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J.R. Aschenbach
Chairman

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16:15	👤	89.	Regulation of glucose transporters and glucose metabolism genes of rumen epithelium in cows transitioned from a forage-based to a high-grain diet **Kreuzer-Redmer S., Pacifico C., Ricci S., Rivera-Chacon R., Castillo-Lopez E., Zebeli Q. – Vienna*	134
16:30	▤	90.	Assessment of plasma and leucocyte derived miRNAs as potential biomarkers for rumen health in cattle fed forage and transitioned to a high-grain diet **Ojo O.-E., Pacifico C., Johanns S., Ricci S., Rivera-Chacon R., Sener-Aydemir A., Petri R. M., Castillo-Lopez E., Reisinger N., Zebeli Q., Kreuzer-Redmer S. – Vienna/Sherbrooke/Tulln*	135
16:35	▤	91.	Effects of dietary phosphorus and calcium supply on insulin signaling in laying hens **Abdi A., Gonzalez-Uarquin F., Sommerfeld V., Rodehutscord M., Huber K. – Stuttgart-Hohenheim*	136

Room 1

Wednesday,

16:40			Session change	
			Section 13: Antimicrobial resistance	page
16:45	👤	92.	Hygiene status of piglet feeding equipment and occurrence of resistant bacteria after cleaning and disinfection in different stable environments *Schmid S.M., Heinemann C., *Steinhoff-Wagner J. – Bonn*	137
17:00	👤	93.	Fecal excretion of ESBL-*E. coli* of calves fed with pooled colostrum **Bachmann L., Liermann W., Hammon H.-M., Delling C., Dengler F., Homeier-Bachmann T. – Neubrandenburg / Dummerstorf/Leipzig/Vienna/Greifswald – Insel Riems*	138
17:15	▤	94.	Investigations of milk feeding practices and the prevalence of antibiotic-resistant bacteria in unweaned calves on Western German dairy farms *Hayer J.J., Heinemann C., Leubner C.D., Sib E., *Steinhoff-Wagner J. – Bonn*	139
17:20	▤	95.	Meta analysis of the impact of antibiotic use in German pig farms on the fecal microbiota **Lührmann A., Palmini A., Vahjen W., Zentek J. – Berlin*	140

17:25	Conversation break
18:00	End professional program day 2

Room 1

Thursday,

			Section 15: Gut health	page
09:00	👤	101.	Weaning age influences the host microbiome, metabolome and the host-microbe interactions in calves **Amin N., Schwarzkopf S., Tröscher-Mußotter J., Camarinha-Silva A., Dänicke S., Huber K., Frahm J., Seifert J. – Stuttgart-Hohenheim/Braunschweig*	146
09:15	👤	102.	The effect of age, breed, sex, and dietary factors on metabolite concentration and immunological traits in the caecum of broilers **Duangnumsawang Y., Goodarzi Boroojeni F., Vahjen W., Tarradas J., Esteve-Garcia E., Alberdi A., Marcos S., Tous N., Zentek J. – Berlin/Constantí/Copenhagen/Leioa*	147
09:30	👤	103.	Investigation of a specific prevention strategy against an *Escherichia coli* infection using a pre- /probiotic combination and parent stock vaccination in broilers **Fuhrmann L., Vahjen W., Zentek J., Günther R., Saliu E.-M. – Berlin/Magdeburg*	148
09:45	👤	104.	Fecal microbiota of vaccinated or non-vaccinated clinically inconspicuous and conspicuous piglets under *Lawsonia intracellularis* infection **Hankel J., Sander S., Galvez E., Strowig T., Kamphues J., Visscher C. – Hanover/Braunschweig*	149
10:00	▤	105.	Impact of colostrum from sows fed different fibers against *Clostridioides* difficile toxin-induced effects on IPEC-J2 cells **Grzeskowiak L., Vahjen W., Zentek J. – Berlin*	150
10:05	▤	106.	Faecal characteristics of old vs younger adult horses under similar housing and feeding conditions **Pisch C., Glatter M., Rechenburg L., Wensch-Dorendorf M., Zeyner A. – Halle (Saale)*	151

9 March 2022

Room 2

16:40			Session change	
			Section 14: Carbohydrates and fibre	page
16:45	👤	96.	Effect of dietary *Tenebrio molitor* larvae cuticles on the gut microbiome and lipid metabolism in obese Zucker rats **Ringseis R., Saeb A., Grundmann S., Gessner D., Schuchardt S., Most E., Wen G., Eder K. – Giessen/Hanover*	141
17:00	▤	97.	Preference for fibre rich feedstuffs in a cafeteria trial with sows **Saliu E.-M., Schulze Holthausen J., Zentek J. – Berlin*	142
17:05	▤	98.	Differences in feed intake duration, faecal consistency and short chain fatty acids in faeces from sows fed dried hemp plants (*Cannabis spp.*) either intact or grinded **Saliu E.-M., Schulze Holthausen J., Zentek J. – Berlin*	143
17:10	▤	99.	Effects of dietary rye and rapeseed on microbiota and electrophysiological parameters of the jejunum in weaner pigs **Ellner C., Röhe I., Zentek J. – Berlin*	144
17:15	▤	100.	Impact of a maternal high-fat or high-protein diet supplemented by 10% inulin/oligofructose on milk oligosaccharide concentrations in mice **Koch F., Hickey R., Slattery H., Langhammer M., Wytrwat E., Tuchscherer A., Hammon H.-M., Mielenz M., Kuhla B., Metges C.C. – Dummerstorf/Fermoy*	145
17:20			Conversation break	

10. March 2022

Room 2

			Section 16: Feed additives	page
09:00	👤	107.	Whole black soldier fly larvae in broiler rations: Impact on carcass characteristics, blood metabolites and fatty acids profiles in plasma, muscle and fat tissues **Seyedalmoosavi S.M.M., Das G., Dannenberger D., Maak S., Mielenz M., Wolf P., Metges C.C. – Dummerstorf/Rostock*	152
09:15	👤	108.	Effects of the probiotic *Bacillus amyloliquefaciens* CETC 5490 on primary cultured chicken immune cells. **Larsberg F., Sprechert M., Hesse D., Loh G., Brockmann G.A., Kreuzer-Redmer S. – Berlin/Halle/Vienna*	153
09:30	👤	109.	Effect of a grape extract on performance, digestibility and microbial metabolites of weaning piglets in comparison to a negative and positive control **Rajković E., Schwarz C., Tischler D., Schedle K., Reisinger N., Emsenhuber C., Ocelova V., Roth N., Frieten D., Dusel G., Gierus M. – Tulln/Vienna/Getzersdorf/Bingen am Rhein/Westerau*	154
09:45	▤	110.	Effectiveness of combined feed additives for inactivation of deoxynivalenol and zearalenone in diets for weaned piglets **Carlson L., Grümpel-Schlüter A., Kluess J., Schatzmayr D., Kersten S., Dänicke S. – Braunschweig/Tulln*	155
09:50	▤	111.	Impact of a combination of fructooligosaccharides and a Bacillus multi-strain probiotic on performance parameters of piglets challenged with *Escherichia coli* **Zeilinger K., Wessels A.-G., Vahjen W., Zentek J. – Berlin*	156
09:55	▤	112.	Effects of sewage sludge recyclate addition to substrate on selected mineral contents of Black Soldier Fly larvae **Mielenz M., Seyedalmoosavi S.M.M., Das G., Schleifer K., Tränckner J., Wolf P., Metges C.C. – Dummerstorf/Rostock*	157
10:00	▤	113.	Effect of increasing doses of phytase on performance, apparent praecaecal nutrient digestibility and intestinal electrophysiological properties of the jejunum in 21-day-old broilers **Martinez Vallespin B., Ader P., Zentek J. – Berlin/Lampertheim*	158
10:05	▤	114.	Investigations into taste preference of dairy cows and its modulation by N-arachidonoylethanolamide **Schwerdtfeger J., Puppe B., Krause A., Kuhla B., Röttgen V. – Dummerstorf*	159
10:10	▤	115.	Effects of a blend of essential oils on zootechnical performance of growing bulls **Brugger D., Salzmann J., Liesegang A., Bolt R. – Zurich/Herzogenbuchsee/Lindau*	160
10:15	▤	116.	Inhibitory agents controlling *Clostridium tyrobutyricum* as fermentation varmint in silages tested via cultivation methods **Glatter M., de Oliveira Silva A., Deising H.B., Wensch-Dorendorf M., Rosner V., Milimonka A., Zeyner A. – Halle (Saale)/Bitterfeld*	161

10:10	Conversation break	
	Workshop: Rearing dairy calves with focus on health and welfare	page
10:45	Introduction *Aschenbach J.R. – Berlin*	
10:50	Importance of colostrum supply for the development of the neonatal immune system *Schuberth H.-J. – Hanover*	165
11:25	Implementation of adequate milk feeding until weaning *Koch C. – Münchweiler an der Alsenz*	167
12:00	Influence of milk supply on calf health *Lorenz I. – Poing*	171
12:35	Improving calf welfare: Effects of social housing and milk volume on behavior, growth and weaning *von Keyserlingk M. – Vancouver*	
13:20	Summary discussion *Aschenbach J.R. – Berlin*	
13:35	Closing remarks *Aschenbach J.R. – Berlin*	
13:45	End of the conference	

10:20	Conversation break

Review lecture

The last 25 years (1997–2021) of the Society of Nutrition Physiology (GfE)

Die vergangenen 25 Jahre (1997–2021) der Gesellschaft für Ernährungsphysiologie (GfE)

Flachowsky G., Kamphues J. – Braunschweig, Hanover

1. Einleitung

Nach dem umfassenden Rückblick von Meyer (1997), in dem er wesentliche Ereignisse der ersten 50 Jahre der Gesellschaft für Ernährungsphysiologie (GfE) Revue passieren ließ, ist es an der Zeit, die vergangenen 25 Jahre einmal näher zu betrachten. Dabei ist allerdings zu ergänzen, dass Wöhlbier (1963) bereits die ersten zehn Jahre der GfE zusammenfassend auswertete und Bartusch (1998) in ihrer Dissertation den Zeitraum von der Gründung bis 1996 umfassend analysierte.

Je drei Jahrzehnte mit folgenden Eckpunkten sollen zusammenfassend betrachtet werden:

- 1953 / 1960: Gründung und Anfangsjahre der GfE
- 1990: Wiedervereinigung. Erweiterung des GfE-Potentials
- 2020: Aktuelle GfE-Aktivitäten
- 2050: Perspektiven; gewagter Ausblick

Schwerpunkt dieses Berichtes sind die vergangenen 25 Jahre von 1997–2021 in ihrer Bedeutung für unser Arbeitsgebiet in Deutschland und darüber hinaus.

Dabei dürfen wir nicht vergessen, dass viele verdienstvolle Tierernährerinnen und Tierernährer im Berichtszeitraum verstorben sind. Bei diesem Rückblick auf die letzten 25 Jahre der GfE ist es den Autoren mehr als nur eine Pflicht, ja ein Herzensanliegen und Bedürfnis, der verstorbenen Mitglieder unserer Gesellschaft zu gedenken, und zwar in Dankbarkeit und Würdigung ihrer ganz persönlichen Verdienste um unsere Gesellschaft, ihres Ansehens im In- und Ausland, der Ermöglichung und Unterstützung wissenschaftlicher Karrieren, d.h. ihres Wirkens um und für den wissenschaftlichen Nachwuchs, von dem u.a. die Zukunftsfähigkeit einer jeden wissenschaftlichen Gesellschaft abhängt. Nicht zuletzt ist es den verstorbenen Mitgliedern zu verdanken, dass sich in der GfE so etwas wie ein Wir-Gefühl entwickelte, eine weitere nicht zu vernachlässigende Voraussetzung für jedes persönliche Engagement der Mitglieder.

Der Zeitraum nach 1996 kann u.a. durch folgende Entwicklungen / Ereignisse mit Konsequenzen für das Fachgebiet charakterisiert werden:

- Weitere Zunahme der Tierbestandsgrößen mit Konzentration in bestimmten Regionen, verbunden mit dem „Verschwinden“ kleinerer Betriebe
- Erheblicher Leistungsanstieg der Tiere in der Praxis (z.B. pro Jahr: >10 000 L Milch / Kuh oder >30 abgesetzte Ferkel pro Sau und Jahr)
- Höhepunkt und Abklingen der BSE-Krise mit verschiedenen Konsequenzen bis zur Mischfutterrezeptur
- Etablierung (2003) der Europäischen Behörde für Lebensmittelsicherheit (EFSA) in Parma (Italien)
- Vereinigung von Lebensmittel- und Futtermittelrecht in einem gemeinsamen Rechtsrahmen (LFGB; 2005)
- Weiterentwicklung des EU-Schnellwarnsystems zu Fragen der Lebens- und Futtermittelsicherheit (RASFF)
- Umorganisation nationaler Behörden, wie z.B. BMEL, BVL, BfR, Landesbehörden etc.
- Verbot antibiotischer Leistungsförderer als Futterzusatzstoffe (2006) und Suche nach alternativen nutritiven Ansätzen
- Neuer Leitgedanke bei Tierversuchen (z.B. **3 R**-Regel; **R**eplacement, **R**eduction, **R**efinement; Russel and Busch, 1959)
- Bedeutsame methodische Entwicklungen (z.B. neue Messtechniken, wie z.B. NIR, Pansenphysiologie / Pansensensoren, Omics-Techniken, Ersatzmethoden für Tierversuche)

- Erweiterung des Spektrums an Futtermitteln (z.B. Insekten, Algen) und Futterzusatzstoffen (z.B. zur Inaktivierung von Mykotoxinen, Gärgasinhibitoren etc.)
- Mögliche Umweltwirkungen der Tierernährung zunehmend im Fokus des öffentlichen Interesses (wie z.B. N, CH_4, P, Cu, Zn)
- Lebenszyklus-Studien (Life Cycle Assessment; LCA) für Lebensmittel tierischer Herkunft (Gesamtaufwand an Ressourcen, Bioökonomie)

Einleitend soll über die Arbeit der GfE mittels einiger statistischer Daten informiert werden. In dem zu betrachtenden Zeitraum (1997–2021) fanden 25 GfE-Jahrestagungen statt (s. Tab. 1); davon wurden 22 Tagungen in Göttingen und je eine in Hohenheim bzw. in Hannover durchgeführt. Corona-bedingt erfolgte die 75. Tagung digital. Die Besucherzahlen je Tagung variierten im Berichtszeitraum zwischen 245 (2020) und 313 (2013). Den Mitarbeiterinnen und Mitarbeitern der Universität Göttingen und vor allem dem Lehrstuhl für Tierernährung mit den Kollegen Frank Liebert und Jürgen Hummel gebührt für die Organisation der Jahrestagungen ein großes Dankeschön.

Ganz herzlich gedankt sei auch den Herren Dr. Walter Staudacher (bis 2017) und Dr. Detlef Kampf (seit 2017) sowie Frau Silke Ausmeier von der Geschäftsstelle der GfE in Frankfurt für die vielfältige Unterstützung in allen Fragen zur jeweiligen Jahrestagung sowie der DLG insgesamt für die Zurverfügungstellung der Ressourcen zur Umsetzung dieser Arbeiten. Walter Staudachers seltene Begabung für die quantitative Tierernährung und sein Verständnis für die mögliche bzw. nötige Umsetzung wissenschaftlicher Empfehlungen in die Fütterungspraxis waren für die GfE ein Glücksfall.

Auf den ersten 50 Tagungen der GfE wurden 1.883 Beiträge präsentiert (Meyer 1997). Auf den 25 Tagungen im Zeitraum von 1997–2021 waren es insgesamt 3.295 Referate und Poster, was fast einer Verdoppelung nahekommt, unter Berücksichtigung der Zeitspanne würde dies sogar fast dem Vierfachen entsprechen. Die Zahl der Beiträge je Tagung variierte zwischen 98 (2003) und 165 (2015). Die stark gewachsene Anzahl der Präsentationen hat vermutlich mehrere Ursachen, wie z.B.:

- Anstieg der Anzahl von Doktorandinnen und Doktoranden an verschiedenen Forschungseinrichtungen
- Erhöhung der Forschungskapazitäten unserer Disziplin durch hinzugekommene Einrichtungen aus der früheren DDR (z.B. Berlin, Dummerstorf, Halle, Jena, Leipzig, Rostock)
- „Zunehmender Druck“ zum Publizieren für die wissenschaftliche Karriere (Beleg für Aktivität – aber nicht automatisch für Qualität)
- Dozentinnen und Dozenten an den FHS und Wissenschaftlerinnen und Wissenschaftler an Landesanstalten sowie Landeskammern (z.B. Grub/Gumpenstein (Österreich)/Haus Riswick/Neumühle) mit höchst relevanten Untersuchungs- und Forschungsergebnissen.

Wie bereits Meyer (1997) feststellte, ist eine umfassende wissenschaftliche Bewertung der Einzel-Beiträge kaum möglich, da jeweils nur eine Seite Kurzfassung (seit 2001 in Englisch, mit erheblichen Vorteilen für die internationale Wahrnehmung) zur Verfügung steht und die Diskussion der Beiträge allein keine fundierte Einschätzung erlaubt.

Auf 20 Tagungen bereicherten Plenarbeiträge mit unterschiedlicher Ausrichtung das Programm (s. Tab. 2). Die jährliche Zusammenkunft der Ernährungsphysiologen und Tierernährer wurde in 18 Jahren durch entsprechende Workshops zu aktuellen Themen der Fachdisziplin ergänzt (s. Tab. 3).

Nicht unerwähnt soll bleiben, dass der Ausschuss für Bedarfsnormen (AfBN) der GfE – das wichtigste Arbeitsgremium der Gesellschaft – den Tagungsband 19-mal nutzte, um aktuelle Aspekte der Fachdisziplin zeitnah an die Interessenten zu bringen (s. Tab. 12). Außerdem hat der AfBN im Berichtszeitraum sieben „Empfehlungen zur Energie- und Nährstoffversorgung“ verschiedener Tierarten und Kategorien erarbeitet, auf die später eingegangen wird (s. Tab.11).

Im Berichtszeitraum bestanden auf nationaler Ebene verschiedene Kontakte zu anderen wissenschaftlichen Gesellschaften, wie z.B. zur Deutschen Veterinärmedizinischen Gesellschaft (DVG), zur Deutschen Gesellschaft für Züchtungskunde (DGfZ), zur Gesellschaft für Pflanzenbauwissenschaften (GPW), zur Gesellschaft für Mineralstoffe und Spurenelemente (GMS), zum Verband der Deutschen Landwirtschaftlichen Untersuchungs- und Forschungsanstalten (VDLUFA), zur Deutschen Landwirtschafts-Gesellschaft (DLG), zum Deutschen Maiskomitee (DMK) oder auch zur Fachagentur für nachwachsende Rohstoffe (FNR). Der Austausch mit der Deutschen Gesellschaft für Ernährung (DGE) hat nach der Jahrtausendwende leider an Intensität verloren.

Tabelle 1: Tagungen der GfE von 1997 bis 2021, alle Tagungsbeiträge erschienen in den „Proceedings of the Society of Nutrition Physiology“ des jeweiligen Jahres beim DLG-Verlag

Tagung Nr.	**Jahr**	**Vorsitz der GfE**[1]	**Zahl der Beiträge**	**Reviews**	**Workshop-Beiträge**	**AfBN-Mitteilungen**
51	1997	Pallauf	141	2	-	3
52	1998	“	116	1	-	1
53	1999	“	116	2	-	1
54	2000	“	114	1	10	-
55	2001	Breves	133	1	13	-
56	2002	“	145	1	11	2
57	2003	“	98	1	-	2
58	2004	“	125	1	11	1
59	2005	Martens	138	1	5	2
60	2006	“	153	1	3	-
61	2007	“	109	1	4	-
62	2008	“	133	1	5	2
63	2009	Rodehutscord	113	2	-	1
64	2010	“	135	-	9	-
65	2011	“	121	1	5	-
66	2012	“	147	1	5	-
67	2013	Zentek	155	1	-	-
68	2014	“	129	1	4	1
69	2015	“	165	1	3	-
70	2016	“	141	1	4	-
71	2017	Windisch	164	1	-	5
72	2018	“	155	1	5	-
73	2019	“	122	1	4	-
74	2020	“	124	1	4	1
75	2021	Aschenbach	103	1	4	-

Tagungsorte: Göttingen; außer 59. (Hohenheim), 70. (Hannover) und 75. Tagung (digital)

[1] Bis zum Jahr 2018 kam immer mindestens ein Vorstandsmitglied (von 8) aus einem Institut von Nachbarländern (Dänemark, Niederlande, Österreich, Polen, Schweiz)

2. Übersichtsreferate und Workshops

In Tabelle 2 sind die Themen der Übersichtsreferate zusammengestellt. Dabei wird offensichtlich, dass die jeweils verantwortlichen Fachkolleginnen und Fachkollegen der GfE erfolgreich waren, sich um international und national ausgewiesene Referentinnen und Referenten zu bemühen. Als vielleicht momentan prominentester Referent soll Prof. Wieler erwähnt werden. Mit dem Namen des heutigen RKI-Präsidenten (s. Tab. 2; 2003) weiß vermutlich auch jeder „Nicht-Tierernährer" etwas anzufangen.

