding of N and/or P alters the hepatic expression of AA transporters in young goats in order to maintain efficient AA uptake.

Methods: Twenty-eight male, growing goats (Bunte Deutsche Edelziege) were randomly assigned to four groups (n = 7 per group), receiving diets with following crude protein (CP) and P contents (percentage of dry matter): control group (16.5% CP, 0.5% P), N-reduced group (8.4% CP, 0.5% P), P-reduced group (16.9% CP, 0.1% P) and N- and P-reduced group (8.1% CP, 0.1% P). All diets were formulated to provide an identical energy content. The feeding period lasted for six weeks, after which the goats were slaughtered and tissue samples were collected for subsequent analyses. Hepatic mRNA expression was quantified by quantitative real-time PCR, normalised to the geometric mean of the reference genes 18S and B2M. Statistical analyses were performed using two-way ANOVA, with $p < 0.05$ considered statistically significant.

Results: Goats fed the N-restricted diet exhibited a significant upregulation of hepatic SNAT2 (1.42-fold, $p < 0.005$), ASCT2 (1.36-fold, $p < 0.01$) and LAT1 (1.46-fold, $p < 0.005$) mRNA compared with controls. In contrast, P restriction alone did not alter any transporter mRNA levels. Two-way ANOVA confirmed a significant main effect of N ($p < 0.01$) but no significant N \times P interaction, indicating that dietary N was the primary driver of hepatic transporter expression changes.

Conclusion: Dietary N restriction induced an upregulation of key hepatic AA transporters, consistent with activation of the amino acid-responsive GCN2-ATF4 pathway. In contrast, P restriction alone had no effect, indicating that hepatic AA transporter expression is primarily regulated by N availability. These findings reveal a compensatory mechanism to maintain AA homeostasis under limited N supply and provide a molecular basis for optimising ruminant feeding strategies to improve efficiency and reduce protein input.

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Effects of mineral phosphorus supply and *myo*-inositol feeding on insulin signaling in adipose tissue of two high-performing laying hen strains at different production stages

Einflüsse der Zulage von mineralischem Phosphor und myo-Inositol auf die Insulinsignalkaskade im Fettgewebe zweier hochleistender Legehennenlinien in unterschiedlichen Phasen des Produktionszyklus

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Introduction: *Myo*-inositol is known to have insulin sensitizing effects in humans and is also discussed to modulate insulin dependent pathways in broilers [1]. For cellular phosphate plays a major role in regulating various cellular processes, dietary supply of mineral phosphorus (P) might also modulate insulin signaling cascade in the laying hen. However, Lohmann selected leghorn (LSL) and Lohmann Brown (LB), commonly used high yielding layer strains, markedly differed in terms of phosphorus utilization and *myo*-inositol metabolism [2]. Thus, the present study aimed to assess the impact of different concentrations of *myo*-inositol or mineral phosphorus in the diet on insulin signaling cascade in LB and LSL hens against the background of different production phases.

Methods: The study was conducted in two trials. Trial 1 comprised 40 Lohmann Brown (LB) and 40 Lohman selected Leghorn (LSL) hens, divided into four feeding groups (LB n = 10; LSL n = 10) supplied with 0g (control), 1g (MI1), 2g (MI2) and 3g (MI3) of *myo*-inositol per kg feed over 4 weeks prior to slaughter and sampling around laying peak at 30 weeks (wks) of age. Trial 2 comprised 40 LB and 40 LSL hens that were fed either 0g (P-; n = 40) or 1g (P+; n = 40) of mineral phosphorus 4 weeks prior to slaughter. Animals were slaughtered and sampled before the onset of egg laying at 19 wks (LB n = 20; LSL n = 20) or at 24 wks of age (LB n = 20; LSL n = 20) after they entered the laying phase. Adipose tissue was taken out of the abdominal fat pad. In both trials, protein abundance of phosphoinositide 3-kinase (PI3K), mammalian target of rapamycin (mTOR), insulin receptor β (INSR), protein kinase B (PKB), adenosin monophosphate activated kinase (AMPK) and their phosphorylated forms (pmTOR, pINSR, pPKB and pAMPK) were assessed by semiquantitative Western Blotting. Data were statistically analysed using a general linear mixed model with the fixed factors strain and diet for trial 1 and strain, diet and age for trial 2. To account for individual variation, the hen father and the slaughter day were included as random factors.

Results: Trial 1: The abundance of mTOR and pmTOR was influenced by strain and diet. LB hens displayed higher expression of pmTOR compared to LSL. Further, LB hens in the MI groups showed higher expression of mTOR than the control group whereas expression in LSL hens was lower in MI than in the control group. A similar interaction was shown for AMPK with higher expressions in MI groups of LB hens compared to control whilst expression in LSL did not change with MI feeding. Concerning pPKB, a tendency for higher expression in LSL was shown. Trial 2: In both strains, expression of mTOR, INSR, and PI3K was lower at wk 19 compared to wk 24 whilst expression of pmTOR and pINSR was higher at wk 19 compared to wk 24. Additionally, P- groups showed higher pINSR abundance than P+ groups. A significant interaction of strain and age was shown in pPKB. LSL hens had higher expressions at wk 24 compared to LB hens at that age.

Conclusions: Abundance of pmTOR in Trial 1 (30 wks) might point to an activated protein synthesis in adipose in LB hens, possibly contributing to their higher body weight gain found in a companion study [3]. In Trial 2, the beginning of egg laying exerted prominent effects on INSR and mTOR suggesting a certain refractoriness of adipose tissue to insulin in that production phase. However this seems to affect only parts of the signaling cascade for expression of PI3K implies the opposite. Further analysis of more downstream effects of insulin signaling would help to get a more complete picture of insulin action in the laying hen

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The bovine hepatocyte cell line BFH12 as a potential *in vitro* model for liver metabolism in dairy cows

Die bovine Hepatozytäre Zelllinie BFH12 als ein potenzielles In vitro-Modell für den Lebermetabolismus im Milchrind

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Introduction: Investigating the liver metabolism of dairy cows in early lactation, the critical period when these animals are prone to diseases like ketosis and fatty liver due to their negative energy balance, is an ongoing challenge. The objective of this study was to investigate if the immortalized cell line BFH12, derived from bovine fetal hepatocytes, might be a suitable tool to investigate hepatic metabolic pathways under such conditions.

Methods: We used quantitative RT-PCR to compare gene expression changes in liver biopsies collected from dairy cows 4 to 2 weeks (wk) before (a.p.) and 2 wk after parturition (p.p.) with the gene expression patterns of BFH12 cells incubated under varying metabolic conditions by modulating the composition of the culture media. The following conditions were used: standard medium according to the protocol from the literature [1] with 100 nM dexamethasone (DEX) and 0.2 U/mL insulin (INS) (+/+), or without both (-/-). In addition, we compared the effect of different glucose concentrations in (+/+) and (-/-) media: either 11.5 mM, 3 mM or 1.5 mM. The target genes chosen were associated with the somatotrophic axis (growth hormone receptor 1A, GHR1A, insulin-like growth factor 1, IGF1), glycolysis (phosphofruktokinase 1, PFK1), gluconeogenesis (pyruvate carboxylase, PC, phosphoenolpyruvate carboxykinase, PCK2), ketogenesis (3-hydroxy-3-methylglutaryl-CoA synthase 2, HMGCS2), fatty acid oxidation (carnitine palmitoyltransferase I, CPT1A, acyl-CoA oxidase 1, ACOX1), and fatty acid synthesis (acetyl-CoA carboxylase, ACC1, fatty acid synthase, FASN, stearoyl-CoA 9-desaturase, SCD1). Data were analyzed using a student t-test for paired observations for liver biopsies and a mixed model with multiple comparisons for fixed effects for BFH12.

Results: In the liver biopsies (N = 5), gene expression of GHR1A ($P < 0.01$) and IGF1 ($P < 0.05$) was downregulated, while ACOX1 was upregulated p.p. compared to a.p. ($P = 0.05$). CPT1A, HMGCS2, PC, PFK1 and ACC1 expression showed substantial variances p.p., probably related to individual differences in energy balance. In BFH12 cultured in -/-, expression of GHR1A, PCK2, PFK1 and SCD1 was downregulated ($P < 0.05$; N = 5). When glucose concentration in the culture medium was lowered from 11.5 mM to 3.0 mM and 1.5 mM, expression of PFK1, FASN, SCD1 and ACC1 was decreased ($P < 0.05$), while PCK2 expression was increased ($P < 0.05$). IGF1 and HMGCS2 were not expressed in BFH12 cells.

Conclusions: It may be concluded that BFH12 cells might be a suitable model to investigate hepatic lipogenesis and with limitations for carbohydrate metabolism, but that these cells cannot be used as a model for fatty acid oxidation, the somatotrophic axis or ketogenesis.

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Total and soluble phosphate content in wet cat food from the US market

Gesamt- und löslicher Phosphatgehalt in US-amerikanischen Feuchttalleinfuttermitteln für Katzen

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Introduction: The majority of complete pet foods on the German market have been found to exceed the recommended daily allowance (RDA) [1] of total phosphate (P_{total}) [2]. Sources of phosphate (P) in commercial products are animal tissue, plant-based diet ingredients, and inorganic phosphate (Pi). Most P containing feed additives are highly soluble P_i salts. Major reasons for adding P_i though are, among other purposes, to enhance flavour, acceptance, and particularly in moist food texture, despite possible health risks. While declaration of added P_i is not mandatory in the EU, the use of P_i containing feed additives must be labelled in the USA. In this study P_{total} and the fractions of P according to solubility [3] in complete moist cat food produced and marketed in the USA were measured in order to compare concentrations in US American with those of German wet cat food.

Methods: Forty different over-the-counter complete, moist cat food products were purchased in different parts of the USA. All samples were homogenised and analysed for P_{total} with standard laboratory methods (photometrically). Fractionation of P regarding solubility was done by the method of Lineva et al. (2019) [3], measuring solubility of P after 1 and 90 minutes in water and 0.4% HCl, respectively. The amount of highly soluble P, i.e., P soluble in water after 1 minute (P_{sol1}), and P_{total} content per metabolic energy (ME) unit was calculated. After testing for normal distribution (Shapiro-Wilk test), results were compared to published data of diets from the German market [2] applying Student's t-test.

Results: In all tested samples, the concentration of P_{total} amounted to more than twice the RDA for total P (cats: 150 mg P/MJ ME [1]). Compared to published data of German products, the P_{total} content was very similar. Mean content of P_{total} in US products amounted to 743 mg/MJ ME (min 338/max 1367), and mean amount in German products came up to 741 mg/MJ ME (min 227/ max 1083). In 63 % of the products, the RDA for P was already met by P_{sol1} while this was true for 84 % of the European products. The amount of P_{sol1} was significantly lower in the US products (mean P_{sol1} : 194 mg/MJ ME (min 73/max 390)) compared to the data of German moist cat food (mean P_{sol1} : 263 mg/MJ ME (min 72/max 500)) ($p \leq 0.001$) [2].

Conclusions: All products exceeded the RDA for P_{total} intake [1] by far. The amount of highly soluble P, represented by the P_{sol1} fraction, varies considerably and exceeds the RDA for total P in most cases. However, P_{sol1} in moist cat food produced in the USA for the US market was significantly lower than in products from Germany [2]. If the different declaration requirements are related to this, it would be another reason to call for mandatory declaration of the source and quantity of phosphate additives.

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Effect of adding phosphate binders Ipakitine® and Renvela® to a high phosphate diet for cats on faecal and renal phosphate excretion

Auswirkungen der Zugabe der Phosphatbinder Ipakitine® und Renvela® zu einer phosphatreichen Diät für Katzen auf die fäkale und renale Phosphatausscheidung

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Introduction: In patients with chronic kidney disease – metabolic bone disorder (CKD-MBD) it is key to normalise serum phosphate (P). P binders are applied to reduce intestinal P absorption, serum P concentrations, and renal P excretion [1]. Ipakitine® (calcium carbonate and chitosan), a P binder for cats and dogs, as well as Renvela® (sevelamer carbonate), a P binding drug for human patients, are approved for treatment of hyperphosphatemia in CKD patients [2,3]. In this study we aimed to test if both P binders effectively reduce apparently digested P and renal P excretion in healthy adult cats fed diets with P containing feed additives.

Methods: Eleven healthy cats (4±2 years, 5f/6m) were fed four different home-made diets (75% beef, 20% rice, 3% rape seed oil, 1% cellulose plus premix) according to their individual energy requirements. A control diet (Ctr; 34 mg P/kg BW/d; no inorganic P (P_i)) and 3 high P (HP) diets with or without P binders (HP_i; HP_{i+Ipa}; HP_{i+Ren}; ~97 mg P/kg BW/d; P_i ~1.06 +/- 0.03 g/Mcal) were fed for 28 days each. Phosphoric acid and sodium phosphate were added to the basic diet as sources of P_i (HP_i, HP_{i+Ipa} and HP_{i+Ren}). The calcium phosphorus ratio in all 4 diets was 1.39 +/- 0.07. From day 19 to 28, faeces as well as urine were collected quantitatively and analysed for P. A washout period of at least 14 days feeding the P_i-free Ctr diet was inserted after each HP diet. Statistical evaluation: All pairwise multiple comparison procedures (Tukey test) were applied after testing for normality (Shapiro-Wilk test).

Results: Adding Renvela® to the HP_i diet had no effect on faecal P excretion (mg/kg BW/d, p=0.602) while Ipakitine® reduced faecal P excretion (p=0.004). The amount of apparently digested P in mg per kg body weight was not influenced by the addition of P binders (HP vs. HP_{i+pa} p=0.247; HP vs. HP_{i+Ren} p=0.075). Renal P excretion was increased in all HP_i diets, regardless of whether phosphate binders were added or not. While addition of Ipakitine in diet HP_{i+Ipa} had no effect on renal P excretion compared to HP_i, adding Renvela® increased renal P excretion further (HP_{i+Ren} 68 vs. HP_{i+Ipa} 31; HP_i 32 mg/kg BW/d). Diet HP_{i+Ren} caused a negative P balance (HP_{i+Ren} -25 vs. HP_{i+Ipa} 10; HP_i 4; Ctr -1 mg/kg BW/d). Increased bone resorption as a reason for the negative P retention in diet HP_{i+Ren} cannot be ruled out. It was not possible to demonstrate the claimed effects of these two P binders in this trial using healthy adult cats.

Conclusions: The unexpected results of this study highlight the relevance of dietetic therapy in CKD patients. The addition of P binders, especially to diets containing highly soluble and therefore available P_i sources, does not appear to replace the need for a restricted dietary P supply to prevent or treat hyperphosphatemia, the main trigger of CKD-MBD progression. These results reinforce current recommendations (International Renal Interest Society, IRIS) that P binders should be administered only after dietary P intake has been appropriately restricted.

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Phosphate fractionation in complementary feed for dental hygiene in dogs

Phosphatfraktionierung in Ergänzungsfuttermitteln zur Dentalhygiene für Hunde

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Introduction: Complementary feed for dental hygiene in dogs advertises not only fresh breath, but also a reduction in plaque formation and even existing dental calculus. Besides mechanical abrasion certain inorganic phosphates (P_i), e.g., sodium hexametaphosphate, are used as active substances. As the possible adverse health effects of soluble P_i -containing food and feed additives are well known, the level of intake from these products should be quantified and considered. The aim of this study was to determine the fractions of phosphate (P) according to their solubility in commercially available products for oral hygiene in dogs.

Methods: Randomly selected products marketed as “dental snacks for dogs” (n=28) were purchased from local or online retailers and analysed for total P content (photometrically, according to standard methods) as well as the fractions of P soluble in water after 1 (P_{sol1}) [1]. Results were related to the estimated metabolizable energy (ME) content, using equations based on labelled crude nutrient concentrations [2] and compared to the recommended daily allowance (RDA) of 0.75 g P/Mcal ME (\pm 0.18 g P/MJ ME) [2]. As dental snacks should be used as a supplement to complete feed, their P contributions to the RDA of a model dog (body weight: 15 kg, maintenance, based on labelled feeding instructions) were then calculated.

Results: Total P concentrations ranged widely with a mean of 2.32 (Min: 0.34 / Max: 5.35) g/Mcal ME (\pm mean 0.55 (Min: 0.07 / Max: 1.29) g/MJ ME). The fraction of highly soluble P accounted for a substantial proportion of total P (P_{sol1} : 45 % (1 / 88)). With 0.59 (0.04 / 4.70) g/Mcal ME (\pm 0.14 (0.01 / 1.12 g/MJ ME)), P_{sol1} was above the RDA for total P in 39 % of the tested products and showed considerably higher values compared to some dry complete diets for dogs (0.52 (0.14 / 1.91) g P_{sol1} /Mcal ME, \pm 0.12 (0.02 / 0.45) g P_{sol1} /MJ ME [3]). When these dental snacks are fed according to their labelled feeding instructions, they provide 2–13 % of the average daily energy requirement and therefore contribute with 28 (1 / 56) % to the RDA of P of a 15 kg model dog.

Conclusions: The content of total P and P_{sol1} vary considerably in the analysed products. As it is not legally required in the EU to declare the P content and P source in pet food, animals for which phosphate restriction is indicated should only be given dental snacks that are guaranteed to be free of phosphate containing additives.

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Comparison of nutrient deposition and mineralization patterns in captive frugivorous bat (*C. perspicillata*) carcasses across developmental stages

Vergleich von Nährstoffgehalten und Mineralisierungsmustern in Körpern von frugivoren Fledermäusen (*C. perspicillata*) in unterschiedlichen Entwicklungsstadien aus einer Zootierhaltung.

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Introduction: Ontogenic changes in body composition and skeletal mineralization are poorly characterized in frugivorous bats [1,2]. This study provides the first length-based reference dataset describing growth-related shifts in nutrient deposition and bone mineral density (BMD) in *Carollia perspicillata*.

Methods: We analyzed 177 *C. perspicillata* frozen carcasses (92 females [F], 85 males [M]) from a single zoological institution, donated post mortem after population management. Carcasses were weighed and measured (snout to pelvic tip). Sex-specific developmental stages were defined using segmented regression of body length versus body mass, identifying breakpoints at 4.41 cm (F) and 4.50 cm (M), and assigning individuals to three length-based groups per sex (F1-F3; M1-M3), representing early, intermediate, and adult life (growth) stages. BMD was assessed using peripheral computed tomography (pQCT) at the orbital, first cervical vertebra (C1), scapular-humeral joint, and midshaft of the left radius [3]. Dried, homogenized carcasses were analyzed for fat, ash, and protein by standard proximate methods modified according to sample size (single determinations) [2,4]. Hydroxyproline was quantified photometrically in duplicate to estimate collagen content as a proportion of protein. Calcium (Ca), phosphorus (P), and magnesium (Mg) were determined following standard protocols [1,2,4]. Group comparisons among developmental stages and sexes, as well as correlation analyses between mineral, ash, collagen, and BMD variables, were performed to assess ontogenic trends.

Results: Body length increased with development in both sexes (F1-3: 4.00 ± 0.46 to 5.26 ± 0.17 cm; M1-3: 4.34 ± 0.32 to 5.25 ± 0.13 cm), accompanied by increasing cranial BMD (F1-3: 226.4 ± 203.4 to 419.5 ± 46.3 mg/cm³; M1-3: 310.6 ± 150.2 to 408.8 ± 47.9 mg/cm³). Cervical BMD remained consistently high (~400 mg/cm³) across stages and sexes, indicating early vertebral mineralization. Wing BMD increased markedly in both sexes with development (F1/M1: 166.6 ± 201.7 to F3/M3: ~420 mg/cm³), reflecting delayed mineralization of appendicular elements. Dry mass increased with development in both sexes (F1-3: 3.93 ± 1.56 to 6.18 ± 0.43 g; M1-3: 4.91 ± 1.36 to 6.63 ± 0.49 g). Absolute fat mass remained stable across stages in both sexes (mean ~1.1 g; 18–19% DM), whereas ash mass increased significantly ($p < 0.05$) from ~0.5 g (F1/M1) to ~0.8 g (F3/M3), corresponding to an increase from 11–13% to ~16% on fat-free dry matter (FFDM). Protein content declined with development, coinciding with reduced collagen proportions (F1/M1: ~20%; to F3/M3: ~16%). Ca, P, and Mg concentrations increased sharply from F1/M1 to F2/M2 (mean: Ca: 38–60 mg/g; P: 23–33 mg/g; Mg: 1.6–2.4 mg/g FFDM) and stabilized thereafter. Strong positive correlations were observed between mineral concentrations and ash content ($r > 0.9$), while collagen showed weak negative associations with mineral content ($r \approx -0.25$).

Conclusions: Developmental stage strongly influences bone mineralization and body composition in *C. perspicillata*. Higher collagen proportions in juveniles are consistent with lower skeletal maturity. Increasing BMD across most anatomical regions is explained by progressive mineral deposition, reflected by rising ash and mineral contents. The associated decline in collagen suggests a shift from matrix-rich skeletal tissues toward mineralized bone, with protein increasingly allocated to muscle tissue. Sex-specific differences were minimal. Ontogenic body composition shifts are primarily driven by structural mineral deposition rather than changes in macronutrient stores. These data provide the first standardized, length-based ontogenic reference for frugivorous bats. Ongoing analyses of additional minerals (Na, K, Fe, and Zn), together with future comparative studies across bat taxa, will further elucidate functional and phylogenetic patterns of nutrient deposition during growth.

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Strain-specific ileal microbiome responses to low-phosphorus diets and their link to phosphorus utilization in laying hens

Stammesspezifische Reaktionen des ilealen Mikrobioms auf phosphorarme Ernährung und ihr Zusammenhang mit der Phosphorverwertung bei Legehennen

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Introduction: Phosphorus (P) is an important mineral in poultry nutrition and production. Organic P stored as phytates in plants is poorly utilized by non-ruminant animals. To compensate for this, poultry diets are often supplemented with mineral phosphate or phytase enzymes. However, with global phosphate reserves dwindling, improving P utilization (PU) in poultry is important [1]. The gut microbiome may contribute to phytate degradation and PU in laying hens, yet the effect of dietary P levels on microbial composition is underexplored, and strain-specific responses to dietary P restriction remain unclear. This study investigated the ileal microbiome of two high-yielding laying hens (Lohmann Brown Classic, LB; Lohmann LSL Classic, LSL) under low P supplementation using shotgun metagenomics to identify the strain-specific microbial and functional adaptations.

Methods: A total of 400 hens (200 LB and 200 LSL) were fed low-P diets (3.6 g/kg dry matter (DM)), and an additional 40 hens (20 LB and 20 LSL) received a P-adequate diet (4.7 g/kg DM). The P concentration in the low-P diet was approximately 1.2 g/kg of non-phytate phosphorus, representing a 50% reduction relative to the standard layer diet. Diets were formulated using corn and soybean meal in accordance with feeding recommendations. After three weeks on the experimental diets, DNA was extracted from ileum digesta and subjected to shotgun metagenomic sequencing. Microbial diversity was assessed using Shannon index and Bray-Curtis dissimilarity. Correlations between ileal species and host traits were determined using Spearman's correlation analysis. Differentially abundant taxa were identified with Analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOM-BC2), while functional differences were analyzed using Microbiome Multivariable Association with Linear Models 2 (MaAsLin2).

Results: Distinct microbiome profiles were observed between the two hen strains, indicating a strong strain effect on the ileal microbiota. At the species level, *Lactobacillus crispatus*, *Ligilactobacillus salivarius*, and *Enterococcus cecorum* were more abundant in LB, while *Limosilactobacillus vaginalis* and *Lactobacillus amylovorus* were enriched in LSL. In LB hens, *L. salivarius* and *E. cecorum* correlated positively with plasma P level, while in LSL hens, *Limosilactobacillus reuteri* and *Limosilactobacillus oris* correlated positively with plasma P, Ca, and myo-inositol. Functional analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) database showed enrichment of inositol metabolism pathways under P limitation, particularly in LB. Low-P diet modulated taxa associated with phosphate transport and phytate hydrolysis, including *L. salivarius* and *E. cecorum* in LB. Enzymes involved in phosphonate transport were more abundant in LSL hens. These functional shifts indicated distinct strain-dependent strategies in phosphate transport and inositol-related metabolism.

Conclusions: Dietary phosphorus restriction induces strain-specific adaptations in the ileal microbiome that are linked with differences in phosphorus utilization. The two hen strains appear to employ distinct microbial and physiological mechanisms to maintain phosphorus homeostasis under restriction, highlighting the microbiome as a potential target for improving phosphorus efficiency and reducing the reliance on inorganic phosphate supplementation in layer production.

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Effects of phosphorus deprivation during the periparturient period on the phospholipid composition of erythrocyte membranes in dairy cows

Auswirkungen von Phosphormangel während der peripartalen Phase auf die Phospholipidzusammensetzung der Erythrozytenmembranen bei Milchkühen

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Introduction: Reduction of phosphorus intake during the dry period may represent a strategy to mitigate the risk of hypocalcemia in early lactation by enhancing calcium mobilization from bone reserves. However, phosphorus depletion during the periparturient phase also increases the risk of developing a syndrome characterized by hemolysis, anemia, and postparturient hemoglobinuria (PPH). The underlying mechanisms contributing to elevated hemolysis rates and the occurrence of PPH under conditions of phosphate depletion remain unclear. The composition of the erythrocyte membrane plays a crucial role in determining membrane properties, including resistance to hemolysis. Therefore, this study investigated the hypothesis that phosphorus depletion during the periparturient period alters the phospholipid composition of the erythrocyte membrane, thereby contributing to an increased susceptibility to hemolysis.

Methods: The study included 18 cows that received basal total mixed rations (TMR) with either adequate phosphorus content (AP; 0.28% and 0.44% P in dry matter during the dry period and lactation, respectively) or identically composed total mixed rations with low phosphorus content (LP; 0.15% and 0.20% P in dry matter during the dry period and lactation, respectively) from four weeks before the expected calving date until four weeks postpartum. The difference in phosphorus content results from supplementing the TMR of the AP group with NaH_2PO_4 . The cows in the AP and LP groups were 4.7 ± 1.2 and 4.5 ± 1.2 years old, respectively, and in their third lactation. The 305-day milk yield from the previous lactation was $9,127 \pm 1,731$ kg for AP cows and $8,935 \pm 2,534$ kg for LP cows. Feed intake was restricted to 12.5 kg DM/day for dry cows and offered *ad libitum* during lactation [1]. Blood samples for the present study were collected two, five, and eight weeks after the onset of the depletion period (i.e., two weeks antepartum, and one and four weeks postpartum). Erythrocytes were isolated by centrifugation, washed three times with phosphate-buffered saline (PBS), and hemolyzed. Membrane fragments were washed with PBS, and lipids were extracted using a chloroform/methanol mixture (1:2, v/v). Concentrations of individual phospholipid species in the lipid extracts were analyzed by liquid chromatography–mass spectrometry. Statistical analysis was performed using a repeated-measures General Linear Model in SPSS [version 29.0.2.0 (20)] to evaluate the effects of treatment and time

Results: Compared to the AP group, cows in the LP group exhibited a reduced plasma phosphate concentration (0.64 ± 0.08 vs. 1.32 ± 0.15 mmol/L, respectively $P < 0.05$, four weeks postpartum). Among the nine cows in the LP group, four cows showed signs of anemia of which 2 cows developed overt PPH. The contents of phospholipid classes in erythrocyte membranes - sphingomyelin, phosphatidylserine, phosphatidylethanolamine (PE), phosphatidylinositol, phosphatidylcholine (PC), lyso-PC, and lyso-PE - did not differ between AP and LP cows. Likewise, the average carbon chain length and the mean number of double bonds in individual phospholipids were similar between the two groups. Only minimal differences were observed in the relative proportions of individual phospholipid species between groups. Sampling time also had negligible effects on overall phospholipid composition and the distribution of individual species. Furthermore, two-dimensional principal component analysis (PCA) score plots (PC1 vs. PC2) with 95% confidence regions for each group, generated for each time point, did not reveal any distinct clustering or separation of cows diagnosed with PPH from the remaining individuals.

Conclusions: The findings of this study indicate that dietary phosphorus deprivation during the transition period does not affect the phospholipid composition of erythrocyte membranes. This suggests that the development of phosphorus deprivation-induced PPH is not attributable to alterations in erythrocyte membrane lipid composition.

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The effect of feeding poplar and willow leaves on blood plasma mineral concentrations in goats

Der Fütterungseinfluss von Pappel- und Weidenblättern auf die Mineralstoffkonzentrationen im Plasma von Ziegen

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Introduction: Silvopastoral agroforestry systems combine pasture grazing and the cultivation of woody plants. Besides grass on pasture, the leaves of trees and hedges may serve as additional feed source for livestock. Poplar and willow are fast-growing, deep-rooting trees, able to acquire substantial amounts of minerals from deeper soil layers. Thus, feeding mineral-rich leaves could reduce mineral mining and contribute to a circular feed concept. Although leaves from willow and poplar trees are readily browsed by small ruminants [1], it is not known whether their intake influences the mineral status of goats. The aim of this study was to evaluate the effect of supplementing a hay-based diet with poplar and willow leaves on blood plasma mineral concentrations in wether goats.

Methods: Four Saanen goat wethers (~1.5 years, 55.7 kg body weight) were fed a hay-based ration (HAY) with or without 36–38% dried leaves from a black poplar hybrid (*P. nigra* x *P. deltoides*; PNIG), a balsam poplar hybrid (*P. nigra* x *P. maximowiczii*; PBAL) and a willow hybrid (*Salix triandra* x *viminalis*; SVIM), each for three weeks in a 4x4 crossover design. Each feeding period was separated by a one-week washout period during which wethers were kept on pasture. Blood was collected from the jugular vein on the last day of each feeding period to obtain EDTA plasma. All elements were analysed by inductively coupled plasma mass spectrometry (ICP-MS): feed Ca, P, Na, Mg, K, Cu, Zn, Mn, Fe and Se at SGS (Jena, Germany); feed Co, Ni, Mo, Ba, Cr, and Hg and all plasma mineral concentrations (same elements analysed in feed) at the German Federal Institute for Risk Assessment (BfR, Berlin, Germany). Statistical analyses were performed in R (v4.3.2; 2023). Data were analysed using a linear regression model including the fixed effects of leaf supplement, feeding period, and animal ID, and pairwise differences were tested with the Tukey Kramer test. Differences were considered significant at $P < 0.05$.

Results: The content of the following minerals was lower in all leaf species than in hay: 7 to 24 times for Na, up to 1.4 times for K, 1.4 to 2 times for Cr and 7 to 8 times for Mo. The Hg concentrations were comparable between the leaves and hay. The Fe content in PNIG was 1.2 times higher than in hay but only half as much compared to SVIM and PBAL. The Cu (1.5- to 2-fold), Ni and Mn (2- to 3-fold), Ca, Co, Zn and Se (3- to 5-fold) contents were higher in all three leaf species than in hay. Although total dry matter intake was comparable in all animals, only plasma Se concentration was affected by the different Se content of the feedstuffs. Goats fed PNIG ($72.6 \pm 2.6 \mu\text{g/L}$) and SVIM ($67.6 \pm 2.6 \mu\text{g/L}$) had Se higher concentrations than those fed PBAL ($59.4 \pm 2.6 \mu\text{g/L}$; $P < 0.05$) and HAY ($61.4 \pm 2.6 \mu\text{g/L}$; $P < 0.05$). Plasma Co, Ni, Mn and Hg concentration could not be detected, and plasma Cr concentrations were found below the detection limits in all groups.

Conclusions: None of the leaf supplements resulted in elevated plasma concentrations of elements of concern. PNIG and SVIM leaves provided superior Se supply compared with HAY and PBAL. Yet, the sample size was too small to detect potential differences in other mineral concentrations.

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Independent and combined short-term effects of ruminal and hindgut acidosis on fermentation and microbiome of dairy cows

Umabhängige und kombinierte Kurzzeiteffekte von Pansenazidose und Dickdarmazidose auf die Fermentation und das Mikrobiom von Milchkühen

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Introduction: In high-yielding dairy production, high grain diets affect the gastrointestinal health causing severe and costly disorders such as subacute ruminal acidosis (SARA) and hindgut acidosis (HGA) [1]. Most of the research on high grain feeding in cattle have focused on the effects of SARA [2]. Additionally, research focusing on the hindgut has indicated that the hindgut plays a crucial role in overall gastrointestinal health in dairy cows [3]. It can be assumed, since both SARA and HGA are caused by high grain diets, that they are concomitant. Yet, investigation of the combined impacts of SARA and HGA as well as possible additive effects on gut function and the microbial communities is missing. The aim of this study was to evaluate the short-term impacts of both SARA and HGA on ruminal and fecal fermentation and microbiome of dairy cows.

Methods: This study to evaluate the short-term impacts of inducing SARA and HGA was conducted as a change-over design with 2 experimental periods of 3 weeks each, using 9 lactating rumen-cannulated Holstein cows (724 ± 79 kg bodyweight, 198 ± 74 days in milk). The SARA was induced by feeding a diet containing 65% concentrate on a DM basis, and HGA was induced through daily abomasal infusion of 3 kg starch (1:1 wheat to corn weight ratio). The treatment groups evaluated were: 1) normal lactation diet containing 40% concentrate (DM basis; normal diet), 2) normal diet with abomasal infusion of starch to induce HGA, 3) a diet containing 65% concentrate (DM basis; SARA diet), and 4) SARA diet with abomasal infusion of starch. Ruminal pH was measured every 15 min using indwelling systems, and the fecal pH was measured 3 times per day (0, 6 and 12 h relative to feeding) using a manual pH meter. The microbiome was analysed through 16S rRNA gene amplicon sequencing in ruminal and fecal samples collected in the morning before first feeding. Additionally, rumen and fecal samples were taken at 0, 6 and 12 h relative to feeding and were analysed for volatile fatty acids (VFA) and lactate to evaluate postprandial effects of treatments. Statistical analysis of pH, VFA, and lactate data were performed as ANOVA using the MIXED procedure of SAS (version 9.4.). Microbiome data were analyzed for abundance and diversity with MAASLIN2 and adonis2 in R (version 4.4.0).

Results: Results showed that changing from the normal to SARA diet lowered ruminal pH (107→250 min/day < pH 5.8, P<0.05) and increased ruminal propionate (18.8→23.5% molar proportions (MP), P<0.01), while decreasing ruminal acetate (67.5→62.7% MP, P<0.01). However, the dietary change did not affect fecal VFA profile or pH. In addition, the SARA diet shifted the ruminal microbiome, increased ruminal propionogenic bacteria such as *Succinibacteriaceae*, and decreased alpha diversity in the rumen and the feces. The abomasal starch infusion decreased fecal pH (from pH 6.56 to 6.30, P=0.01), increased total VFA at 12 h post-feeding (59.9→88.8 mmol, P<0.01), lactate (0.5→7.1 mM, P<0.01), and butyrate (6.8→13.5% MP, P<0.01) in feces at the expense of acetate (75.4→72.7% MP, P=0.11) and propionate (15.7→12.0% MP, P<0.01). Additionally, the abomasal starch infusion shifted the fecal microbiome and increased butyrate producing bacteria such as *Roseburia* and *Lachnospiraceae*, while fibrolytic bacteria such as *Ruminococcaceae* decreased in feces. Furthermore, results showed that SARA in combination with HGA aggravate dysbiosis of the fecal microbiome as revealed by further reductions of microbial alpha diversity indices in cows receiving the SARA diet and abomasal starch infusion.

Conclusions: Considered independently, the SARA diet primarily disrupted the ruminal environment, while the HGA predominantly impacted the hindgut environment. However, SARA in combination with HGA further aggravate dysbiosis in the hindgut. Thus, showing that rumen-bypassing starch may represent energy loss, alters fermentation, and aggravates hindgut dysbiosis with potential long-term impacts in cattle.

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Differences in bile acid metabolism between neonatal calves with or without *Cryptosporidium parvum* infection

Unterschiede des Gallensäuremetabolismus zwischen Cryptosporidium parvum infizierten und nicht infizierten neugeborenen Kälbern

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Introduction: Bile acids are important substances for fat digestion and absorption. The bile acid metabolism of the host is mediated by the intestinal microbial metabolism and colonization and vice versa (1). Moreover, they are associated with a sensing function for the host by giving indications about the presence and abundance of the microbial enzyme activities (1,2). In this context, they play a key role in the intestinal inflammatory and immune response (2). In the present trial the bile acid metabolism after an infection with *Cryptosporidium parvum* as a representative pathogen which causes diarrhoea in the first weeks of life (3) was studied in neonatal calves.

Methods: Five male Holstein calves were infected on day 1 of life with 2×10^7 *C. parvum* oocysts and 5 calves were not. Calves were slaughtered on day 8 of life. Plasma samples and intestinal content of mid jejunum and colon were collected. Targeted metabolomics profiling including bile acid quantification in these matrices was performed using the MxP® Quant 500 kit (BIOCRATES LifeSciences AG, Innsbruck) and LC-MS/MS analytical method. The MIXED procedure of SAS was used for statistical analyses. In case of proportions of total, primary, secondary and conjugated bile acids, the model included the fixed effects infection (yes/no), matrix (plasma, jejunum, colon), and their interactions. In case of specific bile acid concentrations, the model included similar fixed factors, however, plasma was analysed separately.

Results: Only glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glyoursodeoxycholic acid (GUDCA), taurochenodeoxycholic acid (TCDC) and taurochenodeoxycholic acid (TMCA) were detected in plasma of 8-day old calves which were reduced by the *C. parvum* infection ($p < 0.05$) except for GUDCA and TMCA. The infection decreased the sum of total bile acids in jejunum of calves ($p < 0.05$). In control calves the sum of total bile acids was higher in jejunum than in colon ($p < 0.05$). TCDC decreased in control calves from jejunum to colon ($p < 0.05$) but not in infected calves leading to an interaction of both fixed factors ($p < 0.05$). Furthermore, colon showed higher proportions of primary bile acids but lower proportions of conjugated primary as well as glycine and taurine conjugated bile acids compared to plasma and jejunum ($p < 0.05$). Concentrations of cholic acid, chenodeoxycholic acid and deoxycholic acid were lower in jejunum compared to colon ($p < 0.05$). In contrast, proportions of conjugated secondary bile acids and GCA, GCDCA TMCA and TCDC concentrations decreased from jejunum to colon ($p < 0.05$).

Conclusions: The results of this preliminary study give evidence that the infection with *C. parvum* affects bile acid metabolism in neonatal calves, which might be in part a result of delayed abomasal emptying and therefore of a lack of nutritional stimuli in the gut. On the one hand, consequences on fat digestion are suggested. On the other hand, and with particular consideration of the signaling role of bile acids in the interaction between the intestinal immune system and the intestinal microbiota, the development of the intestinal immune system and the response in the neonatal phase could be influenced.

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Comparative effect of β -glucans vs β -glucans and mannan-oligosaccharides on fermentation characteristics of the equine hindgut in a batch culture system

Vergleichende Wirkung von β -Glucanen vs. β -Glucanen und Mannanoligosacchariden auf Fermentationscharakteristika des equinen Dickdarmes in einer Batch-Kultur

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Introduction: The feeding of yeast cell wall components (YCWC) such as β -glucans and mannan-oligosaccharides (MOS) to horses aims at stabilising the microbial community in the hindgut [1]. The mode of action is however not yet fully understood. The aim of the study was to investigate effects of two differently composed yeast cell wall preparations and their interaction with the type of core diet when inoculated with equine faeces in a batch culture system.