Tabelle 2: Übersichtsreferate im Rahmen der GfE-Jahrestagungen

Jahr	Autor(en)	Themen / Titel
1997	MacRae und Beever (UK)	Predicting amino acid supply and utilization in the lactating ruminant
1998	Pabst und Rothkötter (Hannover)	Die morphologischen und funktionellen Grundlagen der Immunfunktion der Darmwand: eine Barrierefunktion im Gegensatz zur Absorption der Nährstoffe
	Meyer (Hannover)	50 Tagungen der GfE; Jubiläumsbeitrag
1999	Danfaer (DK)	Nutrient flow across the liver in dairy cows
	Breves, Praechter und Schröder (Hannover)	Calcium metabolism in ruminants – Physiological aspects and effect of anion rich diets
2000	Sangild et al. (DK)	Perinatal development of digestive enzymes in farm animals
2001	Schemann (Hannover)	Regulation of gastrointestinal functions by the enteric nervous system
2002	Bischoff (Hannover)	Food allergy: New insights into the etiology and pathogenesis
2003	Wieler und Jorges (Berlin)	Evolution of gastrointestinal bacteria with respect to their pathogenicity
2004	Leeb (Hannover)	Genome analysis and performance traits in cattle
2005	Goddeeris (BE)	Crosstalk between nutrition and immunity
2006	Klasing (USA)	Negative consequences of immune response: What can be done by nutritionists?
2007	Spears (USA)	Trace elements and immunology
2008	Metges, Otten und Rehfeldt (Dummerstorf)	Metabolic programming in farm animals
2009	Forbes (USA)	Minimal total discomfort; an integrating framework for feed intake and selection
	Lutz (Schweiz)	Hormonal control of food intake
2011	Kaspers (München)	Energy demand and energy metabolism in the immune system
2012	McNamara (USA)	Principles and applications of systems biology in improving efficiency of dairy cattle
2013	Eder (Giessen)	Oxidative stress: Development and physiological consequences in farm animals
2014	Blaut (Nuthetal)	Host-microbiota interactions in the digestive tract
2015	Groschup (Riems)	Spongiform encephalopathies in humans and animals
2016	Kulling (Karlsruhe)	Biotransformation of secondary plant ingredients
2017	Gerrits (NL)	Nutrition of veal calves: Interactions between milk replacer and solid feeds
2018	Lapierre und Ouellet (Can)	Linking post-absorptive metabolism of amino acids and ration formulation in dairy cows

Jahr	Autor(en)	Themen / Titel
2019	Huber (Stuttgart-Hohenheim)	Metabolic imprinting by pre- and postnatal nutrition in farm animals
2020	Niggli (Schweiz)	Global perspectives and limits to livestock feeding
2021	Windisch (Freising) und Flachowsky (Braunschweig)	Livestock-based circular economy: Perspectives and conflicting goals in the role of livestock in the agricultural production of food of animal origin

Die in den Übersichtsreferaten behandelten Themen dokumentieren eindrucksvoll die inhaltliche Bandbreite unserer Disziplin, die Verbindungen zu anderen Wissenschaftsfeldern (z.B. Mikrobiologie, Immunologie, Biochemie, Medizin), die Bemühungen um einen Import an Wissen, Erfahrungen und Know-how von ausgewiesenen Experten sowie die Bereitschaft der jeweils verantwortlichen Kolleginnen und Kollegen zu einem „Blick über den Tellerrand“ (siehe spongiforme Enzephalopathien oder globale Perspektiven für die Tierproduktion bzw. Lebensmittel tierischer Herkunft). Die in den Proceedings vorhandenen schriftlichen Beiträge dürften noch über Jahre eine Fundstelle wesentlicher Ideen, Vorstellungen und Versuchsergebnisse bleiben, wenn sich der wissenschaftliche Nachwuchs in die entsprechenden Themen einarbeiten will.

Im Rahmen der GfE-Jahrestagungen wurden meist am dritten Tagungstag Workshops durchgeführt (s. Tab. 3). Diese dienten u.a. einer Vertiefung der Thematik der Plenarbeiträge (s. Tab. 2). Im Jahr 2004 waren zwei Workshops der Fütterung von Milchkühen gewidmet, d.h. den Herausforderungen durch hohe Leistungen bzw. den Bedingungen im organischen Landbau.

Tabelle 3: Workshops[1)] im Rahmen der GfE-Jahrestagungen

Jahr	Thema	Anzahl der Beiträge
2000	Einflüsse auf Gesundheit und Leistungsbereitschaft beim Milchkalb	10
2001	Mycotoxins	13
2002	Physiological aspects of genetically modified feed and food	11
2004	Ernährung, Stoffwechsel und Krankheitshäufigkeit der Hochleistungskuh sowie Ernährung der Milchkühe im organischen Landbau	5 6
2005	Aktuelle Aspekte der Futtermittelbewertung	5
2006	Nutrition and fertility	3
2007	Energy and protein evaluation for dairy cows	4
2008	Potenziale der Tierernährung zur Verbesserung der Ressourceneffizienz	5
2010	Evaluation of the adequacy of feeding and metabolic status of dairy cows on the basis of concentrations in blood and other media: Chances, limitations and challenges	9
2011	Effects of feed processing in swine and poultry nutrition	4
2012	Metabolism and efficiency of fermentation in biogas plants	5
2014	What can feedingstuffs perform? Potentials and physiological limits	4
2015	New aspects in physiology and nutrition of sows regarding actual developments in breeding	3
2016	Grain used in animal feed – Insights and perspectives	4
2018	Dietary fiber in animal nutrition	5
2019	Vitamins in ruminants	4
2020	Importance of livestock husbandry and nutrition in a circulatory system	4
2021	Reduce methane losses in cattle through feeding and breeding	4

1) In den Jahren 1998; 1999; 2003; 2009; 2013 und 2017 fanden keine Workshops statt

Wie aus Tabelle 3 hervorgeht, waren die Workshops vom Inhalt und von der Ausrichtung her sehr vielfältig: einerseits ging es um Grundlagen unserer Disziplin (z.B. Energie-/Proteinbewertung), andererseits eher um stark anwendungsbezogenes Wissen (z.B. „Diagnostik der adäquaten Versorgung“ oder auch „Futtermitteltechnologie“), das so eine Vertiefung erfuhr. Die „Fütterung“ von Biogasanlagen und die ablaufenden Stoffwechselprozesse und Gesetzmäßigkeiten waren Gegenstand eines Workshops, der die inhaltliche Vielfalt besonders dokumentiert.

Allen Workshops gemeinsam war und ist das Bemühen um einen stärkeren Austausch mit Fachleuten aus den vor- und nachgelagerten Bereichen (Mischfutter-/Zusatzstoffindustrie), aus angrenzenden Tätigkeitsfeldern (Laboranalytik) oder auch aus anderen Wissenschaftsfeldern z.B. Tierzucht), weil hier auffällige Veränderungen mit teils erheblichen Auswirkungen auf die Tierernährung/Fütterungspraxis verbunden waren/sind (z.B. Wurfgröße beim Schwein und Konsequenzen für den Bedarf in Gravidität und Laktation).

3. Inhaltliches aus den 25 Tagungen

3.1. Tierartenspektrum

Wie in den ersten Jahrzehnten der Gesellschaft wurde das Tierartenspektrum in den Vorträgen und Postern, aber auch in den Übersichtsreferaten und Workshops von den Lebensmittelliefernden Spezies Rind, Schwein und Huhn bestimmt.

Schon seltener, aber dennoch regelmäßig, fanden Kleinwiederkäuer sowie Puten und Enten Berücksichtigung. Pferde, aber auch Hunde und Katzen waren seltener Gegenstand entsprechender Präsentationen. Verschiedene Beiträge aus diesem Bereich beschäftigten sich mit Fragen zum Energie- und Nährstoffbedarf, aber auch zu Auswirkungen des Futters auf Verdauungsvorgänge bzw. die Exkretion.

Beiträge zur Fischfütterung sind zahlenmäßig rückläufig. Dennoch waren vereinzelt Forellen und Karpfen, aber auch Welse und Tilapien Gegenstand tierernährerischer Forschungsaktivitäten. Ein Beitrag zur Shrimp-Fütterung rundet das Bild ab, das der Aquakultur zuzuordnen ist.

Auch Liebhabertiere standen vereinzelt im Fokus wissenschaftlicher Ausführungen, angefangen bei den Aras (Bedarf von Großpapageien) bis hin zu den Zoo- und Wildtieren reichte hier das Spektrum (z.B. bis zur Milchzusammensetzung beim Indischen Rhinozeros). Ebenfalls waren Tierarten, die erst in jüngster Zeit hierzulande eine gewisse Verbreitung fanden (z.B. Neuwelt-Kameliden), bereits Gegenstand von Untersuchungen, die insbesondere aus vergleichender Sicht (Biologie, Verdauungsphysiologie) ein Interesse verdienen.

Mit einem gewissen Bedauern ist rückblickend festzustellen, dass die Versuchstierernährung, die in ihren Anfängen stark von Tierernährern geprägt bzw. zumindest beeinflusst war (u.a. mit den Namen Drepper und Zucker verbunden), auf den Jahrestagungen unserer Gesellschaft nur noch sporadisch vertreten ist.

Nahezu exotisch mutete es an, als es in einem Beitrag um die Fütterung von „Mehlwürmern“ ging (2020). Bereits im Versuchsansatz wurde darauf hingewiesen, dass für Mehlwürmer eine Nutzung als alternative Proteinquelle für Nichtwiederkäuer angestrebt wird. Diese Erwartung wurde bereits durch ein Gesetzblatt (EU, 2021a) Realität, denn es erlaubt den Einsatz von Insektenproteinen in der Tierernährung in der EU (s. auch Hall et al. 2021).

Abschließend zu diesem Block soll erwähnt werden, dass mit der Bearbeitung und Vorstellung von tierernährerisch ausgerichteten Studien an verschiedenen Spezies Grundgesetzmäßigkeiten zu diversen Lebensvorgängen ins Bewusstsein rücken (z.B. Energiebedarf, Futteraufnahme u.a.), die nicht zuletzt ihren Platz in der Lehre haben und weiterhin behalten werden.

3.2 Ernährungsphysiologie

Nicht nur wegen des Namens unserer Gesellschaft gebührt der Ernährungsphysiologie besondere Aufmerksamkeit, sondern wegen der in diesem Bereich erkennbaren Veränderungen und Fortschritte (Tab. 4).

Tabelle 4: Entwicklungen / Fortschritte im Bereich ernährungsphysiologischer Arbeiten

Objekt	**Veränderungen / Kommentierungen / Perspektiven**
Futteraufnahme	Regulation / Interaktionen über Prozesse im / am GIT sowie systemische Einflüsse (s. Entzündungskaskade)
Pansenphysiologie	Unvermeidbare Prozesse, wie Methanbildung, Aminbildung besser quantifizieren, aber auch mikrobielle Protein- und Vitaminsynthese
Lokalisation von Verdauungsprozessen	Dickdarm-Verdauung[1)] nicht nur unter dem Aspekt zu erwartender energetischer Verluste, sondern als Ansatz zur Förderung der Tiergesundheit insgesamt (mehr als nur trophische Funktion)
Magen-Darm-Microbiota (Mikrobiom)	Nicht unidirektionale, sondern bidirektionale Beziehungen (s. „gut-brain-axis“, mikrobiell gebildete Metaboliten beeinflussen Prozesse und Regelkreisläufe des Makroorganismus) → Futter ↔ Mikrobiom ↔ Tier!
„Entzündung“	Grundprozesse einer Erkrankung und davon ausgehende Einflüsse auf den Stoffwechsel (s. Futteraufnahme, Körpertemperatur, Priorisierung u.a.), bzw. Prozesse, die Entzündungsmediatoren freisetzen (z.B. Fettabbau)
„Metabolic programming“	Von Interesse sind „Zeitfenster“, in denen eine bestimmte Versorgung zeitversetzt (d.h. evtl. viel später) besondere Auswirkungen auf den Stoffwechsel des Organismus hat (im Sinne einer Gefährdung bzw. Disposition, aber auch von erhöhter Leistung etc.).

[1)] bei Omnivoren-Spezies, wie Schwein, Geflügel etc.

Angefangen bei den molekularen Vorgängen der Absorption und Sekretion ins Darmlumen und den hierbei wirksamen Einflüssen über die autonome Motorik des GIT und die vielfältigen Interaktionen zwischen Verdauungstrakt und Immunsystem bis hin zu einem vertieften Verständnis des Mikrobioms und dessen Bedeutung für die Prozesse jenseits der Darmwand („systemisch“ bzw. „beyond the GIT“) reichen hier die „Erfolge“ der Ernährungsphysiologie (s. auch Tab. 4), die insbesondere in Fachbüchern (z.B. Gonzalis und Ortis, 2019), in Übersichtsreferaten, aber auch in vielen Beiträgen der GfE-Jahrestagungen zum Ausdruck kamen. Insgesamt rücken bei den ernährungsphysiologischen Arbeiten postabsorptive Vorgänge bzw. „systemische“ Auswirkungen immer stärker und häufiger in den Fokus.

3.3. Futtermittel

Futtermittelkunde und Ernährungsphysiologie sowie Bedarfsableitung und Fütterungseinflüsse auf die Leistung(sprodukte) und Umwelt blieben die originären, d.h. primär selbstbestimmten Arbeitsfelder unserer Disziplin. Diese Bereiche erfuhren traditionell eine weitere Untergliederung im Programm der Vortragsveranstaltungen (Energie, Protein, Mineralstoffe, Vitamine etc.). Interesse verdienen jedoch „neue“ Sektionen, die nicht mehr so einfach den o.g. Feldern entsprachen – und dann eher diplomatisch unter „Freie Themen“, „Fütterungskonzepte“ oder auch „Intermediärstoffwechsel“ und „Ernährung und Entzündung“ ihre Zuordnung erfuhren. Diese Formulierungen lassen aber bestimmte Veränderungen / Entwicklungen in der Ausrichtung von Forschungsaktivitäten in unserer Disziplin erkennen, die über das zuvor beschriebene tradierte Verständnis von Ernährungsphysiologie (z.B. Funktion eines essentiellen Nährstoffs) weit hinausgehen (s. Tab. 5).

Tabelle 5: Herausforderungen und Lösungsansätze aus futtermittelkundlicher Sicht

Objekt	**Veränderungen / Kommentierung / Perspektiven**
Futtermittelarten	Verbot der Schlachtnebenprodukte als Futtermittel für Lebensmittel-liefernde Tiere, Vergeudung von N- und P-Ressourcen, Wiederzulassung in 2021! (s. EU 2021b), Insekten im „Kommen“?
Futtermittelanalytik	Anstelle der Rohfaser zunehmend ADF / NDF sowie „dietary fiber“ als Erweiterung (Hauptkritik: Rohfaser: nur ein Teil der Faserstoffe; „dietary fiber“: alles, was nicht durch körpereigene Enzyme abgebaut wird)
Futtermittelcharakterisierung	Ruminale und postruminale *in situ*, *in vitro* und *in vivo* Techniken, Standardisierung der Verfahren für Schwein und Geflügel; Löslichkeit, Wasserbindung, Extraktviskosität, Partikelgrößen (Verteilung → Siebanalytik)
Tränkwasser	Essentialität auch ohne Nährstoffcharakter: Medium zur Zusatzstoff-Applikation. Entwicklung / Etablierung von „Orientierungswerten“ für die Tränkwasser-Qualität (Kamphues et al. 2007)
Futtermittelhygiene	Biologische, chemische, und / oder physikalische Kontaminationen → Qualitätskriterium mit futtermittelrechtlicher Verankerung; Mykotoxine, Dioxine und andere Kontaminanten als Herausforderung
P-Gehalt in Futtermitteln	Differenzierung in Phytin-P / Nicht-Phytin-P mit Konsequenzen für Phytase-Einsatz bei Monogastriern etc.; Vermeidung unnötiger P-Ergänzung
Futtermittelfermentation	Keine Konservierung, sondern kurzfristige Behandlung (~ 24 h) zur Förderung der Verdaulichkeit / des Hygienestatus
Anstieg der CO_2-Konnzentration in der Atmosphäre	Höhere Erträge, aber „Verdünnung“ des Gehaltes an wert-bestimmenden Inhaltsstoffen (Protein bzw. AS, Mengen- und Spurenelemente, Vitamine; Weigel 2014)

3.4. Futtermittelzusatzstoffe

Nach EU-Verordnung 1831 / 2003 (EG, 2003) werden die Futterzusatzstoffe in 15 Gruppen eingeteilt (3 Gruppen für ernährungsphysiologische bzw. essentielle Zusatzstoffe und 12 Gruppen für nicht essentielle Additive (Antibiotika als 16. Stoffgruppe wurden nach 2006 vom Markt genommen). In Tabelle 6 werden einige neue Entwicklungen zusammengestellt.

Tabelle 6: Effekte von Futtermittelzusatzstoffen (nach EFSA-Systematik)

Zusatzstoffe	**Bewertung von verschiedenen Entwicklungen**
Aminosäuren	Relativ wenig neue Studien; Aminosäurenergänzung ist ein etabliertes Verfahren; die Zahl der supplementierten, auch nicht-essentiellen Aminosäuren nimmt weiter zu
Spurenelemente	Ende der bedarfsüberschreitenden Versorgung mit Cu und Zn; nach „Anfangserfolgen“ bewirkten „Seltene Erden“ (Rare Earth Elements) überwiegend keine reproduzierbaren Befunde
Vitamine / vitamin-ähnliche Substanzen	Konjugierte Linolsäuren (CLA, zunächst der Gruppe der Vitamine zugeordnet) mit positiven Effekten bei Milchkühen); Solanum glaucophyllum-Blätter als Vit. D-Quelle (EFSA 2015)
Enzyme	Weitgehend Standard in Nicht-Wiederkäuermischungen unter Berücksichtigung der Futtermischung, z.B. Phytasen oder auch NSP-spaltende Enzyme (u. a. altersabhängig)
Darmflorastabilisatoren	Lösungsansätze zur Förderung der Magen- / Darm-Gesundheit, relativ wenig neue Studien

Tabelle 6: Effekte von Futtermittelzusatzstoffen (nach EFSA-Systematik)

Zusatzstoffe	**Bewertung von verschiedenen Entwicklungen**
Silierzusatzstoffe	Erstmalige futtermittelrechtliche Verankerung, forcierte Entwicklung von Kombinationspräparaten, aber meist Prüfung der Wirksamkeit ohne Tierversuche
Mykotoxinbindemittel	Nachgewiesene Wirksamkeit für bestimmte Additive (z.B. Fumonisin-Esterase)
Methaninhibitoren	Zahlreiche Studien, über erste Ergebnisse mit 3-Nitrooxypropanol bzw. Acacia mearnsii als Methan-Inhibitoren wurde auf der GfE-Jahrestagung 2020 berichtet

Des Weiteren ist zu konstatieren, dass es wiederholt zu einer Häufung von Beiträgen zu Einzelthemen („Wellen") kam, die schon an eine „Marketing-Offensive" erinnerten (z.B. Phytasen, Kreatin) und vielleicht in Zukunft besser in Form eines Workshops vorgestellt werden sollten.

3.5. Ausgewählte Meilensteine der „speziellen Tierernährung"

In der „speziellen Tierernährung" geht es um die Energie- und Nährstoffversorgung einer bestimmten Spezies in Abhängigkeit von Alter und Leistungsstadium bzw. Nutzungsintensität (z.B. bei der Arbeit). Wie aus Tabelle 7 ersichtlich wird, kam es in den vergangenen Jahren zu teils ganz erheblichen Veränderungen bzw. Fortschritten mit internationaler Ausstrahlung (z.B. Mischfutter-Optimierung nach pcvP). Diese Veränderungen sind dabei maßgeblich den entsprechenden Arbeiten und Veröffentlichungen des AfBN zu verdanken (s. auch Tab. 11). Verschiedene der in Tabelle 7 nur „stichpunktartig" erwähnten Veränderungen belegen beispielhaft die mögliche „Ertragsbildung" einer engagierten Untersuchungs- und Forschungsaktivität des wissenschaftlichen Nachwuchses.

Tabelle 7: Veränderungen in der „speziellen Tierernährung"

Spezies	**„Meilensteine"/Veränderungen mit Kommentaren/Wertung/Perspektiven etc.**
Rinder, Milchkühe	peNDF statt „strukturierter Rfa" (GfE 2014); steigender Bedarf bei zunehmendem Konzentratfuttereinsatz; Verbindung von physikalischen und chemischen Analyten → "artgerechte" Fütterung
Rinder, Kälber	Forcierte Aufzuchtintensität (Tränkemenge/-konzentration/-dauer) → Ziel: Tierwohl und höhere Leistung artgerecht aufgezogener Tiere
Schweine	pcvAS und pcvP in der Bedarfsformulierung (GfE 2006)
Geflügel	Wie bei Schweinen: pcvAS, pcvP in der Bedarfsformulierung und MF-Optimierung
Pferde	ME statt DE (GfE 2014; s. Tab. 11); pcvAS statt vRp; Futtermittel-abhängige Energieverluste über den Harn (Hippursäure!)
Hunde, Katzen	Statt DE seit 2003 ME; beibehalten wurde aber vRp!

3.6. Beiträge im Zusammenhang mit Gesundheitsstörungen

Insbesondere standen hier Erkrankungen und Belastungen für den Stoffwechsel im Fokus, denen bei der Fütterung eine besondere Bedeutung zukommt (z.B. beim Zielkonflikt zwischen Energiebedarf bei steigender Leistung vs. TS-Aufnahme-Vermögen), die entsprechenden Konsequenzen für die Fütterung haben. Hiermit erklärt sich die große Zahl von Beiträgen, die dem Verständnis bzw. der Prophylaxe von Stoffwechselerkrankungen der Kühe zu Beginn der Laktation gewidmet waren bzw. den Auswirkungen und Risiken eines höheren und teilweise immer noch steigenden Anteils von Konzentraten in der Ration. Die Gebärparese bzw. die Herausforderungen der Regulation des Ca-Haushaltes zum Laktationsbeginn und diesbezügliche prophylaktisch günstige Maßnahmen seitens der Fütterung waren über Jahre Gegenstand diverser Beiträge. Verschiedene Studien beschäftigten sich auch mit den Effekten von Glyphosat-Rückständen in Futtermitteln auf Gesundheit und Leistung von Milchkühen.

Unter den Gesundheitsstörungen im Schweinebestand mit entsprechenden Beziehungen zur Fütterung (d.h. zur Struktur / Partikelgrößen-Verteilung) gab es wiederholt Beiträge zur Pathogenese und zur Prophylaxe der Magengeschwüre. Eine ähnliche Zahl von Beiträgen war der Salmonellen-Prävalenz in Schweinebeständen gewidmet (allgemein ohne klinische Erkrankung der Tiere, aber mit erheblichen Risiken für den Eintrag in die Nahrungskette!). Die E-coli-bedingten Probleme in der Ferkelaufzucht verdienen auch weiterhin Aufmerksamkeit, da wirksame Ansätze wie „supra-nutritive“ Zink-Dosierungen nicht mehr möglich sind und der Einsatz von Antibiotika zurückgenommen werden muss.

Bei den Gesundheitsstörungen des Nutzgeflügels waren wiederholt Beiträge dem Problem Fußballen-Erkrankungen (foot pad dermatitis) gewidmet, das ganz wesentlich durch die Exkrement-Qualität bestimmt wird. Nutritive Einflüsse (z.B. Elektrolyt-Aufnahme) können dabei das Vorkommen und die Intensität der Ausprägung sowohl ver- als auch entschärfen. Neben den Salmonellen ist des Weiteren das Vorkommen von Campylobacter (Zoonose-Erreger mit noch größerer Bedeutung für den Menschen als Salmonellen) zu nennen. Erste diesbezügliche Ansätze zur Verhinderung der Erkrankung seitens der Tierernährung wurden vorgestellt.

Was den Bereich der Liebhabertiere (companion animals) angeht, so wurden zu Pferden diverse Beiträge präsentiert, die insbesondere ernährungsbedingte Gesundheitsstörungen betrafen (z.B. atypische Myopathie oder auch Hufrehe infolge einer forcierten Fructanaufnahme).

Ernährungsbedingte Störungen „kleiner Haustiere“ wurden auf den GfE-Tagungen nur sehr selten thematisiert, obwohl das aus vergleichender Sicht (z.B. Adipositas, Urolithiasis, Allergien) wünschenswert wäre.

Für das Grundverständnis diverser Einzelerkrankungen in ihren Beziehungen zur Ernährung / Fütterung hatten wiederholt die Übersichtreferate eine „Leuchtturmfunktion“ (z.B. Immunfunktionen der Darmwand, „Nutrition and immunity“) oder auch Oxidativer Stress (Host microbiota – interaction).

3.7. Weitere Arbeitsfelder

Unverkennbar gab und gibt es bei den Beiträgen, die in Göttingen vorgestellt wurden, auch wirksame „exogene Einflüsse“, wie es aus Tabelle 8 (Beispiele) deutlich wird. Die sich aus derartigen Anlässen ergebenden Forschungsaktivitäten dokumentieren beispielhaft die Bereitschaft von Mitgliedern unserer Gesellschaft, sich diversen neuen Herausforderungen zu stellen, die von außen (z.B. seitens politischer Vorgaben) kommen bzw. kamen und nach „Lösungen“ verlangten. Hierbei wurden u.a. die unerwünschten Stoffe (z.B. Dioxine), Einflüsse auf die Lebensmittel-Qualität, die Mykotoxin-Problematik oder auch Fragen der Nährstoffökonomie (Flachowsky et al. 2019) und des vorbeugenden Verbraucherschutzes behandelt.

Tabelle 8: Beiträge auf den GfE-Jahrestagungen der letzten 25 Jahre, die unmittelbar „exogene Einflüsse“ sowie rechtliche-gesellschaftspolitische Rahmenbedingungen widerspiegeln

Themen / Objekte	**„Treibende Kraft“**
BSE → „feed ban“	Ausbreitung der Seuche im Zusammenhang mit der Fütterung (Ausschluss aus der Lebensmittelkette)
GMO → „Gentechnik“	Politische, europaweite Vorbehalte gegenüber Technologien (am Beginn: „GenMais“, später Soja und weitere Futtermittel), wobei sich neue Entwicklungen andeuten (EC 2021)[1)]
Dioxine / Furane → „Dioxinkrise“	Erkenntnisse aus singulären Vorkommnissen → systemrelevante Risiken mit Auswirkungen auf Mischfutterproduktion und amtliche Futtermittelkontrolle
„Acrylamid“ → „Futter-Erhitzung“	Entstehung bei der Lebensmittelzubereitung, wenn Lebensmittel reich an Monosacchariden und / oder Asparaginsäure sind
„Glyphosat“→„Rückstände“	Medienberichte zu möglichem / notwendigem Verbot aufgrund von Risiken
„Mikroplastik“	Medienberichte über „Umweltrelevanz“, Eintrag in LM-Kette

[1)] Die EFSA hat seit 2004 238 Opinions zu GMO verabschiedet

3.8. Selbstkritik

Nach Vorstellung des auf verschiedenen Feldern Erreichten, d.h. von willkommenen Entwicklungen und Fortschritten, ist im Sinne einer auch selbstkritischen Wertung die Frage berechtigt, ob es nicht auch Anlässe gab, zu denen die GfE schon eher, stärker und wirksamer hätte Kritik und Widerspruch anbringen können und sollen (s. Tab. 9). Dabei ist es nachrangig, ob entsprechende Reaktionen der GfE bestimmte Entwicklungen aufgehalten oder abgeschwächt hätten (vermutlich nicht!). Es wäre jedoch heute im Sinne der GfE ein Vorteil, auf diesbezügliche Stellungnahmen verweisen zu können. Vielleicht ist im Sinne der Glaubwürdigkeit der GfE das Eingeständnis einer fehlenden oder zu späten Reaktion der heute einzig mögliche Ausweg (s. auch Kap. 4).

Tabelle 9: Entwicklungen in den letzten 25 Jahren, die uns als wissenschaftliche Gesellschaft eher / stärker / wirksamen Anlass zu Kritik geben müssen?

Entwicklung / Gegenstand	Sichtbares Zeichen	Anlass zur Kritik
Verlust an Futtermittel-Diversität	„Vermaisung der Landschaft" (Vergleiche mit dem „Klapp-Futterkalender")	Auswirkungen → Diversität von Flora und Fauna in der heutigen „Agrarlandschaft"
Milchleistung bei Kühen	Nutzungsdauer (?), Frequenz von „production diseases" (s. Eutererkrankungen)	Entwicklung von Leistung und Futteraufnahmekapazität verlief nicht parallel
Wurfgröße bei Zuchtsauen	Verhältnis Ferkel- zu Zitzenzahl und Konsequenzen für das Geburtsgewicht ↓	Ursachen und Höhe der Ferkelfrühverluste (1-3 LT!), sind Tiere < 800 g überhaupt lebensfähig?
„Frühabsetzen" bei Ferkeln, Kälbern	Entwicklung von „Verhaltensstörungen" bei „Frühabsetzen" von Säuglingen	Ungestilltes Saugbedürfnis (als Frustrationsreaktionen) → vgl. Säuglingsphase in der Natur
„Foot pad dermatitis" beim Geflügel	Verbreitung von Fußballenerkrankungen in der Geflügelmast	Exkrementenmassen bei hoher Besatzdichte
„Vegane" Ernährung von Hunden / Katzen	Marktangebot für diesen Sektor (ohne jedes vom Tier stammende Futtermittel)	Anspruch der „Art-Gerechtheit"? s. Enzym-Kapazität der Katze, z.B. fehlende Carotinase-Aktivität
„Reduzierte P-Versorgung" als Maxime in Nutztierfütterung	Skelettgesundheit von Sauen zum Laktationsende? (z. B. Frakturen)	Skelettfunktion als Speicher für Phasen eines forcierten Bedarfs, wie z.B. in der Laktation
„Verteufelung" der Tierhaltung (inkl. Weidetierhaltung)	Öffentliche Meinung, Darstellung in Medien	Hoher Ressourcenverbrauch und Umweltbelastung, Tötung von Mitgeschöpfen

Eine gewisse Selbstkritik ist evtl. auch angebracht, was die Formulierung der Titel einzelner Beiträge auf der „Göttinger Tagung" angeht. Es ist vielleicht dem Trend bei internationaler Publizierung geschuldet, dass man häufig im Titel eine Vorwegnahme des Ergebnisses findet, d.h. die „message", was früher als „unseriös" galt. Des Weiteren ist – zumindest vereinzelt – eine eher laxe Handhabung von Termini anzutreffen, wo gerade eine begrifflich exakte Verwendung angebracht gewesen wäre. Diesbezüglich kommt den Reviewern der eingereichten Beiträge auch in Zukunft eine besondere Verantwortung zu.

Schließlich ist bei einem Vergleich mit Proceedings und Journalen von Nachbardisziplinen in anderen Ländern festzustellen, dass für bestimmte Termini – die anderenorts verbreitet genutzt werden – bislang ein entsprechender deutscher Ausdruck im eigentlichen Sinne fehlt und somit auch inhaltlich keine entsprechende Ausrichtung erfuhr (s. Tab. 10).

Tabelle 10: Beispiele für Begriffe / Termini mit hoher „Prävalenz“ im wissenschaftlichen Arbeitsfeld in Journalen, die aber bei GfE-Tagungen bislang kaum vorkamen bzw. fehlten

Beispiele	**Bedeutung**
„residual feed intake“	Von erwarteter Futteraufnahme abweichender Wert (nicht durch Leistung und „normalen“ Erhaltungsbedarf erklärt → besser geeignet als der Futteraufwand?)
„precision farming“ / „feeding“	Auf individuelles Leistungspotential abgestimmte Energie- / Nährstoff-versorgung (Fütterungstechnik mit individuellem Monitoring verbunden)
„production diseases“	Nicht einem bestimmten Erreger, sondern den Produktionsbedingungen zuzuordnender Zustand beeinträchtigter Gesundheit (z.B. Gebärparese)
„resilience“	Fähigkeit eines Organismus, mit verschiedenen Herausforderungen („challenges“) klarzukommen; betrifft nicht nur die „Immun-Antwort“
„Bedürfnis“ (→ „mental health“)	Ein mit hoher Motivation angestrebtes Verhaltensmuster, das bei nicht-möglicher Realisierung zu Gesundheitsstörungen führt (u.a. Ethopathien)

Für die GfE insgesamt dürfte längerfristig – neben dem zentralen Parameter „Bedarf“ – von Interesse sein, auch den Terminus „Bedürfnis“ („mit hoher Motivation angestrebtes Verhaltensmuster, das bei nicht möglicher Umsetzung zu Verhaltensstörungen führen kann“) zu verinnerlichen, d.h. experimentell zu bearbeiten.

3.9. Positiv-Beispiele (Forschungsaktivitäten) mit „Leuchtturmfunktion“

Besonders erwähnenswert erscheinen im Berichtszeitraum Aktivitäten im Sonder-Forschungsbereich (DFG-SFG) „Nutrition and intestinal-host interactions in pigs“ unter der Leitung von Kollegen Zentek (FU Berlin) mit 16 Teil-Projekten sowie die Untersuchungen an Milchkühen mit Konjugierten Linolsäuren (CLA) unter der Leitung von Kollegen Dänicke vom Friedrich-Loeffler-Institut (FLI) in Braunschweig. Die Ergebnisse dieses DFG-Projekts, das sich aus insgesamt sieben Teilprojekten zusammensetzte, flossen wesentlich in die Entscheidungsfindung des FEEDAP-Panels der EFSA in die Bewertung der CLA für Milchkühe ein.

Eine andere herausragende wissenschaftliche Leistung ist das EU-finanzierte und von Kollege Kuhla (Dummerstorf) moderierte internationale Projekt „Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium“. In diesem Projekt haben Wissenschaftler von 14 Einrichtungen aus neun Ländern auf einem sehr hohen Niveau zusammengearbeitet.

Als beispielhaft ist auch das von der BLE von 2011 – 2016 geförderte und von Kollege Rodehutscord (Hohenheim) koordinierte „Grain up“-Projekt zu erwähnen, in dem sieben Einrichtungen kooperierten. Dabei erfolgte eine enge Zusammenarbeit der Tierernährung mit Instituten der Pflanzenzucht mit dem Ziel einer tiefschürfenden Charakterisierung des aktuell als Futtermittel verwendeten Getreides und seiner Nebenprodukte.

Nicht unerwähnt sollen auch verschiedene Projekte zum Einfluss der Tierhaltung auf Emissionen bleiben, wie z.B. das „KLIFF- (Klimafolgenforschung) Projekt“, das vom Niedersächsischen Wissenschaftsministerium finanziert wurde, sowie das von der „Schaumann-Stiftung“ unterstützte „Methan-Projekt“. An beiden Projekten arbeiteten Kollegen von der TiHo Hannover, der Universität Hohenheim, vom FLI in Braunschweig und weiteren Einrichtungen zusammen.

3.10. Themen mit zukünftiger Bedeutung (s. auch Kap. 5)

- Nahrungskonkurrenz zum Menschen (human edible fraction; hef-Werte) oder human edible protein (hep-Werte; s. Tab. 13; Ertl et al., 2015), Bedeutung von „neuen“ Nebenprodukten als Futtermittel
- Bedeutung der Pflanzenzüchtung (einschl. Gen-Technik und Genome editing; s. EC 2021; Upadhady 2021) für die Tierernährung bzw. Wechselwirkung mit der Pflanzenzüchtung (z.B. Baumwollsaatextraktionsschrot; EFSA 2021)
- Tierernährung und Umwelt mit dem Fokus Methan und „traumhaften“ Reduzierungspotentialen (> 80 % durch Braunalgen u.a.; Roque et al. 2021)

- Nährstoffökonomie/Rohstoffeinsatz bzw. Aufwand je erzeugtes Tierprodukt (z.B. Flachowsky und Kamphues 2012; Windisch und Flachowsky 2020)
- Futter bzw. Fütterung als Kostenfaktor (scheint für Workshops geeignet)
- Tierernährung und Produktqualität (Verbraucherwünsche, Zusammenarbeit mit der DGE)
- Liegen noch Erfahrungen bezüglich der Verfütterung von Nicht-Wiederkäuer-Schlachtkörperprodukten nach 20 Jahren Verbot vor (s. Abel et al. 2002 und EU 2021)?
- Sollten wir dem Futtermittelrecht (s. Kruse und Petersen 2021) sowie Politik und Wirtschaft mehr Aufmerksamkeit auf den Tagungen widmen (evtl. Workshops)?
- Gegenwärtig haben wir kein Journal für die Praxis – Medienunternehmen sind nahezu ausschließlich in den Händen rein ökonomisch ausgerichteter Verlage; evtl. „DLG-Mitteilungen“ stärker nutzen (z.B. Windisch 2021; Heft 11; 2021). Für diese Einschätzung spricht auch die aktuelle Response von Taube et al. (2021) auf den Beitrag von Windisch im Heft 11/2021 in Heft 12/2021 der DLG-Mitteilungen.
- Vorstellbar ist auch jährlich ein Vortrags-Thema: „Neues aus der EFSA?“ – mit Bezug zur Ernährungsphysiologie, Futtermittelkunde, Produktqualität etc. (z.B. welche Substanzen, welche Futtermittel/Zusatzstoffe haben im zurückliegenden Jahr die EFSA passiert bzw. wo besteht weiterer Forschungsbedarf?)
- Eine besondere Herausforderung ist auch der fachliche Austausch mit Partner-Gesellschaften in Nachbarländern.

4. Aus der Arbeit des Ausschusses für Bedarfsnormen der GfE (AfBN)

Der AfBN wurde 1975 gegründet und besteht derzeit aus 8 Mitgliedern (Eder, Kamphues, Rodehutscord, Schenkel, Südekum, Susenbeth, Windisch, Zentek). Im Bedarfsfall wird der AfBN durch externe Experten ergänzt. Vorsitzender des AfBN ist Herr Kollege Rodehutscord (Hohenheim). Ihm zur Seite steht als Geschäftsführer Herr Dr. Kampf, der mit persönlichem Engagement und Kompetenz nicht nur administrativ die Arbeit des Ausschusses unterstützt, sondern auch die Kommunikation zwischen den Ausschussmitgliedern in der Corona-bedingt schwierigen Lage durch Einrichtung von Videokonferenzen etc. ermöglichte. Nicht zuletzt die Altersstruktur der Mitglieder des Ausschusses gibt Anlass, über seine zukünftigen Aufgaben und Mitglieder nachzudenken. Im Sinne gesellschaftlicher Entwicklungen wäre es mehr als wünschenswert, wenn in diesem Gremium auch Kolleginnen nicht nur als Sachverständige mitarbeiten würden.

Wesentliche Unterstützung erfuhr der Ausschuss auch durch die finanzielle Förderung des BML/der BLE im Rahmen eines Forschungsvorhabens, so dass phasenweise auch Stellen für wissenschaftliche Mitarbeiter besetzt werden konnten, was die Effizienz der Ausschussarbeit erheblich förderte (angefangen bei entsprechender Literaturrecherche über metaanalytische Auswertungen bis hin zur Abfassung und Überarbeitung erster Text-Entwürfe).

Wiederholt war und ist die Arbeit des Ausschusses dem Vorwurf einer mangelnden Aktualität ihrer verabschiedeten Versorgungsempfehlungen ausgesetzt (gewesen), die in Sprüchen wie „die Praxis ist diesbezüglich viel weiter als die Wissenschaft“ oder „die Nettoenergie ist das Energiebewertungssystem der Zukunft – insbesondere beim Schwein“ oder auch „da nehmen wir lieber unsere eigenen Versorgungsempfehlungen“ gipfelten. Im Nachhinein ist jedoch zu konstatieren, dass es bei nicht wenigen dieser Vorhaltungen an einem fehlte, nämlich der wissenschaftlichen Fundierung.