Methods: Two core diets consisting of either 0.5325 g meadow hay (H) or 0.3975 g H and 0.135 g laboratory-grade wheat starch (HS), both with 0.0675 g of laboratory-grade lignin (LIGN), served as controls (HLIGN, HSLIGN). H and HS were additionally supplemented by 0.0675 g of one of two *Saccharomyces cerevisiae*-derived yeast cell wall preparations (BEMO: 25% β -glucans, 20 - 25% MOS; BETA: > 80% β -glucans, < 2% MOS) to yield four test diets (HBEMO, HSBEMO, HBETA, HSBETA). All six diets were incubated in a batch culture system (ANKOM RF Gas Production System) with mixed faeces from three adult healthy horses and a buffer adapted to equine hindgut conditions [2]. Four experimental runs were conducted with four fermenters per diet and run, as well as four blank fermenters and four fermenters containing only 0.0675 g of LIGN per run to evaluate its use as placebo. Gas production was recorded every 10 s over a 48h incubation period. Afterwards, fermenter liquids were analysed for pH, ammonia (Conway method), short-chain fatty acids (SCFA) and lactate (HPLC). Gas production curves were modelled using the Gompertz function (SAS 9.4), fermentation products and pH were analysed for diet effects using the MIXED procedure (SAS 9.4).

Results: Gas production of the blank- and LIGN-fermenters were similar. Estimated gas production was highest in HSBETA and HSBEMO ($P < 0.05$), followed by HBETA ($P < 0.05$), HBEMO and HSLIGN, and lowest in HLIGN. Among the different rations, time required to produce one-third of the total gas volume was shortest with HBETA, followed by HSBETA, HSBEMO and HBEMO, with the longest time observed in HLIGN ($P < 0.05$). HSBEMO and particularly HSBETA showed lower pH than HSLIGN ($P < 0.01$). In hay-only diets, HBEMO and particularly HBETA showed higher SCFA concentrations than HLIGN ($P < 0.05$). Among SCFA, iso-butyrate and iso-valerate were highest in HBEMO ($P < 0.05$). Moreover, n-butyrate was higher in HBEMO and HBETA than HLIGN ($P < 0.01$), and higher in HSBEMO and HSBETA compared to HSLIGN ($P < 0.01$). In both cases, concentrations were slightly higher in the BETA-rations. Ammonia was overall highest in HBEMO ($P < 0.05$), followed by HBETA and HSBEMO. Lactate was below the limit of detection in all samples.

Conclusions: Results indicate elevated microbial fermentation with both YCWC. The β -glucans alone seem to be superior to the same amount of a β -glucan-MOS-mix in this regard, especially when few readily fermentable carbohydrates are available as with the hay only-diet. Both YCWC elevated n-butyrate, which is assumed to improve the guts epithelial barrier function and reduce inflammation when transferred to *in vivo* conditions [3]. Highest iso-SCFA and elevated ammonia concentrations with the β -glucans-MOS-mix in the hay only diet might be caused by the elevated nitrogen supply through the supplement and an increase of microbial nitrogen turnover. In the hay-starch-diet, more readily fermentable carbohydrates might have supported microbial nitrogen fixation and reduced proteolysis.

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Bacterial and eukaryotic diversity in the gastrointestinal tract of horses fed concentrate containing vs. forage only diets

Bakterielle und eukaryotische Diversität im Gastrointestinaltrakt von Pferden bei Grobfutterfütterung gegenüber einer konzentrathaltigen Ration

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Introduction: In equines, the functionality and stability of the gastrointestinal microbiome is essential for their health. It has been shown that a high supply of starch (e.g., by adding oats to the ration) not only reduces bacterial diversity in the hindgut, but also decreases the stability of the microbiome [1], which in turn might lead to reduced resilience against pathogens. The eukaryotic community (including fungi and protozoa) in the equine gut has received little attention, and their influence through feeding has not yet been well studied. Therefore, the aim of this study was to characterise the effects of forage-only vs. mixed diets on the bacterial and eukaryotic diversity in the digesta of the stomach and subsequent intestinal sections.

Methods: 24 horses (13 males, 11 females; age 11 ± 6.0 years; body weight [BW] 471 ± 63.4 kg; body condition score $5.1 \pm 0.67/9$; cresty neck score $1.0 \pm 0.70/5$) were allocated to one of four rations: 24 h/d pasture (PST; $n = 4$), hay *ad libitum* (HAY; $n = 8$), hay *ad libitum* and oats at 1 g starch/kg BW/meal (OS1; $n = 6$) or hay *ad libitum* and oats at 2 g starch/kg BW/meal (OS2; $n = 6$). The amount of starch in OS2 was twice as high as the upper limit recommended by [2]. The horses had free access to a salt lick and tap water. For the stabled horses, straw was used as bedding. After a minimum of 34 days of the feeding period, horses were euthanised, dissected and digesta samples were taken from the following anatomical sections: stomach pars nonglandularis (PNG) and pars glandularis (PG), jejunum (JEJ), caecum (CAE), colon ventrale dextrum (CVD), colon ventrale sinistrum (CVS), pelvic flexure (PF), colon dorsale sinistrum (CDS), colon dorsale dextrum (CDD), colon transversum (CT) and rectum (R). Genomic DNA was extracted. 16S and 18S rRNA gene amplicons were sequenced using the Illumina MiSeq system. Bioinformatic analysis of amplicon sequences was performed with QIIME 2 including DADA2. The SILVA 138.2 rRNA database was used for taxonomic assignment. The Simpson index was calculated (QIIME 2) and analysed using the procedure MIXED (SAS 9.4).

Results: Regarding the bacterial diversity, the Simpson index was lower in the stomach and small intestine compared to the large intestine ($P < 0.001$), and also lower in OS2 compared to OS1 ($P = 0.028$) with HAY and PST being in between ($P > 0.05$). The eukaryotic diversity index in the foregut could not be calculated due to a low read number in PNG, PG and JEJ. In the hindgut, unlike bacterial diversity, the Simpson index of the eukaryotic diversity was higher in OS2 compared to OS1 ($P = 0.003$), whereas forage only diets showed intermediate values ($P > 0.05$).

Conclusions: The results confirm the previously described reduction in bacterial diversity due to starch feeding. However, this apparently only applies to starch amounts that exceed common dietary recommendations. Small amounts of starch added to *ad libitum* roughage feeding can apparently even increase bacterial diversity under certain conditions. Interestingly, the effect on the eukaryotic diversity appears to be the opposite.

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Comparison of different tannin supplements, tannin extracts and ways of application in Ussing chamber experiments

Vergleich verschiedener Tanninsupplemente, Tanninextrakte und unterschiedlicher Verabreichungsformen in Ussing-Kammer Experimenten

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Introduction: Tannins are discussed as potential preventive agents against post-weaning diarrhoea in piglets [1, 2]. Hence, the effects of tannins and tannin-rich plant extracts in feeding trials or using *ex vivo* approaches on nutrient transport and chloride secretion, using the Ussing chamber (UC) technique was investigated.

Methods: Experiment 1: Jejunum tissues were obtained from weaned piglets (n = 6/group) fed a basal diet or diets supplemented with *Lythrum salicaria* (LSE) or *Bistorta officinalis* extract (BOE) (2 g tannins/kg). Additionally, jejunal tissue from grower pigs (n = 22) fed a basal diet was collected for *ex vivo* UC experiments. Experiment 2: LSE (0.4, 0.8, 1.6 mg/mL) or BOE (1.0, 2.0 mg/mL) were added to the UC (mucosal side). Experiment 3: The pure tannins castalagin (10 µM/L) and procyanidin B2 (10 µM/L) were applied in the UC (mucosal side). Thereafter, electrophysiological data were recorded as basic value and after D-glucose, L-lysine and carbachol or forskolin addition. The 6 minutes around application and the integral of the 36 seconds after application of the substances were analysed. Statistical evaluation was performed using the Welch ANOVA followed by the Games Howell test, with significance set at $P \leq 0.05$.

Results: Dietary supplementation with tannins did not affect *ex vivo* electrophysiological parameters in the UC. In contrast, addition of plant extracts (6 min (except 0.4 mg/mL LSE) and 36 sec) lead to higher ΔI_{sc} ($P < 0.001$) compared to control chambers. Moreover, ΔG , after L-Lysin application (6 min), was lower in 1.6 mg/mL LSE group compared to 0.8 mg/mL group. No significant differences were observed after stimulation with D-glucose, forskolin, or carbachol.

Conclusions: Analysis of the composition of plant extracts is mandatory. Tannins may interfere with the jejunal tissue in *ex vivo* trials, resulting in a higher ΔI_{sc} . The underlying mechanism is not clear and further investigations are warranted.

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Impact of a reduced nitrogen and/or calcium diet on expression of genes involved in epithelial transport in young goats

Einfluss einer reduzierten Stickstoff- und/oder Calciumversorgung auf die Expression von Genen des epithelialen Transports bei jungen Ziegen

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Introduction: Adequate intake of protein and minerals is essential for sustaining growth, fermentation processes, and metabolic homeostasis in ruminants. Nitrogen (N) provides the basis for microbial protein synthesis, whereas calcium (Ca) is central for mineral balance and signaling pathways. Both nutrients influence epithelial transport processes, particularly in the rumen and salivary glands, which play a key role in regulating electrolyte and nutrient exchange. The present study examined how diets reduced in N and/or Ca affect ruminal and salivary fluid composition as well as the expression of genes coding for transport proteins in young goats.

Methods: Twenty-six growing goats were assigned to four groups: control ([N+/Ca+], 21% crude protein (CP), 1% Ca; n = 7), N-reduced ([N-/Ca+], 8% CP, 1% Ca; n = 6), Ca-reduced ([N+/Ca-], 22% CP, 0.4% Ca; n = 6), or combined N- and Ca-reduced ([N-/Ca-], 8% CP, 0.4% Ca; n = 7). After six weeks on the diets, ruminal, abomasal, and salivary fluids as well as epithelial tissues from rumen epithelium and salivary glands were collected. Mineral concentrations, urea, and short-chain fatty acids (SCFAs) were determined, and mRNA expression of key transport-related genes (including Ca-sensing receptor, transient receptor potential channels, plasma membrane Ca ATPase (PMCA), sodium-Ca exchanger 1, urea transporter B (UT-B), sodium-bicarbonate cotransporter (SLC4A4), sodium-potassium ATPase (Na⁺/K⁺-ATPase), aquaporin 5 (AQP5), sodium/phosphate (Pi) cotransporter type IIb, and Pi transporter 1) were quantified by qPCR. Data were analyzed using a two-way ANOVA with dietary N level and Ca level as fixed factors.

Results: Reduced N supply lowered urea concentrations in ruminal and salivary fluids ($p < 0.001$) and led to decreased SCFA levels ($p < 0.05$), indicating impaired microbial fermentation. In the salivary glands, N reduction significantly decreased the expression of SLC4A4 ($p < 0.05$) and AQP5 ($p < 0.001$). In the rumen, N reduction increased PMCA and UT-B ($p < 0.05$) expression and decreased SLC4A4 mRNA ($p < 0.05$). Ca reduction had little effect on the expression of Ca transporters in the rumen, whereas in the salivary glands it elevated Pi levels and upregulated AQP5, Na⁺/K⁺-ATPase, and PiT1 ($p < 0.05$). Abomasal fluids were more sensitive: N reduction decreased Ca and Pi ($p < 0.05$) concentrations, while Ca restriction decreased Ca and increased Pi ($p < 0.01$). Ruminal Pi and Ca concentrations remained stable, indicating effective buffering.

Conclusions: Young goats exhibit distinct, tissue-specific adaptive responses to reduced nutrient supply. N reduction primarily limits microbial fermentation and diminishes transport capacity, whereas Ca restriction triggers compensatory mechanisms to maintain systemic mineral homeostasis. These findings illustrate the plasticity of epithelial tissues in adapting to dietary constraints and provide insights relevant for optimizing nutrient efficiency in ruminant feeding strategies.

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Effects of dietary dried *Laminaria japonica* on growth performance, blood metabolites, and *ex vivo* rumen microbial gas production in dairy sheep lambs during the late growth stage

Einfluss von getrockneter Laminaria japonica in der Fütterung auf Wachstumsleistung, Blutmetabolite und die ex vivo-Gasbildung im Pansen von Milchlämmern im späten Wachstumsstadium

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Introduction: This study examined the effects of feeding dairy sheep lambs in late growth with dried brown algae replacing wheat bran and potassium iodide on growth, inflammatory markers, and *ex vivo* rumen microbial gas production.

Methods: Sixteen mixed-sex dairy lambs (9 mo; 37.8 ± 4.13 kg) were randomly assigned to ALG or CON. All received a hay–concentrate diet according to recommendations [1]. During a 14-d acclimatization, diets contained 2 % wheat bran and potassium iodide to reach $11 \text{ mg I kg}^{-1} \text{ DM}$, approaching the Swiss legal maximum. Thereafter, CON continued this diet for 28 d, while ALG received the same diet with 2 % *Laminaria japonica* ($300 \text{ mg I kg}^{-1} \text{ DM}$) replacing wheat bran and iodide, maintaining equal iodine supply. After 28 d, all animals were bled and slaughtered. Zootechnical and circulatory markers (serum amyloid A [SAA], haptoglobin [Hp], α -tocopherol [α -toc], iodine) were analyzed externally. Rumen fluid from four animals per group (2 m, 2 f) was collected post mortem, sealed anaerobically at 39°C with HFT buffer solution, and used for animal-individual incubations (250 mg DM of hay, concentrate, wheat bran, algae, CON diet, ALG diet; triplicate animal⁻¹) to measure cumulative gas and CH_4 after 24 h via the Hohenheim Gas Test [2]. Data were analyzed by mixed models (SAS 9.4).

Results: Initial live weight did not differ ($P = 0.52$). During 28 d, time ($P < 0.0001$) and sex ($P < 0.05$), but not diet ($P > 0.31$), affected growth. Lambs gained 0.52 and $0.56 \text{ kg wk}^{-1} \text{ animal}^{-1}$ and consumed 6.58 and $6.32 \text{ kg DM wk}^{-1} \text{ animal}^{-1}$ in CON and ALG, reaching 40.5 and 39.6 kg at slaughter. Diet affected SAA ($P = 0.02$) and iodine ($P < 0.0001$) but not α -toc or Hp. ALG lambs showed lower SAA ($0.40 \text{ } \mu\text{g mL}^{-1}$; 95 % CI [0.06, 0.75]; 0.81 vs. $0.40 \text{ } \mu\text{g mL}^{-1}$) and lower serum iodine ($118 \text{ } \mu\text{g L}^{-1}$; 95 % CI [69, 167]; 346 vs. $227 \text{ } \mu\text{g L}^{-1}$). Substrate ($P < 0.0001$), but not donor diet ($P > 0.1$), influenced total gas and $\text{CH}_4\text{:CO}_2$ after 24 h. Hay produced the least gas ($48.9 \text{ mL/250 mg DM 24 h}^{-1}$) and concentrate the most ($80.3 \text{ mL/250 mg DM 24 h}^{-1}$; $P < 0.0001$). Wheat bran and algae (52.0 vs. $52.6 \text{ mL/250 mg DM 24 h}^{-1}$; $P = 0.99$) and CON vs. ALG diets (52.5 vs. $53.0 \text{ mL/250 mg DM 24 h}^{-1}$; $P = 0.99$) did not differ. Algae yielded the lowest CH_4 proportion (14.5 % mol) and wheat bran the highest (18.4 % mol; $P = 0.0001$); CON and ALG diets were similar (16.3 vs. 16.2 % mol).

Conclusions: Previous work in pigs demonstrated gut-health benefits of diets containing ≥ 2 % dried *Laminaria japonica* [3]. The significantly lower SAA observed here indicates mild systemic anti-inflammatory effects in ALG lambs. Reduced gas production from algae compared with wheat bran suggests that parts of algal components might be rumen-protected, potentially showing beneficial activity in the distal gut. The material did not affect ruminal CH_4 output, consistent with current findings for other brown algae species. At equal dietary iodine supply, conventional sodium iodide supplementation resulted in higher circulating iodine concentrations than algal iodine, and it remains to be clarified whether the lower apparent bioavailability of algal iodine represents a disadvantage under more moderate supplementation levels.

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Does the inclusion of duckweed in the diet of common carp (*Cyprinus carpio*) affect growth performance and product quality?

Beeinflusst die Einmischung von Wasserlinsen in die Ernährung von Karpfen (Cyprinus carpio) Wachstumsleistung und Produktqualität?

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Introduction: The aquaculture sector faces the need for more sustainable feed sources for future development and less dependency of common protein sources like fishmeal and soy [1]. Duckweed grows fast on eutrophic water bodies and can be produced in circular systems [2]. Its nutritional profile comprises high protein concentration in % of dry matter, a valuable amino acid distribution and richness in n3-long-chain fatty acids. The present study assessed the growth performance, fish fillet nutrient composition, fillet colour, physical meat quality and sensory characteristics of common carp (*Cyprinus carpio*) fed with duckweed-inclusive diets.

Methods: Four isonitrogenous (CP = 35% of DM) and isoenergetic (GE = 21.5 MJ/Kg DM) diets containing 0 % (DW0), 10 % (DW10), 15 % (DW15), and 20 % (DW20) of duckweed were formulated. A total of 240 Common carp were randomly assigned to the groups. Per group, triplicates of 20 fish were fed in separate tanks. Diets were analysed for nutrient and fatty acid composition and carotenoid concentrations. After 14 weeks the fish were slaughtered. Performance parameters were documented directly or calculated from the obtained data including final weight, fork length, fillet weight, specific growth rate (SGR %/day), Feed conversion ratio (FCR kg/kg) and weight gain (% of initial body mass). Skin colour and fillet colour were measured directly after slaughtering and filleting, respectively. The pH was measured 45 min and 24 h post mortem in one side of the residual fillet of the head. For additional testing 60 fillets (n = 15 fish per group) were assigned to analysis of proximate composition and fatty acid methyl esters (FAME). Fillets of 84 fish (n = 21 per group) were examined for assessment of physical meat quality, including process loss and shear force. Remaining 96 fillets (n = 24 per group) were assigned to a quantitative sensory descriptive analysis (QDA) with nine trained panellists. For statistical analyses, the distributions of variables within each tank and treatment group were assessed for normality and lognormality. A nested experimental design was then implemented to evaluate the effect of each individual tank within the treatment groups before assessing the overall influence of duckweed concentration on the dependent variables. General linear mixed models were employed to investigate both linear and potential quadratic relationships between duckweed concentration and various response variables, including performance metrics, nutritional composition, skin and meat colour, sensory properties, and physical meat quality.

Results: Initial weight of carp did not differ among the groups; performance parameters were negatively affected by duckweed inclusion ($p < 0.05$) but did not differ between groups fed with duckweed. Over the whole experimental period FCR was higher in DW10, DW15 and DW20 (1.55 ± 0.47 , 1.71 ± 0.89 , 1.63 ± 0.56) than in DW0 (1.44 ± 0.33), but after three weeks of experiment differences were less pronounced (1.32 ± 0.17 [DW0], 1.35 ± 0.17 [DW10], 1.37 ± 0.16 [DW15], 1.40 ± 0.18 [DW20]). Compositional analysis revealed higher crude ash and protein concentrations and lower crude lipids and dry matter in fillets of duckweed fed carp ($p < 0.05$). Duckweed inclusion increased n6 and n3 polyunsaturated fatty acid (PUFA) content, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as the n6/n3 ratio were not affected ($p < 0.05$). Physical meat quality parameters were not affected by the diet. Skin colour was affected by dietary duckweed towards higher values of green and yellow, fillet colour shifted towards more yellow colour. The QDA showed an effect of duckweed inclusion towards a fishy taste ($p = 0.001$) together with sweet odour ($p = 0.024$).

Conclusions: Duckweed proved applicable as a feed ingredient for common carp, with potential benefits for product quality and no adverse effects on sensory acceptance. Slight reductions in growth performance may be offset by sustainability gains from its inclusion in aquafeeds.

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Comparison of horse faeces as an alternative inoculum with rumen fluid for measuring gas production in the Hohenheim gas test

Vergleich von Pferdekot als alternatives Inokulum mit Pansensaft zur Messung der Gasbildung im Hohenheimer Futterwerttest

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Introduction: The estimation of metabolisable energy concentration and organic matter digestibility in ruminant feedstuffs can be conducted using the Hohenheim gas test (HGT), which is recognised as a standardised and widely applied estimation method [1]. Due to the limited availability of rumen fluid as a conventional inoculum in the HGT, the use of faecal material from ruminants as an alternative has been proposed. Since horses are hindgut fermenters, equine faeces may also represent a suitable inoculum source.

Methods: In the present study, gas production (GP) from 11 feedstuffs was compared using regression analysis after incubation with rumen fluid (RF) for 24 hours and with horse faeces (HF) for 48 hours. In total, 1 legume grain, 1 rapeseed product, 1 corn-cob mix, 1 molassed beet pulp, 1 wheat bran, 4 forages, and 2 cereal grains were incubated in a HF-based inoculum. At the University of Hohenheim, all samples had previously been incubated with RF from two lactating cows for 24 hours according to the HGT standard procedure [1; 2]. Briefly, the horse was kept on pasture from 9:00 a.m. to 7:00 p.m. and received hay at 7:00 a.m. and 10:00 p.m. Faeces were collected directly after defaecation from the ground after the morning feeding. After transport in a pre-warmed thermos container (39°C), the faeces were immediately homogenised with the buffer solution in the laboratory. Incubation with HF was carried out in three runs, each with three flasks per feedstuff. A linear regression analysis of GP (mL/200 mg dry matter [DM]) was performed using RStudio 4.2.1 (R Core Team, 2022).

Results: For the 11 samples tested, the regression equation for GP of RF (y) and GP of HF (x) was $y = 1.350x - 5.44$, with a coefficient of determination (R^2) of 0.99, a coefficient of variation (CV) of 3.24%, and a root mean squared error (RMSE) of 1.75 mL/200 mg DM.

Conclusions: A close relationship was observed between 24-hour GP with RF and 48-hour GP with HF. This suggests that faeces from herbivorous non-ruminants can be used as a non-invasively obtained inoculum to estimate GP of RF and thus metabolisable energy concentration and organic matter digestibility. Additional feedstuffs and the corresponding GP with RF are available, and further investigations using faeces from multiple donor horses to assess GP variation are currently being conducted using a larger number of feed samples to expand the feed base and data set in order to validate the regression. Likewise, similar relationships with RF were observed when incubating the same feedstuff samples with both cattle and sheep faeces [3]. This approach of replacing RF by faecal inocula in the HGT should be further explored in subsequent studies to establish a standalone method in the future.

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Meta-analysis of the effects of feeding winery byproducts on production performance, carcass yield, and meat quality in lambs and broilers

Meta-Analyse zu den Auswirkungen der Fütterung von Wein-Nebenprodukten auf die Produktionsleistung, Schlachtausbeute und Fleischqualität bei Lämmern und Masthühnern

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Introduction: Winery byproducts, such as grape pomace and seed meal, are commonly used as livestock feed. Their high fiber content allows for significant inclusion in ruminant diets. In non-ruminants, interest in winery byproducts stems from their low cost and bioactive compounds. Recent research has highlighted their potential to enhance meat quality, particularly oxidative stability, though high feeding levels may negatively impact production performance [1, 2]. This meta-analysis examined the effects of feeding winery byproducts on production performance, carcass yield, and meat quality in lambs and broilers.

Methods: Following a systematic literature search and validation, 25 studies were included (9 on lambs and 16 on broilers). Grape extract treatments were excluded. The final dataset comprised 86 treatments (33 for lambs and 53 for broilers). To address the variability in measurements and animal use across studies, units were standardized, such as expressing feed intake relative to body weight. Not all studies reported the same number of target dependent variables. Growth performance was the most reported variables (27 treatments for lambs, 49 for broilers), while fewer studies investigated carcass and meat quality traits. Therefore, subsets of data were used for meta-regression. Data for lambs and broilers were analyzed separately. Various mixed models were applied to assess the effects of winery byproduct feeding levels, considering data characteristics and influential factors (e.g., discrete or continuous predictors, with or without the influence of slaughter age and dietary crude protein levels).

Results: Most broiler experiments included winery byproducts at levels below 10% of the diet (as-fed basis), with only one study (2 treatments) using 20% grape pomace flour. In lambs, 57% of treatments included byproducts at levels below 20% of diet dry matter, while 15% exceeded this threshold. These treatments primarily involved the use of grape pomace silage. Winery byproduct inclusion did not affect feed intake, feed conversion ratio (FCR), carcass yield, or breast meat pH at 24 hours postmortem, however it increased breast meat lightness (L^* , $P=0.019$) without affecting redness (a^*) or yellowness (b^*) values. At high inclusion levels (>3% of the diet), winery byproducts reduced lipid oxidation (lower TBARS values) in broiler breast meat ($P=0.030$). In lambs, increasing winery byproduct levels linearly reduced dry matter intake relative to body weight ($P=0.011$) without affecting FCR, but increased carcass yield ($P=0.046$). The investigated meat quality traits were unaffected in lambs.

Conclusions: This meta-analysis highlights the potential of winery byproducts as a valuable feed resource for lambs and broilers, with added benefits for carcass and meat quality. Specifically, feeding winery byproducts can enhance oxidative stability in broiler meat and improve carcass yield in lambs.

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***In vitro* fermentation characteristics of multi-species hay and silages with ryegrass, alfalfa, and plantain using the RUSITEC**

In vitro-Fermentationscharakteristika von Heu und Silage aus Weidelgras, Luzerne und Spitzwegerich im RUSITEC

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Introduction: In crop rotation, fine-seeded legumes play an important role in maintaining soil fertility. At the same time, these plants can be harvested and preserved for livestock nutrition. Alfalfa (*Medicago sativa*) is widely considered as ‘queen of forages’ for ruminants due to its high crude protein (CP) content. Characteristics such as nitrogen fixation, deep root formation, and drought tolerance increase the plant’s value in terms of climate change. Due to its high CP content, N losses via urine can occur and dietary strategies, like combined growth as alfalfa-grass ley and supplemental provision of energy dense concentrates are used as preventive measure. Another deep rooting forage plant is ribwort plantain (*Plantago lanceolata*), and several studies have indicated its potential to reduce urinary N losses and lower methane emissions due to its plant secondary metabolites [1, 2]. Accordingly, we hypothesized that the combination of alfalfa, grass and plantain might offer a high nutrient value while reducing the environmental impacts, however, effects might depend on preservation method. Therefore, we conducted a RUSITEC experiment to evaluate ruminal degradability and fermentation parameters of both hays and silages of ryegrass (G), alfalfa (A), and plantain (P) in combination.

Methods: The third cut of grass (G), grass plus alfalfa (G+A; G:A=1:1.3 in DM), and grass plus alfalfa plus plantain (G+A+P; G:A:P= 1:8.7:1.6 in DM) was used to make silage and hay. The CP content was 90.5, 165 and 148 g/kg DM for G silage, G+A silage and G+A+P silage, and 83.9, 167 and 163 g/kg DM for G hay, G+A hay and G+A+P hay. The NDF content was 632, 538 and 535 g/kg DM for G silage, G+A silage and G+A+P silage, and 654, 622 and 551 g/kg DM for G hay, G+A hay and G+A+P hay. Ten grams DM of silage and hay of G, G+A and G+A+P was incubated in six RUSITEC fermenters for 14 days (7 days adaptation + 7 days sample collection) for three runs. The buffer solution was prepared according to McDougall [3] and was modified for G silage and hay with 0.695 g/L NH_4Cl to achieve a comparable N availability to silage and hay of G+A and G+A+P. Analyses included degradability, $\text{NH}_4\text{-N}$, CH_4 concentration and protozoa counts. R was used to create a linear mixed model with a fixed effect for forage (G, G+A, G+A+P) and preservation (hay/silage) and the random effect of the experimental run. Sampling days were considered as repeated measurement.

Results: The OM degradability was highest for G+A, and lowest for G+A+P (35.8% and 32.2%; $P=0.05$); hay had a higher OM degradability than silage (38.5% to 29.4%; $P<0.001$). Both forage and preservation affected CP degradability ($P<0.05$); hay had a lower CP degradability than silage (63.2% to 65.0%; $P=0.04$); G+A+P showed a lower CP degradability compared to G+A (62.8% to 67.1%; $P<0.001$). Both, forage and preservation, had no significant effect on CH_4 production per g OM degraded ($P>0.1$). Protozoa number was highest with G ($1.63 \times 10^3/\text{mL}$, $P<0.001$) and higher with hay than that with silage (1.05 to $0.19 \times 10^3/\text{mL}$; $P<0.001$). The $\text{NH}_4\text{-N}$ concentration was significantly affected by forage and preservation ($P<0.001$), with the lowest concentration with G+A+P (10.1 mmol/L) compared to G+A (12.4 mmol/L) and G (13.0 mmol/L); incubations with hay showed lower $\text{NH}_4\text{-N}$ than with silage (10.2 to 12.8 mmol/L).

Conclusions: First results suggest that incorporating plantain into the forage of ruminants reduces OM digestibility, without effects on ruminal CH_4 production. However, plantain’s bioactive compounds might help protecting the dietary CP from ruminal degradation, as apparent in the lower ruminal CP degradability and $\text{NH}_4\text{-N}$ concentration, possibly shifting CP degradation to the post-ruminal digestive tract. Further research is necessary to understand the modes of action associated with feeding plantain.

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A minimalist machine learning approach to analyse *in vitro* gas production profiles obtained with different inocula

Ein minimalistischer Ansatz des Maschinellen Lernens zur Untersuchung von in vitro-Gasbildungsprofilen bei Verwendung verschiedener Inokula

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Introduction: *In vitro* gas production (GP), measured using the Hohenheim Gas Test (HGT), is commonly applied using rumen fluid as the inoculum source to evaluate ruminant feeds [1], while sheep faeces have been demonstrated as a suitable alternative inoculum source [2]. Most GP profiles exhibit S-shaped curves, reflecting a biological transition from lag phase to asymptotic GP. Classical exponential models are simple but lack an explicit inflection point (t^*), oversimplify lag time (tLag), and may fail to capture gradual onset dynamics. These limitations become critical when comparing faecal inoculum (FI) with rumen fluid inoculum (RI), as FI fermentation often develops more slowly [2]. There is growing interest in applying machine learning (ML) approaches, such as artificial neural networks (ANN) in animal research. Recent advances in interpretable ML [3] have suggested that minimalist, designed-for-clarity ML models can yield valuable insights by explicitly estimating biologically relevant parameters while remaining analytically transparent. This concept is critical in nutritional research, where understanding the meaning of model parameters can be as important as predictive accuracy. Therefore, the objective of this study was to develop and evaluate an interpretable ML model for analysing GP data.

Methods: A deliberately simplified ANN comprising a “single artificial neuron”, with a tanh activation function was implemented as: $GP = A \tanh(c t - b) + B$, where time t (h) and the GP (mL/200 mg dry matter; DM) are the input and output of the model, respectively. A , c , b , and B are trainable parameters estimated from the data. This structure resembles a four-parameter sigmoidal function and allows direct calculation of important GP metrics, including t^* ($= -b/c$) and tLag. Uncertainty in estimates and significance were quantified over 1,000-fold bootstrap resampling. A data set including GP profiles (across a 72-h incubation period) of five feeds (wheat grain, rapeseed meal, field beans, grass silage, and wheat straw) determined with the HGT was used. Each feed was incubated with FI prepared from faeces of wether sheep and RI prepared from rumen fluid of lactating dairy cows ($n=5$ replicates per feed and inoculum) [2].

Results: The fits were satisfactory for all GP data (R^2 : 0.97–0.99; error: 1.3–5.7 mL/200 mg DM), with simple and transparent equations. For example, for wheat grain using FI, the resulting model was: FI-based GP of wheat grain = $42.6 \tanh(0.191 t - 1.46) + 35.2$ ($R^2=0.97$, error=5.7 mL/200 mg DM). Distinct differences between GP with FI and RI were observed ($p < 0.01$). For FI, all feeds except rapeseed meal displayed clear S-shaped curves, as indicated by significant t^* : wheat grain $t^* = 7.4$ h; field beans $t^* = 7.9$ h; grass silage $t^* = 8.5$ h; wheat straw $t^* = 30.5$ h. In contrast, RI-based curves, except for wheat straw ($t^* = 19.0$ h), generally showed immediate fermentation onset, with t^* close to zero. Analysis of tLag revealed a clear delay before measurable GP for wheat grain (2.8 h) and wheat straw (10.1 h) with FI, and for wheat straw (2.2 h) with RI, while other feeds showed minimal tLag.

Conclusions: The proposed ML-based model automates curve fitting, parameter estimation, and statistical analysis. It allows direct parameter interpretation and calculation of biologically relevant metrics in *in vitro* GP profiles, thus overcoming limitations of standard exponential models. This is an important prerequisite for a reliable comparison of inocula and thus the evaluation of faeces as an inoculum source in the HGT. This approach accurately captures the distinct GP-kinetics of FI and RI, showing that both inocula follow similar overall GP patterns, differing mainly in rate and extent rather than fundamental progression.

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Functional analysis of faecal samples from cows fed different diets

Funktionelle Kotprobenanalyse von Kühen, denen verschiedene Rationen gefüttert wurden

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Introduction: In dairy cows, great dry matter intake (DMI) of diets characterised by a high energy density can result in an increased flow of fermentable polysaccharides to the hindgut, thereby influencing the microbiome and its products (1). The aim of this ongoing study is to investigate the adaption of the faecal microbiome in dry and lactating dairy cows by analysing the response to different substrates.

Methods: Faecal samples were obtained from ten lactating dairy cows (Holstein Friesian, parity: 3.5 ± 1.1 , days in milk: 90.3 ± 39.3) with an average DMI of 28.4 ± 3.7 kg (36.4% aNDFom, 24.8% starch) and ten dry cows (Holstein Friesian, parity: 1.8 ± 1.1 , days of pregnancy: 268 ± 3.0) with a DMI of 12.4 ± 3.5 kg (48.7% aNDFom, 6.3% starch). Faecal samples were incubated with ultrapure water in a ratio of 1:1 for 24 h at 38°C either without substrate (CON) or after addition of 10% (based on dry matter) glucose (GLU), starch (STA) or cellulose (CEL) and samples were taken at baseline, 6 h, 12 h and 24 h. Concentrations of volatile fatty acids (VFA) were analysed by gas chromatography, pH was determined using a pH meter and both parameters were corrected for time effects independent of substrate using values from CON. A mixed-effects model (time, diet and interaction between time and diet) followed by Sidak's post-test including correction for multiple comparisons was applied to reveal significant differences ($P < 0.05$). Pearson correlation and regression analysis were carried out to reveal relationships between pH and VFA concentrations.

Results: At baseline, pH was negatively associated with absolute VFA in both groups (r: -0.68 in DRY, r: -0.80 in LAC). In comparison to faecal samples from dry cows (DRY) basal, absolute concentration of total VFA was higher in faecal samples from lactating cows (LAC) (30.2 ± 6.32 mM vs. 67.9 ± 18.7 mM, $p < 0.001$). Addition of GLU induced a constant increase in VFA concentration for 12 h (DRY) and 24 h (LAC), resulting in significantly different concentrations of VFA in relation to CON after 24 h of incubation (11.8 ± 4.15 mM vs. 39.7 ± 16.6 mM, $p < 0.01$). This difference mainly stemmed from higher relative concentrations of acetate (9.50 ± 3.29 mM vs. 19.5 ± 10.6 mM, $p < 0.1$) and n-butyrate (0.59 ± 0.62 mM vs. 17.6 ± 16.8 mM, $p < 0.05$) in LAC. In DRY, there was a negative linear relationship between pH and absolute VFA only after incubation for 6 h ($y = -18.7x + 160$, $r^2: 0.89$). In LAC, even positive linear relationships were found after incubation for 12 h ($y = 83.7x - 300$, $r^2: 0.41$) and 24 h ($y = 86.7x - 316$, $r^2: 0.41$). The increase of VFA after addition of STA seemed to be faster in LAC samples, without apparent saturation after 12 h in DRY samples. Significantly higher concentrations of acetate (11.4 ± 4.11 mM vs. 37.7 ± 19.7 mM, $p < 0.01$) in LAC mainly contributed to the higher amount of VFA in after 24 h (17.6 ± 5.23 mM vs. 47.8 ± 23.4 mM, $p < 0.05$). Positive linear relationships between pH and absolute VFA not affected by group were found after incubation for 6 h ($y = -52.0x + 392$, $r^2: 0.89$) and 12 h ($y = -54.3x + 402$, $r^2: 0.86$). After addition of cellulose (CEL), we observed a small increase of VFA in DRY, but a decrease in LAC, resulting in a significantly higher concentration of VFA related to CON in DRY samples after 12 h (5.83 ± 3.78 mM vs. -4.69 ± 3.38 mM, $p < 0.001$).

Conclusions: Lower pH and higher VFA at baseline indicate higher substrate availability and thus fermentation activity in faeces from LAC in comparison to DRY, probably caused by higher starch intake and shorter retention time (2). The differences in pH and VFA related to CON between DRY and LAC after addition of GLU and STA indicate a higher capacity of faecal samples from LAC for fermentation that also depends on the capacity to maintain pH during incubation with GLU. The response to the addition of CEL indicates more cellulolytic species in the faecal microbiome of DRY. Further data on the production of lactate need to be taken into account to support these hypotheses.

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Estimation of ruminal crude protein degradation parameters in concentrates in dairy cows based on literature data

Schätzung von Parametern des ruminalen Proteinabbaus in Konzentraten für Milchkühe basierend auf Literaturdaten

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Introduction: The current recommendations for the nutrition of dairy cattle from the Society of Nutritional Physiology [1] redefine protein evaluation by including ruminal degradation kinetics into the estimation of effective degradation (EDG) and ruminal undegraded crude protein (UDP). For this, ruminal protein degradation parameters a, b, c and L from in situ nylon bag studies are used as reference data [2]. This method is however resource-intensive and more-over subject to ethical and regulatory approvals which limits its applicability in routine. Nevertheless, modern feed evaluation requires the most accurate description possible of protein degradation kinetics also for practical purposes. The objective of this study was to identify relationships between chemical feed analytes and parameters of ruminal protein degradation and to develop regression equations to enable a sufficiently accurate estimation of ruminal protein degradation kinetics in concentrate feeds.

Methods: This evaluation is based on data from seven published studies which followed officially recognized standards to ensure methodological consistency [2]. The data set (94 sets, 24 feed types) contains information about dry matter (DM), crude ash (CA), crude protein (CP), neutral detergent fiber (without ash), acid detergent fiber (without ash; ADFom), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent insoluble crude protein (NDICP), true protein (TP), buffer insoluble crude protein (BICP), and water soluble crude protein (WSCP). WSCP is calculated as the sum of soluble particle and small particles. The data set covers a wide range of chemical parameters, ruminal degradation and feed types to ensure the robustness of the statistical analyses. Statistical analysis was carried out using Pearson correlation coefficient among selected parameters to identify underlying patterns and relationships within the dataset, and multiple linear regression models using Python 3.10 software.

Results: Statistically reliable relations between ruminal protein degradation parameters and crude protein fractions were identified. The washout fraction a showed a negative correlation with BICP ($r = -0.66$), with the opposite for the potentially degradable fraction b and BICP ($r = 0.54$). The degradation rate c correlated positively with ADF ($r = 0.54$). The lag time L showed a moderate positive correlation with starch ($r = 0.47$). The lag time could not be estimated with sufficient accuracy and was therefore excluded from further modeling. The most reliable regression models developed based on these correlations are as follows:

$$a = 1.359 + 0.982 \cdot \text{WSCP} \quad (R^2 = 0.99, n=29)$$

$$a = 56.082 - 0.037 \cdot \text{BICP} - 0.016 \cdot \text{ADL} - 0.028 \cdot \text{ADFom} \quad (R^2 = 0.87, n=32)$$

$$b = 120.833 - 0.979 \cdot \text{WSCP} - 0.316 \cdot \text{CA} - 0.032 \cdot \text{ADF} \quad (R^2 = 0.93, n=20)$$

$$b = 24.749 + 0.062 \cdot \text{BICP} + 0.012 \cdot \text{ADF} \quad (R^2 = 0.59, n=29)$$

$$c = -6.509 + 0.159 \cdot \text{WSCP} + 0.050 \cdot \text{ADF} + 0.031 \cdot \text{CP} - 0.044 \cdot \text{NDICP-DM} \quad (R^2 = 0.75, n=20)$$

$$c = 237.130 - 0.236 \cdot \text{TP} - 0.050 \cdot \text{NDICP-CP} + 0.061 \cdot \text{ADL} \quad (R^2 = 0.76, n=38)$$

a (% CP), b (% CP), c (%/h), WSCP (% CP), CA (g/kg DM), CP (g/kg DM), ADFom (g/kg DM), ADF (g/kg DM), ADL (g/kg DM), TP (g/kg CP), BICP (g/kg CP), NDICP-DM (g/kg DM), NDICP-CP (g/kg CP)

Conclusions: The results of this study demonstrate that regression models based on chemical composition and crude protein fractionation parameters can provide sufficiently accurate estimates of ruminal protein degradation kinetics in concentrate feeds. A positive effect on the coefficient of determination was observed when WSCP was used.

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Change in prediction of milk protein synthesis from the utilizable crude protein at the duodenum to the small intestinal digestible protein evaluation system for dairy cows

Veränderung der Vorhersage der Milchproteinsynthese vom nutzbaren Rohprotein am Duodenum zum dünn darmverdaulichen Proteinbewertungssystem für Milchkühe

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Introduction: To reduce nitrogen (N) emissions from dairy farming caused by cows fecal and urine excretion, both the protein supply from feed and requirements of dairy cows has to be accurately evaluated. The Committee for Requirement Standards of the Society of Nutrition Physiology recently introduced the small intestinal digestible protein (sidP) system, replacing the utilizable crude protein at the duodenum (uCP) system. To evaluate the change in prediction of milk protein synthesis between these two systems, data from a feeding trial with dairy cows were used.

Methods: The study employed 48 lactating German Holstein cows which were assigned to a total of six feeding groups, which differed with regard to concentrate feed proportion and individual components. The study consisted of two periods, each comprising 3 weeks of adaptation and 3 weeks sampling phase. Milk yield (MY) was recorded automatically twice a day. Milk samples were taken twice a week, aliquoted and analyzed for protein content. Feed samples were collected once or twice a week and pooled over 3 weeks. All feed samples were analyzed for chemical composition, *in vitro* criteria and gross energy. uCP content was calculated from equation 1b according to [1]. sidP content was determined according to [2] (recommended procedure). Estimating microbial protein supply was also performed from equation 17 according to [3] (sidP_MP). Rumen undegraded protein (RUP) and small intestinal digestibility (SID) were analyzed enzymatically via 3-step *in vitro* method (sidP_RUPenz). Additionally, sidP was calculated from the combined data of microbial protein supply according to [3] and RUD and SID from 3-step *in vitro* method (sidP_MP_RUPenz). Rations were originally calculated according to [1] to supply adequate uCP for dairy cows of 630kg live weight and a daily MY of 35kg with a ruminal N balance (RNB) of 0. “Performance-independent” protein requirements were calculated from obligatory fecal and urine N, surface N and protein accretion from growth and gestation. Attainable MY was predicted as: (protein intake - “performance-independent” protein requirement) / milk protein content * utilization factor. Prior to statistical evaluation data from the sampling phase were merged to weekly means. Recorded and predicted MY were analyzed with a linear mixed model using “lmer” procedure of RStudio (version 2025.05.1+513). Intercept and slope were tested to differ significantly from 0 and 1, respectively. Lin’s concordance correlation coefficient (CCC) was determined to evaluate concordance between recorded and predicted MY.

Results: Daily dry matter intake and MY ranged from 13.2 to 28.5 kg and from 18.3 to 43.7 kg, respectively. RNB of the rations fed was between -2.3 and 1.2 with a mean of -0.4 g N/kg DM. The range of the protein contents of the ration were: uCP: 130 to 160 g/kg DM; sidP: 94 to 120 g/kg DM; sidP_MP: 89 to 103 g/kg DM; sidP_RUPenz: 98 to 137 g/kg DM; sidP_MP_RUPenz: 93 to 110 g/kg DM. The following regression equations and the corresponding CCC for recorded and predicted MY were determined: uCP: $1.06x$ ($p=0.21$) - 1.13 ($p=0.39$), CCC=0.84; sidP: $1.17x$ ($p<0.01$) - 4.00 ($p<0.05$), CCC=0.76; sidP_MP: $0.81x$ ($p<0.001$) + 2.85 ($p<0.05$), CCC=0.75; sidP_RUPenz: $1.42x$ ($p<0.001$) - 6.79 ($p<0.01$), CCC=0.56; sidP_MP_RUPenz: $0.97x$ ($p=0.54$) + 0.51 ($p=0.70$), CCC=0.82.

Conclusions: The results from the uCP system including RNB indicate that the rations calculated were implemented precisely in the trial. In the sidP system, predicted MY exceeded recorded MY at higher performance, which may be attributed to the procedure of estimating microbial protein supply. The larger differences between the recorded and predicted MY for sidP_MP and sidP_RUPenz could be reduced by combining the two procedures. For a comparison of the accuracy of the different procedures for sidP calculation and the uCP system, feeding trials are required that use the respective procedures as a basis for ration calculation.

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Nutrient composition of regional organic by-products in Eastern Germany

Nährstoffzusammensetzung regionaler ökologischer Nebenprodukte aus Ostdeutschland

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Introduction: The transition towards sustainable and resource-efficient agricultural systems necessitates innovative approaches to the effective valorisation of organic by-products within regional value chains. Such by-products not only contribute to circular economy principles and the reduction of resource waste but also offer a potential strategy to address the protein gap in organic livestock production. Furthermore, there is a paucity of scientific studies that deal specifically with organically produced by-products, and none for the regions considered here. The present study investigates the chemical composition of selected organic by-products from the German federal states of Berlin-Brandenburg and Saxony, focusing on their suitability for animal nutrition.

Methods: The materials were categorised into fruit and vegetable pomaces ($n = 21$), oilseed by-products ($n = 80$), whey ($n = 33$), and cereal brans ($n = 14$). A total of 148 samples were analysed. Chemical analyses were performed according to the Weende method and complemented by determinations of starch. Also an analyses of macro- and trace elements were carried out. All analyses were performed in accordance with VDLUFA standards [1]. All data were subjected to descriptive statistical analysis using SPSS.

Results: The results indicate distinct variations in nutrient and mineral contents both between and within the by-product categories. Oilseed by-products generally exhibited higher crude protein concentrations (365 ± 97.1 g/kg DM (Mean \pm SD)) and substantial variability in fat content (130 ± 42.0 g/kg DM). Among these, oilseed by-products from black cumin demonstrated particularly elevated fat levels (179 ± 43.4 g/kg DM, $n = 9$), while oilseed by-products from sesame exhibited the highest crude protein concentration (502 ± 31.3 g/kg DM, $n = 5$). Similarly, less common oilseed by-products from walnut, almond, pumpkin seed, and cherry stone also showed elevated protein contents. Fruit and vegetable pomaces demonstrated potential as fibrous feed components (154 ± 55.2 g/kg DM), particularly black chokeberry ($n = 2$) and quince pomaces ($n = 2$), which contained more than 200 g/kg DM of crude fibre. Brans were rich in crude fibre (184 ± 102 g/kg DM), neutral detergent fibre (549 ± 155 g/kg DM), with oat bran showing the highest values. Despite generally high fibre levels, some samples also contained notable starch concentrations (179 ± 112 g/kg DM), though with considerable variation. Whey contained little to no crude fat, while crude protein contents varied depending on the whey type (164 ± 38.0 g/kg DM). Regarding mineral composition, oilseed by-products were particularly rich in P (9.86 ± 2.44 g/kg DM), Mg (4.85 ± 1.12 g/kg DM), Cu (22.2 ± 8.68 mg/kg DM), Zn (74.8 ± 21.2 mg/kg DM), and Fe (110 ± 33.8 mg/kg DM). Pomaces exhibited rather low mineral concentrations, especially of P (1.72 ± 0.74 g/kg DM), Mg (1.07 ± 0.61 g/kg DM), and Zn (24.1 ± 11.8 mg/kg DM). Bran contained high levels of P (8.66 ± 5.79 g/kg DM), Mg (3.32 ± 2.11 g/kg DM), Zn (63.9 ± 38.9 mg/kg DM), and Fe (78.6 ± 36.8 mg/kg DM), and was particularly rich in Mn (83.5 ± 47.6 mg/kg DM). In contrast, whey was distinguished by elevated concentrations of P (8.48 ± 3.03 g/kg DM), Ca (12.7 ± 7.69 g/kg DM), Na (6.07 ± 0.89 g/kg DM), and K (22.7 ± 5.00 g/kg DM), but contained very few trace elements.

Conclusions: Overall, the findings highlight the potential of these by-products as valuable feed resources in organic animal nutrition, provided that their heterogeneity is adequately considered. They provide a foundation for assessing the role of regional organic by-products in nutrient recycling and feed formulation in Eastern Germany. Enhancing the integration of these resources into regional feed systems has the potential to reduce external feed dependencies and advance circular nutrient management within the context of organic livestock farming.

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Utilization of lignocellulosic agricultural wastes as sustainable feed for *Zophobas atratus* larvae

Verwertung lignozellulosischer landwirtschaftlicher Abfälle als nachhaltiges Futter für Larven von Zophobas atratus

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Introduction: The increasing global demand for sustainable and resource-efficient animal feed sources has intensified interest in insects as alternative protein ingredients for livestock and aquaculture diets [1]. Among the most promising species, the giant mealworm (*Zophobas atratus* Fab.) offers high nutritional value, digestibility, and feed conversion efficiency [2]. However, large-scale production requires cost-effective and nutritionally balanced diets to ensure optimal growth performance. One promising approach involves utilizing biomass derived from grass- and rewetted peatlands (LPRP) as rearing substrates for insects, supporting peatland conservation, sustainable land use, and circular feed production systems [3]. This study investigates the effects of varying inclusion levels of LPRP up to 50% on the growth performance and survival rate of larvae.

Methods: A total of 900 young larvae (age 90 days; initial weight = 0.05 g/larva) were reared under controlled conditions (25–27 °C, 40–45% relative humidity) for nine weeks. Larvae were randomly assigned to six dietary treatments with three replicates each. The control group received wheat bran only, while the other five diets contained 10–50% LPRP, with wheat bran and distillers dried grains with solubles (DDGS) adjusted to equalize protein content (18%). All diets were provided *ad libitum*, and 4 g of carrots were offered twice weekly as a water source. Feed intake, mean larval weight, and survival were recorded weekly at the box level (three replicate boxes per treatment). Larval weights were obtained by collectively weighing all larvae in each box and dividing by the number of individuals to estimate mean individual weight. All statistical analyses were performed at the replicate (box) level. Data normality was assessed using the Shapiro–Wilk test; normally distributed data were analyzed using ANOVA followed by the Ryan–Einot–Gabriel–Welsch test, whereas non-normal data were analyzed using the Kruskal–Wallis test followed by Wilcoxon two-sample tests. Statistical significance was set at $p < 0.05$.

Results: Larval body weight gain was not significantly affected by LPRP inclusion up to 40%. However, the 50% LPRP group showed a lower final body weight (0.38 g; $p < 0.05$) compared to the control (0.44 g). Feed conversion ratio (FCR, dry matter basis) did not differ significantly among treatments, except for the 30% and 50% LPRP groups (2.92 and 2.86, respectively), which showed significantly lower FCR than the control (3.34). Efficiency of conversion of ingested feed (ECI, dry matter basis) increased with higher LPRP inclusion, ranging from 39.5–39.9% in the 30–50% LPRP groups versus 33.9% in the control ($p < 0.05$), indicating improved biomass conversion. Survival remained high (>98%) and was unaffected by dietary treatments.

Conclusions: These findings demonstrate the potential of LPRP as a sustainable feed ingredient for *Z. atratus* rearing. Moderate inclusion levels ($\leq 30\%$) maintained growth performance comparable to the control while improving feed efficiency. At the highest inclusion (50%), feed utilization improved: FCR was 2.86 versus 3.34 in the control (about 14.4% lower), and ECI reached 39.6% versus 33.9% in the control (about 17% higher); this occurred alongside with a significant reduction in larval body weight gain. These findings support LPRP as a promising component for sustainable insect farming and circular bioeconomy applications.

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Characterizing nutrient composition and *in vitro* methane production of available feed resources used for dairy cattle in Bangladesh

Nährstoffzusammensetzung und in vitro Methanproduktion von Futtermitteln für Milchkühe in Bangladesch

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Introduction: As dairy production in Bangladesh is currently shifting from subsistence-based to enterprise-driven farming [1], achieving optimal nutrient composition of locally available feedstuffs is essential to enhance productivity and mitigate enteric methane (CH₄) emissions. Yet, this remains a challenge in resource limited production system. In the frame of a larger project, this study characterized the nutritional contents and *in vitro* CH₄ production of accessible feeds in Bangladesh.

Methods: In a survey across three representative districts of south-western Bangladesh from May-August 2024, a total of twenty feed samples (11 roughages and 9 concentrates) were obtained. Roughages were sub-divided into three groups: crop residues (n=2), cultivated grasses (n=6) and indigenous grasses (n=3) (shrubs and herbaceous plants). Feed samples were collected and partly pooled to make representative samples. The samples were initially sun-dried during collection and subsequently oven-dried (60°C overnight) in Bangladesh to facilitate transportation for further analyses. The nutritional composition was assessed by proximate analysis including detergent methods following methods of VDLUFA (Association of German Agricultural Inspection and Research Institutes). The Hohenheim gas test (HGT) was conducted according to [2], to evaluate *in vitro* gas production (GP) characteristics; followed by methane quantification via gas chromatography. *In vitro* organic matter degradability (IVOMD) was calculated from organic matter loss after Gerhardt fiber analysis of residues from HGT samples. Metabolizable energy (ME) was calculated using GP data.

Results: The roughages had an NDFom content of 723 ± 55 g/kg DM and a crude protein content of 90 ± 38 g/kg DM on average. Roadside grass stood out in having low NDFom (589 g/kg DM) and high CP content (170 g/kg DM). While *in vitro* methane yield (g/kg DM) was higher for concentrates (26 ± 12 g/kg DM) than for roughages (17 ± 5 g/kg DM; p = 0.04), methane yield related to degradable organic matter (deOM) was similar for roughages (38.2 ± 6.22 g/kg deOM) and concentrates (40.0 ± 11.8 g/kg deOM; p = 0.68). Among roughages, the lowest CH₄ yield was observed in rice straw (*Oryzastiva*) (10 g/kg DM) and the highest in Napier grass (*Pennisetum purpureum*) (24 g/kg DM), while in concentrates, the lowest was evaluated in rice bran (10 g/kg DM) and highest in wheat bran (46 g/kg DM). A positive correlation was observed between CH₄ yield, and IVOMD (r = 0.83; p < 0.001) and total gas production (r = 0.87; p < 0.001), respectively, while this correlation was negative for fibre fractions (r = -0.62 to -0.72, all p < 0.01) and crude protein showed a weak association (r = 0.53; p < 0.05).

Conclusions: The fermentation characteristics observed for typical feedstuffs of Bangladesh were consistent with globally studied feeds, with rice straw and rice bran showing low methane yield comparable to cereal straws and lipid containing brans, while Napier grass and wheat bran exhibited higher methane production like tropical grasses and cereal by-products, highlighting implications for sustainable ration formulation in Bangladesh.

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Effect of feed treats on gastrointestinal parameters in C57Bl/6J mice

Effekte von „feed treats“ auf gastrointestinale Parameter bei C57Bl/6J-Mäusen

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Introduction: In laboratory animal science, medical training and positive reinforcement for handling have become increasingly common, mainly in the context of Refinement approaches to minimize stress. A variety of feed treats is used [1], even though possible effects on experimental outcome have not been investigated and it does compromise standardization. The aim of this pilot study was to investigate how different feed treats affect gastrointestinal parameters of mice to gather first data on this subject. The hypothesis was that the addition of the treats would alter the digestive tract, e.g. due to microbial fermentation (cereals) or higher intake of digestible fat (seeds).

Methods: Twenty-two C57Bl/6J mice (aged 8-18 months) were maintained on a pelleted standard diet (Altromin rat & mouse breeding, based on cereals, *ad libitum*). One group received only this diet (CON, n = 6), while the other groups were offered additional feed treats: oat flakes (OAT, n = 5), sunflower seeds with hulls (SFS, n = 5), hazelnut bread spread (NUT, n = 6) in defined amounts three times per week. The mice were weighed weekly during this period. At the end, they were sacrificed via cervical dislocation (in accordance with § 4 TierSchG) and dissected. Final body weight (BW), liver and caecum weight and the length of the small intestine and the colon were determined. The groups were compared with ANOVA (GraphPad Prism, $\alpha = 0.05$).

Results: All feed treats were consumed completely by the mice and no obvious adverse effects were observed. The weight gain over the final 3 weeks differed significantly between the groups ($p < 0.01$), with the CON and NUT mice losing and the OAT and SFS mice gaining weight. The weight loss of the CON mice might possibly be explained by the relatively high age of the mice. Relative liver weight (in % BW) showed a trend to be higher in the SFS and NUT mice than in the CON and OAT groups ($p = 0.113$), which might be related to the higher fat content of the SFS and NUT treats. There was a numerical, but not statistically significant, observation of lower relative caecum weight in the SFS mice. Small intestinal length showed a near-significant trend ($p = 0.06$) to higher values in the groups fed treats than the CON group. This might be related to absorptive capacity. The appearance of the small intestine was “milky-white” in the dissection in the mice of the SFS and NUT groups. Colon length was numerically highest in the NUT group, but overall the differences were not significant ($p = 0.188$).

Conclusions: The weight development of the groups might suggest a protection against age-related BW loss, especially for OAT. Liver parameters need to be studied in detail to investigate whether there may be a metabolic effect of the treats rich in fat. Surprisingly, intestinal length and weight parameters did not show statistically significant differences. To discern whether the numerical differences are biologically relevant, further studies with a higher number of animals and/or a longer feeding period would be required. Additional samples for microbiome sequencing are currently being processed.

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Effects of *Hermetia illucens* larvae meal as a substitute for soybean meal on performance, egg quality, and yolk lipids in laying hens

Wirkungen von Insektenmehl als Ersatz für Sojaextraktionsschrot auf Leistung, Eiqualität und Dotterlipide bei Legehennen

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Introduction: The global population growth strains natural resources, highlighting the need for sustainable approaches to food production. In animal nutrition, alternative protein sources for monogastric livestock are needed, ideally associated with a reduced environmental footprint. Soybean meal, a predominant protein source, is criticized due to its unfavorable ecological impact. Insect-derived biomass, particularly from black soldier fly (*Hermetia illucens*, HI) larvae, represents a promising alternative. HI larvae exhibit high feed conversion efficiency and can be reared with minimal environmental burden. Previous studies in broilers and pigs have demonstrated that HI larvae meal can effectively replace soybean meal without compromising animal performance or health. However, data regarding its application in laying hen nutrition remain limited. This study investigates the hypothesis that HI larvae meal can serve as a viable substitute for soybean meal in laying hen diets without adversely affecting productive performance.

Methods: 60 LSL-Classic pullets were randomly assigned to three groups (n = 20), each housed separately in one pen under controlled floor-housing conditions. Beginning at week 21, hens were fed isoenergetic and isonitrogenous diets in two phases (21–30 and 31–40 weeks of age), differing only in their primary protein source. The control diet included 221 g/kg and 210 g/kg soybean meal in phases 1 and 2, respectively. In the experimental diets, soybean meal was partially replaced by HI larvae meal, substituting either 33% (HI 33) or 66% (HI 66) of the soybean protein. To ensure nutritional equivalence, concentrations of ether extract, amino acids, calcium, and phosphorus were adjusted across all diets through targeted supplementation. Feed intake and laying performance were recorded at the group level. Egg quality traits (shell strength, albumen height, Haugh units, yolk color) and yolk contents of fatty acids, triglycerides, and cholesterol were analyzed in 20 eggs per/group collected at 30 and 40 weeks of age. Statistical analyses were conducted using SPSS. For egg weight and daily egg mass, the individual egg served as the experimental unit. One-way ANOVA followed by Fisher's LSD post-hoc test was applied, with statistical significance set at $p < 0.05$.

Results: Feed intake between 23 and 40 weeks of age was comparable across all three experimental groups (119 g in the control group, 122 g in the HI 33 group, and 116 g in the HI 66 group.) Laying performance was numerically higher in hens fed diets containing HI meal compared to the control group (94.8%, 96.2%, and 96.3%, respectively). Egg weight was lower in the HI 66 group than in the other two groups (Control: 59.9 ± 5.7^a g; HI 33: 60.4 ± 5.7^a g; HI 66: 58.9 ± 5.5^b g), while daily egg mass was highest in the HI 33 group (Control: 57.0 ± 6.9^b g; HI 33: 58.2 ± 6.2^a g; HI 66: 56.9 ± 6.0^b g). No significant differences were observed among the groups regarding albumen height, Haugh units, or eggshell strength. Yolk color intensity, assessed using the DSM YolkFan™ scale, was slightly lower in the HI 66 group compared to the control (Control: 5.00 ± 0.08^b ; HI 33: 4.77 ± 0.08^{ab} ; HI 66: 4.62 ± 0.08^a). Feeding HI larvae meal decreased the concentrations of total n-6 polyunsaturated fatty acids (PUFA) in the egg yolk at the highest inclusion level. In yolks of eggs collected at 40 weeks of age, the concentrations of total n-3 PUFA in egg yolk were reduced in group HI 66 compared to the other groups. Triglyceride and cholesterol concentrations in the yolk also did not differ between the HI larvae meal-fed groups and the control group.

Conclusions: The present study demonstrates that a substantial proportion of soybean meal in laying hen diets can be replaced by HI larvae meal without adverse effects on feed intake, laying performance, or key egg quality parameters. These findings indicate that HI larvae meal represents a promising alternative protein source for future poultry nutrition strategies.

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Effects of *Hermetia illucens* fat as a substitute for soybean oil on performance, egg quality, and yolk lipids in laying hens

Wirkungen von Hermetia illucens Fett als Ersatz von Sojaöl auf Leistung, Eiqualität und Dotterlipide bei Legehennen

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Introduction: Poultry nutrition is increasingly shaped by the dual challenge of meeting global protein demand while advancing sustainable agricultural practices. Locally available feed ingredients that do not compete with human food resources are gaining relevance. Fat derived from black soldier fly (*Hermetia illucens*, HI) larvae offer a promising alternative to conventional plant oils due to their favorable nutrient profile and low environmental impact. While studies in broilers have shown that fat from HI larvae (HI fat) can replace vegetable oils without negative effects on performance or product quality, data for laying hens are limited. This study investigates the effects of dietary HI fat on laying performance, egg quality, yolk lipid composition, and ovarian follicle development to assess its suitability as a substitute for soybean oil in layer diets.

Methods: 60 LSL-Classic pullets were randomly assigned to three groups (n = 20). Each group was housed separately in one pen under controlled floor-housing conditions. From 21 weeks of age, hens were fed isoenergetic and isonitrogenous diets in two phases (21–30 and 31–40 weeks of age), differing only in fat source. The control group received soybean oil (68 g/kg in phase 1; 64.5 g/kg in phase 2). In the experimental groups, soybean oil was partially (50%, HI 50) or fully (100%, HI 100) replaced with HI fat. Feed intake, laying performance, and egg weight were recorded per group. Egg quality traits (shell strength, albumen height, Haugh units, yolk color) and yolk contents of fatty acids, triglycerides, and cholesterol were analyzed in 20 eggs per group collected at 30 and 40 weeks of age. Statistical analysis was performed using IBM SPSS Statistics 29. For egg weight and daily egg mass, the individual egg served as the experimental unit. One-way ANOVA with Fisher's LSD post-hoc test was used; significance was set at $p < 0.05$.

Results: Between 23 and 40 weeks of age, both experimental groups receiving HI fat exhibited numerically higher feed intake and laying performance compared to the control group. Specifically, average daily feed intake per hen was 119 g in the control group, 126 g in the HI 50 group, and 127 g in the HI 100 group. Corresponding laying rates were 94.8%, 97.2%, and 98.1%, respectively. Egg weight was slightly reduced in the HI-fed groups (Control: 59.9 ± 5.7 g^a, n = 2388; HI 50: 59.6 ± 5.8 g^b, n = 2449; HI 100: 59.3 ± 6.1 g^b, n = 2473), whereas daily egg mass was significantly higher (Control: 57.0 ± 6.9 g^b; HI 50: 58.0 ± 6.3 g^a; HI 100: 58.3 ± 6.5 g^a). No significant differences were observed among groups for albumen height, Haugh units, or shell strength. Yolk color intensity, assessed using the DSM Yolk-Fan™ scale, was slightly elevated in the HI 100 group compared to the control (Control: 5.00 ± 0.08 ^b; HI 50: 5.09 ± 0.08 ^{ab}; HI 100: 5.30 ± 0.08 ^a). In eggs collected at 30 weeks of age, yolk triglyceride content was reduced in the HI 100 group, while cholesterol content was lower in the HI 50 group compared to the control. By 40 weeks of age, no significant differences in yolk triglyceride or cholesterol concentrations were detected among the groups. Feeding HI fat resulted in a dose-dependent reduction in polyunsaturated fatty acid (PUFA) content - particularly linoleic and α -linolenic acids - in egg yolk. In eggs collected 30 weeks of age, additionally, monounsaturated fatty acid (MUFA) levels were decreased in the HI-fed groups, accompanied by an increase in the n-6/n-3 PUFA ratio.

Conclusions: Fat derived from HI larvae constitutes a promising alternative to soybean oil in the nutrition of laying hens. Its inclusion in layer diets enhances daily egg mass without adversely affecting key egg quality parameters. These results underscore the potential of HI fat as a sustainable and functionally effective lipid source in poultry feed formulations.

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Utilization of by-products from certified organic farming and organic food processing as feed substrates for *Hermetia illucens* larvae

Verwertung bio-zertifizierter Nebenprodukte als Futtersubstrate für Hermetia illucens Larven

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Introduction: Protein and amino acid supply is one of the major challenges in feeding monogastric livestock under the regulations of organic farming, particularly in broiler production. The grain legumes currently used in organic animal production, such as peas, faba beans, and lupins, contain relatively low concentrations of the sulfur-containing amino acids (SAA) methionine and cysteine. In contrast, the protein of black soldier fly (*Hermetia illucens*, HI) larvae is characterized by substantially high concentrations of SAA. Therefore, the use of HI meal could significantly enhance the SAA supply for monogastric animals in organic farming systems. However, its application requires that the insect larvae are reared on by-products originating from certified organic production. The aim of this study was to evaluate the suitability of various organic by-products and their combinations as feed substrates for HI larvae.

Methods: Five-day-old HI larvae (30 g per box; represent 6,000 larvae) were reared for ten days under controlled conditions (darkness, 60% relative humidity). Each box contained 300 g organic substrate as fresh matter differing in dry matter content of 20–55%. Each substrate was run in eight replicates. 22 organic by-products were used, including vegetable residues, silages, cereal by-products, oilseed residues, pomace, and plant-based food waste. Substrates that resulted in the highest larval biomass gains were subsequently combined and used for additional growth experiments. After 10 days of rearing, larvae were harvested, freeze-dried, and milled. The larvae were analyzed for crude nutrient composition, their amino acid and their fatty acid profile. Statistical analyses were performed using ANOVA for datasets with homogeneity of variance or Welch's ANOVA for datasets without homogeneity of variance, each followed by appropriate post hoc tests.

Results: The highest larval biomass gains were observed for mixtures containing flaxseed cleaning residues, wheat bran, and broken grains (40:40:20, w/w/w; 140 ± 7 g), flaxseed cleaning residues, wheat bran, brewers' spent grain, and carrot peels (20:20:30:30; 122 ± 5 g), and soybean pulp, plant-based food waste, and wheat bran (50:30:20; 112 ± 23 g). The lowest growth occurred with potatoes and wheat husks (80:20; 25 ± 9 g). Larvae fed brewers' spent grain and wheat husks showed the highest crude protein content (56% DM), while the highest crude fat content (24% DM) was found in larvae fed the flaxseed-based mixture. Larval growth was positively correlated with substrate protein content ($r = 0.72$; $p = 0.002$), while substrate fat content showed a non-significant positive trend. Substrate fat content was also positively correlated with larval fat content ($r = 0.69$; $p = 0.003$). No correlations were found between substrate and larval protein or amino acid concentrations or between substrate and larval fatty acid profiles.

Conclusions: This study shows that specific combinations of organic by-products can effectively support the rearing of HI larvae, and that the protein content of the substrate plays a decisive role in larval growth performance and nutrient composition. These findings highlight the potential of optimized substrate formulations to enhance the efficiency and sustainability of organic insect farming.

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Feed intake of separately offered insects (*Acheta domesticus*, *Hermetia illucens* flies) and macroalgae (*Palmaria palmata*, *Ulva lactuca*) by dual-purpose pullets

Aufnahme separat angebotener Insekten (Acheta domesticus, Hermetia illucens Fliegen) und Makroalgen (Palmaria palmata, Ulva lactuca) durch Junghennen einer Zweinutzungsrasse

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Introduction: The aim of the study was to improve the performance for the rearing of dual-purpose chickens within the organic husbandry system through the resource-efficient optimization of the feed by reducing its nutrient density while simultaneously substituting protein components, such as soy or sunflower press cake, with alternative protein sources. To enable separate provision of these alternative feed materials and to adequately adjust the composition of the additional offered compound feed, their acceptance and intake levels must be known. Against this background, two insects and two macroalgae species were selected and their intake alongside two isoenergetic (based on metabolizable energy) compound feeds with different protein contents by dual-purpose pullets was quantified. The resulting energy and nutrient intakes were calculated and placed in the context of the pullets' growth.

Methods: A total of 360 birds of both sexes of the dual-purpose chicken „Coffee“ (ökologische Tierzucht gGmbH) were reared together for six weeks after hatching. From the 7th week of life, 72 groups were formed, with cockerels and pullets kept separately in groups of five birds each. Six treatment groups were established, each with six replicates distributed across three consecutive trials. The birds were fed complete feed as mash in four phases [Starter 1 (weeks 1-3), Starter 2 (weeks 4-6), Grower 1 (weeks 7-12), and Grower 2 (weeks 13-14)]. Based on the standard Grower 1 diet (as fed/kg; 179 g crude protein (CP), 11.7 MJ AMEn, 0.25 g methionine (Met)/MJ AMEn), a second diet with reduced protein content (as fed/kg; 157 g CP, 12.0 MJ AMEn, 0.22 g Met/MJ AMEn) was created. The control group C1 received only the standard diet, while the second control group C2 received both the standard and the protein-reduced diet. Four experimental groups received the standard and the protein-reduced diets alongside one of the four protein sources: *Acheta domesticus* (AD), *Hermetia illucens* flies (HI), *Palmaria palmata* (PP), or *Ulva lactuca* (UL). Each diet and protein source were provided separately and *ad libitum*, allowing the chickens to self-select their intake. Feed intake was recorded weekly per group, while the body mass (BM) was measured for each bird every two weeks. The evaluation focuses on pullets from week 7 to week 12. Group differences in BM were analyzed using a linear model followed by a one-way ANOVA. Normality of model residuals was assessed using the Shapiro–Wilk test.

Results: The mean intake of AD as a proportion of total intake by the pullets was 7.51% (SD: 9.34; min: 0, max: 33.0), calculated across the six replicate groups over six weeks. No intake was recorded in eleven observations (across groups and weeks). For HI, the mean intake was 6.79% (SD: 5.29; min: 0, max: 18.1), with no intake in three observations. For UL, the mean intake was 3.80% (SD: 2.77; min: 0, max: 14.3), with no intake in two cases. Finally, for PP, the mean intake was 1.03% (SD: 0.934; min: 0, max: 3.22), with no intake in nine cases. Between weeks 7 and 12, birds of the C2, HI, AD, UL, and PP groups ingested diets with average CP contents of 168 ± 1.66 , 200 ± 25.2 , 200 ± 40.4 , 169 ± 2.08 , and 167 ± 1.85 g/kg, respectively, and Met/AMEn ratios of 0.240, 0.303, 0.259, 0.249, and 0.242 g/MJ, respectively. BM at the end of week 12 did not differ significantly among the groups (C1: 1590 ± 142 g, C2: 1638 ± 144 g, HI: 1629 ± 111 g, AD: 1614 ± 156 g, UL: 1638 ± 98.8 g, PP: 1561 ± 151 g; $p = 0.177$).

Conclusions: Insects were ingested in higher proportions than macroalgae, although AD was avoided during several weeks, while HI flies and UL were ingested more consistently. This highlights substantial variability in acceptance by dual-purpose pullets. The present results offer preliminary evidence that reducing dietary protein may not affect BM gain in pullets of the studied dual-purpose breed. However, further research regarding body composition and subsequent laying performance is necessary to draw conclusions.

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Ensiling instead of drying – an alternative to preserve wet-separated *Hermetia illucens* larval protein

Silierung statt Trocknung – eine Alternative zur Konservierung von nass-separiertem Hermetia illucens-Larvenprotein

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Introduction: Sustainable high-quality protein supply is a key challenge in poultry and pig nutrition. *Hermetia illucens* larvae constitute a promising protein source, especially when the protein fraction is isolated. Energy-intensive drying is usually applied for preservation, which reduces sustainability and economic competitiveness. Ensiling may represent an efficient alternative, yet little is known about its feasibility for this type of substrate. This study aimed to identify ensiling conditions that maximize preservation success of wet-separated *Hermetia illucens* larval protein for its subsequent use in livestock feeding.

Methods: Wet-separated *Hermetia illucens* larval protein was ensiled either i) alone [CON], ii) with 15% wheat bran and lactic acid bacteria (LAB; 7 g/t FM, Kofasil Life, ADDCON GmbH) [WHEAT], iii) with 15% molasses and LAB [MOL], or iv) with 3.5% of a chemical additive mix (formic acid, sodium formate, antioxidants; EnsiloX N, ADDCON GmbH) [CHEM]. Choice and inclusion rate of co-substrates were based on our previous findings [1], whereas additives were applied at their respective manufacturer's recommended dosages. All treatments were prepared in quadruplicate in vacuum-packed polyethylene bags and stored at room temperature for 90 days. Additionally, dried larval protein [DRY], dried for one hour at 110°C, was prepared as a further treatment and served as benchmark. The nutrient composition and silage fermentation pattern (except DRY) were determined comprehensively, including standardized precaecal digestible crude protein [2]. Then, data were analyzed using a one-way ANOVA (PROC GLM, SAS 9.4) with treatment as the fixed effect and a Tukey-Kramer post hoc test ($\alpha \leq 0.05$).

Results: Compared with the benchmark DRY (65%), some ensiled treatments (WHEAT and MOL) contained lower crude protein content (53.5-55.5% in DM; $p < 0.01$). Standardized precaecal digestible crude protein of DRY and all silage treatments ranged from 76.1-77.0% without differences. Regarding the silage fermentation pattern, silage pH was lowest in CHEM (3.72; $p < 0.01$) that also showed smallest gaseous weight losses (2.91%; $p < 0.01$). Similarly, pH was sufficiently low in WHEAT and MOL, i.e., 4.39 and 4.30, respectively, whereas pH of CON remained at 6.91. Likewise, CON showed highest gaseous weight losses (4.92%) and elevated butyric acid concentrations (0.71% in DM). The lactate concentration strongly depended on the treatment, with highest level in MOL (6.1% in DM) and lowest in CHEM (0.42% in DM; $p < 0.01$).

Conclusions: Our results show that ensiling of insect larvae protein is successful when applying lactic acid bacteria with co-substrates rich in rapidly fermentable carbohydrates, or otherwise chemical additives. Compared to dried larval protein, ensiled larval protein showed no disadvantages regarding feed value and appears to offer an efficient preservation strategy. From a future perspective, feeding studies are needed to assess the suitability of ensiled wet-separated *Hermetia illucens* larval protein *in vivo*.

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Effects of *Hermetia illucens* larvae meal on growth performance and nutritional quality traits in products of Lohmann Sandy brother roosters

Auswirkungen von Hermetia illucens-Larvenmehl auf Wachstumsleistung und ernährungsphysiologische Parameter in den Produkten bei Lohmann Sandy Bruderhähnen

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Introduction: The increasing demand for sustainable protein sources in poultry nutrition has driven interest in insect meals. Insect biomass, especially from the black soldier fly (*Hermetia illucens*, HI), offers a promising sustainable source of protein. HI larvae can be mass-reared with high feed efficiency and minimal environmental burden. This study aimed to investigate the impact of partially replacing conventional protein sources with HI larvae meal on growth performance and nutritional quality indicators in Lohmann Sandy brother roosters, a genotype suitable for organic farming systems.

Methods: The trial was conducted at the Oberer Hardthof experimental farm of Justus-Liebig University Giessen (Giessen, Germany) under organic farming standards. Day-old chicks were raised on a standard commercial starter diet (CP 22.50%, ME 12.00 MJ/kg) until 6 weeks, after which they were randomly assigned to two groups (n = 65 each) from 7 to 16 weeks. All animals assigned to the same group were housed in a single compartment. The conventional diet (HI0) was formulated with peas and a mixed supplement - including sunflower cake, soybean cake, rapeseed cake, corn gluten, potato protein - as the primary protein sources. In the experimental diet (HI 15), a part of the protein sources was replaced by HI larvae meal (15% of the diet), maintaining comparable energy and nutrient levels (HI0: CP 20.22%, ME 11.96 MJ/kg; HI15: CP 20.24%, ME 11.88 MJ/kg). The HI larvae meal was provided by Probanda (Pfungstadt, Germany). Feed intake was recorded weekly to calculate the feed efficiency ratio. At 16 weeks, birds were slaughtered, and breast, thigh, liver, and plasma samples were collected. Growth performance, carcass traits and vitamin concentrations (Retinol, α/γ -tocopherol), fatty acids, triglycerides (TG), cholesterol (Chol), and plasma metabolites were analyzed. Statistical analysis used one-way ANOVA, and differences were considered significant at $P < 0.05$. Data of the feed-gain-ratio were not statistically treated due to the lack of repetition (n=1).

Results: No significant differences were observed in body weight gains or carcass traits. The feed-gain-ratio (g feed/g gain) was nominally higher in HI0 than in HI15 (HI0: 5.87; HI15: 5.53). Vitamin analysis revealed that HI0 birds had significantly higher α -tocopherol contents in breast (6.88 vs. 6.06 $\mu\text{g/g}$ FM), thigh (8.36 vs. 6.75 $\mu\text{g/g}$ FM), and liver (20.8 vs. 14.1 $\mu\text{g/g}$ FM) tissues compared with HI15. Likewise, hepatic γ -tocopherol concentration was higher in HI0 ($P < 0.05$). The fatty acid composition differed significantly between treatments; HI15-fed birds showed higher proportions of saturated fatty acids (SFA), whereas HI0 birds exhibited higher ratios of polyunsaturated to SFA, a pattern observed in breast, thigh, and liver samples ($P < 0.05$). The concentrations of TG and Chol were only marginally affected by diet. In thigh muscle, TG levels were significantly lower in the HI0 group ($P < 0.05$), while no differences were observed for cholesterol content. Plasma metabolomics analysis using principal component analysis (PCA) revealed clear separation between HI0 and HI15 groups at 95% confidence, with the first two components explaining 39.9% of the total variance.

Conclusions: Replacing 15% of conventional protein with HI larvae meal in Lohmann Sandy brother roosters maintained growth and carcass performance, and altered the fatty acid and vitamin profiles of muscle and liver tissues. These findings contribute to understanding how insect meal inclusion affects metabolic and nutritional quality and support its potential as a sustainable, high-quality protein source in organic poultry meat production.