Wie der Name des Ausschusses besagt, beschäftigt sich dieser mit der Ableitung von Versorgungsempfehlungen für Energie und alle lebensnotwendigen Nährstoffe einschl. des Tränkwassers für diverse Spezies/Kategorien in der Verantwortung des Menschen.

Im Berichtszeitraum wurden vom AfBN sieben „Empfehlungen zur Energie- und Nährstoffversorgung“ verschiedener Tierarten und -kategorien erarbeitet (s. Tab. 11). Der Er- bzw. Überarbeitungszeitraum hing im Wesentlichen von der Thematik und dem Umfang der vorliegenden Daten ab. Die Überarbeitung einer Empfehlung des AfBN benötigt meist weniger Zeit als eine Neu-Erarbeitung.

Wasser ist der Hauptbestandteil des Tierkörpers (bei Jungtieren z.T. >80 %, im Mittel etwa 50 %) und spielt im Stoffwechsel eine wichtige Rolle. Obwohl Wasser keine „Nährfunktion“ erfüllt, ist es ein extrem wichtiger Bestandteil des Körpers. Wassermangel kann nach kurzer Zeit zum Tod der Tiere führen, während die Hauptnährstoffe für ein Überleben auch über längere Hungerperioden aus den Körperreserven rezykliert werden können (Hennig 1971). Diesem Grundsatz folgend, hat nach der Jahrtausendwende der AfBN die Wasserversorgung der Tiere in die Betrachtungen der Versorgungsempfehlungen aufgenommen. Trinkwasser für Menschen muss den Anforderungen der Trinkwasser-Verordnung (2001) entsprechen. Adäquate Rechtvorschriften gibt es für Tränkwasser noch nicht. In der Futtermittelhygiene-Verordnung (EG, 2005) sowie in der Ausarbeitung von Kamphues et al. (2007) sind die Anforderungen an die Eignung von Wasser als Tränkwasser formuliert. Es ist bemerkenswert, dass Wasser auch gegenwärtig auf EU-Ebene noch nicht als Futtermittel angesehen wird.

Die vom AfBN erarbeiteten Empfehlungen für die Energie- und Nährstoffversorgung der jeweiligen Tierart/-kategorie stellen wesentliche Grundlagen für die praktische Fütterung dar und können als wichtigstes Bindeglied zwischen Wissenschaft und Praxis angesehen werden.

Bedauerlicherweise existiert neben dem AfBN international nur noch das National Research Council (NRC) in den USA als ein vergleichbares Gremium, das auf wissenschaftlicher Basis zu entsprechenden Versorgungsempfehlungen kommt. Weitere wissenschaftliche (staatliche) Gremien, wie z.B. das AFRC (Agricultural and Food Research Council; UK), das CSIRO (Commonwealth Scientific and Research Organisation; Australien) und die INRA (Institut National de la Recherche Agronomique; Frankreich), die analoge Zielstellungen wie der AfBN der GfE verfolgten, haben ihre Arbeiten eingestellt und weitgehend der „freien Wirtschaft“ überlassen. Diese Entwicklung ist aus wissenschaftlicher Sicht überaus kritisch zu sehen, da nicht auszuschließen ist, dass neben den wissenschaftlichen Erkenntnissen auch andere Interessen die Versorgungsempfehlungen beeinflussen können.

Die Ausschussarbeit der letzten Jahre (ab 2015) war und ist fokussiert auf die Entwicklung neuer Versorgungsempfehlungen für Milchkühe, die in 2022 zum Abschluss kommen dürften. Dabei erfolgen grundlegende Veränderungen, u.a. in der Bedarfsformulierung (z.B. ME statt NEL; pcvAS anstelle des bisherigen nRp; peNDF statt Rfa).

Nicht zuletzt, um den Entwicklungen in der Praxis sowie den allgemein notwendigen Bemühungen um das „Tierwohl“ gerecht zu werden, sollen in diesem Zusammenhang auch die Versorgungsempfehlungen für die Kälberaufzucht eine Aktualisierung erfahren. Schließlich sind Überarbeitungen weiterer Versorgungsempfehlungen (z.B. von Hunden: seit über 3 Jahrzehnten unverändert) unbestreitbar überfällig.

Tabelle 11: Empfehlungen zur Energie- und Nährstoffversorgung des Ausschusses für Bedarfsnormen (AfBN) der GfE zwischen 1995 und 2021 (Herausgeber: DLG-Verlag)

Jahr/Vorsitz	**Titel**	**Umfang (Seiten)**
1995 (Kirchgessner)	Empfehlungen zur Energie- und Nährstoffversorgung der Mastrinder (Nr. 6)	85
1999	Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler; Nr. 7)	185
2001 (Flachowsky)	Empfehlungen zur Energie und Nährstoffversorgung der Milchkühe und Aufzuchtrinder (Nr. 8)	136
2003	Empfehlungen zur Energie- und Nährstoffversorgung der Ziegen (Nr. 9)	32
2004	Empfehlungen zur Energie- und Nährstoffversorgung der Mastputen[1)]	5
2006 (Rodehutscord)	Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen (Nr. 10; 2008 in Englisch; 2009 in Chinesisch)	247
2014	Empfehlungen zur Energie- und Nährstoffversorgung von Pferden (Nr. 11)	192
In Vorbereitung	Neuauflage der „Milchkuhbroschüre“; Überarbeitung weiterer Broschüren	

[1)] Proc. Soc. Nutr. Physiology 13; (2004), 199-233

Die Aktualisierung von Versorgungsempfehlungen für diverse Spezies und Nutzungsgruppen ist und bleibt ein essentielles Anliegen der GfE, insbesondere des AfBN. Ihre Erklärung findet diese Forderung u.a. in offensichtlichen Veränderungen in der Futteraufnahme (z.B. Milchkühe), im Leistungsniveau (z.B. Wurfgröße bei Sauen), im Leistungsprodukt (s. Milchinhaltsstoffe), in der Körperzusammensetzung (s. Körperfettgehalte) oder auch in der Genetik (vgl. Erhaltungsbedarf der Vollblutpferde und sonstiger Pferderassen).

Der Tabelle 12 ist zu entnehmen, dass der AfBN im Berichtszeitraum 20 „Mitteilungen" in den Proceedings of the Society of Nutrition Physiology publiziert hat. Dabei handelt es sich um ein breites Feld an Informationen, die der AfBN als bedeutsam für die auf dem Fachgebiet arbeitenden Kolleginnen und Kollegen einschätzt, wie z.B.:

- Schätzgleichungen zur Ermittlung des Energiegehaltes verschiedener Futtermittel (Dauergrünland, Maisganzpflanzen; 1998)
- Konsequenzen des Verzichtes der Fütterung von Nebenprodukten von Landtieren in der Tierernährung (in Verbindung mit der BSE-Krise (2002))
- Ermittlung der praecaecalen Verdaulichkeit von Aminosäuren beim Schwein (z.B. 2002; 2005)
- Gleichungen zur Schätzung des Energiegehaltes in Futtermitteln und Rationen (z.B. 2003; 2005; 2008; 2017; 2020)
- Hinweise zur Rationsgestaltung bei Wiederkäuern (z.B. 2014)
- Zur Unerlässlichkeit von Tierversuchen für die Tierernährung (2017)
- Empfehlungen zur Versuchsdurchführung (z.B. 2020)

Längerfristig gesehen ist die GfE bzw. der AfBN gut beraten, wenn bei der Entwicklung von Versorgungsempfehlungen auch Spezies bedacht werden, die nicht der Lebensmittelgewinnung dienen, aber dennoch art- und bedarfsgerecht versorgt werden sollten, nicht zuletzt aus Tierschutzgründen. Schließlich kommen dabei ernährungsphysiologisch vergleichende Aspekte zum Tragen, von denen dann auch wieder die Nutztierversorgung profitieren dürfte (s. Energiebedarf der hochtragenden Stute à Bedarf trockenstehender Kühe!).

Eine noch stärkere Präzisierung des Energie- und Nährstoffbedarfs von Tieren stößt dabei zunehmend an Grenzen (s. Daten zur Ganzkörper-Zusammensetzung), die nicht zuletzt vom Tierschutz, d.h. von den Möglichkeiten für Tierversuche bestimmt werden.

Tabelle 12: Mitteilungen des Ausschusses für Bedarfsnormen

Jahr	Titel	Umfang (Seiten)
1997	– Überarbeitete Empfehlungen zur Versorgung von Schweinen mit Phosphor	8
	– Empfehlungen zur Energieversorgung von Aufzuchtkälbern und Aufzuchtrindern	15
	– Zum Proteinbedarf von Milchkühen und Aufzuchtrindern	20
1998	– Formeln zur Schätzung des Gehaltes an Umsetzbarer Energie in Futtermitteln aus Aufwüchsen des Dauergrünlandes und Mais-Ganzpflanzen	10
1999	– Empfehlungen zur Proteinversorgung von Aufzuchtkälbern	10
2002	– The ban of co-products from terrestrial animals in livestock feeding	9
	– Bestimmung der praecaecalen Verdaulichkeit von Aminosäuren beim Schwein	13
2003	– Schätzung des Gehaltes an Umsetzbarer Energie (ME) in Alleinfuttermitteln für Hunde und Katzen	6
	– Schätzung des Gehaltes an verdaulicher Energie im Pferdefutter	4
2004	– Schätzung des Gehaltes an Umsetzbarer Energie im Mischfutter (TMR) für Wiederkäuer	4
2005	– Standardisierte praecaecale Verdaulichkeit von Aminosäuren in Futtermitteln für Schweine – Methoden und Konzepte	21
	– Bestimmung der Verdaulichkeit zur energetischen Futterbewertung für Schweine	7
2008	– Neue Gleichungen zur Schätzung der Umsetzbaren Energie für Wiederkäuer von Gras- und Maisprodukten	8
	– Schätzung der Umsetzbaren Energie von Mischfuttermitteln für Schweine	6
2009	– Neue Gleichungen zur Schätzung der Umsetzbaren Energie von Mischfuttermitteln für Rinder	4
2014	– Bewertung der Strukturwirkung von Mischrationen für Milchkühe – Stand und Perspektiven	14
2017	– Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Substanz von Grobfutterleguminosen für Wiederkäuer	7
	– Stellungnahme zur energetischen Futterbewertung beim Schwein auf der Basis Umsetzbarer Energie versus Nettoenergie	15
	– Stellungnahme zur Unerlässlichkeit von Tierversuchen und zur Eignung von Ersatzmethoden in der Tierernährungsforschung	7
2020	– Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Masse von Maisprodukten für Wiederkäuer	5

Besondere Erwähnung verdient die Ausarbeitung einer Arbeitsgruppe des AfBN (Abel et al. 2002) zum Verbot der Verfütterung von Schlachttier-Nebenprodukten. Nahezu 20 Jahre benötigten die Kommissionen der EU, um dieses Verfütterungsverbot an Nicht-Wiederkäuer wieder aufzuheben (s. EU 2021b). Bei diesem Beispiel geht es um Ressourceneffizienz und die Verantwortung der Tierernährer im Umgang mit begrenzt verfügbaren Ressourcen. Folgende Frage erscheint durchaus legitim: „Wieviel Hektar Regenwald wurden in diesen etwa 20 Jahren gerodet, um den Proteinbedarf für die Tierernährung in Europa zu decken?“ Erfreulicherweise haben Kastner et al. (2021) kürzlich eine erste Antwort auf diese Frage geben können.

Ähnliche Fragen stellen sich bei der Gesetzeslage im Umgang mit gentechnisch veränderten Pflanzen. Baumwollsaat-Extraktionsschrot ist wegen seines Gossypolgehaltes als Proteinquelle vom Europäischen Markt verbannt. Andererseits gibt es seit mehreren Jahren gossypolfreie, gentechnisch veränderte Baumwollsaat (EFSA 2021), die aber wegen der EU-Gesetzeslage gegenwärtig nicht nach Europa gelangen kann.

5. Perspektiven für die GfE

Nachfolgend werden verschiedene Themen angesprochen, die einer weiteren Bearbeitung bedürfen und vielleicht auch Anregungen für zukünftige Forschungen sein können. Auf manche Themen wird auch im Rahmen von Kurzbeiträgen auf der 76. GfE-Jahrestagung bereits eingegangen.

– **Tierernährung und Tiergesundheit** Unbestreitbar ist die bedarfsgerechte Ernährung eine Grundvoraussetzung für Tiergesundheit und Tierwohl. Die Ausrichtung diesbezüglicher Arbeiten erfordert jedoch eine differenzierte Betrachtung: Bei den Lebensmittel-liefernden Tieren geht es überwiegend um die Gesundheit ganzer Tierbestände und die Qualität der erzeugten Lebensmittel, während bei den „Liebhabertieren“ das einzelne Individuum im Fokus steht. Die zwischen Ernährung und Verhalten bestehenden Beziehungen verlangen zur Klärung hier wirksamer Mechanismen („Mode of action“) innovative, interdisziplinäre Versuchs- und Lösungsansätze.

– **Verminderung der Nahrungskonkurrenz Mensch – Tier** (hef-Fraktionen); Berücksichtigung in Futtermitteltabellen (s. Tab. 13) Die Gruppierung der Futtermittel in vom Menschen verzehrbare und nicht verzehrbare Fraktionen kann wesentlich dazu beitragen, die „Rundum negative Einschätzung“ der Tierhaltung in der Öffentlichkeit zu korrigieren. Für entsprechende Futtermittel sollten Werte bezüglich der „human edible fraction“ (hef; in % des Futtermittels) in die Futtermitteltabellen aufgenommen werden (s. auch Südekum 2020). Mit den hef-Werten könnte durchaus auch die Kommunikation mit verschiedenen Mitbürgern einfacher werden (Reduzierung bzw. Vermeidung von Nahrungskonkurrenz, sinnvolle Nutzung der nicht vom Menschen essbaren Fraktionen etc.).

Tabelle 13: Vom Menschen verzehrbare Fraktionen (human edible fractions; hef; in % des Futtermittels) nach verschiedenen Autoren (Daten nach Ertl et al. (2015) berücksichtigen unterschiedliche Aufbereitung)

Futtermittel	**CAST (1999); Wilkinson (2011)**	**Nach Ertl et al. (2015)**		
		Niedrig	**Mittel**	**Hoch**
Gerste	80	40	65	80
Mais	80	70	80	90
Weizen	80	60	80	100
Sojabohnen	80	50	72	93
Rapssamen	20	30	59	87
Weizenkleie	20	0	10	20
Weideaufwuchs	0	0	0	0
Sonstige Nebenprodukte$^{1)}$	0	0	0	0

$^{1)}$ Sonstige Nebenprodukte wie Trockenschnitzel, Extraktionsschrote sowie Nebenprodukte vom Ackerbau (z.B. Stroh, Rübenblatt)

– **Weitere Verminderung negativer Umweltwirkungen (CH_4, N etc.)** Dieser Thematik wird weiterhin große Aufmerksamkeit zu widmen sein, wobei dem Methan, bedingt durch sein hohes Treibhaus-Gas-Potential (23 – 25 x CO_2) besondere Bedeutung zukommt. Diese Entwicklung ist zu beobachten und die Ergebnisse sollten experimentell überprüft werden.

– **Weitere Erschließung neuer Futtermittel** (z.B. Insekten; s. EU 2021a; Hall et al. 2021), neue Nebenprodukte der verarbeitenden Industrie sowie die Ausnutzung der Potentiale der Pflanzenzüchtung (einschl. Genome Editing; s. Jany et al. 2021) werden zunehmend bedeutungsvoller.

– **Konsequenzen des Climate Change für Futterbau und Tierhaltung** Diese Veränderungen berühren sowohl Pflanzenzüchtung und Pflanzenbau, aber auch die Tiere, wie Baumgard und Rhoads (2013) eindrucksvoll belegten und damit auch die Tierernährung. Längere Trockenperioden, höhere Temperaturen und andere Rahmenbedingungen lassen „neue“ Futtermittel (z.B. Sorghum statt Mais und Futterüben; z.B. Terler et al. 2021) sowie eine veränderte Futtervorratswirtschaft (z.B. wieder zunehmende Bedeutung von Stroh als Futtermittel) als auch Veränderungen in der Tierhaltung erwarten.

Die Gentechnik einschließlich das Genome Editing dürften in Verbindung mit der Trockenheits-Resistenz auch in einem neuen Licht erscheinen. Die höheren CO_2-Konzentrationen in der Atmosphäre können Auswirkungen auf Pflanzenerträge und Inhaltstoffe haben. Beispielsweise schätzen Jägermeyr et al. (2021) ein, dass die Maiserträge bis zum Jahr 2100 im Extremfall um bis zu 24% zurückgehen können, während die Weizenerträge weltweit bis zu 18 % steigen könnten. Die Autoren schätzen ein, dass Weizen als C_3-Pflanze mit der zu erwartenden höheren CO_2-Konzentration besser umgehen kann als die C_4-Pflanze Mais.

– **Wie gehen wir mit der Ernährung von „Heimtieren“ bzw. Zootieren um,** wobei der Futterverbrauch und der ökologische Fußabdruck der Heimtiere nicht übersehen werden dürfen (s. Okin 2017; Bethge 2021).

– **Mitarbeit** (möglichst Mitgliedschaft eines Wissenschaftlers oder einer Wissenschaftlerin) **im FEEDAP-Panel der EFSA** Eine entsprechende Mitgliedschaft weckt Verständnis / Interesse für „europäische und damit auch deutsche Fragen“ und informiert über Entwicklungen (von Futterzusatzstoffen), die demnächst auf dem europäischen Markt (evtl.) erscheinen können. Die Kontakte zu Fachkolleginnen und Fachkollegen können auch für die deutschen Tierernährer bedeutsam bzw. fruchtbar sein. Es wäre auch denkbar, dass ein Mitglied im FEEDAP-Panel der EFSA auf der GfE-Jahrestagung über Entwicklungen bei Zusatzstoffen / Futtermitteln informiert.

– **Gewinnung von Mitgliedern / Referenten für die GfE, vor allem aus Nachbarländern** Während im Berichtszeitraum bis 1996 (Meyer 1997) der Anteil von teilnehmenden Wissenschaftlerinnen und Wissenschaftlern aus den Nachbarländern noch 20 – 30 % betrug, lag er in den letzten Jahren bei < 10 %. Meist war auch ein Wissenschaftler aus den Nachbarländern Mitglied im Vorstand der GfE.

– **Beobachtung und Bewertung von Entwicklungen bei „imitierten Lebensmitteln“** auf der Basis von Pflanzen und / oder von *in vitro* bzw. „lab growing meat“ (s. z.B. „Spiegel“, „Die Zeit“, „FAZ“, „Handelsblatt“, verschiedene Tageszeitungen u.a., aber mittlerweile auch in Fachzeitschriften, wie z.B. in den „DLG-Mitteilungen“ (Lin-Hi 2021) sowie mögliche Schlussfolgerungen.

Ist Letzteres ggf. eine Aufgabe für die Deutsche Gesellschaft für Ernährung (DGE)? Ganz gleich, wessen Aufgabe es ist - für uns kann es existenzbedrohend sein bzw. werden, oder doch nicht? Auf alle Fälle verfügt diese Thematik über erhebliche Brisanz und Zündstoff. Der Traum der Erzeugung vom im „Labor gewachsenem Fleisch“ (artificial cultured oder lab grown oder *in vitro* oder synthetisches / künstliches Fleisch) und anderer „Tierprodukte“ (z.B. Milch, Muskulatur vom Fisch, verschiedene Organe und Gewebe) ist nicht neu. Vor fast 100 Jahren war beispielsweise Winston Churchill (Churchill und Spurrier 1931) diesbezüglich bereits überaus optimistisch, indem er feststellte, dass “Fifty years hence, we shall escape the absurdity of growing a whole chicken in order to eat the breast wing by growing these part separately under a suitable medium”. Etwa 75 Jahre später schlussfolgerte Bill Gates, dass “Remaking meat is one sector of food industry that is ripe for innovation and growth”. Gegenwärtig arbeiten etwa 80 Laboratorien und „Start-ups“ weltweit auf dem Gebiet von *in vitro*-Fleisch bzw. „Clean meat“. Verschiedenen Forschergruppen ist es gelungen, aus Stammzellen (Myoblasten) von Rindern dem Skelett-Muskel adäquates Fleisch zu erzeugen (z.B. Post 2014a,b). Mittlerweile sind auch verschiedene Bücher mit unterschiedlichem Pros und Kontras zum Thema auf dem Markt (wie z.B.: Shapiro 2018; Hassel 2019, Filko 2021).