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ConTrans: A Tool to Predict the Transfer of Contaminants from Feed to Food of Animal Origin

ConTrans: Ein Tool zur Vorhersage des Transfers von Kontaminanten aus Futtermitteln in Lebensmittel tierischen Ursprungs

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Introduction: The presence of contaminants in the food chain exposes consumers to potentially toxic substances such as persistent organic pollutants [e.g., “dioxins” and polychlorinated biphenyls (PCBs); or per- and polyfluoroalkyl substances (PFAS)], as well as natural toxins [e.g. aflatoxins, quinolizidine alkaloids]. Foods of animal origin, such as eggs, meat and milk, may be a source of these contaminants when they enter the food chain e.g., via contaminated animal feed, soil or drinking water for livestock. Predictive toxicokinetic models are mathematical tools that integrate quantitative knowledge on the transfer of contaminants in farm animals. Based on *in vivo*, *ex vivo* and *in vitro* experimental studies, as well as other *in silico* tools, these models can be used to simulate the absorption, distribution, metabolism and excretion of undesirable substances. However, most published toxicokinetic models do not facilitate risk assessment or management directly because applying the complex algorithms requires specialized expertise. sWe present „ConTrans“ (Contaminant Transfer Predictor), a web-based platform that uses toxicokinetic models from various transfer studies to aid farmers, feed manufacturers, risk managers, and scientists in making decisions.

Methods: We demonstrate ConTrans with a toxicokinetic model and a ConTrans module for predicting the transfer of aflatoxin B1/M1 into cow’s milk as an example of its application. We extended a steady-state, two-compartment model for AFB1 metabolism to AFM1 and milk excretion to a dynamic setting. The model was parameterized using hierarchical Bayesian inference with concentration-time data from 10 studies and transfer rate data from 7 additional studies spanning diverse conditions (milk yields: 10.9–44.7 L/d; doses: 50–20,000 µg/d).

Results: The toxicokinetic model provides credible intervals for AFM1 concentrations during the assimilation and elimination phases. Higher milk yields are associated with higher transfer rates: 0.13%-1.40% (5 L/d) versus 1.07%-8.19% (50 L/d) and mean residence times of 2.6-95 hours. Although the transfer rates were consistent across the studies, the non-milk elimination parameters varied widely, likely due to differences in the experimental designs. The predictive model allows risk assessors to estimate AFM1 concentrations under various exposure scenarios and provides a quantitative measure of uncertainty. This improves decision-making by integrating multi-study evidence.

Conclusions: We developed ConTrans, an easy-to-use, web-based, graphical tool that enables risk analysis specialists to perform contaminant transfer estimations from feed to food based on such models, as demonstrated with aflatoxin B1/M1. ConTrans is designed to estimate transfer into a wide variety of substance-animal product combinations using a single interface. Users can configure farm animal exposure scenarios and different depuration periods.

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Effects of feeding Per- and polyfluoroalkyl substances (PFAS) contaminated hay on the liver of dairy goats

Auswirkungen der Fütterung von mit Per- und Polyfluoralkylsubstanzen (PFAS) kontaminiertem Heu auf die Leber von Milchziegen

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Introduction: Small ruminants are often used to graze areas along rivers or industrial wasteland. As these areas pose an increased risk of PFAS contamination these animals are likely exposed. Besides concerns about the transfer of such chemicals from animal feed to human food of animal origin, the effects on animal health are largely unexplored in the affected species. Therefore, the present investigation gathered data on animal health in a feeding experiment with dairy goats on the transfer of PFAS into milk. Special attention was laid on the liver, as investigations on laboratory animals identified the liver as target organ.

Methods: A feeding trial was conducted with eight lactating German Improved White dairy goats assigned to two groups: a control group, fed with uncontaminated hay and an experimental group, fed with PFAS-contaminated hay (mean total of $1310 \pm 536 \mu\text{g}$ PFAS/kg hay at 88%DM with 75% mainly long-chain perfluorosulfonic acids (chain lengths of C8-13) and 24% mainly shorter-chain perfluorocarboxylic acids (C4-C8)) for the first eight weeks, followed by a 12-week depuration period. Blood samples for health monitoring were collected weekly. At the end of the experiment, the animals were slaughtered and dissected and tissue samples were obtained for further analysis. In liver, the tissue composition (protein, RNA, DNA, total lipids), histology and transcriptome (directional mRNA-Seq, Illumina, 2x 150bp, 30M pe reads/sample) were analysed. Tissue composition data were evaluated by analysis of variance (Statistica, version 14.0.0.15, TIBCO Software Inc.). RNA sequencing data was preprocessed (MultiQC v1.23 and Trimmomatic v0.39) and aligned with STAR v2.7.11b against *Capra hircus* genome ARS1.2. Differentially expressed genes were analysed with DESeq2 v1.42.1 with $p_{\text{adjust}} < 0.05$ and \log_2 fold change > 1 or < -1 .

Results: Feeding the contaminated hay resulted in significantly decreased concentrations of total RNA in liver tissue ($p=0.017$) resulting in decreased RNA:DNA ratio as an indicator of transcriptional activity. Concentrations of DNA, protein and lipids were not affected. However, the transcriptome analysis revealed a majority of upregulated genes which enriched the hematopoietic cell lineage, cell adhesion molecules and chemokine signaling pathway in the KEGG pathway -enrichment analysis (clusterProfiler 4.10.1, DAVID). Gene ontology analysis revealed inflammatory response and immune response as the top terms in biological processes while transmembrane signaling receptor activity and integrin binding were identified as the top molecular functions affected. The external side of plasma membrane and plasma membrane were identified as the top cellular components in the gene ontology analysis describing cellular sites where the differentially expressed genes are active.

Conclusions: The observed alterations seem to be driven by blood cells present in the liver tissue. However, PFAS contamination of the hay is not the only possible cause of the described effects. Differences in nutritional value of the hay of both feeding groups and further contaminants may have contributed to the observations. Further investigations under controlled exposure including sufficient replicates are needed to clarify causality.

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Toxicokinetics of per- and polyfluoroalkyl substances (PFAS) in German Improved White dairy goats

Toxikokinetik von Per- und Polyfluoralkylsubstanzen (PFAS) in laktierenden Weißen Deutschen Edelziegen

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Introduction: Perfluoroalkyl acids (PFAAs), a subgroup of per- and polyfluoroalkyl substances (PFAS), are highly persistent synthetic chemicals, many of which bioaccumulate along the soil-(feed)plant-livestock-food pathway. Foods of animal origin may contain PFAS due to the transfer from contaminated feed and drinking water used in animal husbandry. Especially long-chain compounds can accumulate in biological tissues and have been associated with adverse health effects to humans. Knowledge about the contribution of feed contamination to PFAS levels in milk remains scarce. Therefore, a study with dairy goats was conducted to investigate toxicokinetics and transfer of linear (n-) and branched (br-)PFAAs into milk, serum, tissues, organs and excretions.

Methods: Two groups of four German Improved White dairy goats each were housed in straw-bedded stalls. The control group (C) was fed *ad libitum* with PFAS-free hay, while the exposure group (E) was fed hay from a highly contaminated site I for eight weeks (cumulative intake: 4.4 mg PFAAs/kg body weight (bw) with 1.7 mg/kg bw n- and 2.7 mg/kg bw br-PFAA isomers), followed by a 12-week depuration phase with control hay. Milk was collected daily, hay and blood weekly and urine and feces individually during three weeks of single housing. At the end, all goats were slaughtered and organs and muscle tissue sampled for PFAS analysis by LC-MS/MS. A dynamic, compartmental physiologically-based toxicokinetic (PBTK) model with ten interconnected compartments was developed based on the experimental data. The PBTK model consists of representing tissues and organs involved in absorption, distribution, metabolism and excretion (ADME) processes, including blood, liver, kidney, spleen, mammary gland, gastrointestinal tract ((fore-)stomach and intestine), as well as three excretion pools (urine, feces, and milk). The model was built in Python [v3.14].

Results: In the control animals and their excreta, PFAS levels were below the respective LOQs (0.5 µg/kg for serum, organs and excretions, respectively; 0.005 µg/kg for milk). In group E, PFAS levels increased in serum, milk, urine and feces during exposure and declined during depuration, e.g., milk peaked at 64.0±15.2 µg/kg and declined to 5.9±2.4 µg/kg, respectively. Highest mean PFAS levels occurred in organs liver (418.7±82.7 µg/kg) and kidney (49.0±22.7 µg/kg). In milk, the transfer rates (TRs) increased with increasing chain length, reaching maxima for perfluorooctane sulfonic acid (n-PFOS: 15.5±4.8%; br-PFOS: 11.8±4.6%) and n-perfluorodecanoic acid (PFDA: 12.6±4.2%), before declining again. Br-PFAAs consistently showed lower TRs than the linear isomers. A comparable trend was not observed for calculated half-lives (DT50). DT50 were longest for br-perfluoroundecanoic acid (PFUNDA: 89.6±33.1 d) in milk, and for PFOS in serum (n: 25.7±1.4 d; br: 26.0±0.6 d). For most compounds, the long exposure period appeared sufficient to reach near steady-state TRs, suggesting that the estimated values are reliable for modelling purposes.

Conclusions: PFAAs accumulated in organs and serum and were excreted depending on their physicochemical properties, particularly long-chain PFAAs. N- and br-PFOS showed the highest transfer into milk, edible tissues and serum. The proposed PBTK model provides a tool to simulate PFAA kinetics and excretion and aims to provide quantitative predictions of accumulation and depuration of PFAAs in organs and tissues of dairy goats.

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A systematic review and meta-analysis on the use of lupins in fattening pig diets

Ein systematischer Review mit Meta Analyse über den Einsatz von Lupinen in der Mastschweinefütterung

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Introduction: The European livestock sector is heavily reliant on imported protein sources, mainly soybeans, and the search for alternatives is ongoing. Lupins have a high crude protein content, thus being considered an alternative to soybeans for feeding livestock. However, there is uncertainty regarding the safe inclusion rates of lupines to porcine diets. This is partly due to the fact that lupins contain various antinutritional factors (ANF), including quinolizidine alkaloids (QA) [1]. Feed refusal, various gastrointestinal symptoms and even death have been linked to QA intake in pigs after consumption of lupins high in QA. Due to limited data, it has not yet been possible to derive a NOAEL value for QA in farm animals other than salmonids. [1]. Therefore, published literature was reviewed to investigate inclusion rates for lupines and QA in porcine diets and determine whether feed intake or growth performance of pigs could be linked to these factors.

Methods: The systematic review was conducted according to PRISMA guidelines [2]. The review protocol was registered prior to the literature search and contains information about the search strategy, eligibility criteria, risk of BIAS assessment and data extraction [3]. All steps of the systematic review were performed by two reviewers, while a pre-selection of studies was conducted by a single reviewer. This preselection excluded all studies that did not investigate pigs or report in a language other than German or English. The investigated population was limited to growing pigs with intact gastrointestinal tracts that were fed diets containing the most commonly used lupin species (*L. albus*, *L. angustifolius*, *L. luteus*). It was imperative that control groups were fed diets that were isonitrogenous, isocaloric and free of lupins. The outcomes, namely feed intake (FI), average daily gain (ADG), and feed conversion ratio (FCR), were evaluated. Extracted data was used for meta-analysis and meta-regression using the R-package 'metafor'.

Results: The literature search delivered more than 5000 publications, of which 38 met the prespecified criteria. Included studies originated mainly from Polish, Australian and Czech institutions and were published over the last 50 years. Risk of Bias analysis showed at least medium risk of BIAS for all studies mainly attributed to a lack of concealing, blinding, and randomization. Based on previously defined criteria, a total of 207 experimental groups were identified. However, a value of dispersion was not reported for FI in 32 % of cases, for ADG in 10 % of cases and for FCR in 42 % of cases. If the respective value was not reported, the experimental group was not included in the subsequent meta-analysis. The meta-analysis demonstrated that pigs' diets containing lupins had a lower ADG compared to pigs fed control diets (- 17 g/day; $p = 0.007$). Furthermore, with increasing inclusion rate of lupins, ADG (-1.89 g/day, $p = 0.025$) and FI (- 3.35 g/day, $p = 0.042$) decreased, while FCR increased (0.004, $p = 0.039$). However, elevated FI (90.3 g/day, $p = 0.001$) and ADG (34.8 g/day, $p = 0.015$) were observed if *L. luteus* was included in the diet, as compared to diets containing *L. albus*. The QA concentration was not reported in one third of the experimental groups and was below 220 mg/kg DM for 95 % of the groups. Respective meta-regression failed to identify an effect of QA on investigated outcomes.

Conclusions: The findings suggest that effects of lupins as a protein source in porcine diets on feed intake and growth performance may – based on available literature data – depend on the lupin species used and cannot be generalized. Based on the retrieved data a conclusion on the effect of lupin QA on animal performance cannot be stated and should be further investigated.

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Effect of caustic soda treatment of lupin seeds on quinolizidine alkaloid concentrations – preliminary results

Effekt der Behandlung von Lupinenkörnern mit Natronlauge auf die Konzentration von Chinolizidinalkaloiden – erste Ergebnisse

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Introduction. In lupins, quinolizidine alkaloids (QAs) are considered as antinutritional factors that limit their use in animal feeding. Moreover, food of animal origin (e.g., cow milk) can be a potential route for QAs to enter the food chain [1]. Alkaline treatment of feeds is mainly associated with an improved preservation and can be performed on farm with limited technical requirements [2]. In lupins, it also may eliminate QAs when combined with cooking [3]. The objective of the study was to test whether QAs in lupin seeds are susceptible to caustic soda without further hydrothermal treatment.

Methods: The white sweet lupin cultivars “Frieda” (WLF) and “Celina” (WLC) and the blue sweet lupin cultivar “Carabor” (BLC) were used in this preliminary test. All samples were taken from the 2024 harvest season. The air-dry seeds were cracked in a Thermomix TM6 unit using the Turbo setting for 5 s. Caustic soda treatment was carried out following the Sodagrain procedure proposed by Ørskov et al. [2]. In brief, the cracked seeds were mixed with 35 g/kg air-dry weight sodium hydroxide microbeads (technical grade) in the Thermomix at level 1 counterclockwise for 10 min. Then, deionized water was added at 25 % ratio and mixed for another 5 min. The treated samples were stored open for 48 h under a laboratory outlet and then frozen at -20 °C for further analyses. The pH of treated samples was measured using a WTW Multi 3630 IDS device and a WTW SenTix® 980 electrode. The samples were dried to constant weight to determine dry matter (DM) content. The concentrations of QAs were determined before and after treatment by HPLC-MS/MS analysis. In this preliminary test, statistical analysis of the data was not performed.

Results: The pH of the treated lupin seeds was 12.0 (WLF), 12.0 (WLC) and 12.4 (BLC). The total QA concentration, which is the sum of albine, anagryrine, augustifoline, cytisine, lupanine, α -isolupanine, 13-hydroxylupanine, lupinine, multiflorine and sparteine, was 0.46, 0.23 and 0.19 % of DM in WLF, WLC and BLC, respectively. The lupin cultivars contained 570 to 3800 mg/kg DM lupanine, 80 to 150 mg/kg DM α -isolupanine and 200 to 690 mg/kg DM 13-hydroxylupanine. Caustic soda treatment induced a reduction of the concentration of lupanine by 40 (WLF), 11 (WLC) and 5 % (BLC), α -isolupanine by 39 (WLF), 66 (WLC) and 20 % (BLC) and 13-hydroxylupanine by 20 (WLF), 40 (WLC) and 13 % (BLC), respectively. Response to the treatment in other QAs was not congruent among the cultivars. Whereas some QAs were slightly reduced by the treatment, the overall reduction of the total QA concentration was less clear. These results must be interpreted with care as long as no repeated measurements exist.

Conclusions: The results indicate that caustic soda treatment of sweet lupin cultivars may reduce QAs lupanine, α -isolupanine and 13-hydroxylupanine. However, the concentration of some other QAs including the potent toxin sparteine occasionally increased. It cannot be ruled out that metabolites may also be formed that are not currently measurable and whose toxicity is unknown. The method needs further investigation before any recommendation for on-farm application can be derived.

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Transfer of per- and polyfluoroalkyl substances (PFAS) from feed into edible tissues of chicken for fattening (ROSS308)

Transfer von Per- und Polyfluoralkylsubstanzen (PFAS) aus dem Futter in essbare Gewebe und Organe von Masthähnchen (ROSS308)

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Introduction: Perfluoroalkyl acids (PFAAs), a sub-group of per- and polyfluoroalkyl substances (PFAS), are extremely persistent environmental contaminants that bioaccumulate along the soil–plant–feed–livestock–food pathway. Due to their adverse health effects, European maximum levels (MLs) for PFAAs in foods of animal origin have been established [1]. For chicken meat and offal, the limits are 1.3 and 8.0 µg/kg for the sum of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS). To determine the PFAS content in feed and the feeding duration that may lead to exceedance of the EU MLs, a transfer study was conducted in chickens for fattening.

Methods: A total of 80 newly hatched chickens (ROSS 308) were assigned to five exposure groups (E1–E5) with ten animals each and two control groups with 15 animals each (C1 and C2). All animals were fed *ad libitum* with a commercial pelleted feed containing 10% herring fishmeal. The fishmeal for control diets contained background levels of PFAS with a total 1.6 µg ΣPFAS/kg 88% dry matter (DM). The fishmeal for the exposure groups contained a total of 53.7 µg ΣPFAS/kg 88% DM, mainly PFOS (25.6 µg/kg), PFNA (20.1 µg/kg) and PFOA (6.7 µg/kg). The contaminated complete feed contained a total of 6.1 µg ΣPFAS/kg 88% DM. Groups E1 to E5 received the contaminated feed for 15, 22, 27, 31 and 35 days from hatching over the 35-day fattening period, respectively. At the end of each subperiod, five animals per group were slaughtered to determine PFAS content in liver, serum, breast muscle, and kidneys. The remaining animals were switched to control feed and kept until slaughter on day 35. Five control animals were slaughtered alongside the exposure group at each slaughter time. Samples were analyzed for PFAS by LC-MS/MS. Kruskal-Wallis test or ANOVA were used for statistical evaluation depending on data distribution, followed by the respective Bonferroni-corrected post-hoc test.

Results: PFAS levels in liver, kidney and muscle of the control group remained below the limit of quantification (LOQ 0.5 µg/kg), whereas background levels of PFOS in the control fishmeal likely caused slightly elevated PFOS levels in serum. The PFAS intake of the exposed groups E1 to E5 resulted in significantly increased PFAS contents in organs and serum with increasing exposure duration ($p < 0.05$). PFAS accumulated in the order kidney > liver >> muscle tissue. In liver and kidney, PFOS, PFOA, PFNA, perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUNDA) were detected, while serum additionally contained PFHxS. Serum showed the clearest exposure-related increase up to 29.7±1.4 µg/L. In muscle tissue, only PFOS and PFNA were quantifiable, indicating tissue-specific accumulation patterns. After ten days of exposure (group E1: 4.3 µg/animal PFAS intake), PFAS levels exceeded the EU MLs for meat and offal (muscle tissue: 1.8±0.1 µg/kg; kidney: 22.4±3.5 µg/kg; liver: 13.0±1.9 µg/kg), respectively. After 22 days of exposure and 13 days of depuration (group E2: 8.8 µg PFAS intake), kidney PFAS still exceeded the EU ML for offal (10.8±1.2 µg/kg). After 27 and 31 days of exposure and eight and four days of depuration (group E3: 11.9 µg and group E4: 16.4 µg PFAS intake), liver and muscle tissue PFAS still exceeded the EU MLs for offal and meat (liver: 10.8±1.2 µg/kg; muscle: 1.9±0.3 µg/kg), respectively. After 31 and 35 days of exposure, PFAS levels in all matrices stabilized or slightly decreased, suggesting a steady state. PFAS levels declined uniformly across all tissues from exposure end to depuration end by 81±9% (E1), 60±2% (E2), 37±5% (E3), and 24±2% (E4).

Conclusions: Short-term exposure to PFAS-contaminated fishmeal, commonly used for animal nutrition, led to exceedance of EU MLs for PFAS in chicken meat and offal. The results of this study are highlighting the need to define safe feed levels for consumer protection.

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Effects of ensiling jimsonweed (*Datura stramonium* L.) in maize on tropane alkaloid concentrations

Effekte der Silierung von Stechapfel in Mais auf die Konzentration von Tropanalkaloiden

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Introduction: *Datura stramonium* is a plant of the Solanaceae family with high spreading potential. It has been shown that *D. stramonium* is well adapted to survive inhospitable climatic conditions such as drought [1]. According to the Global Biodiversity Information Facility (GBIF) database, the frequency of *D. stramonium* occurrence is increasing in Germany and beyond and it can be observed that the plants grow in the periphery of maize fields and other crops. The plants produce tropane alkaloids, mainly (-)-hyoscyamine and (-)-scopolamine, which evoke intoxication both in humans and animals [2, 3]. Through the contamination of feeds such as maize silages, *D. stramonium* easily enters the food chain. Direct food contamination or indirect contamination through the transfer of the toxins into animal products can pose a risk for the health of consumers [2, 3]. The objective of this study was to test whether the tropane alkaloids (-)-hyoscyamine and (-)-scopolamine present in *D. stramonium* are affected by fermentation in maize silage.

Methods: Chopped maize was either uncontaminated (control) or mixed with 10% dried and ground *D. stramonium* plants on a dry matter (DM) basis and ensiled in 1.5 L WECK jars (3 jars per time point at $t > 0$ h). BIO-SIL[®] was added to obtain 3×10^5 colony forming units of *Lactobacillus plantarum* DSM 8866 and 8862 per gram fresh matter. The jars were stored at 19 °C and 30% humidity. Samples were taken after $t = 0$ h (reference sample), $t = 30$ days and $t = 90$ days, respectively. The DM content and the pH were determined in accordance with VDLUFA methods 3.1 and 18.1, respectively. Organic acids and ethanol were determined by liquid chromatography (LC). Tropane alkaloid concentrations were analysed in dried samples using a QTRAP[®] 6500+ LC-MS/MS system with Hypersil Gold[®] C18 column (150 × 2.1 mm; 1.9 µm particle size). Statistical analysis was carried out using a series of one-sample t-tests in the TTEST procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for (-)-hyoscyamine, (-)-scopolamine or the sum of both and duration of ensiling (30 or 90 days). Gaussian distribution of studentized residuals was verified using the Shapiro-Wilk test. Differences to the reference sample with $p < 0.05$ were considered significant. All values are given as mean and standard deviation.

Results: The DM content of reference samples at $t = 0$ h was 393 (control) and 402 g/kg (contaminated), respectively. The silages had a pH of 3.9 both at $t = 30$ days and $t = 90$ days. The control silages contained 55.3 ± 2.28 and 54.9 ± 2.66 g/kg DM lactic acid, 6.5 ± 0.33 and 6.8 ± 0.29 g/kg DM acetic acid and 12.0 ± 3.33 and 15.2 ± 2.21 g/kg DM ethanol, the contaminated silages 59.1 ± 0.625 and 56.6 ± 2.49 g/kg DM lactic acid, 8.5 ± 0.21 and 8.4 ± 0.63 g/kg DM acetic acid and 8.8 ± 1.6 and 13.5 ± 2.60 g/kg DM ethanol after 30 and 90 days, respectively. (-)-Hyoscyamine and (-)-scopolamine were not detected in the control silages. At $t = 0$ h, the contaminated maize contained 107 ± 4.31 mg/kg DM (-)-hyoscyamine and 14.9 ± 0.949 mg/kg DM (-)-scopolamine. (-)-Hyoscyamine decreased to 92.4 ± 2.44 mg/kg DM after 30 days ($p < 0.05$) and to 85.1 ± 9.45 mg/kg DM after 90 days ($p = 0.083$). (-)-Scopolamine increased to 15.5 ± 1.72 mg/kg DM ($p = 0.686$) and 17.5 ± 1.51 mg/kg DM ($p = 0.131$) after 30 and 90 days, respectively. The sum of the alkaloids decreased from 122 ± 5.26 to 108 ± 2.93 ($t = 30$ days; $p < 0.05$) to 103 ± 11.0 mg/kg DM ($t = 90$ days; $p = 0.132$).

Conclusions: The results indicate that (-)-hyoscyamine is partly degraded during ensiling. However, (-)-scopolamine is thought to be more potent than (-)-hyoscyamine, at least in the central nervous system, and is not degraded during ensiling. Even though the overall concentration of tropane alkaloids slightly decreased, silage contaminated by *D. stramonium* may be a source of exposure and a potential risk to animal health and possibly also to the consumer through the transfer of tropane alkaloids into food of animal origin.

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Impact of replacing starchy feeds by industrial by-products on performance and net food production of high-yielding dairy cows

Einfluss des Austausches stärkehaltiger Futtermittel durch Nebenprodukte auf Milchleistung und Nettolebensmittelproduktion bei Hochleistungskühen

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Introduction: High-yielding dairy cows are typically fed diets containing large amounts of starch-rich components in order to meet the requirements for dietary energy, especially during early and high lactation. However, this feeding strategy does not only challenge the animals' gut health, but also reduces the sustainability of milk production in terms of feed-food competition. Indeed, starchy feeds are characterized by high proportions of human-edible biomass that could be directly utilized in human nutrition. The replacement of starchy feeds by industrial by-products (BP) may therefore represent a viable option to reduce feed-food competition since BP possess only low amounts of human-edible biomass and arise unavoidably during the processing of plant raw materials [1]. In the present study, a feeding trial was conducted to investigate the effects replacing starch-rich components by BP in dairy cow diets on performance, health and net food production. It was hypothesized that feeding a BP-rich diet to high-yielding dairy cows maintains milk yield compared to a grain-rich diet, while being characterized by a positive human-edible feed conversion efficiency (heFCE) and improved rumen health.

Methods: 58 dairy cows were selected for a feeding trial at Hofgut Neumühle. All cows were fed with the same BP diet (<20% starch and sugars/kg DM, dominated by BP) over three weeks [BP1], following of a grain-rich diet (>23% starch and sugar/kg DM, dominated by grains) over six weeks [STARCH], following by a second three weeks lasting BP diet [BP2]. The diets were formulated to be isoenergetic and isonitrogenous and mainly differed in the concentrate composition, with the BP diet being rich in molasses pellets (31.3 %) and low in wheat (4.3 %), whereas the STARCH diet was low in molasses pellets (2.6 %) and high in wheat (35.4 %, all values in % DM). Feed intake, milk performance characteristics and net food production was determined in all cows. The sustainability of each ration was evaluated based on human-edible feed conversion efficiency (heFCE), which was calculated as the ratio of human-edible output from animal products (milk and potential beef) to human-edible feed input under three different scenarios of human-edible nutrient recovery (low, medium, high) [2]. Data were analyzed in a linear mixed model. Experimental phase was included as a fixed effect, while cow was included as a random effect. Days in milk (DIM) and its quadratic term were included as covariates to account for changes in milk production. For dry matter intake (DMI), DIM was included as a categorical variable (DIM class). Repeated measurements within cow were modeled using an autoregressive correlation structure.

Results: DMI was slightly reduced during the starch-rich diet (26.3 kg/d) compared with BP 1 (26.7 kg/d; $p = 0.016$), whereas no differences were observed between STARCH and BP2 (26.3 kg/d; $p > 0.05$). Milk yield was lower during the STARCH diet (38.8 kg/d) compared to the first BP-phase (BP1: 39.7 kg/d; $p = 0.002$) and the second BP-phase (BP2: 39.8 kg/d; $p = 0.002$). No differences were observed between BP1 and BP2 ($p = 1.00$). Energy-corrected milk was reduced from the BP1 to the STARCH period (BP1: 42.3 kg; STARCH: 39.9 kg; BP2: 41.2 kg; $p < 0.05$). Milk fat content decreased from BP1 (4.49 %) to the starch-rich diet (4.27 %) and remained lower during BP2 (4.22 %), with BP1 differing from both STARCH and BP2 ($p < 0.01$). Milk protein content was increased during the grain-rich feeding regime (BP1: 3.55 %, STARCH: 3.66 %, BP2: 3.58 %; $p < 0.001$).

Conclusions: Feeding high yielding dairy cows with different qualities of carbohydrates from feeds with contrasting human-edible proportions affect DMI, milk yield and milk composition. Overall, the milk yield was on a high level and the coming analyses on net food production characteristics will provide valuable insights on the potential of using BP to increase the sustainability of milk production.

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Effects of algae β -glucan supplementation on the performance and intestinal immune system of weaned piglets

Auswirkungen einer Supplementierung mit Algen- β -Glucan auf die Leistung und das intestinale Immunsystem von Absetzferkeln

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Introduction: While numerous studies have investigated β -glucans from plants and yeasts in weaned piglets [1], studies examining β -glucans derived from algae are scarce. The present study aimed to examine the effects of dietary supplementation with β -glucan from the microalgae *Euglena gracilis* on growth performance, apparent total tract digestibility (ATTD) of nutrients, gut morphology, gut integrity parameters, the gut immune system, and the abundance of selected bacterial groups in weaned piglets.

Methods: A total of 66, male, 21-day-old weaned crossbred piglets weighing 6.68 ± 0.99 kg (mean \pm SD) were allotted to three experimental groups in 22 pens with 3 piglets each (conducted in two runs). Pigs were fed either a nutrient-adequate basal diet (control group) or the basal diet supplemented with either 100 g/t (A100 group) or 200 g/t (A200 group) of a β -glucan product from *E. gracilis* for 35 days. At slaughter, fecal samples, small intestinal segments, proximal colon and digesta of mid jejunum and proximal colon and mucosa of mid jejunum and proximal colon were collected for determination of ATTD of nutrients, histological analysis of gut morphology (villus length (VL), crypt depth (CD), VL:CD ratio), gene expression analysis (inflammation, nutrient transport, tight-junctions) and analysis of short-chain fatty acids (SCFA) and selected bacterial groups in the colon digesta. The data were analysed by two-way ANOVA with the fixed factors treatment and experimental run and their interaction, using the general linear model procedure in Minitab statistical software.

Results: Final body weight, daily body weight gain, feed intake, feed:gain ratio, ATTD of nutrients, and gut morphology metrics (VL, CD, VL:CD ratio) in duodenum, mid jejunum and proximal jejunum at the end of the trial did not differ across the three groups. In addition, the mRNA levels of genes involved in inflammation (CCL2, ICAM1, IL8, TNFA), nutrient transport (GLUT2, GLUT5, SGLT1, PEPT1), and tight-junction proteins (CLDN3, OCLN, ZO1) in the jejunum mucosa were not different across the groups. The mRNA levels of genes involved in inflammation and tight-junction proteins with the exception of ICAM1 in the colon mucosa did not differ across the groups; that of ICAM1 was higher in the A100 group and the A200 group than in the control group ($P < 0.05$). The number of intraepithelial lymphocytes, goblet cells as well as lymphocytes, eosinophils and neutrophils in the lamina propria in the proximal jejunum were not different across the groups. In the mid jejunum, the number of lymphocytes in the lamina propria was higher in the A200 group than in the other two groups ($P < 0.05$), whereas the number of the other immune cells did not differ across groups. Mucosa score for villous stunting in the proximal jejunum tended to be lower in the A100 group than in the other two groups ($P < 0.1$), whereas mucosa scorings for mucosa fibrosis and villous epithelial injury in the proximal jejunum and scores for mucosa fibrosis, villous stunting and villous epithelial injury in the duodenum and the mid jejunum were not different across the groups. The concentrations of the main individual (acetate, propionate, butyrate) and total SCFA were not different across the groups. While the abundance of *Prevotella spp.* was lower in the A200 group than in the other two groups ($P < 0.05$), the abundance of *Bifidobacterium spp.*, *Clostridium-leptum*-group, *F. prausnitzii* and *Lactobacillus spp.* as well as the prebiotic and the microbial balance index in the colon digesta did not differ across groups.

Conclusions: Algal β -glucan supplementation at 100 to 200 g/t in healthy weaned piglets has no effect on growth performance and only marginally influences gut morphology, gut integrity, gut-associated immune function, digestibility, and gut microbial community structure. Future research should investigate whether algal β -glucan supplementation might yield beneficial outcomes in weaned piglets under pathogen challenge conditions.

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Effects of different carbohydrates substituting dietary lactose on metabolism in formula fed calves

Einfluss verschiedener Kohlenhydrate als Ersatz für Laktose auf den Stoffwechsel von Kälbern

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Introduction: Lactose represents one of the major nutritional factors in milk fed calves. Since the synthesis of this disaccharide in the mammary gland is complex and requires energy, the question arises how lactose could endure in evolution. In addition, lactase is necessary to cleave lactose in the small intestine into its absorbable monosaccharides glucose and galactose. We investigated the endocrine response on different carbohydrates in milk as a substitute for lactose in formula fed calves.

Methods: Male and female calves (n = 38; age: 16 ± 1 days; body weight: 53.7 ± 1.0 kg) were assigned to 5 different experimental diets (4 kg formula per meal, 2 meals/day) fed for 3 consecutive days. The composition of the milk formula was similar to whole milk (WM; 4% fat, 3,4% protein, 4,7% carbohydrates) and contained the following carbohydrates added to a milk replacer, which was based on reconstituted protein extract with added milk fat: lactose (LAC); glucose (GLU), galactose (GAL), glucose + galactose (G/G) and xylose (XYL). On the last day of the treatment blood samples were taken from the jugular vein, right before the first feeding (T1), two (T2) and seven (T3) hours later. Concentration of glucose, triglycerides (TG), insulin, glucagon, and insulin-like-growth-factor-1 (IGF1) was determined in plasma. Data were evaluated using a mixed model (SAS, version 9.4) with treatment, timepoint of sampling and treatment x time interaction as fixed effects and the individual calf as repeated subject. Significance was assumed at $P < 0.05$.

Results: Plasma glucose concentration increased from T1 to T2 in GLU ($P < 0.05$), but not in the other treatments. Triglyceride concentration increased from T1 to T2 in all treatments (LAC, G/G: $P < 0.1$; GLU, GAL, XYL: $P < 0.05$). Insulin levels increased from T1 to T2 in G/G ($P = 0.03$) and GLU ($P = 0.1$), while glucagon concentrations were greater in XYL ($P < 0.05$) and GLU ($P = 0.08$) at T2 and higher in all groups at T3 ($P < 0.05$) at T3 than in comparison to T1. At T1 IGF1 was greater in GAL than in G/G ($P < 0.04$) and in XYL the IGF1 concentration was always lower than in the other treatments (LAC, GLU, GAL: $P < 0.05$; G/G: $P < 0.1$).

Conclusions: In conclusion, we observed differences in the metabolic and endocrine response towards the different carbohydrates of the milk formula. Even though similar gross nutrient composition of the experimental diets the endocrine baseline and response seem to differ between the different carbohydrates. In calves receiving XYL, no postprandial endocrine response was detectable beside increasing glucagon concentration, as xylose is absorbable, but cannot be used in intermediary metabolism.

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The effect of inulin on faecal bacterial metabolites and blood amino acids in healthy sled dogs

Einfluss von Inulin auf bakterielle Stoffwechselprodukte im Kot und auf die Aminosäuren im Blut gesunder Schlittenhunde

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Introduction: The use of inulin as a prebiotic in pet food is already known. In dogs' positive effects of inulin as a prebiotic on overall health, affecting microbial fermentation, leading to the production of beneficial metabolites in the colon, are described [1;2]. Therefore, the aim of this study was to investigate the effects of inulin in a dry complete dog food on faecal microbial metabolites and blood amino acids.

Methods: Two complete dry diets were fed to 6 Malamute and 14 Siberian Huskey adult dogs in a randomized cross-over design over a period of 14 weeks. The diets either contained cellulose (control diet) or inulin. The trial started with a wash in phase of three weeks with the control containing cellulose feed followed by a cross-over of four weeks with 10 dogs in each group. A wash-out phase of three weeks with the control feed containing cellulose was added in between. After each feeding phase faeces was sampled and blood taken, in total at four sampling timepoints. Statistical analyses were performed with a linear mixed model in SPSS with the diet and feeding period as a fixed factor and the dog ID as a subject, $p < 0.05$.

Results: In dogs fed the inulin diet, the concentration of propionic acid was significantly higher compared to the control diet ($p < 0.05$). The cellulose diet, on the other hand, was associated with a slightly higher faecal butyric acid concentration ($p < 0.05$). No other significant effects on microbial metabolites were detected. No significant effects on blood amino acids were identified.

Conclusions: Further studies are needed to evaluate if this is a result of a differences in absorption of short chain fatty acids in the hindgut or differences in the microbiome of the sled dogs fed with the different diets.

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Fat and protein mass in the body of growing bulls grouped by dairy or dual-purpose and beef breeds

Fett- und Proteinmasse im Körper wachsender Bullen, eingeteilt nach Milch- oder Zweinutzungs- und Fleischrassen

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Introduction: The fattening bulls' nutrient and energy requirements changed during the past decades due to changed production processes and selective breeding, either for milk or meat production. Consequently, an update of nutrient requirements of fattening bulls on base of recent data is urgently needed from a scientific and practical perspective. This meta-analysis aimed to generate basic data on the fat and protein mass in growing and finishing bulls.

Methods: The meta-analysis comprises data from 29 studies, published between 1972 and 2025 and dealing with the empty body composition of fattening bulls with 25-736 kg empty body weight (corresponding to 29-787 kg live weight) and 0-737 days of age. Only studies that performed a complete dissection of the animals' bodies followed by chemical analysis of crude fat and crude protein in the body parts were used. The empty body weight (EBW) was defined as the final live weight (including hide, carcass, blood and all organs) minus the contents of the urinary bladder and gastrointestinal tract. The bulls were grouped according to their EBW in increments of 50 kg from 50 to 650 kg EBW. Holstein and Jersey bulls were allocated to breed group "milk production". Angus, Brown Swiss, Charolais, German Simmental, Limousin, Salers and crosses from these breeds were allocated to breed group "meat production". A two-way ANOVA with interaction (breed group, weight range, breed group x weight range) and a mean value comparison analyses differences between bulls in their production groups within the body weight ranges. Statistical analysis was performed using Proc Mixed of SAS (Version 9.4). Results were compared by the PDIFF option with values of $p < 0.05$ regarded as significant. Furthermore, regression modelling and analysis was performed using Proc GLM of SAS to describe the fat and protein mass in the empty body of growing bulls according to their breed groups.

Results: Bulls bred for milk production and weighing 350 kg EBW or more showed higher empty body fat mass than bulls bred for meat production ($p < 0.05$). Contrary, bulls bred for meat production and weighing 450 kg EBW or more showed higher empty body protein mass ($p < 0.05$). The empty body fat and protein masses were described by quadratic regressions, showing a high coefficient of determination. For milk breeds, Fat (kg) = $5.4 - 0.04 \times \text{EBW} + 0.0005 \times \text{EBW}^2$ ($R^2 = 0.96$) and Protein (kg) = $-2.87 + 0.24 \times \text{EBW} - 0.0001 \times \text{EBW}^2$ ($R^2 = 0.98$). This results in 6 and 157 kg fat and 20 and 104 kg protein in bulls with 100 and 600 kg EBW, respectively. For meat breeds, Fat (kg) = $6.95 + 0.01 \times \text{EBW} + 0.0002 \times \text{EBW}^2$ ($R^2 = 0.91$) and Protein (kg) = $-3.63 + 0.22 \times \text{EBW} - 0.0002 \times \text{EBW}^2$ ($R^2 = 0.97$). This results in 11 and 102 kg fat and 18 and 120 kg protein in bulls with 100 and 600 kg EBW, respectively.

Conclusions: Using a large dataset enabled the fat and protein content in the body of growing bulls to be modelled by regressions. These regressions can be used to calculate the fat and protein accretion in different stages of growth in revised requirement derivations. There are marked differences between the genotypes that are preferred for milk or meat production, which suggests that recommendations for energy and nutrient supply should be diversified according to the genotype of the animals.

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Prediction of the energy value in colostrum and transition milk of Brown Swiss cows

Vorhersage des Energiegehaltes von Kolostrum und Transitmilch von Braunvieh Kühen

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Introduction: The energy content of milk is not only important to calculate lactation requirements of cows, but also for adequate provision of calves with liquid feed. Usually, the energy content of milk is estimated using a variety of formulas. One formula often used for estimation is established by NRC (2001): Gross energy (Mcal/kg) = (Fat% × 0.092) + (Protein% × 0.055) + (Lactose% × 0.039). This formula uses standard heat of combustion for the different energy sources in milk to estimate the gross energy of the sample. However, this formula was adjusted by NASEM (2022) with slightly different heat of combustion for the milk components: Gross energy (Mcal/kg) = (Fat% × 0.094) + (Protein% × 0.056) + (Lactose% × 0.04). Transition milk and especially colostrum vary in their composition of these components compared to mature whole milk. Therefore, using the assumption stated for mature milk could lead to an over- or underestimation of energy content in milk during the first week of lactation.

Methods: Milk previously collected and analyzed in Vorndran et al. (2025), consisting of 16 cows (9 multiparous, 7 primiparous) during the first 7 days after parturition, was used for this study. Dry matter was analyzed in a VWR DRY-Line oven according to method C.35.3 Band IV (VDLUFA, 2020). Protein was calculated by multiplication of the analyzed nitrogen concentrations from the Kjeldahl method (VDLUFA, 2020), with the milk protein specific conversion factor 6.38. Fat was analyzed in a Gerhardt Soxtherm apparatus according to the method of Weibull Stoldt (VDLUFA, 2020). Lactose concentration was determined by photometric detection at 340 nm using a certified enzymatic assay. Gross energy of the samples was then calculated using the formula of NASEM (2022) and NRC (2001). Gross energy measurement of milk samples was conducted using small stamped out proportions of freeze dried milk. This was necessary since the fat concentration in milk did not allow for the pressing of small tablets which are used in standard bomb calorimetric measurements. The stamps were weighed in combustible bags with known energy content and measured in a IKA C2000 basic bomb calorimeter. The data was analyzed using JASP Version 0.95.3 (Amsterdam, Netherlands) and checked for normality and homogeneity of variance before analysis. The association between the measured and calculated energy contents was determined by Pearson's correlation. Significance was set at $p < 0.05$.

Results: Measurement results and estimates of energy concentration using the calculations were highly correlated at all time-points ($r > 0.5$). The NASEM (2022) estimation was closer to the results of bomb calorimetry than the NRC (2001) formula, especially at milkings 1 and 2. While the values of the NRC (2001) estimation numerically tended to underestimate the energy, the NASEM (2022) estimation tended to numerically overestimate energy contents. On average, the results between measurement and estimation differ by only 0.08 MJ/kg DM with the NASEM (2022) formula, but differences of up to 6.012 MJ can be seen in individual samples.

Conclusions: The used method of stamping out a portion of the freeze dried sample worked well compared to the calculated estimates from the equations. Both estimations can be used to estimate energy levels with similar accuracy to that measured for transition milk (> day 2) and mature milk of Brown Swiss cows. However, while the average accuracy is good, there are relatively large differences between estimates and measurements in individual samples. Analyzing energy content of colostrum and early transition milk (day 1), only the NASEM (2022) estimation or measuring with a bomb calorimeter seem to be suitable to determine energy content.

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Investigation of free water intake of dairy calves via water buckets or bowls within the first two weeks of life

Untersuchung zur freien Wasseraufnahme von Milchvieh-Kälbern über Wassereimer oder -schalen in den ersten zwei Lebenswochen

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Introduction: Sufficient water intake is essential for the health and well-being of dairy calves. For newborn calves, milk represents the primary source of water, while voluntary free water intake from open surfaces remains relatively low. However, research has shown that early access to free water can increase overall milk consumption, promote long-term daily weight gain and body development, and influence gut microbiota composition [1, 2]. In West Germany, the most common method of providing free water is through open buckets or bowls [3]. Yet, calves' preference between these two methods has not been evaluated so far. Therefore, the aim of this study was to investigate the preference for free water intake of individually housed dairy calves during the first two weeks of life, comparing buckets and bowls, and to assess their overall water intake.

Methods: The study was conducted at the Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, from October to November 2023. The study followed 19 male and female Holstein Friesian calves from birth until the age of 14 days. Calves were separated from the dam within the first hours after birth, weighed, and received at least 3 L of colostrum checked for quality by using a specific gravity spindle and a digital Brix refractometer. Calves were fed with transit milk of the dam for the first 5 days and afterward with milk replacer (concentration of 140 g/L) twice a day (5 L per feeding). They had *ad libitum* access to a dry TMR, and hay. Free water was offered in a black bucket (12 L maximal volume) and a dark green water bowl (6 L maximal volume) attached to the enclosure, which were daily refilled with 6000 and 3000 g of water, respectively. Transit milk or milk replacer consumption, water intake, and soiling of free water were evaluated daily. Additionally, calf health was assessed weekly using a calf health scoring scheme (18 parameters), and calves were weighed on day 14 to calculate average daily gain. Calves' behavior was evaluated three times per week for the first 30 minutes after changing free water directly by the same trained researcher observing the calves visually and assessing the appearance of 14 different behaviors. Analyzing the effects on free water and total water consumption, a one-factorial ANOVA was conducted, and effects on soiling of the water bucket and bowl were analyzed by using Kruskal-Wallis in Excel. Furthermore, Pearson correlations between evaluated parameters were calculated.

Results: The average (\pm SD) daily water intake for the first two weeks of life was 7038 ± 1973 g of water (6700 ± 1867 g from transit milk or milk replacer, 338 ± 359 g free water). Daily water intake—water in milk and free water intake—increased numerically over time. However, the average daily free water intake showed a large variance between the 19 evaluated calves, ranging from 119 to 612 g of free water. No significant difference was found between water intake from water buckets (daily average: 174.2 ± 258.9 g) and water bowls (163.8 ± 204.8 g, $P = 0.67$). Sex showed a tendency to influence daily free water intake, with a higher free water intake in male calves compared to female calves ($P = 0.11$), which was significant for the time between day 7 and 13 ($P < 0.01$). No association of calves' behavior and health and water soiling with free water intake was found.

Conclusions: This study indicated that dairy calves free water intake varied largely between individual calves and increased over time. Even though higher amounts of milk were fed, significant amounts of free water were consumed from the first day of life. Calves showed no significant preference for water offered in water buckets or water bowls, indicating that both methods are accepted by calves, and farmers can make their choice based on other parameters (e.g., handling or price). More research is needed to evaluate possible effects on free water consumption and thereby improve free water provision for newborn calves.

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Nutrient accretion of fattening boars

Nährstoffansatz von Mastebnern

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Introduction: Nutrient requirements of growing animals are primarily defined by their nutrient accretion. Sound knowledge of the requirements is essential to maintain animal health, sustain growth, and avoid excessive nutrient supply. The fattening of intact boars becomes increasingly relevant, but data on the chemical composition of fattening boars and their nutrient accretion are scarce. Most of the available body composition data on fattening pigs is based on studies with castrates and female pigs. Therefore, the aim of this study was to measure the nutrient accretion of fattening boars from 28 to 125 kg bodyweight (BW).

Methods: A total of 75 animals (offspring of Large White (LW) x Landrace (LR) sows (TN 70) mated with LW sires (Tempo)) were sampled at 4 stages: 28 kg BW (n = 20), 60 kg BW (n = 20), 90 kg BW (n = 16) and 125 kg BW (n = 19). The animals were kept in individual pens at the „Versuchs- und Bildungszentrum Landwirtschaft Haus Düsse“. They received pelleted commercial diets, which were offered for *ad libitum* consumption in three feeding phases (28-60 kg, 60-90 kg and 90-125 kg). Individual feed consumption and BW were recorded. The animals were euthanised at the Forschungsinstitut für Nutztierbiologie (FBN, Dummerstorf) and processed into samples representing the whole body of the animals. Nitrogen (N), ether extract (EE), phosphorus (P), calcium (Ca) and amino acid (AA) concentrations in the samples were determined following freeze-drying and further processing at the University of Hohenheim. The concentration of the nutrients in the BW gain was calculated by fitting regressions to the nutrient mass in the animal body against the BW of the animal using proc reg in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results: The mean feed conversion ratio (FCR) over the whole fattening period was 2.1 (n=19) and the average daily BW gain was 1227 g/d (n = 19). Per 1 kg BW gain, 29.2 g N (SE = 0.21), 13.1 g Lys (SE < 0.01), 3.7 g Met (SE = 0.02), 1.6 g Cys (SE = 0.02), 13.1 Leu (SE = 0.07), and 5.9 g His (SE = 0.05) were accreted by the animals. The N accretion was 14% greater than in the literature reviewed by the GfE in 2006 [1] and greater than recently published data of castrates by Beckmüller et al. [2]. In contrast, the amino acid pattern was consistent with values published by the GfE [1]. The higher N accretion was associated with a lower EE accretion compared to the literature. The EE mass in the body followed a quadratic function: $EE \text{ (kg)} = 0.001 \cdot BW \text{ (kg)}^2 + 0.051 \cdot BW \text{ (kg)}$. For example, this equation leads to an EE mass of 12.7 kg at 90 kg BW. At an empty body weight of 90 kg equations of [1] lead to a EE mass of 21.7 kg. This indicates massive differences between contemporary intact boars and standard model accretion data with potentially large differences in the animal's energy and nutrient requirements. The P and Ca accretion was 5.1 g/kg BW gain (SE = 0.04) and 7.8 g/kg (SE = 0.09) BW gain, respectively, consistent with the literature [1].

Conclusions: The results of the current study demonstrate that the body composition and nutrient accretion of contemporary fattening boars differ from published data found in the literature, specifically protein and fat accretion. This highlights the importance of re-evaluating the chemical composition of intact boars from contemporary breeds. Differences in nutrient accretion of the animals affect their requirements, but further studies with a more diverse genetic background of the fattening boars are needed before feeding standards can be revised.

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On-farm assessment of external traits to monitor body condition and nutritional status of milk-deprived and milk-fed calves in the first four weeks after birth

Betriebsbasierte Erfassung äußerer Merkmale zur Beurteilung der Körperkondition und des Ernährungszustands von nicht Milch gefütterten und Milch-gefütterten Kälbern in den ersten vier Lebenswochen nach der Geburt

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Introduction: Adequate milk intake during the early postnatal period is essential for calf development. In cow-calf contact or early group housing systems, where birth and feeding are often unobserved, farmers face challenges in assessing whether calves have consumed sufficient colostrum or milk. Currently, animal nutrition and welfare indicators needed for assessing the body condition and nutritional status of suckling calves are lacking. This study aimed to identify external traits that can be used to assess body condition and nutritional status of suckling calves between days 1 and 28 postpartum.

Methods: To develop practical indicators for assessing body condition and nutritional status in suckling calves, a two-part study was conducted combining expert input and field-based measurements and scorings. First, expert interviews ($n = 11$) with calf professionals from Germany were conducted via Zoom using a structured questionnaire containing 20 rearing specific questions, including visual identification of relevant body regions for a Body Condition Score (BCS-calf). Based on these interviews and a literature review, an evaluation protocol was developed and tested on two Bavarian research farms (BaySG Achselschwang and TUM Veitshof). In study 1, 85 healthy calves were assessed between days 1 and 28 postpartum. The primary focus was on external body measurements in the flank area that were expected to respond rapidly to changes in feed intake, therefore, some parameters ($n = 5$) were recorded in both the milk-deprived and milk-fed states. To minimize handling and enhance the practical applicability of the protocol, selected body condition traits that are expected to change only long-term and the general health status of the calves were assessed once in the milk-fed state. In study 2, 10 Brown Swiss calves were monitored from day 8 to 10 postpartum, with controlled milk intake and repeated flank circumference measurements before and after feeding. Statistical analyses included Kruskal-Wallis non-parametric ANOVA to evaluate age and sex effects, and ROC analysis to determine threshold values for distinguishing nutritional status. The Youden index was used to define optimal cut-offs.

Results: Health parameters of all calves remained within physiological ranges, and no major impairments were observed. In study 1, several external body measurements ($n = 12$) and scorings ($n = 9$) showed significant age-dependent differences. These findings indicate a progressive enhancement of musculoskeletal development and body width in the shoulder and flank area, hindquarters, and pelvic region as animals mature. The ROC analysis identified flank circumference, flank width, and the left and right hunger pits as reliable indicators for distinguishing between milk-deprived and milk-fed states. In contrast, rear body width showed limited diagnostic value. In study 2, flank circumference increased proportionally with the volume of milk consumed. The ROC analysis demonstrated that changes in flank circumference were more accurately detectable at lower milk intake levels ($< 5\%$ of body weight). However, diagnostic performance declined at higher intake volumes ($\geq 6\text{--}10\%$ BW), as reflected by reduced true-positive rates and increased false-positive rates.

Conclusions: This study demonstrated that external body measurements, particularly in the flank region, can serve as practical indicators for assessing the nutritional status of suckling calves in the first month of life. Flank circumference showed strong diagnostic potential, while other traits such as flank width and hunger pits further supported differentiation between nutritional status. Establishing a standardized scoring system for body condition and nutritional status for calves independent of rearing system and feeding management should be a key objective to improve nutritional and welfare assessments.

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Preliminary data on the validation of the RumiWatch System for automated detection of short-term feeding behavior and estimation of feed intake in pastured dairy goats

Vorläufige Ergebnisse zur Validierung des RumiWatch Systems zur automatisierten Erfassung des kurzzeitigen Fressverhaltens und Schätzung der Futteraufnahme bei Ziegen auf der Weide

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Introduction: The RumiWatch System (RWS) has been validated for automated recording of feeding behavior in grazing dairy [1] and beef cattle [2], but no validation of the RWS currently exists for goats. This study aims to evaluate the accuracy of the RWS for detecting short-term feeding behavior of pastured dairy goats using an adapted halter for small ruminants, and to estimate daily herbage dry matter intake (HDMI) from RWS data.

Methods: Six female goats (LW = 51.9 ± 5.0 kg) were kept in a rotational pastoral system without supplementary feed. Artificial microswards (AS) were used to validate RWS detection of short-term feeding behavior within 10-min sessions and to determine bite size [3]. Each session, the goat was offered 59 ± 9 g DM of fresh cut grass (DM content = $36.7 \pm 10.4\%$). Sessions were videorecorded and replicated three times per goat on three consecutive days. Grass DM was measured before and after feeding to calculate DM intake. In addition, the goats wore RWS halters for 24 h, followed by one day without the devices, yielding at least four days per goat. Daily HDMI was calculated by multiplying herbage intake per feeding minute (from AS) with the daily amount of time spent foraging (from RWS). To compare the RWS-derived estimates, three enclosure cages (EC, 1 m² each) were placed on the pasture and allocated every 3–4 days to a new pasture stripe. Herbage DM yield was determined both inside and adjacent to each cage, and used to calculate HDMI on herd-level (6 animals). Video recordings were analyzed by one observer, noting total eating time and number of prehension and mastication bites. Raw data of the RWS was converted using converter versions V0.7.3.36 (V3) and V0.7.4.13 (V4). In total, 14 out of 18 AS observations of 6 goats were obtained for analysis (in four cases, goats refused the offered forage). Agreement between RWS output and video analysis was evaluated using the Spearman's rank correlation (r_s) and the Concordance Correlation Coefficient (CCC; asymptotic approach).

Results: Goats consumed on average 22.5 ± 6.7 g DM per 10-min-period, with a mean bite size of 412.3 ± 112 mg per prehension bite. Of the 600 s observation period, goats spent on average 403 ± 31 s eating, and total bite rate (sum of prehension and mastication bites) averaged 86 ± 8 chews min⁻¹ feeding time. For the determination of feeding time per 10 min session, a low, non-significant correlation and concordance were detected between video analysis and RWS V3 ($r_s = 0.43$, $p > 0.05$, CCC = 0.21), and RWS V4 ($r_s = 0.43$, $p > 0.05$, CCC = 0.30), while negligible relation and concordance was discerned between the two versions ($r_s = 0.16$, $p > 0.05$, CCC = 0.09). For total chews, correlation was low for video analysis and RWS V3 ($r_s = 0.34$, $p > 0.05$, CCC = 0.35), while moderate for RWS V4 ($r_s = 0.55$, $p < 0.05$), but agreement remained low (CCC = 0.22). Similarly, the two converter versions correlated moderately ($r_s = 0.60$, $p < 0.05$), while remaining poorly concordant (CCC = 0.14). Daily HDMI estimated by RWS V3 (1819 ± 216 g DM) correlated significantly with EC measurements (1505 ± 430 g DM, $r_s = 1.0$, $p < 0.05$), while V4 showed a weaker trend (1060 ± 183 g DM, $r_s = 0.9$, $p < 0.1$). For the AS, converter V3 tended to overestimate eating time and chews, occasionally misclassifying non-feeding behavior (e.g., nibbling wooden frames) as feeding, whereas in some cases, V4 often underestimated or mislabeled feeding as rumination.

Conclusions: Both RWS converter versions showed limited accuracy for detecting short-term feeding behavior in goats. However, accuracy improved for long-term intake estimation, particularly with V3, likely because misclassified behaviors (e.g., wood nibbling) are rare in natural pastures. Further refinements like a converter version specifically developed for goats could improve the system's reliability for small ruminants. Additionally, visual inspection of daily chewing activity graphics in RWS may also allow a manual estimation of 24 h feeding duration.

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Skeletal Muscle Myokines, Myogenic Regulators, and Satellite Cell Markers in Dairy Cows from Late Pregnancy to Early Lactation

Myokine, myogene Regulatoren und Satellitenzellmarker in der Skelettmuskulatur von Milchkühen vom Spätstadium der Trächtigkeit bis zur frühen Laktation

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Introduction: During the transition from late gestation to early lactation, dairy cows experience negative energy balance, leading to mobilization of body reserves. Skeletal muscle is central in the homeorhetic adaptation to lactation, providing amino acids for gluconeogenesis, immune function, and milk protein synthesis. Beyond substrate supply, muscle acts also as an endocrine organ via myokines (1). It was unknown whether the mobilization and subsequent regain of muscle mass in the transition period is limited to myofiber size and protein turnover or might concern also activation of the adult myogenic progenitor, the skeletal satellite cells. We therefore examined the expression of myokines, and markers of myogenesis and satellite cells in muscle samples collected during the transition period.

Methods: Biopsies of the semitendinosus muscle were analyzed in two experiments. In Experiment 1, 46 Holstein cows were sampled at weeks (wk) -7 and +4 relative to calving to quantify mRNA abundance of 11 myokines, one transcriptional regulator, and the satellite cell marker paired box 7 (PAX7). During wk 2-3 of lactation, 23 cows underwent incomplete milking (TRT; ~11% reduced yield) to alleviate metabolic load, while the remaining cows served as conventionally milked controls (CON; 2). In Experiment 2, two groups of Holstein cows (n = 19 each) were managed to achieve divergent body condition score (BCS) and backfat thickness (BFT) targets by dry-off at wk -7: high BCS (>3.75; BFT > 1.4 cm) and normal BCS (<3.5; BFT < 1.2 cm; 3). Muscle biopsies were collected at wk -7, +1, +3, and +12, and mRNA abundance of PAX7, myostatin (MSTN), and the myogenic regulatory factors MRF4 and myogenin (MYOG) was determined. The mRNA data were analyzed using repeated measures in SAS (MIXED procedure, v9.3), with treatment, time (wk relative to calving), and their interaction as fixed effects, and cow as a random effect. No treatment or interaction effects were detected in Experiment 1, so groups were combined for final analysis. Multiple comparisons were adjusted using the Tukey-Kramer method. Significance was set at $P \leq 0.05$; $0.05 < P \leq 0.10$ indicated trends.

Results: Between wk -7 and wk +4, mRNA abundance of apelin (APLN), meteorin-like (METRNL), and MSTN declined ($P \leq 0.02$), while follistatin (FST) increased slightly ($P = 0.05$); other myokines, peroxisome proliferator-activated receptor γ coactivator 1-alpha (PGC-1 α), and PAX7 remained unchanged. In the BCS experiment, no group or group \times time effects were detected, except for a trend in MYOG ($P = 0.08$). Across time, PAX7 expression rose progressively to ~2.2-fold above prepartum levels by wk 12, MSTN decreased sharply at wk 1 then returned to baseline by wk 12, and MRF4 increased ~2-fold postpartum and remained elevated, whereas MYOG was stable.

Conclusions: The transition into lactation was marked by downregulation of APLN, METRNL, and MSTN mRNA, alongside a reciprocal rise in FST, suggesting enhanced amino acid mobilization with concurrent muscle-sparing mechanisms. Independent of BCS, early lactation featured MSTN suppression with progressive increases in PAX7 and MRF4, reflecting acute muscle remodeling to meet metabolic demands while preserving regenerative capacity. Collectively, MSTN repression emerges as a hallmark of periparturient muscle adaptation.

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ProBioHuhn: Temporal variation in the organic chicken gut microbiota and influencing factors*ProBioHuhn: Zeitliche Variation im Mikrobiota des Darms von Biohühnern und Einflussfaktoren*

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Introduction: The intestinal microbiota plays a vital role in chicken performance and health [1]. Both host-associated factors, such as genetics and physiology, and other factors, including management and housing conditions, are recognized as key determinants shaping microbiota development [2]. In this study, we characterized the gut microbiota of organically raised chickens from three fattening types across the entire fattening period in Germany. By integrating longitudinal sampling with farm- and fattening-type metadata, this study identifies when during the fattening period, and under which conditions, key shifts in the chicken gut microbiota occur.

Methods: Sampling was conducted throughout the entire fattening period at five defined time points. Meconium samples were collected on the first day of life directly from transport boxes. Cloacal swab samples were collected at approximately 12 and 30 days of age, two weeks after outdoor access, and at the end of the fattening period. A total of 8 farms were visited, covering fattening types of slow-growing broiler, dual-purpose cockerels, and male layer. Each farm was sampled three or four fattening flocks. All samples underwent DNA extraction, and the V1-V2 region of the 16S rRNA gene was sequenced. QIIME2 [3] and the R programming language were used for bioinformatic analysis. Alpha diversity was assessed using the Shannon diversity index, and statistical significance was determined by the Wilcoxon rank-sum test with the Benjamini-Hochberg correction. Beta diversity was based on Bray-Curtis dissimilarities and visualized using Principal Coordinate Analysis (PCoA). Differentially abundant taxa were identified with analysis of compositions of microbiomes with bias correction (ANCOM-BC) with Holm–Bonferroni false discovery rate correction.

Results: A clear temporal shift in chicken gut microbiome was observed throughout the fattening period. Meconium samples had a lower Shannon diversity than cloacal swab samples, indicating the initially low microbial richness and evenness at hatching. Chicks from different hatcheries also differed significantly in Shannon diversity, indicating that early environmental or parental influences may affect microbial colonization. The initial microbiota genera were *Enterococcus*, *Escherichia-Shigella*, and *Clostridium*. During the fattening period, both farm and fattening type significantly influenced microbial diversity and community composition. Over time, *Lactobacillus*, *Ligilactobacillus*, and *Limosilactobacillus* became dominant across samples. Differential abundance analysis revealed an increase in *Lawsonia* after chickens gained outdoor access (P-value < 0.05), indicating potential exposure to environmental or soil-associated microorganisms. Substantial variation was detected between flocks, even within the same farm, highlighting the influence of batch- or management-related factors on microbiota composition.

Conclusions: The hatchery environment may play a key role in shaping the initial gut microbiome while farm conditions, fattening type, and outdoor access further modulate its composition throughout fattening. The marked variation between flocks within the same farm suggests that additional, yet unidentified, factors influence microbial dynamics in chickens.

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Heat stress in rats: Evaluation of the antioxidant characteristics of a wood based complementary feed

Hitzestress bei Ratten: Bewertung der antioxidativen Eigenschaften eines auf Holz-basierten Ergänzungsfuttermittels

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Introduction: Heat stress imposes substantial physiological strain and disrupts oxidative balance in livestock, thereby compromising animal health, welfare, and productivity [1]. Polyphenolic compounds present in wood and bark, particularly lignans and tannins, exhibit well-documented antioxidant and anti-inflammatory properties [2]. This study investigated the effects of a lignan- and tannin-rich complementary feed on growth performance and antioxidant traits in rats as the model animal exposed to thermoneutral and heat stress conditions. We hypothesized that dietary inclusion of wood rich in lignans and tannins would enhance thermotolerance and antioxidant capacity in rats compared to the unstressed control and mitigate the adverse effects of sustained exposure to high ambient temperature.

Methods: A 3×2 factorial design was applied to test the impact of increasing concentrations of wood rich in lignans and hydrolysable tannins (0, 0.75, 1.5 g/kg feed as fed) in a semi-synthetic AIN-93G diet [3] under two continuous temperature levels (normal temperature, 23°C, vs. heat stress, 32°C). Forty-eight male Sprague Dawley rats (184.7 ± 2.5 g body weight) were housed in pairs and assigned to six treatments for 21 days. Experimental diets (16.2 MJ ME/kg, 176 g CP/kg) and water were provided *ad libitum*. Performance variables (average daily gain [ADG], average daily feed intake [ADFI], and feed conversion ratio [FCR]) were recorded weekly, and subcutaneous body temperature was monitored daily via subcutaneously implanted transponders. On day 22, the rats were euthanized, and intestinal and hepatic tissues were collected for analyses of lipid peroxidation (TBARS assay), total antioxidant capacity (DPPH assay), and the enzyme activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). Statistical analyses were performed using the MIXED procedure of SAS 9.4. The statistical model included diet, temperature, and their interaction. Least square means (LS-means) were compared using the Tukey–Kramer test, with significance declared at $p < 0.05$ and trends at $0.05 \leq p < 0.10$.

Results: Dietary treatment had no significant effect on overall ADG, ADFI, or FCR ($p > 0.05$); however, in week 1, rats receiving the high-wood inclusion diet showed a smaller reduction in ADG under heat stress, whereas by week 3, their ADG decline exceeded that of the control group. Heat stress significantly reduced growth performance parameters and increased body temperature. Notably, rats receiving the high wood diet exhibited a significantly lower mean subcutaneous body temperature compared to the other groups ($p < 0.05$), indicating improved thermoregulation. Although heat stress increased manganese SOD activity in both intestinal and hepatic tissues ($p < 0.05$), total antioxidant capacity was reduced in the liver. Despite these changes, lipid peroxidation levels in both tissues remained unaffected ($p > 0.05$). Dietary wood rich in lignans and tannins did not significantly affect antioxidant enzyme activities or total antioxidant capacity in intestinal or hepatic tissue ($p > 0.05$), and no diet × heat stress interactions were observed.

Conclusions: Dietary supplementation of heat-stressed rats with wood rich in lignans and tannins did not improve overall growth performance or antioxidant capacity but promoted body cooling. These findings suggest that such complementary feed may support thermoregulation under heat stress. However, the absence of effects on lipid peroxidation and antioxidant parameters highlights the need for further research to determine optimal dietary inclusion levels.

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Impact of 3-Nitrooxypropanol on methane production, blood biochemical parameters and immune response in dairy cows throughout an entire lactation

Auswirkungen von 3-Nitrooxypropanol auf die Methanproduktion, die biochemischen Blutparameter und die Immunreaktion von Milchkühen während der gesamten Laktation

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Introduction: The feed additive 3-nitrooxypropanol (3-NOP) has shown proven efficacy in lowering methane emissions from ruminants for on average 30% [1]. However, its methane-reducing efficacy depends on factors such as diet composition and the frequency of 3-NOP administration. To date, limited research has addressed potential long-term effects of 3-NOP, its practical application on pasture-based systems, or potential archaea-mediated effects on the immune system. Therefore, the aim of this study was to evaluate the effects of 3-NOP supplementation over an entire lactation within a forage-based feeding system, which includes night grazing during summer, focusing on animal performance, methane production, fecal microbiota, and blood biochemical and immunological parameters.

Methods: The experiment spanned 12 months and involved 28 Holstein cows. The cows began the experiment in early lactation and completed it either in the dry period or early lactation of the subsequent calving. The experiment was divided into four 3-month experimental periods (P1-P4). During P1 and P2, cows were housed in the barn and fed a partial mixed ration (PMR) *ad libitum*. In P3, all cows grazed at night and were housed in the barn during the day with PMR provided. During P4, cows were again housed in the barn and fed PMR *ad libitum*. Throughout the experiment, half of the cows (3-NOP, n=14) were offered 3-NOP via concentrate, administered up to 5 times daily (targeted 80 mg/kg feed dry matter (DM)). The control group (CON, n=14) received the same concentrate without 3-NOP. Feed intake (except in P3), milk yield, and methane production (via the GreenFeed® system) were continuously recorded. Ruminal fluid and blood samples were collected monthly to analyze volatile fatty acids and biochemical parameters. Fecal and additional blood samples were taken every three months and analyzed by flow cytometry for microbiota and immune cells, respectively. Data were analyzed using linear mixed models with treatment, experimental period, their interaction, and days in milk as fixed effects, and animal as a random effect.

Results: The 3-NOP concentration in the concentrate decreased over time, with 1691, 1583, 1248 and 1269 mg/kg DM in P1, P2, P3 and P4, respectively. Total dry matter (DMI) and NDF intake was greater in P1 and P2 than P4 ($P < 0.001$). Non interaction occurred for DMI ($P = 0.78$) but for NDF intake ($P < 0.003$). Daily 3-NOP intake varied between periods ($P < 0.001$) and was highest in P1 and P2 (1340 mg/d), followed by P4 (1050 mg/d), and lowest in P3 (561 mg/d). Milk yield varied by period ($P < 0.001$) and was unaffected by treatment and interaction ($P \geq 0.45$). The 3-NOP cows had, varying in its magnitude by period, lower ruminal acetate and greater propionate and n-butyrate percentage than CON cows (interaction: $P \leq 0.025$). Total methane production and methane yield per kg of DMI were lower in 3-NOP than CON cows (on average -8% and -12%, respectively; $P \leq 0.013$), most pronounced in P4 (-9% and -18%, respectively; interaction: $P \leq 0.041$). All biochemical blood parameters, except creatinine, varied by period ($P < 0.001$). Monocytes, NK cells and CD4 T cells varied by period ($P \leq 0.002$). All blood biochemical and immunological parameters were unaffected by treatment and interaction ($P \geq 0.084$). The 3-NOP cows had lower fecal archaea abundance and a higher percentage of IgA-coated archaea compared to CON cows ($P \leq 0.012$).

Conclusions: The supplementation of 3-NOP in a forage-based diet over an entire lactation had no effect on feed intake and milk production, shifted the ruminal fermentation profile, and reduced methane emissions without loss of efficacy over time. The relatively low methane reduction is likely explained by the high dietary NDF content, the interval-fed 3-NOP, and partly by the over time decreasing 3-NOP concentration in the concentrate. A possible IgA-mediated mechanism in the reduction of archaea by 3-NOP treatment requires further investigation.

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Seasonal variation in methane production of lactating cows in pasture-based feeding systems

Saisonale Schwankungen der Methanproduktion von Milchkühen in Weidehaltungssystemen

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Introduction: Enteric methane (CH₄) production in ruminants is affected by several factors, including intake, nutritional composition, and maturity of their forage [1], which vary seasonally in pasture-based feeding systems [2]. Hence, this study aimed (1) at estimating the CH₄ output of lactating cows using the GfE (2023) equation [3], and (2) at identifying the animal- and diet-level factors driving the variation in the CH₄ estimates across seasons. We hypothesized that CH₄ yield (g/kg dry matter intake; DMI) would be lower during the spring season due to higher feed intake and digestibility compared to the summer and fall seasons.

Methods: Monthly data on body weight, DMI in the barn, milk yield, and milk composition of lactating cows were gathered on two pasture-based dairy farms in Northern Germany during the grazing season of 2024. While cows (n=105) on one farm grazed day and night and were fed total mixed rations directly fed after milking, those of the second farm (n=94) grazed for 8 h/d and were otherwise housed and fed indoors. The DMI from the barn was recorded as the difference between the feed offered and refusals collected from the feed bunk. Samples of the pasture vegetation (n = 74) were taken at 4 cm residual height in 0.25-m² quadrats randomly positioned on pastures and oven-dried at 60 °C for 48 h. The pasture samples were then pooled per season (spring, summer, and fall) and farm. Additionally, samples of total mixed rations (n = 12) were taken monthly and freeze-dried at -90°C for 48 h. Gross energy, proximate nutrients, and fiber fractions were analyzed (VDLUGA, 2012). Digestibility of organic matter (OMD) was estimated from *in vitro* gas production to calculate metabolizable energy (ME) concentrations according to GfE (2023). The OMD and ME concentrations were corrected for the expected DMI level. Total daily ME requirements were estimated according to GfE (2023). The DMI from pasture was then calculated as the difference between total ME requirements and the ME intake in the barn, divided by the ME concentrations of the pasture forage. Finally, CH₄ energy (MJ/kg organic matter), CH₄ production (g/d), intensity (g/kg milk), and yield (g/kg DMI) were estimated according to GfE (2023) for each cow, farm, and season. Data were analyzed using a linear mixed-effects model in R (version 4.4.2), with season as fixed effects and animal and farm as random effects to account for repeated measures. Significance was declared at p < 0.05. A random forest analysis was performed to identify and rank the animal- and diet-level factors contributing to variations in CH₄ estimates across seasons, using the percentage increase in mean square error (%IncMSE) as an indicator of variable importance, where higher values indicate stronger influence.

Results: The CH₄ energy differed across seasons, being highest in fall (1.21 MJ/kg organic matter) and lowest in spring (1.16 MJ/kg organic matter; p < 0.01). In contrast, daily CH₄ production was highest in spring (396 g/d) and lowest in fall (347 g/d; p < 0.01). The CH₄ intensity (summer: 13.8 g/kg milk; fall: 14.1 g/kg milk) and yield (summer and fall: 19.3 g/kg DMI) were greatest during summer and fall, and lowest in spring (13.2 g/kg milk and 19.1 g/kg DMI; p < 0.01). The DMI was the most important driver for CH₄ production in spring (%IncMSE = 35.1), whereas OMD explained the largest variation in CH₄ energy (%IncMSE = 38.9 and 30.7) and CH₄ yield (%IncMSE = 37.3 and 39.0) in summer and fall. The CH₄ intensity was affected mainly by milk yield across all seasons.

Conclusions: The CH₄ production, intensity, and yield of lactating dairy cows in pasture-based feeding systems vary greatly with season, mainly due to variations in their feed intake and feed digestibility. These findings suggest seasonal-specific feeding and forage management strategies to reduce CH₄ emissions.

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Food Conversion Efficiency of different diets in Simmental steers: Implications for sustainable beef production

Nachhaltigkeitsbewertung der Fleckvieh-Ochsenmast unter Berücksichtigung der Lebensmittel-Konversions-Effizienz variierender Rationstypen

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Introduction: The food conversion efficiency (FCE) complements existing sustainability indicators in livestock production by quantifying the ratio of human-edible (he) output to human-edible input, thereby evaluating the potential food competition posed by livestock systems. The study aimed to analyze the human edible food conversion efficiency (heFCE) of organic beef production and to investigate how animal husbandry can contribute to resilient and resource-efficient food systems without competing with human nutrition.

Methods: Seven feeding scenarios were defined for the fattening of Simmental steers, including three organic grassland-based diets and three organic cropland-based diets. The scenarios varied in intensity, as reflected by daily body weight gain, concentrate supplementation, and the inclusion of by-products from industrial food production. The most extensive grassland-based diet consisted entirely of non-edible components (silage and pasture grass) and was not supplemented with concentrate. The other grassland-based diets were supplemented with 15 % concentrate in the form of a by-product (wheat bran) and 14 % concentrate from grains (corn and triticale). One organic cropland-based scenario consisted of 57 % clover grass silage and 14 % pasture grass, supplemented with 29 % wheat bran as concentrate. Another cropland-based diet included 25 % clover grass silage and 12 % pasture grass, supplemented with 44 % corn silage and 20 % wheat bran. A further cropland-based diet contained 66 % clover grass silage and 11 % pasture grass, supplemented with grains (12 % corn and 12 % triticale). Additionally, a conventional control diet consisting of 80 % maize silage, 13 % soybean meal and 4 % grains was included. The quantity of human-edible components in the feedstuff was estimated for three levels (high h, medium m, low l). The digestible indispensable amino acid score (DIAAS) was used to calculate the protein quality ratio (PQR) from feed to food, which was multiplied by the $\text{heFCE}^{\text{Protein}}$ ($\text{heFCE}^{\text{Protein}} \times \text{PQR}$). To compare the average values between the organic diets and the control diet, a paired two-sample t-test was conducted.

Results: None of the fattening diets examined achieved efficient utilization of human-edible gross energy ($\text{heFCEGE} > 1$). On average, the forage-based organic diets reached significantly higher $\text{heFCE}^{\text{Protein}} \times \text{PQR}$ values than the conventional control diet (3.12 vs. 0.32, $p = 0.013$, one-tailed), due to their higher share of non-human-edible forage such as pasture or grass silage. In summary grassland scenarios achieved higher $\text{heFCE}^{\text{Protein}} \times \text{PQR}$ than cropland-based scenarios (88.8 % higher (m); 139.2 % higher (h)). Replacing cereal with by-products doubled $\text{heFCE}^{\text{Protein}} \times \text{PQR}$ and at least tripled heFCE^{GE} in both types of agricultural practice.

Conclusions: Forage-based production systems, as typically found in organic farming, compete less directly with human food resources than conventional intensive systems, which may lead to trade-offs when considering other sustainability parameters. The use of by-products represents an effective strategy to increase food conversion efficiency.

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Impact of the temperature-humidity-index on dairy cow performance at two contrasting German regions (Baltic Sea and Central Region) during Summer 2025

Einfluss des Temperatur-Luftfeuchtigkeitsindex auf die Leistung von Milchkühen an zwei kontrastierenden Standorten in Deutschland (Ostsee und Zentralregion) im Sommer 2025

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Introduction: Dairy cows are particularly susceptible to hot environmental conditions due to their body size, high milk yields and endogenous heat production. Most studies investigating the impact of increasing ambient heat on the performance of dairy cows have been conducted by comparing seasonal differences between summer and autumn or spring using large datasets from milk recordings alongside weather data [1]. However, this way of heat stress assessment is confounded at least by photoperiod length. The aim of this study was to quantify the influence of varying temperature-humidity-index (THI) on dry matter intake (DMI), milk yield (MY), and milk composition within summer months only.

Methods: Forty-seven Holstein dairy cows from two research herds (Baltic Sea & Central Region), 30 from FBN-Dummerstorf (FBN; 2.5 parities, 136±90 days in milk (DIM), 34±7 kg milk/d) & 17 from Oberholz-Leipzig (OHL, 2.9 parities, 163±81 DIM, 37±7 kg milk/d) were involved in this study conducted in 2025. Cows were fed a total mixed ration (incl. concentrate) for *ad libitum* intake (ME FBN 10.1 MJ/kg dry matter (DM), OHL 11.6 MJ/kg DM; crude protein: FBN 133 g/kg DM, OHL 143 g/kg DM). Feed intake and MY were recorded daily. Ambient temperature and relative humidity were recorded in 15 min intervals using indoor sensors to calculate daily mean THI [2]. Feed samples were taken weekly to determine total mixed ration DM content. Individual milk samples were taken on the afternoon of 27th May (THI1 with THI 59-63), 15th June (THI2 with THI 67-72), 26th June (THI3 with THI 65-75) and 15th July 2025 (THI4 with THI 64-69), before night cooling. Before milk sampling, respiratory frequency (RF) was measured visually by counting flank movements (breaths/min). Milk composition was analysed using mid-infrared spectroscopy and flow cytometry. For statistical analysis, MY and DMI were assessed in two ways: (1) on the day of milk sampling, and (2) calculated as mean of three consecutive days (mean 3d-MY and mean 3d-DMI) immediately preceding milk sampling. Data were analyzed with repeated measures ANOVA using MIXED procedure of SAS (v9.4) with fixed effects of timepoint (THI1, THI2, THI3, THI4), location (FBN, OHL) and their interactions with DIM as covariates.