Da sich auch das Fachorgan der Deutschen Landwirtschaft (die „DLG-Mitteilungen“ Nr. 8 / 2021) mit der Thematik beschäftigt hat (z.B. Lin-Hi 2021), wird die Fragestellung vermutlich demnächst auch die GfE erreichen. Lin-Hi (2021) ist der Ansicht, dass „ein Klammern am Bekannten und Bestehenden die große Gefahr mit sich bringt, die eigene Zukunftfähigkeit zu gefährden“. Nach seiner Ansicht hat es die Branche in Deutschland bisher versäumt, sich mit der „zellulären Landwirtschaft“ zu beschäftigen. Demnach sind keine Strategien vorhanden, wie die deutsche Landwirtschaft Teil der neuen Geschäftsmodelle rund um kultiviertes Fleisch werden kann. Auch die Wissenschaft (Mikrobiologie, Ernährungsphysiologie, Tierernährung und Veterinärmedizin) und die vor- und nachgelagerten Industrien sind nach Ansicht des Autoren gut beraten, sich mit den neuen Wertschöpfungsketten auseinander zu setzen und ihre Rollen zu finden. Nach seiner Ansicht wird der Standort Deutschland langfristig nur dann erfolgreich sein, wenn er sich auf den anstehenden Paradigmenwechsel einlässt. Für Lin-Hi (2021) existiert nicht die Frage, ob Laborfleisch traditionell erzeugtes Fleisch vom Markt verdrängt, sondern wann?

Bereits 2018 veranstaltete der Wissenschaftliche Dienst des Deutschen Bundestages ein Meeting zur Einschätzung des Standes der Forschung bei der Herstellung von *„in vitro* Fleisch“ (s. Deutscher Bundestag, 2018). Dabei ging es vor allem um den Stand der Forschung, die Stammzellen-Gewinnung und die Ernährung der Spendertiere und der Zellen, die Notwendigkeit des Antibiotika-Einsatzes, umweltpolitische Bedenken, Life Cycle Bewertungen der verschiedenen Verfahren, Preisentwicklung usw. (s. auch Bhat und Bhat 2021). Es mangelt nicht an mutigen Kalkulationen, wie man beispielsweise weltweit statt 1,4 Mrd. Milchkühen nur noch 40 000 Tiere als Stammzellen-Spender benötigt und das der CO_2-Fußabdruck um 17 (Broilerfleisch) bis 92 % (Rindfleisch) gesenkt werden könnte (Peters 2021). Bill Gates als eine finanzstarke „treibende Kraft" dieser Entwicklung (Temple, 2021) schätzt ein, dass möglichst zeitnah die Bürger in reichen Ländern ausschließlich „Lab grown meat" konsumieren sollten, da die Preise für „Lab grown Meat" längerfristig sehr hoch bleiben werden (>10 US $/kg) und die Menschen in ärmeren Ländern sich dieses Fleisch nicht leisten können. Neben klassischen Tierprodukten sieht Gates auch im „plant based meat" eine potentielle Konkurrenz von *„in vitro*"-Fleisch. Diese Aussagen klingen bei weitem nicht mehr so optimistisch wie in den Anfangsjahren der Entwicklung – man „rudert" also im Vergleich zu früheren Aussagen deutlich zurück.

Bevor sich die sog. „zelluläre Landwirtschaft" weiter durchsetzen kann, sind dringend Untersuchungen in Richtung Ressourceneinsatz und anderer Themen erforderlich, die an der GfE nicht vorbeigehen sollten. Beispielsweise zweifelt Muraille (2021) die oben erwähnten CO_2-Reduzierungen stark an. Andere Autoren (z.B. Chriki und Hocquette 2020; Le Coutre 2021) stellen kritische Fragen zur neuen Technologie, wie z.B. von der Erzeugung des Fleisches bis zur Akzeptanz seitens der Verbraucher. Auch das „System Bioökonomie" (Windisch und Flachowsky 2020) kann in diesem Zusammenhang an Bedeutung gewinnen.

Bei aller Tradition und dem Glauben an die traditionelle Landwirtschaft/Tierproduktion sollte jedoch nicht übersehen werden, dass ein Großteil der städtischen Bevölkerung dem Trend „weg vom Tier" aus verschiedenen Gründen aufgeschlossen gegenübersteht. Mehrere Begriffe wie Massentierhaltung, Tierquälerei, Ressourcen- und Flächenverbrauch, Nahrungskonkurrenz, Emissionen, Tötung von Mitgeschöpfen usw. belegen die schwindende gesellschaftliche Akzeptanz der Landwirtschaft bei großen Teilen der Stadtbevölkerung. Tierhaltung steht leider für manche Mitbürger in Deutschland auf der „roten Liste" der verbotenen Dinge, unmittelbar hinter dem Begriff „Gentechnik".

Aus den angeführten Schwerpunkten dürften sich vielfältige interdisziplinäre Forschungsansätze und auch Themen für Review Lectures und/oder für Workshops ergeben. Wenn man das oben Geschriebene liest oder hört, resultiert daraus die Frage:

Gibt es (dennoch) Perspektiven für unsere Disziplin? Die Nutzung von Ressourcen zur Fütterung von Tieren wird zukünftig – unabhängig von der Bedeutung von Tieren als Lebensmittel-Lieferanten – allein schon aufgrund der wirtschaftlichen Konsequenzen – zunehmend hinterfragt bzw. kritisiert werden. Arbeiten zur möglichst exakten Bedarfsdeckung werden dabei aber nicht überflüssig, sondern sogar dringlicher. Die Vermeidung jeder Verschwendung von Ressourcen wird an Bedeutung gewinnen. Eine stärkere Fokussierung auf den nicht vom Menschen verzehrbaren Anteil von pflanzlichen Produkten wird dabei erforderlich (s. hef-Werte in Tab. 13).

Je nach dem Selbstverständnis der Disziplin bzw. der darin aktiven „wissenschaftlichen Personen" werden weitere Arbeitsfelder zu erschließen sein bzw. Ergebnisse tierexperimenteller Arbeiten in Nachbardisziplinen ausstrahlen können (s. Tab. 14), so dass sich dabei auch neue Herausforderungen und Chancen für die GfE ergeben können.

Wissenschaftlicher Fortschritt, d.h. eine Erweiterung und Vertiefung von Kenntnissen sowie die Aufdeckung von Zusammenhängen und Wirkungsmechanismen oder Regulationsprozessen erfordern auch in unserer Disziplin kontinuierliche Bemühungen um ganz neue versuchstechnische Ansätze und/oder methodische Innovationen. Dabei ist es eher von nachrangiger Bedeutung, ob diese in der „eigenen" Disziplin entwickelt oder in benachbarten Wissenschaftsbereichen generiert wurden (z.B. Mikrobiom-Untersuchungen von Chymus bzw. Kot). Methodisch Neues zu importieren und/oder zu adaptieren ist nicht ehrenrührig – so lange die Quellen/Autoren genannt sind – sondern eine die Zukunftsfähigkeit mitbestimmende Voraussetzung. Dem intra- und interdisziplinären Austausch (s. auch Wissenschaftsrat 2020) über „methodische Fragen" gebührt demnach auf den GfE-Tagungen eine größere Aufmerksamkeit, die evtl. bereits durch eine Sektion unter der Überschrift „Methodische/Analytische Innovationen" erreicht werden könnte (s. Tab. 14).

Tabelle 14: Zukünftige Arbeitsfelder und mögliche Bedeutung für „Nachbar-Disziplinen“

Arbeitsfelder	**Ausstrahlung auf… / Bedeutung für…**
Ressourcen-Effizienz	Nutzung von nicht vom Menschen verwertbarer Biomasse
Bioökonomie	Kalkulationen zum Ressourceneinsatz und zur Effizienz verschiedener Verfahren (LCA-Vergleichs-Studien)
Emissionen	Verminderung von CH_4; N_2O und anderer Emissionen, aber auch von Cu, Zn u.a.
Pflanzenzüchtung / Futterbau / -konservierung / Tierernährung	Optimierung der Nahrungskette Futter – Tier – Lebensmittel
Ernährungswechsel (Phasen von Unter- / Überversorgung)	„Natürliche Ernährung“ war / ist gekennzeichnet durch dauernden Wechsel
Ernährung und Verhalten (s. Ethopathien bei Nutztieren)	Ethologie insgesamt bzw. Vermeidung von Verhaltensstörungen
Technologie von Rohwaren / Futtermitteldekontamination	Futtermittel-Produktion; Enzymgebrauch, Stärke- / Protein-Technologie
Bedeutung des „Unverdaulichen“ für die GIT-Entwicklung	„Mechanische Stimuli“; s. peNDF-System oder Torf als Substanz in der Positivliste
Tiere als Modell, z.B. hinsichtlich Humanerkrankungen	Diätetik in Human- und Tiermedizin; s. exokrine Pankreasinsuffizienz
Ernährung und Alter	Human- und Tierernährung bzw. -medizin („geriatrische Patienten“)
Methodische / Analytische Innovationen	Einführung neuer Methoden; Austausch von Ergebnissen; Verfahren

Die Ausstrahlung bzw. Wahrnehmung von Forschungsaktivitäten der GfE-Mitglieder sowie von Beiträgen auf unseren Jahrestagungen könnte / dürfte erheblich davon profitieren, wenn dabei stärker das Potential der Nutztiere (und ggf. auch Heimtiere) als Modell betont würde. Fragen zur Bedeutung des Ernährungsniveaus (einschl. Nahrungskarenz), des Alters oder des Alterns, der physikalischen Struktur der Nahrung, bestimmter Organerkrankungen und Insuffizienzen oder auch Auswirkungen einer spezifischen Versorgung mit bestimmten Nähr- oder Futterzusatzstoffen in einem definierten Lebensabschnitt oder Zeitfenster lassen sich in standardisierten Studien mit Tieren deutlich besser untersuchen als mit Menschen. Verschiedene von uns vorrangig beforschte Spezies reflektieren die Physiologie des Menschen oft deutlich besser als die allgemein als Modell verwendeten Labornager. Bisweilen dürfte schon ein gezielter Verweis auf Parallelen zum Menschen die öffentliche Wahrnehmung unserer Bemühungen erheblich fördern.

Eine weitere, für den Bereich der Tierernährung insgesamt bedeutsame Indikation für Untersuchungs- und Forschungsaktivitäten dürfte dem Spannungsfeld „Ernährung und Gesundheit“ (Überschuss / Mangel) gewidmet bleiben, wobei hier weniger monokausale Beziehungen als vielmehr die generellen Dispositionen für bestimmte Störungen in ihrem jeweiligen „Mode of action“ interessieren dürften.

Unter Berücksichtigung der oben zusammen getragenen Fakten kann die Schlussfolgerung nur lauten, dass es „ohne Tiere nicht geht“ (Windisch 2021) und dass die konventionelle Erzeugung von Lebensmitteln tierischer Herkunft sich im Wettbewerb zur „zellulären Landwirtschaft“ entwickeln wird / muss.

6. Schlussfolgerungen und Zusammenfassung

In 25 „GfE-Jahren“ (von 1997 bis 2021) wurden im Rahmen der Jahrestagungen 25 Plenarvorträge, 115 Vorträge im Rahmen der Workshops, sowie 3.295 Abstracts für Kurzvorträge und Poster-Darstellungen präsentiert.

Außerdem dienten die Tagungen der Information über die Arbeit des Ausschusses für Bedarfsnormen (AFBN) – dem wichtigsten Organ der GfE. Im Berichtszeitraum wurden vom AfBN sieben Empfehlungen zur Energie- und Nährstoffversorgung der Tiere sowie 19 Stellungnahmen zu aktuellen Fragen der Ernährungsphysiologie, der Tierernährung und der Futtermittelkunde erarbeitet.

Bei selbstkritischer Reflexion der Tätigkeit unserer Fachdisziplin ist ihre Daseinsberechtigung – ganz banal gesehen – an eine Prämisse gebunden, nämlich an die Haltung von Tieren, mit welchem Ziel auch immer. Das war und ist insbesondere die Erzeugung von Lebensmitteln von und mit Tieren. Andere Nutzungen von Tieren sind hier auch eingeschlossen, wie z.B. die Verwendung als Zug-, Last- und Versuchstiere, im Sport oder auch als Heim-/Begleittiere bis hin zur Haltung aus reiner Freude (Zootiere) am Tier oder als Statussymbol. Der Mensch als Tierhalter schuldet dabei dem Tier eine art-, alters- und bedarfsgerechte Versorgung mit Tränkwasser, Energie und Nährstoffen, und zwar nicht zuletzt aus Verantwortung gegenüber dem von ihm abhängigen Mitgeschöpf. Damit hat die Tierernährung im wohlverstandenen Sinne eine „dienende Funktion". Es geht also auch in Zukunft in der Fachdisziplin um die Erarbeitung von Versorgungsempfehlungen, wozu es entsprechende Grundlagen und Daten braucht. Unsere Arbeit soll und muss vor diesem Hintergrund einen „Impact" haben, d.h. den Tieren, den Tierhaltern, den Verbrauchern und „Nutzern" oder der Gesellschaft unter Berücksichtigung der jeweiligen Standards dienen, weil damit die Existenzberechtigung einer ganzen wissenschaftlichen Disziplin verbunden ist.

Ansehen, öffentliche Wahrnehmung und schließlich politische Akzeptanz der Tierernährung dürften erheblich davon profitieren, wenn diese ihre möglichen Beiträge zur Schonung von Ressourcen und Umwelt, zur Förderung von Tierwohl und Tiergesundheit sowie zur Gewährleistung von Lebensmittelsicherheit und Verbraucherschutz medienwirksamer aufbereiten könnte und würde.

In diesem Sinne wünschen wir der GfE ein herzliches **„Ad multos annos!!!"**

7. Literatur

ABEL HJ., RODEHUTSCORD M., FRIEDT W., WENK C., FLACHOWSKY G., AHLGRIMM H.-J., JOHNKE B., KUHL R.,

BREVES G. (2002): The ban of by-products from terrestrical animals in livestock feeding: Consequences for feeding, plant production, and alternative disposal ways. Proc. Soc. Nutr. Physiol. 11: 201-229

BARTUSCH M. (1998): Entwicklung der Gesellschaft für Ernährungsphysiologie von 1953–1996. Vet. Med. Diss., TiHo Hanover, 135 S.

BAUMGARD L.H., RHOADS R.P. (2013): Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1: 311-337

BETHGE P. (2021): Hasso, der Ökosünder. Der Spiegel 42 (16.10.2021): 102-104

BHAT Z.F., BHAT H. (2021): Tissue engineered meat – Future meat. Journal of Stored products and Postharvest Research 2 (1): 1-10

CAST (Council for Agricultural Science and Technology) (1999): Animal Agriculture and global food supply. Task Force Report No. 135; July 1999, Cast, Ames, IA, USA

CHRIKI S., HOCQUETTE J.-F. (2020): The myth of cultured meat: A Review. Front Nutrition, 07.02.2020

CHURCHILL W., SPURRIER S. (1931): Fifty years hence. Strand Magazine 82 (492)

DEUTSCHER BUNDESTAG (2018): „*In vitro* Fleisch" WD 5 – 3000 – 009/18. Wissenschaftlicher Dienst des Deutschen Bundestages

EC (European Commission) (2021): Study on new genomic techniques. Study on the status of new genomic techniques under Union law and in the light of the Court of Justice ruling in Case C-528/16 (PDF). Europea Commission, Brüssel, 29.04.2021

EFSA (European Food Safety Authority) (2015): Scientific Opinion on the safety of Solanum glaucophyllum standardised leaves as feed material. EFSA Journal 13(1): 3967

EFSA (European Food Safety Authority) (2021): Assessment of genetically modified cotton GHB811 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-ES-2018-154) EFSA Journal 19(8): 6781

EG (2003): Verordnung (EG) Nr. 1831/2003 des Europäischen Parlaments und des Rates vom 22. September 2003 über Zusatzstoffe zur Verwendung in der Tierernährung

EG (2005): Verordnung (EG) Nr. 183/2005 des Europäischen Parlaments und des Rates vom 12. Januar 2005 mit Vorschriften für die Futtermittelhygiene

ERTL P., KLOCKER H., HÖRTENHUBER S., KNAUS W., ZÖLLITSCH W. (2015): The net contribution of dairy production to human food supply: The case of Austrian dairy farms. Agricultural Systems 137: 119-125

EU (2021a): Durchführungsverordnung EU 2021/882 zur Genehmigung des Inverkehrbringens getrockneter Larven von Tenebrio molitor (EU 2015/2283 vom 01.06.2021)

EU (2021b): Verordnung EU 2021/1372 der Kommission vom 17. August 2021 zur Änderung des Anhanges IV der Verordnung (EG) Nr. 999/2001 des Europäischen Parlaments und des Rates hinsichtlich der Fütterung von anderen Nutztieren als Wiederkäuern, ausgenommen Pelztiere, mit tierischem Protein. Regelwerk, EU 2021, Naturschutz/Tierschutz; ABI. L 295 vom 18.08.2021

FILKO N. (2021): Clean Meat: Fleisch aus dem Labor: Die Zukunft der Ernährung? Langen-Müller Verlag, 224 S.

FLACHOWSKY G., KAMPHUES J. (2012): Carbon footprints for food of animal origin: What are the most preferable criteria to measure animal yields? Animals 2: 108-126

FLACHOWSKY G., SÜDEKUM K.-H., MEYER U. (2019): Protein tierischer Herkunft: Gibt es Alternativen? Züchtungskunde 91: 178-213

GESSNER D.K., MEYER S., MOST E., BEREZINA N., HUBERT A., LIEBISCH G., EDER K. (2018): Effects of insect meal from *Tenebrio Molitor* L. on the phospholipid composition and desaturation of fatty acids in the liver of obese rats. Proc. Soc. Nutr. Physiol. 27: 65