Results: Location effects were found between FBN and OHL for MY, mean 3d-MY, and energy-corrected-milk yield (ECM; F-test $P < 0.01$, respectively). RF was higher at THI2 and THI3 compared to THI1 and THI4 in both herds ($p < 0.05$, respectively). Mean DMI was higher at THI2 compared to THI1 and THI4 in OHL and FBN ($p < 0.05$), whilst mean 3d-DMI decreased from THI2 to THI4 ($p < 0.05$, respectively). Mean 3d-MY and MY were lower at THI3 than THI2 in FBN ($p < 0.05$, respectively), whereas MY tended to be lower at THI4 than THI2 in OHL ($p < 0.09$). Milk urea concentrations (MUC) were higher at THI1 and THI4 than THI2 and THI3 in FBN ($p < 0.05$). Percentage of milk fat, ECM and the fat-protein-quotient were or tended to be lower at THI4 compared to THI1 in FBN ($p < 0.09$) but not in OHL. Percentage of milk lactose was higher at THI4 compared to THI1 in FBN, whilst milk protein concentrations were not altered by THI levels. The opposite was found in OHL with highest MUC at THI2 and THI3 compared to THI1 and THI4 ($p < 0.05$). Somatic cell counts were not different between the time points, but numerically increased from THI1 to THI4.

Conclusions: Despite an 8 to 9 unit increase from THI1 to THI2, DMI increased, suggesting that the rise in RF allowed cows to adapt to the first heat wave of the year to sustain MY. When THI > 65 lasted 10 more days, increased RF could no longer compensate for heat burden as indicated by the decrease in DMI and MY from THI2 to THI3. Production parameters remained on a lower level from THI3 to THI4 although THI slightly decreased by 1 to 6 units, however, this allowed cows to decrease RF and suggests a delayed ability to recover from THI > 65 . Higher MUC in OHL at THI2 and THI3 may suggest an increase in protein catabolism to maintain energy demand at THI > 72 .

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Epigenetic and transcriptional responses in the liver of primiparous Holstein dairy cows to short-term heat stress or feed restriction

Epigenetische und transkriptionelle Reaktionen der Leber von erstkalbenden Holstein-Milchkühen auf kurzfristigen Hitzestress oder Futterrestriktion

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Introduction: Extreme weather events with high ambient temperatures are placing unprecedented pressure on farm animals. Hyperthermia disrupts energy balance and attenuates lipolysis, shifting the hepatic metabolic adaptation in dairy cows [1]. Hepatic metabolism is regulated among others by gene transcription, which is controlled by chromatin accessibility, and access to chromatin by post-translational modifications (PTM) of histones. These modifications are part of the epigenetic regulation of gene transcription; however, they have not been investigated in response to heat stress in dairy cows. Therefore, this study aims to elucidate the short-term effect (7 days) of heat stress on the hepatic methylation and acetylation pattern of histones and their related transcription.

Methods: Thirty non-pregnant, primiparous German Holstein cows (169±9 days in milk (DIM)) were adapted to climate chambers in a predefined thermoneutral (TN) zone at 16°C, 63% relative humidity (RH), temperature-humidity-index (THI) 60, for six days (d). Then, cows were randomly allocated into either a heat-stressed (HS, n=10), control (CON, n=10) or pair-feeding (PF, n=10) group. In the subsequent experimental phase, HS cows were subjected to 28°C for 7 days (52% RH, THI 76) with *ad libitum* feeding and water [2]. PF and CON groups were kept in the TN zone where, CON cows had *ad libitum* access to feed and water but PF cows were fed restrictively to mimic energy intake of HS cows. After 7 days of treatment, liver samples were subjected to histone isolation and free lysines (K) of histone H3 and H4 proteins were identified for methylations or acetylations using LC-MS. RNA was isolated to perform RT-qPCR for genes regulating histone acetylation and methylation. Data analysis was conducted with linear mixed model in R (Version 4.5), accounting for treatment and block as fixed effects and DIM as random effect.

Results: We analysed 36 of over 51 PTMs detected- 8 unmodified, 15 methylated and 13 acetylated K sites. H3 acetylation at K4 and K23 were lower in PF than in CON and HS cows ($P<0.01$, respectively). At K36 of H3.3 variant, it was higher in HS than in PF and CON cows ($P<0.05$). Additionally, H3K14ac and H3K18ac were lower in PF than in CON cows ($P<0.05$, respectively). H4 acetylation at K5, K8, K2 and, K16 were lower in PF compared to CON and HS cows ($P<0.01$, respectively). H3 mono methylation at K4 and K9 was lower in PF than HS cows ($P<0.01$), and K9 was lower in CON too ($P<0.05$). H4 mono methylation at K20 was higher in HS than PF cows ($P<0.01$) and tended to be higher in the HS than in the CON cows ($P<0.09$). Relative mRNA abundance of histone deacetylase HDAC1 was higher in HS than in CON cows ($P<0.05$), whereas HDAC2 and HDAC5 tended to be higher in HS than in PF cows ($P<0.1$, respectively). Transcription co-activator CITED1 was higher in HS compared to PF cows ($P<0.001$). Additionally, CITED1 and the methyltransferase KMT5A tended to be higher transcribed in HS than CON cows ($P<0.09$, respectively). Higher expressed CITED2, the histone methyltransferase SETDB1, and genes regulating histone acetylation (EP300 and CREBBP) were observed in HS cows vs CON and PF ($P>0.1$).

Conclusions: Hypoacetylation of H3K4 and H3K23 as well as of all four H4 lysines under restricted feeding compared to HS and CON conditions, associated with the lower tendency of deacetylase HDAC2 and HDAC5 suggested the effect of feed restriction in PF cows, leading to insufficient substrate - acetyl CoA for acetylation. However, greater H4K20me1 under hyperthermia indicates its involvement in chromatin accessibility and transcription, in turn, might alter hepato-lipolytic capacity during heat stress. Further research is required for deeper understanding of the underlying epigenetic mechanism.

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Decreasing feed-food competition and environmental impact: By-products in diets of fattening pigs

Nahrungsmittelkonkurrenz und Umweltbelastung reduzieren – Nebenprodukte in der Schweinemast nutzen

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Introduction: Increasing per capita consumption of animal derived products, will increase the pressure on livestock and food production. Livestock production is a relevant source of greenhouse gases (GHG), for pigs especially from imported protein feed, mainly soybean meal (SBM). These emissions will likely increase with higher meat production. Diets of fattening pigs typically contain 70-80% cereals and 10-15% soybean meal, resulting in a human-edible fraction (HEF) of ~75%, causing strong feed-food competition and environmental impact through feed production, deforestation and nutrient excretion (P, N, CO₂). Feeding pig diets with low climate impacts and reduced feed-food competition will be an important option for future pork production. To decrease feed-food competition and enhance circularity in pig production, replacing ingredients with high HEF such as cereal grains with industrial by-products (BPs) and edible biomass not suitable for human consumption has gained attention. Substitution by regional protein feed as well as supplementation of synthetic amino acids are further strategies.

Methods: The partial replacement of cereal grains by cereal BPs and total replacement of SBM by alternative protein feeds in fattening pig diets were studied. In total, 96 pigs (BW: 27.3 kg) were allocated to 4 dietary treatments, with 24 animals, 2 pens per feeding group. An automatic single-space feeding system recording feed intake per animal. The study included two phases (p) feeding-program: 30-75 and 75-120 kg BW. The first 3 diets were calculated iso-nitrogen and iso-caloric. Diet 4 was reduced in MJ ME and pcv lysine. The diet of the control group (CON) included 81 and 89% grains in p 1 and p 2, respectively. In the moderate HEF (HEF-M) group, grains were reduced to 68 and 83%. In low (HEF-L) and very low (HEF-VL) groups, grains were further reduced to 51 and 52, as well as 38 and 34%, respectively. Conventional SBM for CON-fed pigs was totally replaced by canola meal, DDGS, and sunflower meal. In HEF-L and HEF-VL, a high amount of wheat bran, sugar beet pulp, wheat gluten feed, and wheat middling were included. Performance, carcass and meat quality were measure, while N and P excretion, and dietary carbon footprint (CFP) were calculated. The CFP in kg CO₂-eq (GWP100) is based on economic allocation and Agri-footprint data. Human-edible feed conversion efficiency (heFCE) was assessed following to Ertl et al. (2016) as a mixture between 'current' and 'potential' levels of protein edibility. Furthermore, digestible indispensable amino acid score (DIAAS) was calculated. Data were analysed using ANOVA and GLM procedure.

Results: High levels of BPs in the diets of fattening pigs resulted in marginal effects on performance (ADG: 942a, 892b, 918ab, 880b g/d, p<0.05). Lower ADG was caused by reduced ADFI (2.32^a, 2.19^b, 2.19^b, 2.18b kg/d, p<0.05), while FCR was not affected (2.47, 2.46, 2.44, 2.46, p>0.05). Calculated N excretion showed no difference (p>0.05), while P excretion markedly increased by feeding higher levels of BPs (351^d, 494^c, 764^b, 905^a g P per pig, p<0.05). HEF-VL showed lowest slaughter weight (p<0.05), while the amount of lean was unaffected (59.48, 59.53, 60.39, 60.27 kg, p<0.05). The high amount of fibre in HEF-L and HEF-VL diets reduced dressing percentage (p<0.05) due to higher weight of gastrointestinal tract. Increasing levels of BPs in the diets reduced calculated CFP per pig (193, 135, 123, 118 g CO₂-eq per kg feed). Also, HEF of protein per diet and phase (p 1 and p 2, 68.7 and 69.1, 57.2 and 64.4, 45.6 and 46.2, 37.8 and 34.6) decreased, while calculated heFCE (0.411, 0.455, 0.625, 0.784) increased. heFCE (DIAAS) markedly increased (0.878, 1.600, 2.053, 2.231).

Conclusions: Increasing BPs in diets for fattening pigs to reduce feed-food competition and improve circularity of pig production may impair growth performance and P excretion. Thus, detailed characterisation of the nutritive value of the BPs is required to minimize these trade-off effects.

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The relationship between Carcass Yield in Fattening Pigs and Performance and Carbon Footprint

Über den Zusammenhang zwischen Ausschlagungsgrad von Schlachtschweinen und Leistungsdaten und CO₂-Fußabdruck

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Introduction: Germany has set itself the aim of becoming climate neutral until 2045. Under typical housing conditions, feed accounts for 50% to over 60% of greenhouse gas emissions in pig fattening. For this reason, feed efficiency has a major impact on the amount of greenhouse gases produced. The aim of this study was to describe the relationship between carcass yield, feed efficiency and carbon footprint.

Methods: The analysis was based on data from a representative sample of 19 fattening groups (grower to finisher) from a total of 11 conventional pig finishing farms located in northwestern Germany. The data were from 2015 to 2024. All farms were connected to the same piglet production system and operated under similar management practices. The recorded data included the raw components used in the compound feeds, their average consumption per animal, and the contents of crude protein and phosphorus. In addition, performance data such as mortality, live weight at entry and exit, and slaughter weight were documented, allowing the calculation of the carcass yield for each fattening group. The life cycle assessment was carried out using the software SimaPro Craft, version 10.2.0.2, with the GFLI database [1]. The soybean meal used originated from Brazil and it was assumed that this soybean meal was not produced without deforestation. Depending on the fattening group, the pigs were fed using a three- to six-phase feeding regime. Emissions of methane (CH₄), nitrous oxide (N₂O), nitrogen dioxide (NO₂), and ammonia (NH₃) out of the barn and outside manure storage with natural crust cover were calculated according to literature [2]. The utilization of crude protein, phosphorus, and potassium was determined by applying body composition data of growing-finishing pigs and slaughter pigs from the literature [2]. The calculated nutrient excretion of nitrogen, phosphorus, and potassium are contained in the slurry. As an organic fertilizer, slurry can reduce the need for synthetic fertilizers in crop production. The production of synthetic fertilizers is highly energy-intensive. In the pig production model used in this study, the amount of synthetic fertilizer that can be replaced by applying the produced slurry to cropland was credited. Electricity consumption for the production of 1 kg of feed was set at 0.0033 kWh. The assessment was carried out based on a cradle-to-factory-gate model with a functional unit of 1 kg of slaughtered pig. Energy and water consumption in the barn could not be considered due to incomplete data. Resource use at the slaughterhouse was obtained from the literature [3].

Results: A higher carbon footprint per kilogram of carcass weight was associated with a lower carcass yield (Pearson $r = -0.60$, $p = 0.01$). In addition, fattening groups with higher mortality tended to exhibit a lower carcass yield ($r = -0.34$, $p = 0.16$). Similarly, a higher feed conversion rate tended to exhibit a lower carcass yield, although correlations were weak and not significant ($r = -0.20$, $p = 0.42$). A limitation of the study is the small number of fattening groups per farm. More included farms would also have improved the robustness and representativeness of the study.

Conclusions: The higher carcass yield was associated with a lower carbon footprint of the final product. In addition, the results may reflect the high importance of herd health, as indicated by lower mortality, in fattening groups with higher carcass yield. Groups with higher carcass yield may tended to show more efficient feed utilization. However, this aspect requires further investigation using a larger sample size to confirm the findings.

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Effects of stress of the endoplasmic reticulum on genome-wide gene expression in a bovine liver cell model

Auswirkungen von Stress des endoplasmatischen Retikulums auf die genomweite Genexpression in einem Rinder-Leberzellmodell

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Introduction: Endoplasmic reticulum (ER) stress occurs in the liver of high-yielding dairy cows during early lactation. In rodents, ER stress promotes inflammation and induces hepatic steatosis. However, its specific effects on hepatic metabolism in cattle remain unknown. This study aimed to examine ER stress effects on bovine liver cell metabolism using BFH12 cells, an established model for fatty liver research in cattle. Genome-wide gene expression was analyzed after treatment with two ER stress inducers, tunicamycin (TM) and thapsigargin (TG). The central hypothesis was that ER stress significantly alters cellular metabolism, promoting inflammation and lipogenesis in BHF12 cells.

Methods: The cells used in this study (BFH 12 cells) are hepatocytes of a bovine foetus (Holstein Frisian) with a crown-rump length of 80 cm, equivalent to the 8th to 9th month of pregnancy, which were used for transduction with the retroviral recombinant vector construct pRetro-E2-SV40 [1]. The cells were cultured in Williams' E medium containing 5% heat-inactivated FBS, 2 mM L-alanyl-L-glutamine, 100 nM dexamethasone, 0.2 U/mL insulin and 1% penicillin/streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. After reaching 70-80% confluency, cells were treated with medium containing either vehicle only (0.1% DMSO) or TM or TG (both dissolved in DMSO) at different concentrations and for different time periods (4-24 h). Effects of TM and TG on cell viability were assessed by the MTT assay, while effects on gene expression were analyzed by whole-genome transcript profiling, qPCR and immunoblotting. Statistical analysis was performed using the Minitab statistical software. Data were subjected to one-way ANOVA. For statistically significant F values, individual means of the treatment groups were compared by Fisher's multiple range test. Effects were considered significant if P<0.05.

Results: Based on results from cell viability testing, TM and TG were used at concentrations of 100 ng/mL and 100 nM, respectively. At these concentrations, the mRNA levels of several ER stress target genes including HSPA5 and DDIT3 were markedly higher in BFH12 cells treated with TM and TG than in cells treated with vehicle at all incubation periods (P<0.05). In addition, the protein levels of HSPA5 and DDIT3 were higher in BFH12 cells treated for 24 h with TM (8- and 2.4-fold, respectively) and TG (9.5- and 2.5-fold, respectively) than in cells treated with vehicle (P<0.05). Transcript profiling revealed that 220 and 218 genes were differentially expressed between cells treated with TM and TG, respectively, compared with vehicle (P<0.05). In line with the activation of ER stress, a large number of ER stress-regulated genes were identified amongst the upregulated genes in BFH12 cells treated with TM and TG (P<0.05). Transcript profiling revealed that treatment of BFH12 cells with TM for 24 h causes downregulation of a large number of genes involved in lipid synthesis, while treatment with TG does not. Genes involved in fatty oxidation were found to be either upregulated or unaltered in cells treated for 24 h with TM and TG. Genes involved in cytoprotection and antioxidant defense were downregulated in cells treated for 24 h with TM and TG (P<0.05).

Conclusions: While ER stress can be successfully induced in BHF12 cells by incubation with TG or TM, several hallmarks of ER stress observed in rodent models - such as the activation of lipogenesis, inhibition of β -oxidation, and induction of cytoprotective and antioxidant genes - could not be replicated in this bovine liver cell line. Extrapolating these findings to the bovine organism suggests that ER stress may not play a central role in the development of fatty liver in dairy cows. However, our data provide indications that ER stress might contribute to the establishment of a pro-oxidant and pro-inflammatory state during early lactation in dairy cattle.

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***In ovo* administration of myo-inositol and its implications for metabolic programming in broilers**

In-ovo-Applikation von myo-Inositol und ihre Bedeutung für die metabolische Programmierung bei Broilern

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Introduction: Myo-inositol (MI) is a cyclic sugar polyalcohol with essential roles in cellular signalling, osmoregulation, and metabolic control, including modulation of glucose homeostasis and insulin sensitivity [1, 2]. These functions position MI as a potential contributor to metabolic pathway programming during embryonic development, a period of high physiological plasticity. In the present study, *in ovo* MI supplementation was applied as a targeted strategy to transiently increase MI concentration in the amniotic cavity during late embryogenesis in broiler chickens, a stage at which substances delivered into this compartment are ingested and absorbed into the circulation. This transient increase in MI availability to the embryo was expected to interact with MI-dependent pathways involved in energy metabolism, mitochondrial function, and oxidative stress responses, thereby influencing postnatal metabolic regulation. Accordingly, the aim of the present study was to determine whether MI-induced developmental adaptations occur and lead to persistent changes in metabolic phenotype, consistent with the concept of metabolic imprinting.

Methods: A total of 480 fertilized Ross 308 broiler eggs were distributed into four equal groups and incubated under standard conditions. Non-fertile and dead embryos were removed after candling on days 11 and 16, resulting in 110 viable eggs per group. On day 17, eggs were manually injected with 1 ml of either 12 $\mu\text{mol/ml}$ MI (MI 12), 24 $\mu\text{mol/ml}$ MI (MI 24), 0.9 % saline (positive control, PC) into the amniotic fluid, or left non-injected (negative control, NC). After hatching, 384 chicks (96 per group) were housed in floor pens (8 pens per treatment, 12 birds per pen) and fed standard starter (days 0-14) and grower (days 14-35) diets for *ad libitum* consumption. Growth performance was recorded weekly, including body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and survival rate (SR). On day 35, one bird per pen was slaughtered for blood and tissue collection. MI concentrations in plasma and tissues were quantified spectrophotometrically, the expression of inositol monophosphatase 1 (IMPase 1) in liver and muscle and MI oxygenase (MIOX) in kidney was determined by ELISA, and plasma metabolite profiles were analysed using the AbsoluteIDQ™ p180 kit. Statistical analyses were performed using the MIXED procedure in SAS, with planned contrasts between MI-injected (MI 12 + MI 24) and No-MI (PC + NC) groups.

Results: MI *in ovo* administration did not affect hatchability. MI-injected groups showed lower BW from day 14 onward, reduced ADG ($P < 0.050$), and a tendency to lower ADFI, while FCR remained unchanged compared to No-MI groups. SR was higher in MI-injected groups during d 0-21 ($P = 0.028$) and tended to remain higher until day 35. MI concentrations in plasma and tissues and the expression of IMPase 1 and MIOX did not differ among groups. Metabolomics revealed lower plasma serotonin and increased concentrations of acylcarnitines (C2, C9), threonine, sarcosine, some glycerophospholipids, and sphingomyelins in MI-injected compared to No-MI groups. These changes may reflect alterations in diacylglycerol-related pathways, mitochondrial β -oxidation, and shifts in nutrient partitioning and methylation-related metabolism.

Conclusions: Transient MI exposure during late embryogenesis caused modest changes in growth performance and plasma metabolite profiles, potentially affecting mitochondrial function and membrane-associated signalling. This may be important for improved survival and metabolic adaptability rather than maximal growth in broiler chickens and therefore deserves closer investigation.

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MicroRNA expression profiling in sows and piglets during the periparturient period*MicroRNA-Expressionsprofile bei Sauen und Ferkeln in der peripartalen Phase*

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Introduction: The periparturient period represents a critical window of immune and metabolic transition for both sows and piglets. Previous research has demonstrated marked shifts in the maternal and neonatal microbiota during this period, influencing nutrient transfer, immune priming, and microbial colonization of the offspring. Based on lactation-related shifts in maternal microbial composition and their influence on neonatal gut colonization [1], we hypothesized that colostrum exposure and maternal microbial imprinting shape circulating plasma miRNA signatures in sows and piglets.

Methods: Plasma samples were collected from sows and piglets immediately after farrowing and following colostrum ingestion. Small RNA libraries were prepared by adaptor ligation, cDNA synthesis, and PCR enrichment, followed by Illumina sequencing. After quality control and adapter trimming, reads were mapped to the reference genome using Bowtie, and known as well as novel miRNAs were identified with miRDeep2 and miRevo. Differential expression analysis was performed using DESeq2 and edgeR ($p < 0.05$). Predicted target genes of differentially expressed miRNAs were subjected to Gene Ontology (GO) and KEGG pathway enrichment analyses using ClusterProfiler and KOBAS.

Results: Distinct miRNA expression profiles were observed between sows and piglets plasma and colostrum. In piglets, 92 miRNAs were upregulated and 66 downregulated ($p < 0.05$) when comparing plasma to colostrum, indicating activation of ion transport, cytoskeletal organization, and transmembrane signaling. KEGG enrichment highlighted MAPK, Calcium, and Rap1 signaling, as well as chemokine and cell adhesion pathways. In piglet plasma versus sow plasma (98 up, 48 down, $p < 0.05$), enriched pathways related to leukocyte migration, cellular remodeling, and neuroendocrine regulation (adrenergic, oxytocin, and relaxin signaling). In contrast, the sow plasma–colostrum comparison revealed fewer regulated miRNAs (41 up, 82 down, $p < 0.05$), mainly associated with lipid metabolism, signal transduction, and transcriptional regulation. Functionally, piglet miRNA upregulation was linked to enhanced immune readiness and epithelial reorganization, whereas in sows, downregulation of lipid- and transcription-related miRNAs indicated metabolic reorientation toward lactation and immune stabilization.

Conclusions: Plasma miRNA profiles in sows and piglets around parturition reflect complementary physiological priorities. Piglets exhibit miRNA signatures promoting rapid postnatal immune and barrier adaptation, whereas sows display regulatory patterns supporting lactation-related metabolic reorientation. These findings suggest that circulating miRNAs act as a functional interface between maternal microbial dynamics and neonatal adaptation and may serve as biomarkers of coordinated immune–metabolic transitions during the periparturient period.

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Towards standardization in pig microbiome research: Biases introduced by DNA extraction kits are more prevalent in 16S rRNA gene datasets compared to shotgun metagenomic profiles

Standardisierung in der Mikrobiomforschung beim Schwein: DNA-Extraktionskits verursachen stärkere Verzerrungen in 16S-rRNA-Gendatensätzen als in Shotgun-metagenomischen Profilen

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Introduction: In pigs, an animal model of agricultural biomedical relevance, DNA extraction protocols remain unstandardized, thus limiting reproducibility and cross-study comparability. We investigated the impact of several commercial DNA extraction kits and protocols on the bacterial community recovered from porcine fecal samples. We further examined how these effects vary across commonly targeted 16S rRNA gene regions in pig microbiome studies and compared them with profiles obtained from shotgun metagenomic sequencing of the same samples.

Methods: Fecal samples from 50 male German Landrace × Pietrain pigs were subjected to DNA extraction using 12 commercial kits following manufacturer protocols. Eluted DNA was used to amplify the V1–V2, V1–V3, V3–V4, and V4 regions of the 16S rRNA gene, including negative controls. Amplicons were pooled equimolarly and sequenced (250 bp paired-end) on an Illumina MiSeq by Biomarker Technologies (BMK GmbH, Münster, Germany). In parallel, metagenomic libraries were prepared from 30 µL of DNA (≥ 20 ng/µL) and sequenced (150 bp paired-end, PCR-free) on an Illumina NovaSeq. Quality control was performed using DADA2 in QIIME2 [1] (16S amplicons) and Trim Galore [2] (metagenomics), followed by bioinformatic analysis in QIIME2 (amplicon and moshpit distributions v2025.4). Numeric variables compared between extraction kits were tested with Kruskal–Wallis or ANOVA, followed by paired Wilcoxon or t-tests, with P-values corrected using the Benjamini–Hochberg method.

Results: The choice of DNA extraction kits significantly affected DNA concentration and purity metrics, with the highest yields obtained using QIAamp Powerfecal pro, DNeasy Powersoil, Maxwell RSC Fecal Microbiome kit (median concentrations = 606.4, 212.2, 179.2 ng/µl; IQR = 398.3, 172.6, 89.5, respectively (all P-adj < 0.05)). The Zymo DNA Mini kit produced the lowest yield (median = 7.5 ng/µl; IQR = 8.4; P-adj < 0.05). DADA2 output was significantly affected by the amplified 16S rRNA gene region. The V4 region yielded the fewest unique ASVs (2,755), while the merged V3–V4 and V1–V2 datasets yielded 5,267 and 8,068 ASVs, respectively. Bacterial community in both metagenomic and amplicon datasets, as well as the functional profiles of metagenomes, were significantly affected by extraction kit choice (all P-values < 0.01). Almost all pairwise comparisons between the kits were significant for each 16S rRNA gene region, while approximately half of the comparisons were not significant for MG samples and their functions. Alpha diversity metrics, namely Shannon’s entropy and Pielou’s evenness, were significantly affected by the DNA extraction kit in amplicon data (all P-values < 0.01), but not in metagenomic data (all P-values > 0.05). Defining the core microbiome as taxa annotated at a given taxonomy level in at least 95% of samples within a specific group (defined by sequencing approach, region and DNA extraction kit), and with an average relative abundance $\geq 1\%$, we observed that among metagenomic samples, 11 out of the 12 DNA kits recovered 30 genera with one kit lacking a single core genus. At the genus level, the core genera in amplicon samples varied among targeted regions and extraction kits. In the V1–V2 region, only 4 kits detected 28 core genera. In the V3–V4 region, 3 kits reported ≥ 30 core genera, and in the V4 region, only two kits reported ≥ 30 core genera.

Conclusions: The selection of a DNA extraction kit has a stronger influence on amplicon-based microbiome profiles than the choice of hypervariable region, affecting diversity estimates and core taxon detection but these differences were not significant in the metagenomic microbiome profiles. These results indicate that 16S rRNA datasets are particularly sensitive to extraction-dependent bias and emphasize the need for standardized protocols to ensure reproducible and comparable pig animal microbiome studies.

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The kidney proteome of Holstein dairy cows with divergent milk urea concentrations

Das Nierenproteom von Holstein-Milchkühen mit unterschiedlichen Milchkharnstoffkonzentrationen

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Introduction: Milk urea concentration (MUC) is a valuable indicator for monitoring the level of dietary crude protein intake and urinary nitrogen excretion in dairy cows [1]. Within a herd, dairy cows with comparable milk yield consuming the same diet may exhibit different MUC. Cows with high MUC reveal lower or tending lower urea, creatinine, uric acid and creatine clearance rates and lower kidney weights than cows with low MUC [2, 3]. However, the renal molecular mechanisms underlying the different clearance rates are far from understood. Therefore, the objective of the present study was to elucidate the kidney proteome of Holstein dairy cows with divergent MUC.

Methods: A total of eighteen non-pregnant, multiparous Holstein cows (2.8 parities, 276±89 days in milk) were screened for high (HMU: 285.9 ± 11.5 mg/L) and low MUC groups (LMU: 190.5 ± 33.7 mg/L) with following plasma urea concentrations: HMU: 3800±270 µmol/L; LMU: 3455±263 µmol/L [2]. Cows had comparable milk yields (HMU: 32.9 ± 1.0 kg/d; LMU: 31.5 ± 1.4 kg/d; P = 0.807). Each one HMU and one LMU cow per block was fed a total mixed ration (11.0 ± 0.8 MJ metabolizable energy/kg DM 14.9 ± 0.1% CP on DM basis) for 2 weeks. Then, cows were fitted with a customized urinal to collect urine for 4 days. Dry matter intake (DMI), milk yield, milk composition and body weight were measured. Urine samples were analysed for free amino acid concentrations using HPLC and for chloride by Fuji Dri-Chem4000i. After the collection period, dairy cows were slaughtered, the left renal cortex was sampled and stored at -80°C. Protein extracts of the renal cortex were run on a 1D gel. One lane per animal was cut into 10 slices and subjected to HPLC and mass spectrometry analysis. Group differences were analyzed in R with DESeq2 package and pathway enrichment was performed with DAVID. Remaining data were analyzed with ANOVA using the MIXED procedure of SAS (version 9.4) with the fixed effects of MUC (HMU, LMU) and block (1-9). Days in milk served as covariates.

Results: Dry matter intake, milk yield, body weight and urine volume were comparable between HMU and LMU cows. Overall, 200 out of 4463 kidney proteins were differentially abundant between HMU and LMU cows, whilst 80 proteins were up-regulated and 120 were down-regulated in LMU vs HMU cows. The top functions of proteins differing between LMU and HMU cows were related to an up-regulation of metabolic pathways (MCCC2, ACOT8, PCYT1A, ARG1, GMPS, OPLAH, FMO4, PFAS, MTM1, GGCT, PFKL, ND1, ACOT4), thyroid hormone signaling pathway, aminoacyl-tRNA biosynthesis and down-regulation of glycerine, serine and threonine metabolism, endocytosis, glycolysis/gluconeogenesis and proteasome. Notably, the transporter for neutral amino acids SLC7A8, peptides (SLC15A2), chloride (SLC12A9), choline (SLC44A1), glucose (SLC2A2), lactate (SLC16A7), and organic anions (SLC22A8) were lower, whilst the ion exchange protein SLC4A1 and the citrate transporter SLC25A1 were higher expressed in HMU than in LMU cows (P<0.05). The protein expression of urea transporters as well as urinary urea, pH and chloride concentrations did not differ among groups. Urinary glutamate concentrations tended to be lower in HMU than in LMU cows (P<0.1), whereas all other urinary amino acids concentrations were comparable between groups.

Conclusions: The earlier observed different urea, creatinine, uric acid or creatine clearance rates between HMU and LMU cows [2, 3] could not be attributed to differences in the expression of the respective transporters. Moreover, the lower SLC7A8 and SLC12A9 expressions in HMU compared to LMU cows are not reflected by different urinary amino acid and chloride concentrations. Lower SLC7A8 and SLC12A9 expression may indicate less effective neutral amino acid and chloride reabsorption, respectively, but their plasma concentrations should be analysed to prove this assumption.

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Comparison of the efficacy of a phytogetic feed additive composed of pomegranate polyphenols, aromatic essential oils and quillaja saponin compared with zinc oxide on growth performance and postweaning diarrhea indices in weaned piglets

Vergleich der Effizienz eines phytogeten Futterzusatzstoffs aus Granatapfel-Polyphenolen, aromatischen ätherischen Ölen und Quillaja Saponin mit Zinkoxid auf die Wachstumsleistung und Indikatoren von Absetzdurchfällen bei Ferkeln

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Introduction: Since 2022, dietary zinc oxide (ZnO) as a growth promoter and to prevent post-weaning diarrhea in piglets has been banned in the European Union. The scientific evaluation of plant-derived molecules resulted in numerous phytogetic feed additives (PFA) as viable ZnO alternatives. Aromatic essential oils, for example, reduce enterotoxigenic *E. coli* virulence through quorum sensing inhibition [1]. Pomegranate polyphenols unroll strong anti-inflammatory effects and inhibit bacterial biofilm formation [2]. Specific saponin fractions from Quillaja enhance both innate and adaptive immune responses [3]. Consequently, the current study evaluated the effectiveness of a phytogetic feed additive, comprised of 10.0% matrix-encapsulated aromatic essential oils (Cinnamaldehyde and Thymol), 8.0% Pomegranate Extract, and 1.75% Quillaja Saponin Extract, compared with ZnO, on the performance, diarrhea incidence and faeces consistency in weaned piglets under practical conditions. The individual compounds in the PFA were chosen based on their specific modes of action.

Methods: At 24 days of age 216 weaned Danbred x Duroc piglets (108 f/ 108 m) from Asmussen Livestock Feedtests GmbH, Bylderup-Bov (Denmark) with an average body weight of 6.76±1.25 kg were allocated to three experimental treatments: (1) Negative Control (NC = no additive), (2) Positive Control (PC = 3000 mg/kg diet ZnO) and (3) PFA (150 mg/kg diet). Each treatment consisted of 8 repetitions with 9 piglets each. The piglets were fed commercial diets without (NC) or with (PC, PFA) the additives, respectively. The feeding- and observation study lasted 42 days and consisted of a starter phase (d 25 to d38 = 14 days) and a grower phase (d39 to d66 = 28 days). The study was accompanied by the Institute of Animal Nutrition, Free University, Berlin. During the experiment, performance, diarrhea incidence and faeces consistency were controlled weekly. Results are shown as Means ± Standard Deviation. The study used a randomized complete block design with the pen as the experimental unit. All performance and diarrhea parameters were analyzed by a one-way ANOVA using SPSS (IBM, Version 21).

Results: Initial body weight did not differ among groups (6.76±1.79 kg). After the 14 days starter period, PC piglets showed the highest body weight (10.17±1.84 kg), followed by PFA piglets (9.97±1.83 kg) and NC animals (9.51±1.99 kg). In this period daily weight gain was lower in tendency in the NC group (0.19±0.057 kg/d) compared to groups PC (0.24±0.050 kg/d; P=0.085) and PFA (0.23±0.045 kg/d; P=0.096). In contrast, FCR was significantly worse in group NC (1.478±0.137 kg/kg) compared to groups PC (1.337±0.062; P= 0.006) and PFA (1.301±0.081; P=0.002). During the 28d grower period the PFA unrolled the strongest growth promoting effects within groups. With 28.12±2.25 kg PFA piglets achieved the highest final body weight compared to groups PC (26.95±3.10kg; P=0.098) and NC (26.80±3.24 (P=0.095) at highly significant differences in FCR between PFA piglets (1.417±0.054) and both, PC piglets (1.527±0.037; P<0.001) and NC animals (1.531±0.042; P<0.001). Due to illnesses 3% of piglets must be removed in group NC, compared to 2% in group PC and 0% in group PFA. After fourteen days of feeding ZnO or the PFA, fecal scoring revealed significantly softer faeces in group NC (0.69 ±0.38) compared to the treated groups PC (0.11±0.20; P<0.01) and PFA (0.22±0.23; P<0.03). This effect persisted throughout the entire experimental period.

Conclusions: This study demonstrates that phytogetic feed additives, when formulated based on their specific modes of action, had a comparable potential to ZnO in weaned piglets. Notably, the ZnO dosage used in this trial was deliberately high in order to reveal the full potential of the tested PFA under challenging conditions.

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Prececal disappearance of phytate and digestibility of phosphorus and amino acids in nursery piglets

Untersuchungen zum Zusammenhang zwischen prae-caecalen Phytatabbau und prae-caecaler Verdaulichkeit von Phosphor und Aminosäuren

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Introduction: Phytate (myo-inositol hexakisphosphate; InsP_6) is the principal storage form of phosphorus (P) in plant-based feed ingredients commonly used in swine nutrition. However, in piglets as in all monogastric animals phytate is largely indigestible because endogenous phytase activity in the gastrointestinal tract is negligible. To improve nutrient utilization, exogenous microbial phytases are routinely incorporated into piglet diets. These enzymes increase the availability of phytate-phosphorus (PP) and reduce the antinutritional effects of phytate which otherwise binds to proteins and interferes with enzymatic digestion. However, the relationship between disappearance of phytate and digestibility of phosphorus and amino acids remains unknown in nursery piglets.

Methods: This study was carried out to investigate the relationship between prececal disappearance of PP (prececal Dis of PP; i. e. disappearance of all inositol-phosphates) and prececal digestibility of total phosphorus (P) and amino acids in nursery piglets. A total of 192 male weaned piglets with initial body weight of 8.7 ± 1.1 kg were allocated to one of four treatments using a randomized complete block design (2 pigs/pen, 24 pens/replicate) for 42 days. The basal experimental diets (post-weaning 1 and 2) were formulated on corn, wheat and soybean meal to be deficient in P and calcium (Ca; STTD of P/total Ca = 0.21%/0.58% and 0.19%/0.54%, in post-weaning 1 and 2 diets, respectively; all basal diets contained 0.22% PP). These diets were supplemented with a novel bacterial 6-phytase at four different levels, including 0 (control diet), 500, 1,000 or 1,500 FTU (phytase units)/kg. On day 42 of the experimental period, all piglets from 12 pens per treatment were euthanized and ileal digesta samples were collected for prececal Dis of PP and digestibility of P and amino acids determination. Pearson correlation coefficients were analyzed using JMP software to assess the relationship among prececal Dis of PP, digestibility of P and digestibility of amino acids. Statistical significance was set at $P < 0.05$.

Results: Increasing phytase dose from 0 to 1,500 FTU/kg diet increased (linearly and quadratically, $P < 0.001$) prececal digestibility of P (from 45.0%, control, up to 76.0%, 1,500 FTU/kg) and prececal Dis of PP (42.2% to 90.6%, 1,500 FTU/kg). A strong positive correlation ($r = 0.97$; $P < 0.001$) was observed between prececal Dis of PP and prececal digestibility of P. Increasing levels of phytase supplementation resulted in quadratic increases of prececal digestibility of Arg, His, Ile, Leu, Lys, Phe, Thr, Trp, Val, Ala, Asp, Cys, Glu, Ser, Tyr and total amino acids. Phytase supplementation increased prececal digestibility of Met and Pro linearly ($P < 0.001$), with prececal digestibility of Met showing a tendency ($P = 0.09$) for a quadratic increase. Positive correlations were also found between prececal digestibility of total amino acids and prececal digestibility of P ($r = 0.83$; $P < 0.001$) and total amino acid and prececal Dis of PP ($r = 0.74$; $P < 0.001$).

Conclusions: This study demonstrated that supplementing P- and Ca-deficient diets with an exogenous novel bacterial 6-phytase can improve prececal phytate disappearance, digestibility of P and amino acids in a dose dependent manner. It also highlighted that progressive phytase dosing not only maximizes phosphorus release but also substantially improves amino acid availability, highlighting the central role of phytate hydrolysis in optimizing nutrient efficiency especially of P and amino acids in nursery piglets.

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Effects of dietary microalgae species on growth performance, health, and physiological responses in broilers

Auswirkungen verschiedener Mikroalgenarten in der Ernährung auf das Wachstum, die Gesundheit und die physiologischen Reaktionen von Broilern

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Introduction: Growing consumer demand for natural and sustainable animal production has driven interest in alternative feed ingredients with functional benefits. Microalgae represent a promising and sustainable feed source rich in proteins, fatty acids, vitamins, and bioactive compounds [1]. Their cultivation does not compete with arable land or freshwater resources, making them environmentally attractive [1]. Among microalgae, *Spirulina* (*Arthrospira*), *Chlorella*, and *Schizochytrium* species are particularly valued for their high nutrient content and potential health-promoting properties in poultry. Previous studies have shown that some microalgae can enhance broiler performance, immunity, intestinal morphology, and meat quality [2-3]. However, most research has focused on single species under differing conditions, limiting direct comparison. Comparative data on the effects of different dietary microalgae species in broilers are therefore still scarce. The present study aimed to evaluate the effects of *Spirulina* platensis, *Chlorella vulgaris*, and *Schizochytrium limacinum* supplementation on growth performance, health parameters, and meat quality in broiler chickens.

Methods: One-day-old broiler chickens (Ross 308, male, n=240) were randomly allocated to six dietary treatments with five replicate pens each. The trial lasted 35 days. Treatments included a control (basal diet) and five experimental diets (A1-A5) where 1% of the basal diet was replaced by one of the following microalgae: A1) *Spirulina* platensis; A2, A3, A4) three differently cultivated/processed *Chlorella vulgaris* products; A5) *Schizochytrium limacinum*. Performance parameters (body weight, feed intake, feed conversion ratio) were recorded weekly. Welfare indicators (such as foot pad dermatitis score, litter dry matter) were also assessed weekly. At the end of the trial (day 36), three birds per replicate (n=15 per group) were randomly selected, euthanized, and dissected. Sample analyses included serum fatty acid profiles, intestinal histomorphometry (villous height and crypt depth in jejunum and ileum), and meat quality. Breast meat quality was evaluated 24 h post-mortem for pH, electrical conductivity, and color (CIE L, a*, b*). Data normality was assessed using the Shapiro-Wilk test. Normally distributed data were analyzed by one-way ANOVA followed by the Ryan-Einot-Gabriel-Welsch multiple range test; non-normal data and footpad scores were analyzed by the Kruskal-Wallis test followed by pairwise Wilcoxon tests.

Results: No significant differences ($p > 0.05$) were observed among groups for overall growth performance (final body weight, feed conversion ratio), litter quality, foot pad health, or intestinal histomorphology. However, significant differences were found in serum fatty acid profiles and breast meat color. Serum γ -linolenic acid (2.81-3.56 vs. 1.27 $\mu\text{g/mL}$) and arachidonic acid (216-257 vs. 124 $\mu\text{g/mL}$) levels were higher in groups A1-A4 than in A5. Conversely, docosahexaenoic acid was markedly elevated in group A5 (117 $\mu\text{g/mL}$) compared to others (16.7-18.9 $\mu\text{g/mL}$). Breast meat color differed significantly: groups A3 and A4 had the lowest redness (a*: 1.13 and 1.18, respectively) compared to control, A1, and A5 (a*: 2.19-2.25), while group A4 exhibited the highest yellowness (b*: 12.1).

Conclusions: The results indicate that dietary microalgae can be a valuable functional feed ingredient for modifying meat quality parameters and fatty acid profiles with no adverse effect on growth performance. *Schizochytrium* (A5) is particularly effective in enriching broiler meat with omega-3 fatty acids (docosahexaenoic acid). These findings support the use of specific microalgae as targeted supplements in broiler nutrition.

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Investigations on the effects of 3-nitrooxypropanol on performance and methane emission of dairy cows

Untersuchungen zu den Auswirkungen von 3-Nitrooxypropanol auf die Leistung und Methanemission von Milchkühen

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Introduction: Methanogenesis in the rumen of dairy cows, which is an unavoidable consequence of feed digestion, is climate relevant and results in energy losses for the animal. Supplementation of 3-NOP results in a reduction of methane emissions in dairy cattle of about 30% (Kebreab et al. 2023), depending on diet composition and 3-NOP dose. Given that there are contradictory results on long-term effects of 3-NOP supplementation on methane emission, the present study aimed to examine the effects of feeding 3-NOP on performance and methane emission of Brown Swiss and Simmental cows over the entire lactation period.

Methods: The trial was conducted with thirty-two Simmental and sixteen Brown Swiss cows at Bayerische Staatsgüter, Research and Education Center for cattle Feeding Achselschwang. The animals were divided evenly into a control group (CON) and an experimental group with a 3-NOP supplement (NOP) from the 10th day of lactation on basis of lactation performance, breed and age. The cows received a maize- and grass silage-based TMR. The forage to concentrate ratio depended on lactation stage. In the NOP group, each cow received 185g/d of a mineral premix containing 0,8% 3-NOP per day, while in the CON group the corresponding amount of a placebo consisting of wheat bran and calcium carbonate was added. The methane emission of the cows was measured using two GreenFeed stations (C-Lock Inc., Rapid City, South Dakota, USA). Daily feed and nutrient intake was recorded via weighing troughs along with daily milk yield and weekly composition, weight development, body condition score and backfat thickness (three times per trial). The statistical analysis was performed using the SAS statistical package (version 9.4, SAS Institute, Cary, NC, USA) using the 'MIXED' procedure and Bonferroni correction. In the model, the date, treatment, lactation number, lactation week and correction factors according to Ali and Schaeffer (1987) were taken as fixed effects. Cow, clustered by group membership, was considered a random effect. The significance level was set at $p < 0.05$.

Results: 3-NOP did not affect total DM intake (24.3 kg/d for CON and 23.4 kg/d for NOP; $P=0.219$). Since diet composition was identically for the feeding groups except for the addition of 3-NOP, the daily intake of energy and nutrients did not differ between the experimental groups. 3-NOP did not affect daily milk yield (35.9 and 34.3 kg/d for CON and NOP; $P=0.185$). The milk fat content was 3.93% in the NOP group and 4.10% in the CON group ($P=0.119$). The milk protein content (CON: 3.58; NOP: 3.63%; $P=0.314$) and the ECM yield (CON: 35.9 kg/day; NOP: 35.2 kg/day; $P=0.572$) did not differ between the groups. Daily methane emission was 290 g/cow for NOP and 394 g/cow for CON ($P < 0.001$). Methane intensity was 11.3 and 8.3 g/kg ECM for CON and NOP ($P < 0.001$). Methane yield was 17.2 and 13.2 g/kg DM for CON and NOP ($P < 0.001$). 3-NOP had no effects for weight development, BCS and backfat thickness. **Conclusions** Overall, effects of 3-NOP addition on methane emissions were within the expected range given in literature. Effects were constant over the whole lactation and therefore the present results do not indicate any adaptation of ruminal microorganisms on 3-NOP addition. The project was financially supported by the Bavarian State Ministry of Food, Agriculture, Forestry and Tourism.

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Effects of gallic acid on rumen fermentation, nutrient digestibility and rumen bacterial community in beef steers at low and high dietary crude protein levels

Auswirkungen von Gallussäure auf die Pansenfermentation, Nährstoffverdaulichkeit und die Pansenbakteriengemeinschaft bei Mastrindern mit niedrigen und hohen Rohproteingehalten im Futter

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Introduction: Gallic acid (GA) is a monomer of hydrolysable tannin with a small molecular weight. Our earlier research indicated that dietary addition of GA effectively decreased the urinary nitrous oxide (N₂O) emission from beef steers (Bao et al. 2018). However, it is unclear whether adding GA in diets affects rumen fermentation and nutrient digestibility in beef steers.

Methods: Eight Simmental steers (liveweight 310.5 ± 21.5 kg) were used as experimental animals. Two levels of dietary crude protein (CP) [(113.5 and 150.8 g/kg dry matter (DM))] and two levels of gallic acid (GA) (0.0 and 15.2 g/kg DM) were assigned in a replicated 2 × 2 crossover design. Each experimental period included a 14-d adaptation phase and a 5-d sampling phase. During each sampling phase, the faeces and urine from the animals were totally collected daily and rumen fluid samples were collected 2 h after morning feeding on the second day of each phase. The DM, total nitrogen (N), ether extract (EE) and crude ash of feeds and faeces were analyzed according to AOAC (1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) of feeds and faeces were analyzed on an Ankom 2000 fibre analyzer. The CP content was calculated as N × 6.25 and the organic matter (OM) was calculated as DM minus crude ash. The volatile fatty acids (VFA) in rumen fluid were determined using gas chromatography. The ammonia N (NH₃-N) in rumen fluid was analyzed using a colorimetric method on a spectrophotometer. The metabolites of GA, i.e. pyrogallol and resorcinol and GA in rumen fluid and faeces were analyzed on HPLC. The total genome DNA from ruminal fluid samples was extracted using the method of Porebski et al. (1997) for bacterial community analysis. The data were statistically analyzed using SAS 9.2 (SAS Institute, Inc., Cary, USA).

Results: Dietary addition of GA increased the ruminal concentration of acetate (P = 0.003) and the ratio of acetate to propionate (P = 0.007), tended to decrease the ruminal concentration of total VFA (P = 0.096), but it did not affect the pH and NH₃-N concentration of rumen fluid (P > 0.10) and the digestibility of DM, OM, EE, NDF or ADF (P > 0.10) at two dietary CP levels. GA increased the ruminal concentrations of GA and its metabolites including pyrogallol and resorcinol (P < 0.001) but GA, pyrogallol and resorcinol were undetectable in the rumen fluid samples from steers without feeding GA and undetectable in the faeces from all steers at the limit of 2 × 10⁻³ mmol/L. Gallic acid did not affect the diversity of ruminal bacterial community (P > 0.10), but decreased the relative abundance of Cyanobacteria and increased Fibrobacteres at the phylum level (P < 0.05), and decreased the relative abundances of *Prevotella_1* and *Ruminococcaceae_NK4A214_group* and increased that of Fibrobacter at the genus level (P < 0.05).

Conclusions: Dietary addition of GA had a minor impact on rumen fermentation and rumen bacterial community and had no influence on nutrient digestibility.

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Supplementation of the human milk oligosaccharide 2'-Fucosyllactose to calves milk replacer

Ergänzung des humanen Milcholigosaccharids 2'-Fucosyllactose zum Milchaustauscher für Kälber

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Introduction: The foundation for high-performing cattle and successful beef production is the rearing of healthy, vital calves. Human milk oligosaccharides (HMOs) are naturally occurring, soluble milk sugar derivatives that serve as fermentable oligosaccharides. Among them, 2'-fucosyllactose (2'-FL) is one of the most abundant components in human milk and is characterized by prebiotic, anti-adhesive, antimicrobial, and immunomodulatory properties [1]. When added to calf feeding systems, 2'-FL may support digestive function, the gut microbiota, and contribute to a balanced diet in early life. Recent studies also indicate that milk oligosaccharides have beneficial effects on performance, gut development, the immune system, and calf health [2].

Methods: A practical trial was conducted with 181 male Simmental calves, purchased from a local calf market and housed simultaneously in two identical calf barns, each equipped with two automatic milk feeders. During the milk replacer phase, the 181 calves were divided into two groups. 89 calves (split into 2 treatment groups) received 11.4 g of pure 2'-FL per calf per day from day 3 until weaning at day 37, administered via 2 automatic milk feeders. The control groups (92 calves, also reared in 2 groups) were kept under identical conditions without supplementation. At the beginning of the study, the control group was 43 days old (SE = 9.6), and the treatment group was 46 days old (SE = 10.5). Calves were weighed on days 0, 13, 28, 44, 93, and up to day 133. Average daily weight gain was calculated for the intervals between weighings.

Results: From day 0 until day 93, all calves showed comparable growth rates (2'-FL: 0.95 kg/d (SE: 0,242) vs. control: 0.99 kg/d (SE: 0,249)). During the final weighing period (day 93 to day 133), the treatment groups achieved a significantly ($p < 0.05$) higher daily gain compared to the control groups (1.69 kg/d (SE: 0,278) vs. 1.59 kg/d (SE: 0,317)).

Conclusions: The supplementation of 2'-FL during the milk replacer phase resulted in a significant improvement in growth performance in the last weighing period from day 93 to 133. As a novel feed material, 2'-FL may serve as a source of fermentable oligosaccharides for young animals. Further long-term studies are needed to confirm its effect and explore the potential of this human milk oligosaccharide in calf rearing.

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Individual promotion of calf health using specific colostrum additives and phytogetic substances

Tierindividuelle Förderung der Kälbergesundheit durch Nutzung spezifischer Kolostrumadditive und phytogener Substanzen

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Introduction: Since calves only must be registered and reported from the 7 days of life onwards, it is difficult to generate valid data on calf mortality rates, it is assumed that these mortality rates are still too high and need to be reduced further. The most common causes of calf mortality are diarrhea and respiratory diseases [1]. These are diseases whose incidence could be reduced through improved management. The overall objective of the “CalPhy” project is to reduce the incidence of diseases requiring treatment in calves and thus counteract increased losses in calf husbandry. Using a colostrum supplement and a phytogetic additive, we aim to improve the overall care of calves and thus promote animal health, welfare, and performance. The aim is to develop an individualized feeding concept for agricultural practice for particularly sensitive animals within animal groups. Colostrum supply in the first 24 hours after birth is important for the survival chances of calves. Phytogetic additives are intended to increase the resilience of calves to stressors by improving intestinal health through modulation of the immune system.

Methods: TiHo and Phytobiotics conduct a series of comparative feeding trials on a conventional dairy farm with 1,000 milking cows, to examine the supplements both individually and in combination on several hundred calves in the first four weeks of life (2243/2-19-2024-20-E). In consecutive trials 4 x 60 calves are housed individually in four trial groups (group 1 – group 4) and 4 control groups of 30 calves each. Each group receives a different supplementation consisting of colostrum supplement (Trial 1 - 300g within 3 hours after birth / Trial 2 - 300g over 28 days), phytogetic additive or both supplements. Blood, fecal samples, body weight, and various animal welfare parameters from each calf on days 1, 14, and 28 of life were collected or recorded. The data was recorded for each calf using a specially designed questionnaire via “RedCap®” application. All groups were housed in two identical calf barns, each with 50 individual places. The milk replacer / phytogetic additive was fed in both barns using the Förster Technik® CalfRail. Water and calf mixed ration were available *ad libitum*. Statistical analyses were performed with the program R, normally distributed data (body weight) were compared with a T-test, non-normally distributed data (total protein in blood serum (Tp) and IgG) were compared with Kruskal-Wallis-Test and Wilcoxon-Test.

Results: The course of the trial so far shows a tendency towards higher body weights at day 28 of life in the trial groups (Control 1: 58.5 ± 7.32 kg vs. Trial 1: 59.4 ± 7.09 kg; Control 2: 64.3 ± 6.35 kg vs. Trial 2: 67.2 ± 6.86 kg). Tp and IgG at day 28 were equal between groups in Trial 1 and 2 (Trial 1: Tp 55.4 ± 2.81 g/l vs. 54.2 ± 2.78 g/l and IgG 725 ± 181 mg/dl vs. 722 ± 202 mg/dl; Trial 2: Tp 56.9 ± 3.23 g/l vs. 55.1 ± 3.62 g/l and IgG 797 ± 22 mg/dl vs. 806 ± 273 mg/dl for control- and trial groups respectively).

Conclusions: The results to date show a clear positive trend in terms of calf body weight regarding supplementary feeding with additional colostrum powder. A comparison of the groups also shows a slightly positive trend in higher total protein. More Conclusions regarding other test parameters recorded for the calves can only be made after a final statistical evaluation of all test groups.

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Supplementation of rumen-protected choline during warm summer days reduces body surface temperatures and improves production traits of dairy cows

Die Zugabe von pansengeschütztem Cholin an warmen Sommertagen verringert die Oberflächentemperatur und verbessert Leistungsparameter von Milchkühen

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Introduction: When the daily mean temperature–humidity index (THI) in Western European barns increases above 56 to 60, dairy Holstein cows display first signs of heat stress [1]. It has been shown that dietary supplementation of rumen-protected choline (RPC) is a promising nutritional strategy to mitigate negative effects of heat stress and improve feed efficiency and milk production in dairy cows [2], and goats [3]. The mode of action includes choline's role in supporting energy metabolism and immune function, but its role as acetyl choline precursor regulating blood circulation in the skin, thus improving heat dissipation and reducing surface temperature, has not been studied. Therefore, the main objective of the study was to investigate the impact of RPC supplementation on body surface temperature in Holstein dairy cows.

Methods: Eighteen multiparous Holstein cows (average milk yield: 32.2 kg/d; days in milk: 85) kept in a free-ranging barn were randomly allocated to a control (CON; n = 9) and RPC supplemented (RPC; n = 9) group. In the pre-experimental period, both cow groups were fed the same total mixed ration (TMR) for one week (week -1), starting in July 2025. In the experimental period, the RPC group were fed the same TMR supplemented with 60 g RPC/cow/day for 4 weeks. Rectal temperature was measured in week -1, and muzzle, ear and eye temperatures were measured on day 20 of the experimental period using an infrared camera. Air temperature, relative humidity, wind speed and solar radiation were recorded throughout the trial to calculate the temperature humidity index (THI) and comprehensive climate index (CCI). Individual feed intake, water intake (WI), body weight (BW) and milk yield (MY) were recorded daily. The TMR dry matter and milk composition were analysed weekly to calculate dry matter intake (DMI) and energy-corrected milk yield (ECM), respectively. The statistical analysis was performed by repeated measures ANOVA (fixed effect: group, time and their interaction) or student's t-test. Significance was detected at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.1$.

Results: In the pre-experimental phase, the weekly mean THI was 67 and the weekly mean CCI was 24. During the experimental phase, THI ranged between 65 to 68 and CCI between 22 and 26. No significant differences ($P > 0.1$) were noticed for rectal temperature, BW and DMI between the groups in the pre-experimental period, but ECM and WI were greater in the RPC than CON group ($P < 0.05$). During the experimental period, the eye temperature of RPC cows was significantly lower ($P = 0.05$) than in the CON group. The RPC group tended to have lower values of ear ($P = 0.07$) and muzzle temperatures ($P = 0.1$). Relative to week -1, DMI, BW, ECM, milk protein and lactose yields were higher in the RPC compared to the CON group ($P < 0.01$). Furthermore, RPC cows tended to have higher fat yield ($P = 0.06$), milk fat ($P = 0.09$), and lactose percentages ($P = 0.08$).

Conclusions: The lower body surface temperatures in dairy cows supplemented RPC are likely due to increased heat dissipation facilitating feed intake and milk production during warm summer days.

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Impact of the green alga *Ulva intestinalis* on ruminal methanogenesis and fermentation parameters *in vitro* using the Rumen Simulation technique (RUSITEC)

Einfluss der Grünalge Ulva intestinalis auf die ruminale Methanogenese und weitere Fermentationsparameter in vitro mithilfe der Rumen Simulations Technik (RUSITEC)

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Introduction: The rapid increase in global greenhouse gas emissions, dominated by methane (CH₄) and carbon dioxide (CO₂), is making it crucial for environmental protection to find sustainable mitigation strategies. An emerging approach might be the use of potential feed materials that could influence the ruminal microbiome and therefore might have an impact on methane mitigation. This study aimed to investigate the effect of the green alga *Ulva spp.* on different fermentation parameters using the *in vitro* rumen simulation technique (RUSITEC).

Methods: The experiment was conducted according to the general incubation procedure of the RUSITEC described by Czerkawski and Breckenridge (1). It was arranged as a completely randomized, 2 x 2 factorial design with a high (HC)- and a low (LC)-concentrate diet that was either incubated with (AL+) or without (AL-) the addition of the alga *Ulva spp.* at a concentration ratio of 4% dry matter (DM). The experimental diets on a DM basis consisted of a forage: concentrate ratio of 70:30 for the LC and 40:60 for the HC, with a forage composition of 70% maize silage and 30% grass silage. In total, three incubation trials with a 2 x 6-vessel RUSITEC were conducted (N=36). In every trial, each diet x algae combination was tested in triplicate (n=9) and one trial lasted for 12 days with 8 days of equilibration followed by a sampling period of 4 days. Prior to each trial, liquid and solid rumen content were collected from five fistulated German Holstein cows. Each fermenter was then inoculated with 700ml of pooled ruminal fluid, 80g solid rumen content and 12g DM test substrate. The test substrate was incubated for 48h and then replaced. During the sampling period, the fermented substrates were analyzed for DM and dry matter degradability (DMD) was calculated. Every day, pH and redox potential were measured and volatile fatty acid- (VFA) and ammonia-N- (NH₃-N) concentration were analyzed. The fermentation gases were collected over the sampling period. Total gas volume was measured and gas samples were analyzed for CH₄, CO₂ and H₂ concentration. Statistical analysis was done using PROC MIXED (SAS, version 9.4) with diet, alga, time and their interactions as fixed effects and incubation vessel nested in run as random effect.

Results: Preliminary analysis of the parameters showed a significant ($p < 0.001$) more negative redox potential for the HC diet compared to the LC diet. With the AL+ treatment, the redox potential was lower for both the LC and HC diet compared to the AL- treatment, indicating a generally more negative redox potential for AL+. The HC also showed a significant higher DMD ($p < 0.001$) compared to the LC and there is a tendency ($p = 0.062$) for the AL+ to have a higher DMD than AL-. The NH₃-N concentration was higher ($p < 0.001$) for the HC compared to the LC diet, but no impact of the alga could be detected. An examination of the acetate: propionate ratio revealed a shift towards propionate for the HC compared to the LC, where the ratio shifted towards acetate ($p < 0.001$), but there were no significant changes when adding *Ulva spp.* However, alga addition lowered valeric acid (C5 mmol/L) concentration ($p = 0.003$) and tended to reduce butyric acid (C4 mmol/L) concentration ($p = 0.053$). Overall, no significant impact of the alga or the diet on total gas production and CH₄-concentration could be observed.

Conclusions: The effects of the addition of the green alga *Ulva spp.* on the redox potential and the VFA-concentration, in combination with the tendency to improve the DMD, could indicate that the alga might influence the rumen microbiome, without having an effect on gas- and methane production. Further studies are needed to evaluate the potential impact of the addition of *Ulva spp.* on animal health and performance.

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Impact of increasing dosages of a phytase in broiler feed on nutrient digestibility and performance parameters.

Einfluss steigender Dosierungen einer 6-Phytase in Broilerfutter auf Nährstoffverdaulichkeit und Leistungsparameter

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Introduction: In practice, phytases are mainly used to increase the phosphorus and calcium digestibility of plant-based feedstuffs whereas the effect of this additive class on digestibility of energy and above all protein is still often questioned. The focus of the present study was to evaluate the effect of increasing doses of a 6-phytase on digestibility of these two but as well of other nutrients and on animal's performance.

Methods: One-hundred-fifty, one-day-old male broiler chicks (Ross 308) were randomly distributed to 5 treatments with 10 replicates per treatment and 3 birds per replica. The duration of the study was 26 days, while a standard commercial crumbled diet was provided *ad libitum* from day 1-6 and an experimental, no inorganic P-containing, pelleted diet, from day 6-26. Access to water was given *ad libitum* through nipple drinkers. The treatments consisted of a control diet (T1) and the following four treatments that were obtained by adding a hybrid bacterial 6-phytase (Natuphos® E 10000 G, BASF SE) at levels dosed for 500 FTU/kg (T2), 1000 FTU/kg (T3), 1500 FTU/kg (T4) and 2000 FTU/kg (T5) to T1. T1 fulfilled all nutrient requirements except P. The diet formulation was as follows: [%]: corn (61.08), soybean meal (24.4), sunflower meal (5.0), rapeseed meal (5.0). Performance parameters (FI, BWG, FCR) were measured and calculated, respectively, for pre-experimental and experimental phases. On day 26, birds were euthanized by electric stunning and digesta samples were taken from the middle of the ileum, frozen at -80°C, and later on analyzed for Ca-, P-, N- and energy for calculating the apparent ileal digestibility (AID). TiO_2 was used as a dietary marker at a concentration of 0.5%. The experiment was conducted as a completely randomized design, and digestibility data were analysed using variance analysis using the general linear model procedure of JMP Pro 18. Data are presented as means with root means square error. The least square means separation was done by the Dunnett post hoc test.

Results: No difference in performance parameters was found between the treatments during and at the end of the pre-experimental phase. During the following experimental phase, the performance parameters were significantly increased by phytase supplementation compared to T1 ($p < 0.05$), and the dose response was also significant ($p < 0.001$). The AID of Ca, P were significantly increased by all dose rates ($p < 0.05$), while for crude protein (CP) and digestible energy (digE), that was only the case for T4 and T5 and for T3 and T5, respectively. At the highest dose rate AID of CP was increased from 75.35% (T1) to 78.83% (T4) (+4.6%), digE was increased from 2927 kcal/kg of DM (T1) to 3063 kcal/kg of DM (T4) (+136 kcal). A significant dose effect was observed for AID of all nutrients ($p < 0.001$) and for digE ($p = 0.003$).

Conclusions: The study proved that the applied bacterial 6-phytase not only significantly increases performance parameters and AID of Ca and P, but potentially also crude protein and energy digestibility. Considering this, using the feed additive clearly provides further practical cost savings and benefits the environment.

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Impact of pastures composition on calves microbiota in saliva, rumen and faeces*Einfluss der Weidezusammensetzung auf die Mikrobiota von Kälbern in Speichel, Pansen und Kot*

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Introduction: The rearing of male calves from dairy herds poses considerable economic and sustainability challenges [1]. Utilizing underused pasture areas for calf rearing may represent an opportunity to improve resource efficiency and environmental performance. Because diet composition strongly influences the gastrointestinal microbiota and, consequently, ruminal fermentation efficiency, it is essential to assess how different pasture types affect microbial community development. This study investigated the effects of three contrasting pasture compositions and one indoor system on the bacteria in saliva, rumen, and fecal samples of calves between 3 and 6 months of age.

Methods: 72 male calves of three breeds (Brown Swiss, Swiss Fleckvieh, Limousin × Brown Swiss crossbreed) were raised under same conditions for the first three months and then allocated to alpine, permanent or intensive pastures and to an indoor control group. All animals received hay *ad libitum*, while grazing times were measured in hours (771 alpine, 769 permanent, 611 intensive). Additionally, all groups were fed with lucerne and maize pellets as well as concentrate feeding. CP and NDF (g/kg DM) content levels are known for all supplementary feed. Sampling was done between May and September 2024. Saliva and fecal samples were collected at three time points every months, from 3 to 6 month of age, and rumen fluid was obtained once via esophageal probe at six months of age. Bacterial communities were characterized by 16S rRNA amplicon sequencing, processed in QIIME 2 (DADA2), and taxonomically assigned using GTDB (v226). Diversity metrics (Shannon index and Bray–Curtis dissimilarities) and statistical tests (Kruskal–Wallis, PERMANOVA) were calculated in R (vegan package).

Results: Across all sample types, phyla of *Bacteroidota* and *Pseudomonadota* dominated, with minor contributions from *Fusobacteriota*, *Fibrobacterota*, and *Spirochaetota*. In the rumen, genera *Succinimonas* and *Succinivibrio* were key carbohydrate fermenters converting sugars to succinate and short-chain fatty acids, contributing to energy supply and rumen fermentation efficiency. Among *Bacteroidota*, genera *Prevotella* was predominant, reflecting its central role in degrading polysaccharides and proteins. The presence of *Fibrobacterota* and *Spirochaetota* suggests fibrolytic and cellulolytic potential. Fecal samples were mainly composed of *Bacteroidota* and *Spirochaetota*, and a high proportion of low-abundance taxa (<1%). Calves raised on alpine pastures showed the highest alpha diversity, which increased over time, while the indoor group displayed the lowest. Saliva-associated bacteria exhibited the highest variability among animals, and rumen-associated bacteria the highest overall diversity. Beta diversity analyses revealed clear clustering by sample type. For saliva and faeces, alpine and permanent pastures formed distinct clusters, while intensive and indoor groups overlapped. PERMANOVA confirmed significant effects of pasture types on microbial composition across all sample types ($p < 0.001$) and time effects for saliva and faeces ($p < 0.001$), whereas breed has no significant influence.

Conclusions: Pasture composition significantly affected microbial composition and diversity. Calves grazing alpine and permanent pastures developed richer, more diverse microbiota compared to indoor or intensive systems. Repeated sampling enabled the characterization of microbial community dynamics and the identification of taxonomic differences among pasture systems, sampling times, and genotypes. Individual-level observations further allowed assessment of inter-animal variability and microbial stability during calf development. Given the calves' young age, these findings suggest ongoing rumen microbial maturation influenced by pasture type and feeding environment.

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WiMiQ: Metabolic adaptation patterns in dairy cows following changes in dietary energy supply: a multivariate approach to analyzing classical feeding trials

WiMiQ: Stoffwechselanpassungsmuster bei Milchkühen nach Veränderungen der Energiezufuhr: Ein multivariater Ansatz zur Analyse klassischer Fütterungsversuche

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Introduction: Previous research has shown that the amount of energy in a dairy cow's diet has a strong effect on metabolic and phenotypic responses. These alterations are closely linked to energy partitioning and adaptation processes that follow dietary changes. The present study aimed to induce contrasting metabolic states by varying the energy content of the diet. We hypothesised that such dietary shifts would lead to distinct metabolic response patterns emerging over time between feeding groups and between individuals, which could be identified through multivariate analysis.

Methods: A six-week feeding trial was conducted with 46 mid-lactation Holstein cows, divided into two groups. Cows were initially fed either a high-energy (HE; 11.45 MJ ME/kg dry matter (DM)) or a low-energy (LE; 10.85 MJ ME/kg DM) diet, with energy levels adjusted via concentrate proportion using automated feeders. At the start of the trial, an abrupt switch was made: HE cows received a diet with lower energy content 10.8 MJ ME/kg DM, and LE cows received a higher energy content of 11.5 MJ ME/kg DM, resulting in the group designations HE/LE and LE/HE. All cows had *ad libitum* access to water and a partial mixed ration (66% maize silage, 32% grass silage, 2% barley straw). The trial included a three-week adaptation period followed by three weeks of sampling. DM intake (DMI), body weight and milk yield (MY) were documented daily and milk composition was determined once a week. Before the trial (week (w) -1) and during the sampling period (w 4, w 5, w 6), blood samples were taken by needle puncture from an external jugular vein and serum was obtained by centrifugation (Heraeus Varifuge 3.0R, Germany). Serum was used to determine levels of glucose, beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Statistical analysis was performed using Proc MIXED in SAS (9.4), including time (T), group (G), and their interaction (TxG) as fixed effects, individual cows as random factor, and w -1 as covariate. Principal component analysis (PCA) based on a set of metabolic and performance variables was performed using R package mixOmics and combined with hierarchical clustering to explore multivariate patterns among the different feeding groups and to identify individual adapting patterns in response to changes in energy intake.

Results: DMI and MY increased in LE/HE and decreased in HE/LE over time ($p_{TxG} < 0.001$), while DMI increased in HE/LE again in w 6. Glucose levels tended to vary between feeding groups over time ($p_{GxT} = 0.054$). Both groups reached their nadir in w 5 with a subsequent increase in LE/HE to the baseline value and remaining lower levels in HE/LE. Significant group differences over time were observed for BHB and NEFA ($p_{TxG} \leq 0.026$). BHB levels in HE/LE increased significantly by w 4 and reached its maximum at w 5 (0.94 mmol/L). While BHB levels remained unaffected by the feed change in LE/HE, NEFA levels decreased significantly by w 5 in this group. In HE/LE, NEFA levels remained stable with variation over time reaching a similar level to that of the LE/HE by the end of the trial. PCA and cluster analysis revealed distinct temporal response patterns between feeding groups. The procedure partially separated the groups, indicating that dietary energy shifts induced specific metabolic profiles, while individual variability remained evident, as some cows did not align with the main group clusters.

Conclusions: The observed differences between feeding groups reflect the typical metabolic and performance adaptations to changes in dietary energy content. Multivariate analyses confirmed distinct group-related response patterns, while also revealing individual variability. These findings emphasise the dynamic nature of metabolic adaptation processes, demonstrating the potential of novel approaches to capture the diversity of responses in dairy cows.

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Influence of different dietary energy levels on the rumen microbiota of dairy cows*Einflüsse verschiedener Energieniveaus in der Ration auf die Pansenmikrobiota in Milchkühen*

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Introduction: Dietary energy supply plays a key role in rumen function and microbial ecology in dairy cows by influencing rumen fermentation and nutrient availability for microorganisms. Therefore, this study aimed to investigate how changes in dietary energy levels affect the structure and temporal stability of the rumen microbiome, with the goal of improving feeding strategies and supporting rumen health in dairy cows.

Methods: A total of 48 mid-lactation Holstein dairy cows (178.2 ± 23.5 days in milk; primi- and multi-parous cows) were randomly assigned at week -1 to a 2×2 design with two dietary energy levels, high-energy (HE; 11.45 MJ ME/kg dry matter [DM]) and low-energy (LE; 10.85 MJ ME/kg DM) and two partial mixed ration (PMR) types: a grass silage-based PMR (57% grass silage, 22% sugar beet pulp silage, 12% brewers grain silage, 2% straw, 7% concentrate) and a maize silage-based control diet (63% maize silage, 29% grass silage, 2% straw, 6% concentrate). From week 1, dietary energy levels were reciprocally exchanged between groups, such that cows initially fed the high-energy diet were transitioned to the low-energy diet, and those receiving the low-energy diet were switched to the high-energy diet, while all cows were offered a common diet supplemented with fava beans and *ad libitum* access to a PMR consisting of 66% maize silage, 32% grass silage and 2% barley straw. Diet formulation and nutrient supply were conducted in accordance with the GfE (2023) recommendations. The trial lasted a total of 7 weeks (from week -1 to week 6), including a 3-week adaptation period to the new diet. Rumen fluid samples were collected in weeks 4 and 6 using an orogastric tubing technique. DNA extraction and sequencing were performed by BMK Gene, targeting the V4 region of the 16S rRNA gene for bacteria and the V3-V4 region of the 16S rRNA gene for archaea. Raw reads were processed in QIIME2 (v2024.10) using the SILVA 138.2 reference database for taxonomic classification and downstream analyses were conducted in R. Microbial α -diversity was calculated using the Shannon index, whereas β -diversity was assessed based on Bray-Curtis dissimilarity and visualized using principal coordinates analysis (PCoA). Differences in microbial composition between energy levels and the temporal persistence of supplement-associated patterns observed at week -1 were tested across subsequent weeks using PERMANOVA. Bray-Curtis dissimilarity was also used to quantify microbiome shifts from week -1 to week 6 to assess individual animal adaptation over time.

Results: Principal coordinate analysis (PCoA) revealed a clear separation of bacterial communities according to energy levels, which was statistically significant ($P < 0.05$). Archaeal communities showed a less pronounced clustering, though the separation between energy levels remained significant ($P < 0.05$). Analysis of the supplement effect indicated that rumen fluid samples were clearly separated by supplement groups at week -1, but this distinction was no longer observed in weeks 4 and 6. A similar pattern was seen for the archaeal community. While the main effect of the supplement was significant ($P < 0.05$), a significant week \times supplement interaction indicated that the microbial response to supplementation changed over time. Bray-Curtis dissimilarity values varied among animals, with some individuals exhibiting more pronounced microbial shifts than others.

Conclusions: Energy level was the primary driver of microbial community composition, highlighting the strong impact of dietary energy on the gut microbiome. Previous dietary effects had disappeared by later weeks, indicating full adaptation to the new diet. Individual variability in microbiome shifts suggests differences in the rate and extent of adaptation among animals.

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Milk-Derived Extracellular Vesicles: Characterization and Proteomic Profiling of their Response to Dietary Energy Transitions in Dairy Cows

Extrazelluläre Vesikel in Milch: Charakterisierung und Proteomprofiling ihrer Reaktion auf Energiezufuhränderungen bei Milchkühen

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Introduction: Extracellular vesicles (EV) are nanosized carriers of bioactive components such as proteins, lipids, and nucleic acids that mediate intercellular communication between different organs, such as between the gastrointestinal tract and the mammary gland. Dietary interventions and associated shifts in the gut microbiome can influence systemic metabolism and be reflected in altered milk EV (mEV) cargo composition [1, 2]. This study aimed to comprehensively characterize mEV, including their particle characteristics and proteomic composition, in response to changes in diet composition in mid- to late-lactation cows. We hypothesized that changes in the dietary concentrate : roughage ratio would alter both the abundance and protein cargo of mEV, reflecting metabolic remodelling in response to the altered energy availability.

Methods: Twenty German Holstein cows (182 ± 15 days in milk) were kept either on a high-energy (HE; 45% concentrate, $n = 10$) or low-energy (LE; 25% concentrate, $n = 10$) diet. At the start of the experiment (Week 1), the diets were switched: HE cows received a low-energy diet (20% concentrate; HE/LE), and LE cows received a high-energy diet (50% concentrate; LE/HE). Feeding lasted 6 weeks, with milk sampling at Week -1 (baseline) and Week 6 (post-transition). The mEV were isolated by differential centrifugation and ultracentrifugation, purified using size exclusion chromatography, and characterized by nanoparticle tracking analysis, transmission electron microscopy (TEM), and western blotting to demonstrate the presence of an EV marker (CD9). Proteomics were profiled by LC-MS/MS (Orbitrap Fusion Lumos), and processed in Proteome Discoverer 3.2 (Chimerys node) against the *Bos taurus* UniProt proteome (UP000009136) at 1% false discovery rate (FDR). Statistical analysis in R (4.4) included variance stabilization (VSN), missing-value imputation (msImpute v2-mnar), and differential analysis using Limma. Functional enrichment was performed with STRING (v12), ClueGO (v2.5.10), and summarized with REVIGO.

Results: TEM showed typical round and cup-shaped vesicles (~100 nm), and western blot confirmed the EV marker CD9 in all samples, validating successful mEV isolation. No significant differences in EV concentration or size were observed among groups, indicating that dietary energy transitions did not affect EV abundance or morphology. Proteomic profiling identified 1,248 proteins, including 74 from Vesiclepedia's top-100 EV proteins, indicating high vesicular purity. Comparative analyses revealed 33 differentially abundant proteins (DAPs) between the groups at Week 6, with 17 downregulated and 16 upregulated in LE/HE versus HE/LE. Downregulated proteins were mainly related to amino acid biosynthesis, endoplasmic reticulum organization, and glutathione peroxidase activity, while upregulated proteins were associated with methionine synthesis, ossification, and neuron recognition. Within the LE/HE group, 64 DAPs (27 downregulated, 37 upregulated) were identified when comparing week 6 with week -1, indicating enhanced vesicular trafficking and protein sorting (e.g., flippase activity, protein localization to endosome) and reduced endocytic and phosphorylation-mediated signaling. Among the top 100 EV proteins, those encoded by BSG (Basigin) were upregulated, whereas proteins encoded by RAB10 (Ras-related protein 10), PRDX1 (Peroxiredoxin-1), YWHAE (14-3-3 protein epsilon), and YWHAB (14-3-3 protein beta/alpha) were downregulated in Week 6.

Conclusions: Dietary energy transitions significantly modulated the proteomic composition of mEV, with stronger effects observed during the low-to-high-energy (LE/HE) transition. Alterations in amino acid metabolism, vesicle trafficking, and redox-related proteins indicates adaptive remodelling of EV cargo to energy supply. These results show that mEV reflect nutritional and metabolic states, providing new insight into molecular mechanisms underlying the adaptation to different diets in dairy cows.

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WiMiQ: Rumen fermentation patterns and ruminal pH values reflect host–microbiome interactions after energy-related dietary shifts in dairy cows

WiMiQ: Fermentationsmuster und pH-Werte im Pansen spiegeln die Wechselwirkungen zwischen Wirt und Mikrobiom nach energiebezogenen Futterumstellungen bei Milchkühen wider

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Introduction: The rumen microbiome is central to host energy metabolism, and shifts in dietary energy content are known to alter its fermentation activity. This study aimed to evaluate functional changes in response to energy-related dietary transitions, using dietary energy level as the experimental input, rumen pH as the environmental condition, and volatile fatty acids (VFA) as main microbial fermentation products.

Methods: A six-week feeding trial was conducted with 46 mid-lactation Holstein cows, divided into two groups. Cows were initially fed either high-energy (HE; 11.45 MJ ME/kg dry matter (DM); 45% concentrate, mainly composed of 34% soybean meal, 39% faba bean) or low-energy (LE; 10.85 MJ ME/kg DM; 25% concentrate, with a main composition of 15% rapeseed/soybean meal, 14% maize, 11% wheat/barley/rye) diets via automated feeders. At the trial start, HE cows switched to 10.8 MJ ME/kg DM (20% concentrate), LE cows to 11.5 MJ ME/kg DM (50% concentrate), resulting in the group designations HE/LE and LE/HE. All cows had *ad libitum* access to water and a partial mixed ration (PMR) (66% maize silage, 32% grass silage, 2% barley straw). The trial included a three-week adaptation period followed by three weeks of sampling. Feed intake was recorded daily. Dry matter intake (DMI) was calculated from fresh matter intake of PMR and concentrate, adjusted to their analyzed DM contents. The ruminal pH was measured and VFA concentrations were determined by gas chromatography in week (w) -1, w 4 and w 6 from oro-ruminal samples. Statistical analyses were performed using the MIXED procedure in SAS (9.4), including time (T), group (G), and their interaction (TxG) as fixed effects, individual animals as a random factor, and w -1 as a covariate.