GONZALEZ-ORTIZ M.R., BEDFORD M.R., BACH KUDSEN K.E., CORURTIN C.M., CLASSEN H.L. (2019): The value of fibre – Engaging the second brain for animal nutrition. Wageningen Academic Publisher, Wageningen, The Netherlands, 383 p.
HALL H., FITCHES E., SMITH R. (2021): Insects as animal feed: Novel ingredients for use in pet, aquaculture and livestock diets. CAB eBooks, 138 p.
HASSEL H. (2019): Neues Fleisch: Essen ohne Tierleid – Berichte aus der Zukunft unserer Ernährung. Gütersloher Verlagshaus, 176 S.
HENNIG A. (1971): Grundlagen der Fütterung, Deutscher Landwirtschaftsverlag, Berlin, Teil 1, S. 19
JÄGERMEYR J., MÜLLER C., RUANE A., +35 weitere Autoren (2021): Climate impacts on global agriculture emerge earlier in new generation of climate and crop models. Nature Food 2: 873-885
JANY K.-J., FREYSSINET G., DUMONT P. (2021): Genomeditierung für eine nachhaltigere Landwirtschaft in Europa. European Scientist. https://www.europeanscientist.com/de/features-de/genomeditierung
KAMPHUES J., BÖHM R., FLACHOWSKY G., LAHRSSEN-WIEDERHOLT M., MEYER U., SCHENKEL H. (2007): Empfehlungen zur Beurteilung der hygienischen Qualität von Tränkwasser für Lebensmittel liefernde Tiere unter Berücksichtigung der gegebenen rechtlichen Rahmenbedingungen. Landbauforschung Völkenrode 57: 255-272
KASTNER T., CHAUDKARY A., GINGRICH S., BIDOGLIO G. LA PROVOST G., SCHWARZMÜLLER F. et al. (2021): Global agricultural trade and land system sustainability: Implications for ecosystem carbon storage, biodiversity ans human nutrition. One Earth 4 (10): 1425-1443
KRUSE S., PETERSEN U. (2021): Wegweiser Futtermittelrecht. Grundlagen kompakt und verständlich. Agrimedia-Verlag, 151 S.
LE COUTRE J. (2021): Cultured meat – Are we getting it right? Front Nutrition. 14.06.2021. https://doi:10.3389/fnut 2021.675797
LIN-HI N. (2021): Eine Revolution! DLG-Mitteilungen 8/21: 26-29
MEYER H. (1997): 50 Tagungen der Gesellschaft für Ernährungsphysiologie – ein Rückblick. Proc. Soc. Nutr. Physiol. 6: 173-190
MURAILLE E. (2021): Cultured meat? This could create more problems than it solves. Green Recovery 2021
NUCCIO M.L., CLAEYS H., HEYNDRIKS K.S. (2021): CRISPR-Cas technology in corn: A new key to unlock genetic knowledge and create novel products. Molecular Breeding 41: 11
OKIN G.S. (2017): Environmental impacts of food consumption by dogs and cats. PLoS ONE 12 (8): e0181301
PETERS A. (2021): What is the carbon footprint of lab growing meat? World Changing Ideas, 09.03.2021
POST M.J. (2014a): Cultured beef: Medical technology to produce food. J. Sci. Food Agric. 94: 1039-1041
POST M. J. (2014b): An alternative animal protein source: cultured beef. Ann. N. Y. Acad. Sci. 1328: 29-33
ROQUE B.M., VENEGAS M., LINLEY R.D., DE NYS R., DUARTE T.L., YANG X., KEBREAB E. (2021): Red seaweed (Asparagopsis taximirmis) supplementation reduces enteric methane by over 80 percent in beef steers. PLoS ONE 16(3): e0247820
RUSSEL W.M.S., BUSCH R.L. (1959): The principles of Humane Experimental Technique. London, Methuen & Co LTD: 230 p.
SHAPIRO P. (2018): Clean meat: How growing meat without animals will revolutionize dinner and the world. Gallery Book, 256 p.
SÜDEKUM K.-H. (2020): Lebensmittelerzeugung mit Nutztieren in einer ökologisierten Landwirtschaft – Ziele und Zielkonflikte. Agrarspektrum 53, Ökologisierung der Landwirtschaft, DLG-Verlag: 38-46
TAUBE F., GATTINGER A., HEISSENHUBER A. (2021): Es muss mit weniger Tieren gehen! DLG-Mitteilungen 12/2021: 81-82
TEMPLE J. (2021): Bill Gates: Rich nations should shift entirely to synthetic beef. MIT Technology Rev., Febr. 2021
TERLER G., RESCH R., GAPPMAIER S., GRUBER L. (2021): Nutritive value for ruminants of different fresh and ensiled sorghum (Sorghum bicolor (L.) Monch) varieties hervested at varying maturity stages. Arch. Anim. Nutr. 75: 167-187
TRINKWASSER-VERORDNUNG (2001): Verordnung über die Qualität von Wasser für den menschlichen Verbrauch. Bundesgesetzblatt, Bundesanzeiger; Inkrafttreten der letzten Änderung am 09.01.2018
UPADHYAY S. (2021): Genome engineering for crop improvement. John Wiley & Sons, 416 p.
WEIGEL H.J. (2014): Plant quality declines as CO_2 levels rise. eLIFE: 3e03233
WILKINSON J.M. (2011): Re-defining deficiency of feed use by livestock. Animal 5, 1014-1022
WINDISCH W. (2021): Ohne Tiere geht es nicht! DLG-Mitteilungen 11/2021: 14-19
WINDISCH W., FLACHOWSKY G. (2020): Tierbasierte Bioökonomie. In: Das System Bioökonomie (D. Thrän und U. Moesenfechtel (Hrsg.)), Springer-Verlag, Kap. 5: 69-85
WISSENSCHAFTSRAT (2020): Wissenschaft im Spannungsfeld von Disziplinarität und Interdisziplinarität. Positionspapier, Köln, Oktober 2020, 80 S.
WÖHLBIER W. (1963): 10 Jahre Gesellschaft für Ernährungsphysiologie der Haustiere 1953 – 1963; Schrift der Gesellschaft, 35 S.

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Abstracts

1.

Individual differences in digesta retention and chewing behaviour in cattle: a pilot study

Individuelle Unterschiede in der Digesta-Passage und im Kauverhalten bei Rindern: Pilotversuch

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The time digesta takes to pass the gastrointestinal tract – the 'mean retention time' (MRT) – is an important characteristic of digestive physiology. Because it is one of the factors influencing digestive efficiency, it has been suggested that genetic selection for MRT characteristics could be important for improving overall livestock efficiency [1]. Yet, characterizing individual animals by their MRT is impractical on a large scale, given the influence of diet and intake on MRT, and the 14 days per animal required for a state-of-the-art MRT measurement. MRT might critically depend on inter-individual differences in chewing behaviour: a higher chewing intensity (chews/kg dry matter intake [DMI]) should lead to more salivation and hence shorter MRTfluid, as well as – assuming similarity in dental anatomy – to a higher degree of particle size reduction and hence shorter MRTparticle. Therefore, the easier-to-perform measurements of chewing characteristics might be a possible alternative to direct MRT assessment. The opportunity of a study on the influence of a sialogogue, pilocarpine, was used to assess inter-individual differences in these traits in cattle.

Methods: We used Co-EDTA as a fluid and Cr-mordanted (<2 mm) and La-mordanted (10 mm) hay particles as particle markers, following a standard protocol [2] as well as chewing halters [3] in four dry nonpregnant cows (age > 5 years; 670-850 kg) fed grass hay and a small portion of maize silage for 4 weeks per treatment. Animals were kept as a group with restricted food intake during the first 2 weeks, and kept individually in the last 2 weeks, with daily DMI kept constant at 87 g/kg$^{0.75}$. We used a 4×4 Latin square design with four different pilocarpine treatments (0, 1, 2.5 and 5 mg/kg). Analysis of variance was conducted with animal and experimental run as random factors and treatment as fixed factor to assess the effect of treatment, and additionally using treatment and runs as random factors and animal as fixed factor to test for inter-individual difference. Pearson's correlations were used to assess correlations between measurements.

Results: Animals maintained a stable body mass irrespective of treatment. There was no effect of treatment on DMI (as intended by study design), or on total ingestion time, ingestion chewing intensity, total rumination time, rumination chewing intensity, boli per DMI, or the ratio of rumination:ingestion chewing, and MRT for any marker in the reticulorumen (RR). However, the animals differed distinctively in these characteristics among each other (e.g. mean total chewing intensity ranging from 3700 to 5400 chews/kg DMI; MRTCrRR ranging from 30 to 38 h). These differences were not consistently linked to differences in body mass. Total chewing intensity was correlated to MRT in the RR of the various markers (MRTCoRR: R2 = 0.45, P = 0.005; MRTCrRR: R2 =0.61, P < 0.001; MRTLaRR: $R^2 = 0.71$, P < 0.001).

Conclusions: To our knowledge, larger-scale assessments of individual animals for chewing behaviour have not been performed so far, but the existence of inter-individual differences in MRT has been demonstrated in studies that explored individual differences in methane emissions. When assessing chewing characteristics in future studies, it is recommended to document the dental status as increased chewing might be used by animals to compensate for a compromise of functional dental chewing surface. Our results support the concept that differences in chewing behaviour may explain differences in MRT between individual animals. Tests with a large number of individuals are required to determine whether chewing characteristics – logistically much easier to perform as MRT measurements – can be used to reliably predict MRT across individuals.

Bibliographical references

[1] HEGARTY R.S. (2004): Aust. J. Exp. Agric. 44:459-467

[2] LECHNER I., BARBOZA P., COLLINS W., FRITZ J., GÜNTHER D., HATTENDORF B., HUMMEL J., SÜDEKUM K.-H., CLAUSS M. (2010): Comp. Biochem. Physiol. A 155:211-222

[3] BRAUN U., ZUERCHER S., HAESSIG M. (2015): BMC Vet. Res. 11:231

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2.

Effect of a saliva stimulant on measures of intake and digestion in cattle

Effekt eines Speichelstimulans auf Charakteristika von Futteraufnahme und Verdauung bei Kühen

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Ruminants evolved a digestive system with a voluminous forestomach that selectively retains large particles for rumination and where symbiotic microbes ferment plant materials and produce volatile fatty acids, CO_2 and CH_4. Symbiotic microbes flow into the lower digestive tract and are digested as main N source. There are both *in vitro* and *in vivo* investigations suggesting that higher digesta dilution might be associated with a higher microbial yield [1, 2]. Theoretical considerations also indicate that an increase in microbial yield is linked to a decrease in CH_4 production, which was also confirmed in a continuously operating *in vitro* culture system [3]. However, whether this effect is also operative in live animals has, to our knowledge, not been investigated systematically so far.

Methods: We tested the effect of orally given pilocarpine (a saliva stimulant) on digesta retention, chewing behaviour, digestibility, ruminal microbial N synthesis, CH_4 emission and rumen fluid variables. Four dry nonpregnant cows (age>5 years; 670-850 kg) fed grass hay (150 g crude protein / kg dry matter [DM], 595 g neutral detergent fibre [aNDForg] / kg DM, 316 g acid detergent fibre [ADForg] / kg DM and 9.5 MJ metabolizable energy / kg DM) in a restricted amount were used in a 4×4 Latin square design with four pilocarpine treatments (0, 1, 2.5 and 5 mg / kg body weight per day) given orally at 0600, 1400 and 2200. For better acceptance, pilocarpine was fed with maize silage (total amounts of ~5% DM of diet). Dry matter and water intake were recorded for 14 days; urine and faeces were collected for 7 days and respiratory data for 48 hours by open-circuit chamber respirometry. Ruminal microbial N synthesis was estimated via urinary purine derivates. Chewing behaviour was recorded by chewing halters. Reticular contractions were counted via ultrasound for rumen motility. Mean retention time (MRT) of fluid and particles were assessed using a solute and two particle markers. Rumen fluid was obtained via oesophageal tubes and characterized for fermentation parameters. Analysis of variance was conducted with a linear mixed model with treatments of saliva stimulant as fixed factor and animals and experiment rounds as random factors. Tukey's procedure was used for multiple comparisons.

Results: No differences between treatments were observed for relative daily feed intake (86.8 ± 2.09 g / kg $BW^{0.75}$). Pilocarpine visibly increased salivation, reduced the dry matter content of leftovers (due to drooling) (P=0.005) and faeces (P=0.013), and increased water intake (P=0.021). Fluid MRT in total digestive tract and CH_4 emissions per digested dry matter were reduced by pilocarpine (P=0.002 and 0.045, at magnitudes of 8.8% and 7.3%, respectively). No effect of treatment was found on chewing activity, reticular contractions, particle MRT, nutrient digestibility, ruminal microbial N synthesis, or the pH and the concentrations of VFA and ammonia of rumen fluid. There was no significant correlation between CH_4 yield and microbial N yield per unit of digested dry matter.

Conclusions: Based on our visual observation of saliva drooling and the related measurements, we suggest that salivation was successfully increased. Both fluid MRT and CH_4 yield were decreased by applying the saliva stimulant, though at magnitudes smaller than expected from other studies. The inverse relationship between CH_4 and microbial biomass production reported from *in vitro* studies is not as distinct in cattle *in vivo*, at least not on a forage-dominated (= saliva stimulating) diet.

Bibliographical references
[1] ISAACSON H.R., HINDS F.C., BRYANT M.P., OWENS F.N. (1975): J. Dairy Sci. 58:1645-1659
[2] SMUTS M., MEISSNER H.H., CRONJÉ P.B. (1995): J. Anim. Sci. 73:206-210
[3] PFAU F., HÜNERBERG M., ZHANG X., HUMMEL J. (2019): Proc. Soc. Nutr. Physiol. 28:120

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3.

Measurement of apparent dry matter digestibility in cattle: comparison of the quantitative sampling technique with the TiO_2 indicator method

Messung der scheinbaren Verdaulichkeit der Trockenmasse bei Rindern: Vergleich der quantitativen Sammelmethode mit der TiO_2-Indikatormethode

*Peschel M., Buffler M., Bolduan C., Windisch W. – Freising

Standard method of measuring the apparent dry matter (DM) digestibility in ruminants comprises quantitative collection of faeces for at least 7 days. This rises problems with animal welfare legislation and stimulates application of alternative methods. The present study compares experimental data from an animal trial where the standard collection was applied simultaneously with the indicator method (titanium dioxide (TiO_2)).

Methods: 9 non-lactating dairy cows were fed restrictively (6.5 kg DM/day) a TMR consisting of grass silage, corn silage and concentrates containing 0.1% TiO_2. The feed was offered in two equal portions (morning and evening) and was consumed completely. The study consisted in 3 identical replicates of each 4 weeks adaptation followed by 1 week quantitative faecal collection, resulting in 27 collection records. Daily faecal excretions were recorded quantitatively and two sets of aliquots were sampled, of which one set was merged to the 7 day pool sample. Besides, faecal grab samples were derived twice per day after the morning and evening feeding. Pool samples, daily faecal samples, and grab samples were analysed for contents of DM and TiO_2. Since experimental treatment effects were absent in this study, the data was considered to reflect typical variations caused by individuality of animals and method of sampling and analysis, respectively. Different methods were analysed for the potential to detect differences in DM digestibility (least significant difference [LSD]) at $p<0.05$ and statistical power of $b<0.20$, respectively.

Results: With pool samples (standard method) DM digestibility averaged 75.3±2.3% with LSD=2.5%. Daily excretions of faecal DM was lower at day 1 ($p<0.05$) and higher for day 6 and 7 ($p<0.05$) compared to the overall mean. Shortening the sampling period to day 2 to 5 did not alter the DM digestibility (75.7±2.7%), but increased LSD to 3.0%. Achieving the same LSD as with the 7-day collection methods would have required 40 instead of 27 sampling records. The 7-day cumulative recovery of ingested TiO_2 in faeces was virtually complete (96.9%). Concentrations of TiO_2 in DM of daily faecal samples remained constant except for day 1, where values tended to be somewhat lower ($p<0.07$). Therefore, only data from day 2 to 7 were evaluated further. Concentrations of TiO_2 in morning and evening grab samples correlated to their corresponding levels of complete daily sampling with $r=0.83$ and 0.86, respectively. Combining corresponding morning with evening samples improved the correlation to 0.91. When using only one day of combined grab sampling, DM digestibility on base of TiO_2 averaged at 74.5±2.2% with a LSD of 2.6%. LSD further decreased asymptotically when rising the numbers of collection days. A virtual plateau of LSD=2.2% was reached with 4 days.

Conclusions: The determination of DM digestibility by the quantitative collection method seems to be susceptible to fluctuations in total DM excretion via faeces, probably along with weekly working rhythms. Obviously, this does not apply to the concentration of faecal TiO_2. The indicator method based on 4 days morning and evening grab samples seems to provide at least the same reliability as the quantitative collection method.

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4.

Digestibility of fat and calcium changes during and after a low carb, moderate protein diet in pigs

Verdaulichkeit von Fett und Calcium von Schweinen während und nach Fütterung einer Ration mit niedrigem Kohlenhydrat- und moderatem Proteingehalt

Schmitz M., *Böswald L.F. – Munich-Oberschleißheim

Contribution was withdrawn at short notice.

5.

Effects of different dietary Ca levels on precaecal and postileal phytate degradation, P digestibility, and faecal microbiota in pigs

Einfluss der Ca-Konzentration im Futter auf den praecaecalen und postilealen Phytatabbau, die P-Verdaulichkeit und die Mikrobiota im Kot von Schweinen

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Introduction: The gastrointestinal phytate breakdown has often been reported reduced with increased Ca concentrations in the feed of broiler chickens but such effects were barely studied in pigs. Pig diets are usually supplemented with microbial phytase and contain little or no inorganic feed phosphate while the addition of limestone and consequently the Ca concentration can markedly vary. Therefore, the objective was to study the effects of different Ca concentrations on phytate degradation, P digestibility, and microbiota composition in pigs, including interactions with added phytase.

Methods: A diet based on maize (59.1%), soybean meal (25%), rapeseed meal (10%), and TiO_2 (0.5%) was mixed with either 0.6% or 1.4% finely ground limestone to achieve Ca levels of 5 g / kg (BD_{low}) and 8 g / kg (BD_{high}). Both diets were supplemented or not with a hybrid 6-phytase at 1,500 FTU / kg (+BDlow and +BDhigh). No mineral P was added to the feed. The intended concentrations of Ca and P and phytase activity were confirmed by analyses. Eight barrows with an initial BW of 27 kg were fitted with a simple T-cannula at the distal ileum and assigned to the four dietary treatments in a completely randomized row column design. Feed was provided twice per day in mash form and daily feed allowance was 4% of BW. Each of the four periods had five days of adaption, four days of semi-quantitative faeces and urine collection, and two 12-h periods of continuous ileal digesta collection. Data were analysed in a two-factorial analysis of variance using the Mixed procedure of SAS 9.4.DNA from faecal samples was extracted using a commercial kit and submitted to 16S rRNA gene target amplicon sequencing to study the faecal microbiota composition. Bioinformatics analysis followed the MOTHUR pipeline [1], followed by multivariate statistical analysis.

Results: Precaecal phytate degradation was reduced at high Ca when phytase was included (90 vs. 85%) but not without phytase (22 vs. 23%), indicated by a significant interaction (P=0.02). Faecal phytate degradation was reduced by high Ca from 96 to 87% without phytase (P<0.01) and 99 to 96% with phytase (P=0.02). Faecal P digestibility was not affected by Ca but increased by phytase (27 vs. 60%; P<0.001). PERMANOVA of faecal microbiota data showed a trend for the interaction between phytase and limestone (P=0.085). The relative abundance of Bifidobacterium in the faeces was higher at high Ca and phytase inclusion (25.3%) compared to high Ca alone (17.7%) and low Ca and phytase inclusion (17.2%). High Ca and phytase inclusion led to an increase in the relative abundance of Limosilactobacillus and Ligilactobacillus (3.6% and 1.6%) compared to high Ca alone (2.2% and 0.6%) and low Ca and phytase inclusion (2.2% and 0.6%).

Conclusions: Limestone addition to pig feed can reduce phytate degradation in the hindgut without affecting P digestibility. Together with added phytase, limestone addition can increase the abundance of bacteria known to produce short-chain fatty acids and discussed as phytate degraders.

Bibliographical references

[1] KOZICH J.J., WESTCOTT S.L., BAXTER N.T., HIGHLANDER S.K., SCHLOSS P.D. (2013): Appl. Environ. Microbiol. 79:5112-5120

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6.

Can differences in $InsP_6$ disappearance in broilers and turkeys be attributed to different endogenous mucosal phosphatase activity?

Können Unterschiede im $InsP_6$-Verschwinden bei Broilern und Mastputen durch unterschiedliche endogene mucosale Phosphatase-Aktivität erklärt werden?

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About two thirds of P in plant feedstuffs are present in the form of inositol hexakisphosphate ($InsP_6$). Before $InsP_6$-P can be absorbed in the digestive tract it needs to be released by enzymatic hydrolysis. Precaecal $InsP_6$ disappearance has been reported to be higher in broilers than turkeys [1,2], but the diets used in those studies were different for the species. When gnotobiotic broilers were fed diets with low Ca and P content and without a phytase additive, 42 % of dietary phytate disappeared by the end of the ileum [3]. This indicated substantial endogenous mucosal activity of phosphatases in broilers, but mucosal phosphatases have barely been studied in turkeys. The objective of this study was to compare broilers and turkeys using the same feed for both species and evaluate the effects of Ca, P, and phytase addition on precaecal $InsP_6$ disappearance and endogenous mucosal phosphatase activity.

Methods: The experiment had a 2x2x2-factorial arrangement of treatments with 2 species, 2 CaP levels, and 2 phytase levels. The diets were based on maize and soybean meal, and contained TiO_2 as indigestible marker. They were formulated to meet supply recommendations for turkeys (except for P and Ca). The diets were supplemented without (CaP-; 4.7 g P / kg DM, 6.2 g Ca / kg DM) or with (CaP+; 10.2 g P / kg DM, 13.6 g Ca / kg DM) monocalcium phosphate and additional limestone, and without (Phy-) or with (Phy+) 1500 FTU / kg of an *E. coli*-derived 6-phytase (Quantum™Blue). At 36 days of age, 240 broilers and 240 turkeys were allocated to 24 pens per species with 10 birds per pen, following a randomised complete block design with 6 replicates. After 7 d of feeding the experimental diets, all animals were sacrificed. Mucosa samples of the jejunum were obtained and frozen in liquid nitrogen. Digesta samples from the last two-thirds of the ileum were pooled on pen basis and frozen at -20 °C. Digesta was analysed for $InsP_6$ and TiO_2. Mucosal phosphatase activity was assayed in enriched brush border membranes (BBM) by incubation with pure phytate at pH 5.5 and 40 °C for 15 min and subsequent photometric analysis of released phosphate. Data was analysed with three-way ANOVA using MIXED procedure of SAS.

Results: Species × phytase interaction was significant ($P < 0.001$) for precaecal $InsP_6$ disappearance. If no phytase was added, disappearance was higher in turkeys (30.0 %) than broilers (21.4 %). With added phytase, $InsP_6$ disappearance increased more in broilers than turkeys (to 78.6 % vs. 67.5 %). Across both species and phytase levels, $InsP_6$ disappearance was higher in CaP- than CaP+ ($P < 0.001$, 52.9 % vs. 45.8 %). Mucosal phosphatase activity was higher in turkeys than broilers; it increased by phytase in turkeys but not in broilers, resulting in a species × phytase interaction ($P = 0.010$, broilers Phy-: 0.9, broilers Phy+: 1.7, turkeys Phy-: 4.2, turkeys Phy+: 7.1 [µmol phosphate / g BBM protein / min]). The increase in CaP level of the feed reduced mucosal phosphatase activity regardless of species and phytase supplementation ($P = 0.034$, CaP-: 3.9, CaP+: 3.1 [µmol phosphate / g BBM protein / min]).

Conclusions: In diets without added phytase, higher precaecal $InsP_6$ disappearance in turkeys than broilers coincided with a higher mucosal phosphatase activity in jejunum, indicating that a causal relationship might exist. In diets with added phytase, such relationship did not exist and may have been masked by the added phytase. A higher efficacy of the added phytase in broilers may be attributed to better conditions in the digestive tract for the enzyme to be active. This could be due to a moister environment in the crop of broilers observed during sampling.

Bibliographical references

[1] INGELMANN C.J., WITZIG M., MÖHRING J., SCHOLLENBERGER M., KÜHN I., RODEHUTSCORD M. (2019): Poultry Science 98:912-922

[2] OLUKOSI O.A., GONZÁLEZ-ORTIZ G., WHITFIELD H., BEDFORD M.R. (2020): Poultry Science 99:1528-1539

[3] SOMMERFELD V., VAN KESSEL A.G., CLASSEN H.L., SCHOLLENBERGER M., KÜHN I., RODEHUTSCORD M. (2019): Poultry Science 98:5562-5570

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7.

No size-dependent particle retention in the hindgut of horses

Keine grössenabhängige Partikelretention in Blind- und Dickdarm von Pferden

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The peculiar anatomy of the hindgut of horses, with narrow passages between the caecum and the proximal colon, and between the dorsal layer of the proximal colon and the Colon transversum, as well as previous reports on an accumulation of fine particles in the colon, have led to the theory that particles of different size are retained differently during their passage through this part of the gastrointestinal tract [reviewed in 1, 2]. Because in contrast to ruminants, feeding passage markers of different particle size is probably unreliable in horses due to intensive ingestive chewing, other ways to either measure or apply markers representing different particle sizes are required [2]. In a classic study, this was done by inserting plastic particles of different size into the caecum via the cannula in fistulated animals, and relevant differences in the 'mean retention time' (MRT) between these particles were reported [3]. Because this is in contradiction to results obtained by assessing the MRT or particle size classes as derived from feeding a whole, marked hay and sieve analysis of faeces [2], we repeated the classic study, applying various (non-plastic) passage markers directly to the caecum. We expected a significant difference between the MRT of the solute vs. the particle markers, but no difference among the particle markers.

Methods: Five caecum-cannulated geldings (body mass 562 ±31 kg) were fed a constant amount (6.81 kg dry matter/d) of grass hay (CP and NDF 151 and 592 g/kg DM, respectively) in three equal portions per day for a ten-day adaptation period. Co-EDTA was used as a solute, Yb-acetate as a small (size not specified; mostly assumed to bind to fine particles), as well as Cr-mordanted (1-2 mm) and Ce-mordanted (8 mm) hay particles as particle markers, applied simultaneously as a pulse-dose to the caecum cannula. Faeces were collected at regular intervals for four consecutive days. Analysis for faecal marker concentration and calculation of MRT for the individual markers followed standard procedures [2]. Results were analyzed by repeated-measurements ANOVA and Bonferroni post hoc comparisons.

Results: The MRT in the hindgut were 22.2 ±2.4 h for Co (solute marker representing fluids), 25.0 ±3.4 h for Yb (representing fine particles), 26.2 ±1.6 h for Cr (small particles) and 26.3 ±1.5 h for Ce (large particles). Differences between the particle marker MRTs were all not significant ($P_{adj.} > 0.05$). The only significant differences were between the solute marker Co and each of the particle markers Cr and Ce ($P_{adj.} < 0.009$).

Conclusions: Although our sample size was small with only five animals, a difference in MRT of less than 3 h was detectable, confirming the well-known, albeit small, difference in MRT in horses between the fluid and the particle digesta phase, with ratios of particle:fluid MRT at a magnitude of 1.1 to 1.2. Even if, hypothetically, differences in MRT between particle size classes (of even smaller magnitude) were detectable with larger numbers of experimental animals, their relevance in terms of fermentative digestion would be slight. In contrast to previous reports in fistulated horses [3], no difference in MRT between markers representing different particle sizes were detected. Notably, in the previous study, plastic markers were used that may not correctly represent digesta properties in horses. However, the findings are consistent with previous ones generated by another method indicating little differentiation between particle size classes during digesta passage in horses [2]. Given the congruence of these findings, no differential retention of different particle size classes in the digestive tract of horses should be assumed.

Bibliographical references

[1] VAN WEYENBERG S., SALES J., JANSSENS G.P.J. (2006): Livestock Sci. 99:3-12

[2] HUMMEL J., SCHEURICH F., ORTMANN S., CROMPTON L.A., GERKEN M., CLAUSS M. (2018): J. Anim. Physiol. Anim. Nutr. 102:429-439

[3] ARGENZIO R.A., LOWE J.E., PICKARD D.W., STEVENS C.E. (1974): Am. J. Physiol. 226:1035-1042

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8.

Influence of European seaweeds on gas production, nutrient degradation and microbiome in the Rusitec

Einfluss europäischer Algen auf Gasproduktion, Nährstoffabbau und Mikrobiom im Rusitec

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Microbial fermentation in ruminants is related to the release of methane [1]. The addition of some seaweeds to ruminant diets was shown to reduce methane production markedly *in vitro*. For instance, a reduction of up to 99% was observed for the red seaweed Asparagopsis taxiformis (AT) grown in the Pacific [2]. Seaweeds are a very heterogeneous group, not only the species but also sampling time and growing location can influence their content of nutrients and bioactive compounds. Therefore, the present study investigated the effect of different European seaweeds on *in vitro* total gas and methane production, nutrient degradation and microbiome composition.

Methods: Five seaweed samples were tested at 2.5% inclusion in a total mixed ration (TMR) using the rumen simulation technique Rusitec with the unsupplemented TMR as the control. Besides AT sampled in the Azores, the brown seaweeds Ascophyllum nodosum (AN) and Fucus vesiculosus (FV) were used, both sampled in Scotland and in Iceland, to give five seaweed additions. Each of the six treatments was replicated twice in two consecutive Rusitec runs, each with seven days of adaptation and seven days of sampling. In the sampling period, feed residues were pooled per fermenter to determine nutrient degradation (dry matter, organic matter, crude protein, acid and neutral detergent fibre on an ash-free basis) and total gas and methane production were measured daily. In addition, 16S rRNA amplicon sequencing was used to assess the microbiome present on d 7 and 13, followed by bioinformatic analysis via Qiime2 [3].

Results: The supplementation of all seaweeds except the Icelandic AN decreased total gas production by 7% (Scottish AN) to 19% (Scottish FV; $p < 0.001$). Methane concentration of total gas production was decreased by AN (8%) and FV (19%) from Scotland and AT (74%) compared to TMR alone ($p < 0.001$). The degradation of all nutrients was decreased by both FV samples by 8% (dry matter and organic matter for Icelandic FV) to 25% (acid detergent fibre for Scottish FV; $p \leq 0.001$). For AN and AT, only the crude protein degradation (AN: 13%, AT: 11%) and in the case of the Icelandic AN the degradation of acid detergent fibre (11%) was decreased ($p < 0.001$). Among all seaweeds, AT affected both archaeal and bacterial community compositions, as well as archaeal methanogens abundances. Particularly, AT supplementation resulted in decrease of Methanobrevibacter and Methanomicrobium relative abundances, meanwhile Candidatus Methanomethylophilus proportions increased.

Conclusions: The addition ofAT markedly reduced methane production and altered microbial composition without affecting nutrient degradation other than a reduced degradation of crude protein, which could be beneficial for intestinal supply of amino acids. The effect of seaweed on microbial fermentation and methane emission strongly depends on species and location of origin.

Bibliographical references

[1] DIFFORD G.F., PLICHTA D.R., LØVENDAHL P. et al. (2018): PLoS Genet. 14:e1007580
[2] MACHADO L., MAGNUSSON M., PAUL N.A., KINLEY R., DE NYS R., TOMKINS N. (2016): J. Appl. Phycol. 28:1443-1452
[3] BOLYEN E., RIDEOUT J.R., DILLON M.R. et al. (2019): Nat. Biotechnol. 37:852-857

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9.

Impact of ambient heat on leucocytic HSP70 expression, NF-κB p65 translocation, and hematology in primiparous Holstein dairy cows

Der Einfluss von Hitze auf die leukozytäre HSP70 Expression, NF-κB p65 Translokation und Hämatologie in primiparen Holstein Kühen

*Koch F., Hansen C., Viergutz T., Kuhla B. – Dummerstorf

Environmentally induced hyperthermia jeopardizes farm animal health, among others by compromising the immune system. Leucocytes are important immune cells, which respond to hyperthermia by increasing heat-shock protein 70 (HSP70) expression. HSP70 has been shown to inhibit the translocation of the transcription factor nuclear factor-κB p65 (NF-κB p65) into the nucleus thereby reducing the transcriptional activation of the immune response. However, it is not known whether heat stress to dairy cows inhibits the translocation of NF-κB p65 in leucocytes. Therefore, our objective was to elucidate the effects of chronic heat stress on the number of leucocytes, the NF-κB p65 translocation to the nuclei and the HSP70 protein expression in leucocytes of primiparous dairy cows.

Methods: Thirty primiparous, non-pregnant German Holstein cows (169±9 days in milk) were evenly allocated into either a heat-stressed (HS, n=10), control (C, n=10) or pair-feeding (PF, n=10) group. During the adaptation phase, all animals were kept in a climate chamber at conditions defined earlier to be thermoneutral (TN) for lactating dairy cows [1,2]: 16°C, 63% relative humidity (RH), temperature-humidity index (THI) 60 for 6 days (d) and received a total mixed ration twice daily (0730 and 1730 h). In the subsequent experimental phase, HS cows were exposed to 28°C for 7 d (52% RH, THI 76) with ad libitum feeding and access to water, both tempered to 28°C. The C group was exposed to 16°C (63% RH, THI 60) with ad libitum feeding for 7 d. The PF cows were exposed to the same environmental conditions as C cows, but were offered only the amount of feed the HS cows ingested. Feed allowance to PF cows was calculated as percentage of daily mean feed intake per kg body weight (BW) of HS cows. Dry matter intake (DMI), water intake, milk yield, rectal temperature (RT), heart rate (HR), and respiration frequency (RF) were measured daily. Body surface temperature was measured by infrared thermography on -1 d and 6 d of the experimental phase. Blood samples were taken daily from day 0 to 7 and analyzed by flow cytometry for abundance of leucocytic heat shock protein 70 (HSP70) and nuclear-stained NF-κB p65 using fluorescent-labeled antibodies. Differential blood count and hemoglobin were measured with VetScan HM5. Daily measurements on the same animal were analyzed by repeated measurement ANOVA using the MIXED and UNIVARIATE procedures of SAS (Version 9.4).

Results: From d 2 to 7, DMI was lower in HS and PF than C cows ($P<0.001$). From d 5 to 7, water intake per DMI was higher in HS than C and PF cows ($P<0.05$). On d 7, milk yield was lower in HS than C and PF ($P<0.05$). From d 1 to 7, RT and RF were higher in HS than C and PF cows ($P<0.001$), whereas HR did not differ between groups. Body surface temperatures of the side area and the lateral and posterior udder were higher under HS than TN conditions ($P<0.001$, respectively). Leucocytic HSP70 abundance were not different among the groups. However, the nuclear NF-κB p65 fluorescence intensity was lower on d 5, but higher on d 6 in HS than C and PF cows on ($P<0.05$). On d 3, the number of lymphocytes was lower in HS and C than PF cows ($P<0.05$). Moreover, the number of lymphocytes remained higher in PF than C cow on d 4 and 5 ($P<0.05$). On d 7, the number of erythrocytes were lower in HS than PF cows ($P<0.05$). The hemoglobin concentration and hematocrit content tended to be lower in HS than PF on d 7 ($P<0.09$).

Conclusions: Our data suggests that HSP70 is not involved in the regulation of NF-κB translocation to the nucleus during heat stress and that the leucocytic immune response underlies dynamic changes. The lower number of lymphocytes, erythrocytes, and a tendency for lower hemoglobin and hematocrit might to be associated with the hemodilution effect due to increased water consumption under HS. Further studies are required to understand the underlying immunological mechanism.

Bibliographical references

[1] NARDONE A., RONCHI B., LACETERA N., BERNABUCCI U. (2006): Vet. Res. Commun. 30:75-81

[2] ZIMBELMAN R., COLLIER R. (2011): Proc. Dairy Nutri. Conf., Fort Wayne, Indiana (USA). 111-126

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10.

Alterations in the ruminal microbiome diversity and composition of periparturient dairy cows supplied with 3-Nitrooxypropanol and varying concentrate feed proportions in the ration

Veränderungen in der ruminalen mikrobiellen Diversität und Gemeinschaft von peripartalen Milchkühen bei Fütterung von 3-Nitrooxypropanol und variierenden Kraftfutteranteilen in der Ration

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3-Nitrooxypropanol (3-NOP) inhibits the last step of CH_4 formation (1). In the experiment underlying this investigation (2), interactive effects of 3-NOP and varying concentrate feed proportion (CFP) on the magnitude of CH_4 inhibition have been examined in periparturient cows. Aside from CH_4 inhibition, 3-NOP and CFP influenced the pattern of rumen volatile fatty acids (VFA). In this context, it was hypothesized that feeding 3-NOP combined with varying CFP influenced rumen microbial community structures which led to already described changes in CH_4 emissions and VFA profile (2).

Methods: From d 28 antepartum (a.p.) until d 120 postpartum (p.p.), 45 pluriparous German Holsteins were grouped in a 2×2 factorial design by low (LC) or high (HC) CFP with (NOP-LC, 48.4 mg/kg DM; NOP-HC, 51.2 mg/kg DM) or without 3-NOP supplement (CON-LC, CON-HC) in the ration (2). After parturition, CFP of LC groups was maintained at 30%, while that of HC groups was gradually increased to 55% until d 21 p.p. where it remained for the rest of the experimental period. Rumen fluid was collected on d 28 a.p., d 49 p.p. and d 120 p.p. before morning feeding by an oro-ruminal probe and a suction pump. After DNA extraction from the rumen fluid, V4 region of 16S rDNA was amplified using primers 515F and 806R. Amplicons were sequenced using IonTorrent 5S. Data analyses were conducted using the QIIME2 framework. Alpha-diversity measures were further analyzed in a linear mixed effects model (PROC MIXED SAS v9.4), while beta diversity measures were analyzed by Adonis tests and Mantel tests.

Results: A reduction in community richness was observed in 3-NOP and HC groups over time (Shannon, 3-NOP×TIME; $p<0.01$; CFP×TIME; $p<0.01$). Distribution of rumen microbiota was influenced by 3-NOP and CFP through the course of the trial (Pielou evenness; 3-NOP×TIME; $p<0.025$; CFP×TIME; $p<0.01$). Faith´s phylogenetic diversity was significantly reduced in 3-NOP ($p<0.01$) and HC supplemented cows (CFP×TIME; $p<0.01$). Unweighted UniFrac distance between microbial communities was affected by 3-NOP ($p<0.01$) and CFP×TIME ($p<0.01$), even though only a limited proportion of the distances between samples were explained by CFP or 3-NOP ($R^2=0.02$). However, differences in distances within experimental groups were smaller than distances between groups. Interactive effects between both dietary factors were not observed in diversity metrics. In contrast to CH_4 production (spearman rho (ρ)=-0.048; $p=0.06$), CH_4 yield ($\rho=0.273$), ruminal acetate-propionate ratio ($\rho = 0.627$) and molar VFA proportions were positively correlated to unweighted UniFrac distances in microbial communities between the samples ($p<0.01$) indicating that cows with similar structure of rumen microbiota also display similar patterns of rumen microbial products.

Conclusions: We confirmed our hypothesis that both, dietary CFP and 3-NOP, influenced the rumen microbiome. High CFP and 3-NOP reduced microbial diversity, whereby interactive effects were not apparent.

Bibliographical references
[1] DUIN E.C., WAGNER T., SHIMA S. et al. (2016): Proc. Natl. Acad. Sci. 113: 6172-6177
[2] SCHILDE M., VON SOOSTEN D., HÜTHER L., MEYER U., ZEYNER A., DÄNICKE S. (2021): Arch. Anim. Nutr. 75: 79-104

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11.

Utilization of pomegranate peel as functional feed in dairy cows – effects on milk production, methane and urinary nitrogen emissions

Einsatz von Granatapfeltrester als funktionelles Futtermittel für Milchkühe – Effekte auf Milchproduktion und Methan- sowie Harnstickstoffemissionen

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Food industry by-products may serve as feed ingredients for dairy cows to increase sustainability of milk production. The aim of the present study was to evaluate the effects of the by-product pomegranate peel (PP) for dairy cows on milk yield and milk composition. Furthermore, considering the rather high prevalence of polyphenols and especially tannins in PP, which may inhibit methanogens and increase rumen bypass of feed protein, thus lowering urinary nitrogen (N) losses, the methane mitigation potential and effects on urinary N of dietary PP were evaluated.

Methods: The experiment was approved by the Cantonal Veterinary Office of Zurich (ZH229/2020) and performed at AgroVet-Strickhof (Eschikon, Lindau, Switzerland). Four late-lactating dairy cows (Brown Swiss, fourth lactation, 796 ± 30 kg body weight) were fed a basal mixed ration (composed of grass silage, hay, straw, alfalfa, and concentrate) that was not supplemented (control) or supplemented with two concentrations (5% and 10% of dry matter (DM), top-dressed) of dried PP (obtained from Alfred Galke, Bad Grund, Germany) in an incomplete Latin Square design in three periods. The order in which the animals received the experimental feed was randomized and each treatment was included at least once in each period. Each period lasted for 23 days, with an adaptation period of 14 days, 7 days for total urine and faeces collection, and 2 days for respiration chamber measurements to quantify methane emissions. On day 15, plasma was obtained from blood collected from the jugular vein. Nutrient contents in (pooled) samples of feed, faeces, and urine were analysed by standard methods. Daily milk yield was measured and per period, milk gross composition was analysed in milk samples pooled according to milk yield by MIR spectrometry (MilkoScan FT6000, Foss, Hillerod, Denmark). Plasma urea was quantified by a Cobas® MIRA Plus (Roche Diagnostics GmbH, Mannheim, Germany). Experimental data were analysed by fitting mixed models to the lmer procedure of R statistical language (R Core Team 2016; version 4.0.2) with treatment as fixed and animal and period as random effects.

Results: The concentrations of total tannins in PP and basal mixed ration were 19.9% and 0.31% DM, thereof 18.5% and 0.3% hydrolysable tannins in DM, respectively. Feed intake, absolute and energy corrected milk yield were not affected by the experimental diets. The proportions of fat, protein, and lactose in the milk did not differ but urea concentration was significantly lower in milk from animals fed 10% PP compared to the control feeding (14.9 vs. 19.1 ± 1.03 mg/dl). Plasma urea concentration was also significantly lower in animals fed 10% PP compared to control animals (2.78 vs. 3.87 ± 0.303 mmol/l). Urinary nitrogen excretion decreased by 27% and 36% when the animals were fed 5% and 10% PP as compared to the control feeding. Methane emissions did not differ ($P>0.05$) between experimental groups.

Conclusions: The preliminary results of the present study suggest that PP can be included into diets of dairy cows in a proportion of up to 10% DM without negatively affecting feed intake and productivity. Despite an apparent lack of methane mitigation potential, the considerable reduction of urinary nitrogen by the PP polyphenols is highly favourable from an environmental perspective. The analyses of the antioxidant status of dairy cows‘ plasma and their milk are underway.