Results: Feed change resulted in a contrasting DMI in both groups ($p_{\text{TxG}} < 0.001$). LE/HE showed an increased DMI by w 3, which remained stable. By w 4, the DMI of the HE/LE group had decreased and remained stable. There was a significant increase in pH values ($p_{\text{TxG}} = 0.018$), whereby HE/LE remained unchanged until week 4, while LE/HE exhibited a consistent increase. Findings on VFA concentrations demonstrated that there were no significant differences between groups over time in the concentrations of acetic acid and butyric acid, but both concentrations were constantly higher in LE/HE. Significant difference in propionic acid concentrations were observed between the two groups ($p_{\text{TxG}} < 0.001$), with LE/HE exhibiting higher levels, reaching a peak in w 4 that subsequently returned to baseline levels. HE/LE showed no significant differences over time. The acetate: propionate ratio demonstrated a significant group by time interaction ($p_{\text{TxG}} < 0.001$), with HE/LE maintaining higher values that continuously increased (up to 3.75), while LE/HE reached a nadir at w 4 (2.55).

Conclusions: Dietary transitions elicited distinct rumen responses, reflecting the interplay between feed intake, rumen pH, and microbial fermentation. HE/LE adapted with a delayed pH increase, initially reduced feed intake that later stabilized and rose, and consistently elevated acetate and butyrate levels, suggesting stable fibre-driven fermentation. LE/HE exhibited a continuously rising pH and a pronounced propionate peak, indicative of a glucose-oriented fermentation pathway. These group-specific fermentation patterns reflect microbial metabolic outputs and provide a context-specific functional perspective on host–microbiome interactions following dietary energy transitions.

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Workshop

■ Successfully implementing recommendations for energy and nutrient supply in dairy cows! Implementation of GfE (2023) in DLG 2025 – Concept for science, industry and practice

Empfehlungen zur Energie- und Nährstoffversorgung bei der Milchkuh erfolgreich umsetzen!
Umsetzung von GfE (2023) in DLG 2025 – Konzept für Wissenschaft, Wirtschaft und Praxis

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Die Gesellschaft für Ernährungsphysiologie (GfE) hat im September 2023 ein Buch mit den neuen Versorgungsempfehlungen des Ausschusses für Bedarfsnormen (AfBN) für Energie und Nährstoffe von Milchkühen herausgegeben, das im DLG-Verlag erschienen ist. Hiermit wurde dem kontinuierlich gestiegenen Milchleistungsniveau der Tiere ebenso Rechnung getragen wie der Notwendigkeit, eine diverser gewordene Futtermittelgrundlage angemessen zu bewerten. Die neuen Empfehlungen berücksichtigen den aktuellen Stand des international verfügbaren Wissens und lösen die Empfehlungen der GfE aus dem Jahre 2001 ab, die ihrerseits auf Konzepte und Daten aus den siebziger und achtziger Jahren des letzten Jahrhunderts zurückgingen. Bei der Auswahl der Daten und den neuen Auswertungen wurde darauf geachtet, dass sie die Futtergrundlage und die Entwicklungen der Produktionsbedingungen speziell in Mitteleuropa abbilden. Dies schließt die Eignung und Anwendbarkeit der neuen Empfehlungen unabhängig von den Produktionssystemen, in denen Milch erzeugt wird, ein. Die Auswertungen ergaben, dass für die meisten Nährstoffe das bisherige Vorgehen fortgeschrieben werden kann und die bislang verwendeten Faktoren des Bedarfs weiterhin zutreffend sind. Für einige Nährstoffe waren jedoch Anpassungen erforderlich, die zu Veränderungen bei einzelnen Faktoren zur Herleitung des Bedarfs oder der empfohlenen Konzentration in Rationen für Milchkühe geführt haben. Besonders weitgehend sind die Veränderungen bei der Energie und beim Protein; denn hier wurden nicht nur die Faktoren des Bedarfs verändert, sondern es wurden neue Systeme zur Bewertung der Futtermittel vorgestellt.

In Ergänzung zur Energie und zu den Nährstoffen werden im Buch weitere Themen behandelt. So wurde ein besonderes Augenmerk auf die Höhe der Futteraufnahme und die Futteraufnahme beeinflussende Faktoren gelegt. Hiermit steht die Wiederkäuergerechtigkeit der Ration und die Berücksichtigung der Strukturwirksamkeit über die physikalisch effektive Neutral-Detergenzien-Faser (peNDF) in einer engen Beziehung. Die Erläuterungen hierzu helfen, möglichen Zielkonflikten zwischen Gesundheit und Leistung von Tieren vorzubeugen. Zudem adressiert das Buch an verschiedenen Stellen Zusammenhänge, die die Umweltwirkungen der Milchkuhfütterung betreffen, z. B. im Hinblick auf Stickstoff und Phosphor. Der Abgabe von Methan wird ein separates Kapitel gewidmet, in dem die biologischen Zusammenhänge und Beeinflussungsmöglichkeiten ebenso behandelt werden wie die Möglichkeiten und Verfahren zur Schätzung der Methanabgabe. Des Weiteren geht das Buch auf artgerechte Ernährung, Gesunderhaltung, Einflüsse auf die Zusammensetzung und Qualitätseigenschaften der Milch sowie auf Sonderwirkungen bestimmter Futtermittel und Futtermittelzusatzstoffe ein.

Die Empfehlungen des AfBN zur Versorgung der Nutztiere bilden die Grundlage der Fachaussagen in der angewandten Forschung, dem Laborwesen, der Futtermittelwirtschaft, der Beratung, der Schule und der landwirtschaftlichen Praxis. Die Aktivitäten zur Umsetzung werden durch die Gremien der Deutschen Landwirtschafts-Gesellschaft (DLG), dem Verband der landwirtschaftlichen Untersuchungs- und Forschungsanstalten (VDLUFA) und dem Verband der Landwirtschaftskammern (VLK) koordiniert. Die Federführung liegt beim DLG-Arbeitskreis Futter und Fütterung. Im Weiteren wird das Konzept des DLG-Arbeitskreises Futter und Fütterung für eine mit allen Beteiligten abgestimmte Einführung und Implementierung der neuen Empfehlungen in die praktische Anwendung nach einer kurzen Darstellung der wichtigsten fachlichen Neuerungen zur Sicherstellung der Energie- und Proteinversorgung erläutert.

Energie

Die Umsetzbare Energie (ME, metabolisable energy) ist seit der Ablösung der „Stärkeeinheiten“ die Stufe, auf der die Energie für Wiederkäuer universell bewertet wird. Speziell für die Anwendung bei Milchkühen wurde bislang aus der ME die Nettoenergie für Laktation (NEL) berechnet. Diese Umrechnung entfällt und auch für die Milchkuh werden zukünftig der Bedarf und die energetischen Futterwerte auf der Stufe der ME ausgedrückt. Für die Bestimmung der ME von Futtermitteln wurde aus Originaldaten ein neues Vorgehen entwickelt und geprüft, das sogenannte **dreistufige Verfahren**. Dies löst das bisherige Vorgehen ab, bei dem die ME mittels Regressionsgleichungen aus verdautlichen Rohnährstoffen berechnet wurde. Die mit dem dreistufigen Verfahren ermittelten ME-Werte werden in der Wiederkäuerfütterung zukünftig generell verwendet, unabhängig von der Tierart und der Nutzungsrichtung. Das dreistufige Verfahren führt im Vergleich zum bisherigen Vorgehen zwar nicht grundsätzlich zu anderen ME-Werten. Die Relationen zwischen einigen Futtermitteln verschieben sich jedoch, was für die Kalkulation von Rationen und die relative Vorzüglichkeit der Futtermittel relevant ist.

Für die Ermittlung der ME mit dem dreistufigen Verfahren werden nur wenige Variablen benötigt: die Bruttoenergie (**GE**, gross energy), die bombenkalorimetrisch gemessen oder aus Rohnährstoffen berechnet wird, das Rohprotein und die Rohasche, die beide zum Analysenstandard gehören, und die Verdaulichkeit der organischen Masse (**OMD**, organic matter digestibility). Im Referenzverfahren wird die OMD weiterhin mit Hammeln bei einer Versorgung im Bereich des ME-Erhaltungsbedarfs ermittelt. Die Untersuchungspraxis bestimmt die OMD mit Schätzgleichungen, für deren Anwendung die Ergebnisse aus Nährstoffanalysen und *in-vitro*-Kenngrößen (Gasbildung bzw. Enzymlösliche Organische Masse, ELOS) erforderlich sind. Für verschiedene Gruppen von Grobfuttermitteln und Mischfuttermitteln sind Schätzgleichungen publiziert bzw. zusammengestellt worden (GfE 2023, 2024, 2025).

Aminosäuren und Protein

Für die Proteinversorgung des Tieres ist ausschlaggebend, welche Mengen an Aminosäuren bis zum Ende des Dünndarms absorbiert werden und somit für die Bedarfsdeckung zur Verfügung stehen. Dies gilt für einzelne potenziell limitierende Aminosäuren und die Summe aller Aminosäuren. So sind die Kenngrößen des neuen Proteinsystems für Milchkühe das dünn darmverdauliche Protein (**sidP**, small intestinal digestible protein) als die Gesamtheit aller dünn darmverdaulichen Aminosäuren (**sidAA**, small intestinal digestible amino acids) sowie die **Ruminale-Mikrobielle-Differenz (RMD)**. Die Ableitung des sidP anhand der Summe der Aminosäuren bringt es mit sich, dass das Niveau der Futtermittel- und Bedarfswerte niedriger ist als im System des nutzbaren Rohprotein am Duodenum (nXP), weil nXP-Werte auch Nicht-Aminosäuren-Stickstoff enthalten. Beim sidP erfolgte eine sachlogische Zuordnung der Faktoren zum Futterwert auf der einen und zum Bedarf auf der anderen Seite, womit eine Fortschreibung des sidP-Systems bei Vorlage neuen Wissens zu einzelnen Faktoren und/oder bei veränderten Anforderungen leicht möglich sein wird. Die Menge des im Pansen gebildeten mikrobiellen Rohproteins (**MCP**, microbial crude protein) wird von der Energie bestimmt, die den Mikroorganismen aus der Fermentation zur Verfügung steht und die durch die Menge an verdauter organischer Masse gut beschrieben wird. Somit ist die OMD eine entscheidende Größe nicht nur für die Ermittlung der ME, sondern auch für das sidP. Für die Ermittlung des im Pansen nicht abgebauten Futterrohproteins (**UDP**, ruminally undegraded crude protein) werden Daten verwendet, die mit dem *in-situ*-Verfahren mit pansenfistulierten Tieren ermittelt wurden und werden. Die Anwendung des neuen Systems und der Daten ermöglicht es, die Höhe der ruminalen Passagerate zu berücksichtigen, die durch die Höhe der Futteraufnahme der Tiere bestimmt wird und den UDP-Anteil beeinflusst. Im neuen System der GfE (2023) sind auch die Faktoren hinterlegt, die zur Kalkulation von Versorgung und Bedarf für einzelne Aminosäuren erforderlich sind. Die Dünndarmverdaulichkeit des Proteins und einzelner AA ist spezifisch für die UDP-Quellen, was im sidP-System berücksichtigt wird.

Bedeutung des Futteraufnahmeniveaus

Für Einzelkomponenten werden die Werte für die ME und das sidP für ein Futteraufnahmeniveau (FAN) von 1 berechnet. Das FAN 1 entspricht einer Futteraufnahme von 50 g Trockenmasse (TM) je kg^{0,75} Körpermasse (KM), näherungsweise dem energetischen Erhaltungsbedarf. Die aktuelle Höhe der Futteraufnahme einer Ration wird als Vielfaches des FAN 1 kalkuliert. Mit einem Anstieg der Futteraufnahme ist eine Erhöhung der Passagerate des Futters im Pansen verbunden, was das Ausmaß des Abbaus von Kohlenhydraten und Proteinen im Pansen verändert. Die resultierenden Effekte auf die ME und das sidP werden bei der spezifischen Kalkulation der Rationen berücksichtigt. Hierfür stehen Gleichungen zur Verfügung, die die Effekte des FAN auf die OMD und die Methanbildung berücksichtigen. Bis zu einem FAN von circa 3 kompensieren sich diese beiden Effekte auf die ME weitgehend. Bei einem hierüber hinausgehenden FAN wird ein deutlicher Rückgang in der ME-Konzentration der Ration relevant. Der Einfluss des FAN auf die sidP-Konzentration ergibt sich in zweifacher Weise: Die bei steigendem FAN zurückgehende OMD bewirkt bis zu einer gewissen Grenze einen Rückgang der mikrobiellen Proteinmenge. Hingegen führt das steigende FAN zu einer höheren UDP-Menge, insbesondere bei Proteinfuttermitteln mit einem langsamen ruminalen Proteinabbau. Sowohl bei der Energie als auch beim Protein und den Aminosäuren sind somit Systeme verfügbar, die mit der systematischen Zuordnung von Einflussfaktoren auf den Bedarf einerseits und den Futterwert andererseits die Voraussetzungen dafür schaffen, neue Erkenntnisse zu Einzelaspekten in der Zukunft einfacher aufgreifen zu können. Die neuen Systeme legen einen besonderen Wert auf die Einbeziehung von *in-vitro*-, *in-situ*- und Laborverfahren. Der zweite Workshop-Beitrag geht auf die Konzepte und insbesondere auf die neuen Leitfäden der DLG ein (Leitfäden der DLG zur sachgerechten Energieberechnung und Proteinversorgung - C. Böttger, L. Meßmer, E. Gerster).

Umsetzung – aktueller Stand und weiteres Vorgehen

Die neuen Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe sind unter den Wissenschaftlern abgestimmt und auf die Verhältnisse in Europa (Futterbasis, Leistungsvermögen, Betriebsorganisation etc.) ausgerichtet. Im nächsten Schritt gilt es, die Empfehlungen in der Praxis erfolgreich in Anwendung zu bringen. Bewährt hat sich

hier die Federführung durch den DLG-Arbeitskreis Futter und Fütterung. Dieses Gremium ist unabhängig und arbeitet nach den Grundsätzen von Max Eyth für den Fortschritt in der Landwirtschaft. Im Arbeitskreis sind Fachleute aus der Analytik, der Beratung, der Wirtschaft und der Wissenschaft vertreten. Der Arbeitskreis hat sich mit den Fragen der Umsetzung der neuen Normen der GfE befasst und einen Arbeits- und Zeitplan erstellt. Zur Umsetzung sind folgende Maßnahmen erforderlich:

1. Neufassung der DLG-Futterwerttabellen

Zur Futteroptimierung und Rationsplanung müssen die von der GfE (2023) z. B. für den Bereich des Proteins zusammengestellten Tabellen erweitert und aktualisiert werden. Für die wichtigsten Futtermittel sind aktuelle Werte für die Rohnährstoffe, die OMD, die aNDFom und die Proteinkennwerte zum ruminalen Abbau des Proteins und zur Dünndarmverdaulichkeit des UDP zu erarbeiten und zu tabellieren. Die Tabellen sollen auch aktuelle Werte für Lysin und Methionin enthalten. Dies erfolgt auch online, was den Vorteil einer laufenden Aktualisierung und der digitalen Anbindung hat. Die DLG-Futterwerttabellen stehen inzwischen zur Verfügung (DLG 2025a), die Futtermitteldokumentation ist weiter zu entwickeln und konsequent mit Forschung, Analytik und Praxis zu vernetzen, dazu besteht die Notwendigkeit des kontinuierlichen Einpflegens neuer Daten. Der dritte Workshop-Beitrag geht vertiefend auf die Rationsoptimierung und -kontrolle ein (Rationsoptimierung und Fütterungskontrolle neu ausgerichtet - B. Losand).

2. Neuausrichtung der Futteruntersuchungen in den wissenschaftlichen und den anwendungsorientierten Laboren

Die Futteranalytik und -bewertung sind entsprechend der neuen Maßgaben anzupassen und mögliche Innovationen auf den Weg zu bringen. Da zur Energiebestimmung und zur Ableitung des MCP die OMD von zentraler Bedeutung ist, steht die Ermittlung der OMD im Labor unter Verwendung von Gasbildung oder ELOS im Fokus. Beim Protein gilt es, die Abbaubarkeit des Rohproteins unter Verwendung der chemischen Fraktionierung oder dem erweiterten Hohenheimer Futterwerttest (eHFT) bzw. weiterer Verfahren abzuschätzen. Angaben zu ME und sidP sollen auf den Analysezertifikaten auf Basis FAN 1 erfolgen. Ergänzende Informationen zu den Aminosäurekonzentrationen wie z. B. Methionin und Lysin sind wünschenswert. Hier kann auf die Erfahrungen für Futter in der Schweine- und Geflügelfütterung, ergänzt um Grobfutter, zurückgegriffen werden. Der vierte Workshop-Beitrag befasst sich eingehender mit den Belangen der Laboranalytik (Anwendung in der Analytik - W. Richardt).

3. Anpassung der Software zur Rationsplanung und Futteroptimierung

Die Software für Rationsplanung und -kontrolle ist grundsätzlich fachlich und programmtechnisch anzupassen. Neben der Einführung der neuen Kenngrößen zur Futterbewertung und den geänderten Versorgungsempfehlungen sind digitale Rechenwege (Algorithmen) zu entwickeln und zu integrieren, die die Dynamik des Futterabbaus in Abhängigkeit vom FAN einbeziehen, da sich die Werte für ME und sidP je nach FAN ändern und daher in der Rationsberechnung und -kontrolle zu berücksichtigen sind. Dazu sind zur Berechnung des FAN die Werte zur Körpermasse, z. B. der Herde oder Fütterungsgruppe, und die veranschlagte Futteraufnahme erforderlich. Die Abschätzung der Futteraufnahme erfolgt auf Basis der in GfE (2023) angegebenen Gleichungen. Hierbei spiegelt sich die Qualität der Grobfutter, z. B. der Gras- und Maissilagen, in der ME-Konzentration in der Trockenmasse wider, so dass die Anpassung an die neuen Maßgaben gegeben ist. Eine Differenzierung nach Rassen entfällt zukünftig, da neuere Untersuchungen zeigen, dass Fleckvieh und Deutsch Holstein bei gleicher Milchleistung und Körpermasse sich nicht unterscheiden (Gruber et al. 2021). Der fünfte Workshop-Beitrag stellt die digitalen Ansätze anhand eines Beispiels vor (Digitale Umsetzung in der Rationsplanung und -kontrolle am Beispiel Zifo2 - P. Rauch und H. Schuster).

4. Anpassung von Lehrbüchern, Unterrichtsmaterialien und Beratungsunterlagen sowie der Lehrinhalte

Lehrbücher und Unterrichtsmaterialien für Berufs- und Fachschulen sowie Universitäten und Hochschulen sind zu überarbeiten und aktuell zu gestalten, gleiches gilt für Beratungs- und Schulungsunterlagen aller Art. Hierbei müssen ebenso die Anknüpfungen an Pflanzenbau, Tiermedizin sowie zur Produktions- und Umweltökonomie beachtet werden. Da die dynamische Betrachtung von ME und sidP eine besondere Herausforderung darstellt, muss gerade in Berufsschulen und im Bachelorstudium zeitnah mit der Umsetzung begonnen werden. Der digital gestützte Rationsplanung und -kontrolle kommt in allen Bereichen dabei eine besondere Bedeutung zu.

5. Umstellung der Futteroptimierung in der Mischfutterindustrie

In der Mischfutteroptimierung sind die Ziel- und Kontrollgrößen auf die neuen Bewertungsmaßstäbe anzupassen. Dies betrifft sowohl die Bewertung der Rohwaren als auch die Mischfutterkonzeption und -optimierung. Die Beurteilung der ME und des sidP erfolgt auf Basis **FAN 1**, ebenso die Deklaration von ME und weiteren Angaben. Da sich je nach Einsatz der Mischfutter die erreichten Futterwerte in der Ration insbesondere in Abhängigkeit vom FAN verändern, gewinnt die Passgenauigkeit der Mischfuttermittel bei niedrig- und hochleistenden Kühen weiter an Bedeutung. Die Konzeption der Futter dürfte sich in fast allen Futtersegmenten vom Mineral- bis zum Milchleistungsfutter oder TMR-Ergänzungsfuttermittel verändern.

6. Schulung der Lehr-, Fach-, und Beratungskräfte, Landwirte, Tierärzte etc

Alle Beteiligten und Interessierten sind in weiteren Schritten zu schulen, wenn die letzten noch offenen Fragen geklärt und die Rationsberechnungssoftware und die weiterführenden Beratungsunterlagen zur Verfügung stehen. In diesem Zusammenhang wird ebenso großes Augenmerk auf die Vereinheitlichung/Neufassung von Begriffen (z. B. Grobfutter, Protein, Konzentrat etc.) und Abkürzungen (z. B. TM, CP, sidP, FAN etc.) gelegt und deren konsequente Anwendung wird empfohlen.

Die angeführten Punkte zeigen, dass eine erhebliche Kraftanstrengung erforderlich ist, um die Chancen und Möglichkeiten der neuen Empfehlungen für die Milchkühe, die Land- und Futterwirtschaft sowie die Gesellschaft nutzbar zu machen. Hierzu ist die aktive Beteiligung des gesamten Sektors notwendig. Alle Beteiligten werden eingebunden, um an der Umsetzung mitzuwirken. Dies betrifft Organisationen wie z. B. den Verband der Landwirtschaftskammern (VLK), den Verband der landwirtschaftlichen Untersuchungs- und Forschungsanstalten (VDLUFA), das Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL) aber auch Verbände wie z. B. Deutscher Verband Tiernahrung (DVT), Bundesverband Rind und Schwein (BRS), Bundesverband der Pflanzenzüchter (BDP), Arbeitsgemeinschaft Wirkstoffe in der Tierernährung (AWT) und Bundesverband Praktizierender Tierärzte (BPT), darüber hinaus die offizielle Seite mit den Einrichtungen auf Bundes- und Länderebene sowie die Landwirtschaft und die gesamte Futterbranche.

Der bisherige Fahrplan und die noch ausstehenden Arbeiten sind wie folgt:

- Einrichtung von Arbeitsgruppen durch den DLG-Arbeitskreis Futter und Fütterung für ME und Protein. Bei der **AG-ME** ging es dabei insbesondere um die Klärung offener Fachfragen und die Aktualisierung des DLG-Leitfadens zur Energieberechnung und -angabe (DLG 2025b). Die **AG-Protein** erarbeitete ein Merkblatt zur Sicherstellung der Proteinversorgung. In beiden Fällen erfolgte dies in enger Zusammenarbeit und Abstimmung mit Einrichtungen in Österreich sowie der Einbindung des Bereichs Analytik (DLG 2025c). Basierend auf diesen Arbeiten wurde die DLG-Information **1/2023** zur Milchkuhfütterung aktualisiert (DLG 2025d).
- Vom Ausschuss für Bedarfsnormen der GfE (AfBN) wurden die Arbeiten zur Schätzung der Verdaulichkeit der organischen Masse (OMD) bei Mischfuttermitteln und Konzentraten weitergeführt und zum Abschluss gebracht (GfE 2025). Die fachlichen Voraussetzungen zur Anpassung der rechtlichen Vorgaben zur Deklaration der ME im dreistufigen System nach GfE (2023) sind damit bereitgestellt. Zeitnah wird die englischsprachige Fassung der neuen Empfehlungen der GfE veröffentlicht, was die internationale Harmonisierung des Vorgehens in der Rationsplanung und -kontrolle unterstützt.
- Die neuen Empfehlungen werden im Bereich des Versuchswesens zur Futterbewertung und zur Fütterung der Milchkuh eingeführt. Hierdurch besteht die Möglichkeit, weitere konkrete Erfahrungen zur Anwendung zu gewinnen, die dann noch in die Ausgestaltung der Empfehlungen und die Ausgestaltung der Beratungs- und Fütterungspraxis einfließen können.
- Die Stakeholder wurden über eine spezielle Plattform zum Austausch eingebunden. Mit den Softwareherstellern bzw. -anbietern wurden Workshops zur Neugestaltung der Software zur Rationsplanung durchgeführt.
- Die weiteren fachlichen Grundlagen und Empfehlungen zur Anwendung von GfE (2023) in Forschung, Wirtschaft und Praxis wurden weitestgehend erarbeitet. Hierzu dienen auch Zuarbeiten z. B. aus der Neuauflage des Praxishandbuchs Futter- und Substratkonservierung und Workshops zur Thematik wie z. B. beim Forum Fulda zur angewandten Forschung in der Rinder- und Schweinefütterung.
- Neugestaltung der Futteruntersuchung. Die Analyse der Ernte **2025** und der Ausweis von Energie- und Proteinwerten auf Basis GfE (2023) ist in Laboren möglich.
- Alle Arbeitsmittel in Industrie, Landwirtschaft, Tiermedizin, Schule und Beratung werden angepasst.
- Die Umstellung in der Futterbewertung in Schule, Beratung, Mischfuttermittelherstellung soll zu einem

allgemein abgestimmten Stichtag in 2026 erfolgen. Die weiteren Arbeiten sind Gegenstand des sechsten Workshop-Beitrags (Agenda zur weiteren Implementierung - D. Kampf, H. Schuster).

In 2025 wurden folgende Arbeiten abgeschlossen:

DLG [Deutsche Landwirtschafts-Gesellschaft] 2025a. **DLG-Futterwerttabellen** Wiederkäuer, DLG e.V., Frankfurt am Main. https://www.dlg.org/fileadmin/downloads/Landwirtschaft/Tierhaltung/Futtermittel/Fachinfos/Rinder/Futterwerttabellen_fuer_Wiederkaeuer/DLG_Futterwerttabellen.pdf.

DLG [Deutsche Landwirtschafts-Gesellschaft] 2025b. **Leitfaden** zur Berechnung der Energiekonzentration von Einzel- und Mischfuttermitteln für die Schweine- und Wiederkäuerfütterung. DLG-Arbeitskreis Futter & Fütterung. DLG-Merkblatt **503**, DLG e.V., Frankfurt am Main. https://www.dlg.org/fileadmin/downloads/Merkblaetter/dlg-merkblatt_503.pdf.

DLG [Deutsche Landwirtschafts-Gesellschaft] 2025c. **Leitfaden** zur Proteinbewertung und -versorgung von Milchkühen nach den Empfehlungen zur Energie- und Nährstoffversorgung (GfE 2023). DLG-Arbeitskreis Futter & Fütterung. DLG-Merkblatt **504**, DLG e.V., Frankfurt am Main. https://www.dlg.org/fileadmin/downloads/Merkblaetter/dlg-merkblatt_504.pdf.

DLG [Deutsche Landwirtschafts-Gesellschaft] 2025d. **Rationsoptimierung** und Fütterungskontrolle bei Milchkühen. DLG-Arbeitskreis Futter & Fütterung. DLG-Information **1/2025**, DLG e.V., Frankfurt am Main. https://www.dlg.org/fileadmin/downloads/Landwirtschaft/Tierhaltung/Futtermittel/Fachinfos/Rinder/DLG_Information_01_25.pdf.

VLK/DLG 2025a. Begrifflichkeiten und Abkürzungen neu gefasst. Mitteilungen des Bundesarbeitskreises der Fütterungsreferenten in der DLG. in: Tagungsunterlage „25. Forum angewandte Forschung in der Rinder- und Schweinefütterung“ 28./29.04., Fulda, Verband der Landwirtschaftskammern, Bad Sassendorf, 244-246.

VLK/DLG 2025b. Betriebszweigkontrolle in der Milcherzeugung: Fortschreibung der Maßgaben von 2001 unter Berücksichtigung der neugefassten Empfehlungen zur Energie- und Nährstoffversorgung der Milchkuh (GfE 2023). Mitteilungen des Bundesarbeitskreises der Fütterungsreferenten in der DLG. in: Tagungsunterlage „25. Forum angewandte Forschung in der Rinder- und Schweinefütterung“ 28./29.04., Fulda, Verband der Landwirtschaftskammern, Bad Sassendorf, 237-243.

In **2026** ist die allgemeine Einführung der neuen Empfehlungen in der Begleitforschung, der Analytik, der Wirtschaft und der Praxis auf Basis der vorgestellten Arbeiten (DLG 2025 a-d) vorgesehen. Speziell zum Grobfutter sind Orientierungswerte in Spiekers et al. (2024) und erweitert in DLG (2026) ersichtlich.

Fazit und Ausblick

Die Neufassung der Normen zur Milchkuhfütterung ist fachlich geboten und verbessert die Fütterung mit Vorteilen für Tier, Landwirtschaft und Gesellschaft. Von der Umstellung der Systeme sind große Bereiche der Land- und Futterwirtschaft betroffen. Dies gilt insbesondere für den Futterbau, das Grünland, Futter und Fütterung und die Produktionsökonomie. Die koordinierte Umstellung gewährleistet eine geordnete und effiziente Einführung in die Fütterungspraxis. Die Federführung liegt beim DLG-Arbeitskreis Futter und Fütterung in enger Abstimmung mit allen Stakeholdern. Mit der Umstellung und der erfolgreichen Anwendung der neuen Empfehlungen ist die Milchkuhfütterung up to date und international konkurrenzfähig.

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Spiekers H, Schneider M, Böttger C, Resch R 2024. Umsetzung der Grobfutterbewertung nach GfE (2023) und Ableitung von Orientierungswerten. in: **66.** Jahrestagung der AGGF „Klimaschutz und Klimawandelanpassung im Grünland“, 4.-6.09.2024, Eberswalde, 21-24.

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Index

A

Abd El-Wahab A.	86, 129
Abousoliman I.	134
Ader P.	136
Ahmadi H.	81
Albrecht D.	126
Altemeier M.	28
Amerah A.M.	136
Armstrong R.	130
Arnold C.	133
Atif R.M.	70

B

Bachmann L.	71
Bachmann M.	73, 99, 101
Baidlowi A.	114
Bajrami E.	122
Bakiasi M.	138, 139, 140
Bannert E.	84
Bao Y.	131
Becker S.	109
Becklönne M.S.	28
Bennewitz J.	53
Bernert S.	58
Biber P.	70
Bierlein G.F.	104
Billenkamp F.	139
Bliese L.	112
Blümel F.	130
Bochnia M.	73
Bode L.	96, 97, 100
Boehlen R.	127
Böswald L.	56, 88
Böttcher F.	37, 43
Brandes-Samak J.	59
Braun K.	29, 30
Brouwers J.F.	68
Bruckmaier R.M.	104
Brugger D.	76
Brunnbauer S.	135
Buchallik-Schregel J.	98, 99, 100
Bühler S.	96
Buhrow F.	34
Büngener-Schröder S.	121
Burmester M.	75

C

Calisici O.	49, 133
Camarinha-Silva A.	40, 51, 52, 67, 113, 125
Castillo-Lopez E.	70
Cermak R.	62
Cheng Y.-C.	113
Chien Y.-H.	67
Clauss M.	27
Cohrs I.	33, 68
Consuegra J.	128

D

Dänicke S.	34, 84, 96, 135, 138, 139, 140, 141
Delling C.	71
Dengler F.	71
Dickhoefer U.	116
Diepold D.	76
Diesing L.E.	29, 30
Dietz L.I.	62
Dobenecker B.	63, 64, 65
Dohme-Meier F.	115
Dou W.	46
Dreyer C.	41
Durkalec M.	69
Dusel G.	102, 108, 132

E

Ebersbach L.	74
Eder K.	42, 68, 89, 90, 91, 94, 103, 122
Eggerschwiler L.	115
Egler C.-Y.	75
Engel P.	42, 94
Enokela S.	125
Erkinger H.	49, 55
Ernst A.	56
Ettle T.	106, 130

F

Faas J.	130
Fazel Dehkordi N.	86
Fenrich L.	108
Fingerhut A.	138, 139, 140, 141
Fischer-Tenhagen C.	96, 97, 100
Flaga J.	27
Foggi G.	76
Frahm J.	34, 96, 138, 139, 140, 141
Funk H.	48

G

Gemmeke P.	58
Geßner D.	42, 89, 90, 91, 94, 122
Ghaffari M.H.	33
Ghoreishi N.	98
Gierus M.	36, 79, 114
Godglück A.	92
Górka P.	27
Görs S.	126
Grabowski N.	86
Gregesen E.	77
Gressley T.	70
Grimm A.	128
Groß C.	133
Gross J.J.	32, 49, 55, 104
Gruber L.	106
Grünberg W.	62, 68
Grundmann S.M.	68, 89, 90
Grzeškowiak Ł.	57
Gutöhrlein F.	103

H

Haas V.P.	53
Hammerl G.	110
Hammon H.M.	29, 30, 71
Hanauska A.	53
Hankel J.	92
Harms J.	31
Hartinger K.	93
Hartinger T.	70, 78, 93, 102
Hauling F.	132
Haus A.	111
Hauschild J.	58
Hautzinger T.	117
Hayer J.	102, 108
Heidelberg M.	75
Heidemann C.K.	93
Heiden T.	57
Heidysz M.	136
Heinichen K.	73, 82
Helffenstein K.	69
Hemmert K.J.	33, 50
Henning A.-K.	71
Henrich M.	103
Herz R.	83
Hilgers B.	78
Hofs I.	112
Honig A.C.	106
Hoppe A.	99
Hornfeck F.	89, 90

Hörtenhuber S.	120
Hosseini Ghaffari M.	50, 112, 140
Htoo J. K.	103
Huber K.	53, 61, 123, 138, 139, 140
Huber V.	110, 130
Hummel J.	38, 41, 59, 77, 87, 111
Hünerberg M.	38, 87
Hustedt K.	60, 75

I

Ismita J.	87
-----------	----

J

Jahnke A.	97, 100
Jamrogiewicz M.	27
Jlali M.	128
Jung C.	78
Just H.	96, 97, 100

K

Kaczmarek S.	136
Kaiser A.	43
Kaiser A.M.G.	37
Kampf D.	145
Kański J.	27
Kehraus S.	78
Kersten S.	34, 96, 97, 100, 138, 141
Kessler E.C.	32
Khiaosa-ard R.	79, 93
Klähn J.	58
Klein N.	39, 40, 51, 52
Kleinstauber S.	73
Klevenhusen F.	80
Koch C.	33, 50, 102, 132
Koch F.	118, 119, 126, 134
Koger S.	124
König S.	42, 94
Kostenko J.	74
Kowalczyk J.	69, 96, 97, 98, 100, 101
Kraus V.	114
Kreuzer-Redmer S.	124
Krieg J.	109
Krischek C.	129
Krone B.	77
Krüsselmann P.	74
Kuerbis C.	97

Kuhla B. 38, 69, 118, 119, 126, 134
 Kurek A. 31, 110
 Kurek J. 31, 110

Müller I. 130
 Müller-Kiedrowski C.B. 126
 Müller R. 89, 90
 Muscher-Banse A.S. 60, 75

L

Lange F. 37, 43
 Lange I. 72
 Lauer-Weich C. 137
 Lauridsen C. 23
 Leiber F. 137
 Lewan L. 65
 Liegsalz T.M. 110
 Liermann W. 29, 30, 71
 Liesegang A. 66, 76
 Lingens J.B. 86, 129
 Lizardo R.C. 116
 Löffelmann S.F. 63
 Loibl P. 128
 Losand B. 41

M

Maak S. 109
 Maenner K. 127
 Malik A. 138, 139, 140
 Mateus Vargas R. 77
 Mathaiyan G. 116
 Melzer N. 118
 Mercier Y. 45
 Mertens P. 84
 Mesbahi G. 137
 Metzler-Zebeli B. 124
 Mewes S. 82
 Meyer A. 47, 107
 Meyer I. 112
 Meyer U. 34, 84, 135, 138, 141
 Middendorf L. 28
 Miltko R. 27
 Mitterhuber M. 99
 Moenning J.-L. 95, 97
 Mörlein D. 77
 Mörlein J. 77
 Moscoso D.R. 66
 Moscoso F.F. 66
 Mosler A.M. 118
 Most E. 42, 89, 90, 94, 103
 Muche S. 83
 Mueller A.S. 127
 Mueller U. 112

N

Neumer F. 105
 Nieradt A. 58
 Nieschulze T. 29, 30
 Niu M. 27
 Noh L. 43, 117
 Numata J. 95, 97

O

Ortmann S. 27
 Ostendorf C.S. 33, 50, 107
 Oster M. 126

P

Padberg K. 84
 Pankratz C. 64, 65
 Paschen M. 135
 Paßlack N. 48
 Ped S. 102
 Peschel M. 54, 84
 Philippi H. 45
 Pieper R. 95, 96, 97, 98, 99, 100, 101
 Pisch C. 73
 Piwowarski J. 74
 Plötz M. 86
 Preißinger W. 120
 Priepke A. 41
 Przybyło M. 27
 Przygoda L. 97
 Puntigam R. 120

Q

Quinger F. 40, 51, 52

R

Rath A.	86
Razenberger D.	36
Rehman K.U.	86
Reiche A.-M.	115
Reich M.	135
Reisinger N.	70
Resch R.	93
Rettig A.M.	37, 43
Richardt W.	72, 83
Ringseis R.	103, 122
Rippstein L.	78, 81
Rodehutscord M.	39, 40, 45, 51, 52, 53, 61, 78, 81, 106, 109, 123, 145
Rosenau S.	77
Rosengart S.	121
Rownicki M.	74
Ruda L.F.	61
Rüegg-van den Broek P.	66
Rühl E.	85

S

Sadri H.	50, 112
Saengiem S.	57
Salewski M.	95
Saliu E.-M.	37, 43, 58, 74, 117
Santo M.M.	73
Sarfo T. S.	38
Sarkar S.M.	119
Sauerwein H.	33, 50, 71, 102, 112, 138, 139, 140, 141
Schäfer L.	42, 89, 90, 94
Scharch C.	44
Schedle K.	35, 36, 93
Schemmann K.	91
Scherneck S.	132
Scheu T.	102
Schmidle L.	39
Schmid M.	53
Schneeberger E.	120
Schneider M.R.	134
Schnepel N.	60, 75
Schollenberger M.	109
Scholz P.	34
Schomaker T.	130
Schönleben M.	132
Schubert D.C.	129
Schuchardt S.	48, 50
Schuh-von Graevenitz K.	112

Schulz A.	58
Schulz C.	135
Schulze Holthausen J.	74, 105
Schulz J.	118
Schusser G.F.	73
Schwarz C.	114
Schweder T.	135
Schwennen C.	86
Schwerdtfeger J.	134
Sebold E.	64
Seemann L.	84
Sehlen K.	121
Seifert J.	40, 51, 52, 67, 137, 138, 139, 140
Shomina N.	123
Siebenmorgen C.	77
Siebert D.-C.	44
Siegenthaler R.	115
Siegert W.	45, 46
Silacci P.	115
Sizzano F.	115
Sommerfeld V.	53, 61, 123
Spiekers H.	110, 145
Spieß F.	92
Starke S.	129
Steinhoff-Wagner J.	31, 47, 54, 56, 107, 110
Stoschus A.	29, 30
Strack M.	77
Sünder A.	77
Syriopoulos K.	127
Szentgyörgyi A.	61

T

Tetens J.	77
Timme C.	121
Tona R.	127
Trei G.	58

U

Uddin M.M.	87
Ulrich R.	71
Unruh C.	84

V

van Asten A.L.	109
van den Brink L.M.	68
van der Lee A.	57
van Milgen J.	45
Veit H.	48
Verhovsek D.	124
Visscher C.	86, 121, 129, 133, 135
Vlasova I.	74
Voelker-Albert M.	119
Voltenauer H.	133
von Riedheim M.A.	32, 49, 55
von Soosten D.	34, 84, 106, 135, 141
Vorndran A.M.	47, 107, 110

W

Wagner B.	98, 100, 101
Wandel R.	127
Wang J.	42, 94
Watzke L.	58
Weindl P.A.	117
Welter H.	78
Wemhoff S.	132
Wen G.	122
Wensch-Dorendorf M.	73
Werner J.	137
Whelan R.	103
Wilkens M.R.	62, 69, 77, 82, 118
Wimmers K.	126
Windisch W.	54, 106
Wittenberg K.L.	60
Woitow G.	73
Wojtysiak D.	27

Y

Yergaliyev T.	67, 125
---------------	---------

Z

Zebeli Q.	70, 93
Zeiler E.	31, 117
Zeitj J.O.	103
Zentek J.	48, 57, 74, 85, 105
Zeyner A.	34, 72, 73, 83
Zhang X.	80
Zhao G.	131
Zhao X.	27
Zhou K.	131

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