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12.

How to calculate water demand in pigs – which parameters provide the optimum basis?

Wie berechnet man den Wasserbedarf bei Schweinen – welche Parameter bilden die optimale Grundlage?

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For successful pig fattening it is very important to know the water requirements of pigs. Water demand can either be calculated based on pig's live weight or on their feed intake and is closely related to climatic conditions in the barn. Thus, we examined whether temperature-humidity-index (THI) is a good indicator for sufficient water supply in pig production and concluded that there was indeed a close relationship between THI and actual water consumption in fattening pigs (1). Elaborating on this topic, we investigated further the relationship between feed consumption, live weight and water requirements and THI.

Methods: Barrows were allocated to 4 pens (n = 80, 20 pigs / pen) and data collection was conducted during starter and grower fattening phase (start = 24.3 kg ± 4.3 kg, end = 72.5 ± 8.8 kg BW). Feed was composed of barley, wheat, soya bean meal, mineral feed and soya oil (starter feed: 13.0 MJ ME / kg air-dry matter [ADM], crude protein 175 g / kg ADM; grower feed: 13.0 MJ ME / kg ADM, crude protein 171 g / kg ADM) and provided ad libitum via dry feeders with an animal-to-feeder space ratio set at 4:1. Two nipple drinkers were installed per pen, with a flow rate between 0.8 – 1.2 l / minute. Daily water consumption was recorded for each pen using water meters on the nipple drinker. Once a week pigs were weighed individually, while their feed consumption was recorded on pen-basis. Ambient temperature and relative humidity were continuously recorded in each pen over the entire study and THI was calculated. Further information is provided in (1). The following equation was used to calculate the water requirement of fattening pigs based on live weight: $Y = 0.076x + 1.96$ (2).

Results: Feed consumption (n = 4 pens, 20 pigs / pen) was 2.3 ± 0.5 kg / pig*d and feed-to-gain ratio at 2.4 ± 0.5.The average daily water consumption (n= 4 pens, 20 pigs / pen) was 7.2 l / pig*d. Lowest water consumption was 4.1 l / pig*d, concurrently with the lowest THI (67.5), at the highest THI (74.7) water consumption amounted to 9.1 l / pig*d. When relating water consumption to live weight of the pigs, it could be determined that measured water consumption was above the calculated water consumption (acc. to equation) in all 7 experimental weeks (1). The measured average daily water consumption was 163.9 ± 20.8 ml / kg body weight (BW), whereas according to the equation of SCHIAVOON and EMMANS (2), only 119.3 ± 1.9 ml / kg BW would be sufficient in meeting water demand. With increasing live weight development, this difference between calculated and measured water consumption widens further, from ~14 % at the start to ~35 % at the end of the trial. Water requirement based on daily feed intake was calculated on average 3.2 ± 0.6 l / kg feed intake. Overall, water consumption was highest in week 4 with an average of 3.9 l / kg feed and lowest in week 1 with 2.2 l / kg feed. The calculated water consumption according to the equation correlated significantly with THI in all pens (pen 1: rank correlation coefficient spearman (rs) = 0.79, pen 2: rs = 0.81, pen 3: rs = 0.77, pen 4: rs = 0.78). These data are in agreement with the correlation between measured water consumption and THI (1).

Conclusions: THI correlated well with the actual water consumption of fattening pigs (1). The measured water consumption was generally higher than the calculated water consumption based on BW. One possible explanation could be the difference in pig genotype: in our study we used high-performing fatteners, whereas the equation was based on an "unimproved pig genotype" (2). In modern pigs, capacity for feed intake and body accretion was dramatically increased and thus likely their respective water demand. The water consumption according to (2) might not be an optimal indicator for water requirement in modern pigs. However, the ratio between water and feed intake (~3:1) was within the reported range in literature (3), suggesting this to be the preferred indicator for water requirement.

Bibliographical references

[1] SCHALE P., DÄNICKE S., HESSEL E.F., KLUESS J., GRÜMPEL-SCHLÜTER A. (2021): Proc. Soc. Nutr. Physiol. 30:73

[2] SCHIAVOON S., EMMANS G.C. (2000): British Journal of Nutrition (84), 873-883

[3] KAMPHUES J., WOLF P., COENEN M., EDER K., IBEN C., KIENZLE E., LIESEGANG A., MÄNNER K., ZEBELI Q., ZENTEK J. (2014): Supplemente zur Tierernährung für Studium und Praxis. 12. Aufl., Hanover: M. & H. Schaper GmbH

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13.

Physiological responses of grazing dairy cows to increasing ambient temperature in moderate climates

Physiologische Reaktionen von weidenden Milchkühen auf steigende Umgebungstemperaturen in gemäßigten Klimazonen

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Heat stress of grazing dairy cows occurs even in temperate climates (1). Continuous monitoring using heat stress-sensitive indicators may help to take timely preventive measures. Despite the existing literature, examining physiological indicators for heat stress in general and mostly focusing on acute heat stress (2), it is unclear how well and how early they can indicate physiological changes in grazing cows in temperate climates. The objective of the present study was to study short-term physiological responses to increasing ambient temperature in grazing dairy cows that may help to identify heat stress before animal wellbeing might be compromised.

Methods: The study was performed with 38 lactating Holstein dairy cows during summer 2018 and 2019 at the experimental farm of Agroscope (Posieux, Switzerland). At the start of the data recording in 2018 and 2019 cows were on average 103 ± 26 and 125 ± 20 days in milk and produced 35.2 ± 5.4 kg and 32.1 ± 6.1 kg of milk per day, respectively. Cows grazed full-time using a set stocking system and were supplemented with an energy-rich concentrate according to their current milk yield with a minimum of 1.8 kg / day and a maximum of 4.2 kg / day. Climate conditions were recorded every min and were used to calculate the comprehensive climate index (CCI), which reflects the felt temperature in °C. Data were collected in 12 experimental periods, each consisting of 3 consecutive days with increasing CCI. The vaginal temperature (VT) of each cow was measured every 10 minutes with a microprocessor temperature logger (DST micro-T logger, Star-Oddi, Garðabær, Iceland) and was used as a physiological indicator of heat stress. Heart rate data were automatically collected using the Polar Team Pro system (Polar Electro Oy, Kempele, Finland). Jugular venous blood and milk were sampled once daily in the afternoon before and during milking, respectively. The plasma concentrations of hormones thyroxine (T4) and triiodothyronine (T3), and the concentrations of milk cortisol and milk electrolytes (Cl^-,K^+ and Na^+) were analyzed. For the data analysis, the averaged CCI (CCIMEAN) and the maximal VT (VTMAX) recorded between 0830 and 1430 h were considered. Correlation analysis were conducted using the R platform (version 4.0.2) applying linear mixed-effects models (lmer).

Results: The VTMAX correlated positively with CCIMEAN ($P < 0.001$). The average heart rate correlated positively with CCIMEAN and with VTMAX ($P < 0.001$). The concentrations of plasma T4 and T3 correlated negatively with CCIMEAN ($P < 0.01$) and with VTMAX ($P < 0.01$). Milk cortisol concentration correlated positively with CCIMEAN and with VTMAX. No correlations could be detected between CCIMEAN and the concentration of milk K+ ($P = 0.64$), Cl− ($P = 0.48$) and Na+ ($P = 0.13$) and between VTMAX and the concentration of milk Cl^- ($P = 0.62$). The concentration of milk K+ correlated positively ($P = 0.05$) and milk Na^+ concentration correlated negatively ($P < 0.01$) with VTMAX.

Conclusions: The increase in VT showed a response of the cows to increasing environmental temperature. In addition, changes in blood and milk hormones, some milk electrolytes, and heart rate related to increasing VT suggest that cows may have perceived the increasing heat load as a stressor.

Bibliographical references

[1] AMMER S., LAMBERTZ C., VON SOOSTEN, D., MEYER U., DÄNICKE S., GAULY M. (2018): J. Anim. Physiol. Anim. Nutr. 102: 103

[2] HOFFMANN G., HERBUT P., PINTO S., HEINICKE J., KUHL B., AMONT T. (2019): Biosyst. Eng. 199: 83

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14.

Impacts of concentrate supplementation and horn status on responses to mild heat stress in Brown Swiss dairy cows

Einfluss von Kraftfutterzulage und Hornstatus auf Reaktionen von Braunviehkühen auf milden Hitzestress

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Feeding strategies may mitigate heat stress (HS) in dairy cows. As concentrates have a lower heat increment than forages, the supplementation of concentrates is one nutritional strategy to lower negative effects of HS on performance and physiology of cattle, as shown in intensive production systems (1). If reduced dietary neutral detergent fiber (NDF) contents also lower HS symptoms in dairy cows in forage-based systems has not yet been studied. Further - albeit not yet studied - it has been repeatedly assumed that the cow's horns may dissipate heat and therefore increase heat tolerance (2). The present work investigated the impacts of supplementing concentrates or alfalfa pellets to a hay-based diet and horn status on feed and water intake, milk production and physiological responses to mild heat stress.

Methods: Nine horned (H+) and 10 disbudded (H-) Brown Swiss dairy cows (milk yield: 18.8 ± 0.2 kg / d; 150-180 d in milk) with similar genetic indices were used in a cross-over design with two isoenergetic hay-based diets. Cows received either ad libitum hay and alfalfa pellets (90:10, C-, NDF content: 41.0%) or restricted amounts of hay and concentrates (70:30, C+, NDF content: 34.5%). The restricted diets were calculated based on the individual intake of the C- diet in order to obtain an equal NEL intake. Each crossover period included a 10-d adaptation and a 5-d sampling period. During the latter, cows were housed in respiration chambers under varying climatic conditions (CC). The ambient temperature was 10°C (considered as thermoneutrality for cows with a daily milk yield of about 20kg (3); TN) and 25°C (HS) for the first and last 48 h, respectively. Feed and water intake, milk yield and composition were measured daily. Respiratory (RR) and heart rate (HR), and body temperature (BT) were measured twice daily. Data were analyzed using linear mixed models, including the fixed effects diet, CC, horn status, their interactions, and the time of the day (where applicable) and the random effects crossover, replicate and animal.

Results: The C- cows had greater dry matter (+12%), NDF (+33%), crude protein (+11%) and water intakes (+16%) than C+ cows (all $P<0.05$). At TN, cows consumed less water (-13 %, $P<0.05$) than under HS. Milk fat contents were greater (+3%, $P<0.05$) and milk yield and milk lactose contents were lower (–5% and -0.5%, both $P<0.05$) in C+ cows compared to C-. At TN, milk lactose contents were greater (+1%, $P<0.05$) than under HS, whereas milk yield stayed the same. The C+ cows had a slower HR (-4%, $P<0.05$) compared to C- cows. At TN, cows showed slower RR (-74%, $P<0.05$) and HR (-7%, $P<0.05$) than under HS, whereas BT did not differ by CC. Horn status did not influence any of the measures ($P>0.10$), except for RR that was slower for H+ cows than H- cows under HS (interaction: $P<0.05$).

Conclusions: In the present study, the HS condition caused greater water intake and physiological responses, but did not affect performance and body temperature, suggesting that HS was relatively mild or in its beginning. Under such conditions, concentrate supplementation did not mitigate HS responses, but the slower RR points towards a possible role of the horn in thermoregulation. Further ongoing analyses of heat production, serum and milk samples will determine possible diet- and horn status-related influences on the cows' metabolic responses to mild heat stress.

Bibliographical references
[1] WEST J. (1999): Journal of Animal Science 77 Suppl 2(2):21-35
[2] KNIERIM U., IRRGANG N., ROTH B.A. (2015): Livestock Science 179
[3] HAHN G.L. (1999): Journal of Animal Science 77 Suppl 2:10-20

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15.

Procedure for updating the default values for the average body weight of different farm animal types in the model for environmental risk assessment of veterinary medicinal products

Vorgehensweise zur Überarbeitung der Annahmen für das durchschnittliche Körpergewicht verschiedener Nutztier-Kategorien im Modell zur Umweltrisikobewertung von Tierarzneimitteln

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When calculating the predicted environmental concentration (PEC_{soil}) of veterinary medicinal products (VMPs), the model used in the environmental risk assessment (ERA) of the EU contains the average BW for calculating possible VMP loads originating from intensive animal husbandry. The existing default values (absolute numbers published in Tables 3-6 [1]) for the different farm animal types originate from a discussion of experts 15 years ago. Default values for farm animal weights are frequently questioned, because it is likely that they have changed. Therefore, a transparent procedure for revising or adding new default values is necessary.

Methods: Growth curves of all relevant farm animal types were reviewed and simplified into three phases: I. linear gain from birth to puberty, II. a transition from puberty to fully grown (logarithmic curve) and III. fully-grown with a growth rate of zero at maximum BW. Age and weights were collected from recent literature at birth, weaning, end of linear gain, and beginning of fully grown for all different animal types. The average BW was calculated over the production period of each animal type [2] and was defined as the sum of the weight at the start of the production period and the weight at the end of the production period divided by 2. This applies for all linear growing animal types (e.g., calves or weaner pigs, phase I). For all animal types that exceeded the end of linear gain (e.g., dairy cow or sow, phase II and III), using the average will lead to an underestimation. Therefore, average BW was interpreted as a lower limit. Recalculated average BWs were compared with existing default values. Furthermore, current trends in animal feeding and management practice were reviewed and possible effects on the default values in the future were described.

Results: Systematically, all newborn farm animals in the ERA model were covered by the animal type of the mother until the separation from her. Consequently, the production period for calves and weaner pigs started at separation, which led to higher default values for average BW of calves and weaner pigs. Default values appeared to be realistic for fattening pigs, broiler, ducks and rabbits. For laying hens, replacement layer, broiler breeder, turkeys and horses, recalculated average BW needed to be adjusted for underestimation, but matched previous default values. Due to new animal types, new default values for cattle (> 0.5 years), dairy goats and sheep were suggested. Several feeding and management practices in livestock production will be mirrored in the growth curves. A larger frame of animals will lead to an increase of maximum BW. Breeding goals, management decisions or increased health status aim for higher longevity of the animals. These trends resulted in higher default values for average BW in the future of fully-grown animal types. Contrary, lower growth rates as required by some organic standard providers would counter this.

Conclusions: In order to assess the environmental risks as realistic as possible and, above all, to avoid their underestimation, the current default values for average body weight should be slightly adjusted upwards for the following animal types: calves, dairy cows, weaner pigs and sows. Availability and access of current data could be improved.

Bibliographical references

[1] EUROPEAN MEDICINES AGENCY (2016): GL on EIA for VMPs in support of the VICH guidelines GL6 and GL38: EMA/CVMP/ERA/418282/2005-Rev.1-Corr

[2] MONTFORTS M.H.M.M. (2003): ERA for VMP Part 1, RIVM report 320202001/200. NIPHE, Bilthoven, Netherlands

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16.

O_2 consumption, CO_2 and NH_3 emission of Black Soldier Fly larvae grown on different substrates

O_2-Verbrauch, CO_2- und NH_3-Emission von Larven der Schwarzen Soldatenfliege, aufgezogen auf unterschiedlichen Diäten

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Black soldier fly larvae (BSFL) are known for their capacity to produce protein from low-quality nutrient sources. As mini-livestock, they may help to close the dietary protein gap. However, for effective research on these insect species standardized protocols are necessary, allowing e.g., the calculation of nutrient conversion efficiencies [1]. Currently, comparison of conversion efficiencies of diets between different laboratories is difficult because of differences in dietary energy and nutrient contents and physical structure of the feeding substrates. A ring test was performed in the framework of the HORIZON 2020 project SUSINCHAIN, comparing BSFL growth performance either on a local chicken diet (CF) or on a local standard Gainesville fly diet (GD), with a single batch (SB) of CF and GD diet at different labs [2]. The GD diet contains wheat bran (50 %), corn meal (20 %), and alfalfa hay (30 %). Our objective was to compare the greenhouse gas emission derived from BSFL reared on these different diets.

Methods: Newly hatched larvae were fed up to day (d) 5 with the local CF before they were separated from their substrate, counted and distributed to one of the experimental diets. The BSFL were reared on a local CF (Trede & von Pein, Germany), the SB-CF (Hobby first, Belgium), a local GD, and the SB-GD diet prepared by Università degli Studi di Torino, Italy (5.2, 6.4, 5.0, 5.5 % ash; 17.8, 17.6, 13.3, 13.5 % crude protein; 2.1, 4.4, 2.8, 3.3 % crude fat; 3.8, 5.1, 14.5, 12.6 % crude fibre; 41.4, 32.5, 20.8, 25.2 % starch; 3.8, 4.1, 4.7, 3.6 sugar; 10.9, 10.2, 7.1, 7.9 MJ ME / kg DM). Per insect growth box 150 larvae were spread on 114 g of CF or GD substrate (70 % water; 30 % feed), prepared one day before and incubated at 27.5°C. The larvae were reared at 27.5°C and 70 % humidity (4 diets, 6 replicates each) between d 5 and 8. At d 9 the growth boxes were transferred to respiratory chambers (27.5°C, 40 l air flow / h), were the larvae were reared for another 7 d. Values for carbon dioxide (CO_2) and ammonia (NH_3) production, and oxygen (O_2) consumption were recorded continuously every 24 min. At d 16, the larvae were harvested and weighed. The experiment was performed in four runs with 6 replicates each. Accumulated gas exchange data (ml / 7 d) per mg of body weight were analysed with ANOVA considering fixed effects of diets and runs using the GLM procedure of SAS ($P < 0.05$). Multiple comparisons between groups were performed with the Tukey test.

Results: The larval weight (5th to 6th instar) was highest in the CF groups (SB-CF = 212 mg, local CF = 198 mg), which was higher than in the SB-GD (122 mg) and the local GD (106 mg) group. On average the larval weight was increased 500- and 885-fold from d 5 to d 16 post-hatching with the GD and the CF diets, respectively. The CO_2 production over 7 d was highest with the local GD (90.7 ml / mg), followed by the SB-GD (68.1 ml / mg), the local CF (42.3 ml / mg) and the SB-CF diet (34.1 ml / mg). The CO_2 production did not differ among the two CF diets. A similar pattern was found for the O_2 consumption with highest values in the local GD group (158.5 ml / mg) followed by the SB-GD (123.3 ml / mg), the local CF (75.8 ml / mg) and the SB-CF group (64.7 ml / mg). The NH_3 production was highest in the SB-GD group (1.2 ml / mg), which differed from the local CF (0.5 ml / mg), but not from the local GD (1.0 ml / mg) and the SB-CF dietary group (0.7 ml / mg).

Conclusions: Highest values for larval CO_2 production and O_2 consumption were found in the groups grown on the less nutrient dense GD diets compared to the CF diets. The CO_2 production and O_2 consumption differed between both GD groups, which reflect differences in the dietary nutrient and energy contents. However, in spite of differences in their nutrient composition this was not the case for the two CF diets. Our data allow to compare greenhouse gas emission of BSFL reared on similar but not identical diets.

Bibliographical references

[1] BOSCH G., OONINCX D.G.A.B., JORDAN H.R. ZHANG J., VAN LOON J.J.A., VAN HUIS A., TOMBERLIN J.K. (2020): JIFF 6: 95-109

[2] GASCO L., DERUYTTER D., GLIGORESCU A., YAKTI W., COUDRON C.L., MENEGUZ M., GROSSO F., SHUMO M., FROONINCKX L., NOYENS I., BELLEZZA ODDON S., BIASATO I., SPRANGHERS T., VANDENBERG G., OONINCX D.G.A.B., BOSCH G. (2021): EAAP Book of abstracts no. 27, 72nd Annual Meeting, Davos, Switzerland, 471

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17.

Organic matter digestibility of total mixed rations (TMR) at restricted feed intake in standardised sheep digestibility trials and ad libitum intake in lactating dairy cows

Verdaulichkeit der organischen Masse von Gesamt-Mischrationen (TMR) bei limitierter Futteraufnahme im standardisierten Verdaulichkeitsversuch mit Hammeln und ad libitum-Futteraufnahme bei laktierenden Milchkühen

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Energy concentrations of total mixed rations (TMR) for ruminants can be estimated from standardised digestibility trials with wether sheep or using prediction equations based upon such trials [1]. Standardised digestibility trials are conducted at or slightly above feed intake corresponding to maintenance energy requirements, yet it is known that digestibility is generally reduced with increasing feed intake. In the current German feed evaluation system for dairy cows, this fact is considered via additional energy requirements depending on milk yield [2]. However, future feed evaluation systems may require a direct estimation of the digestibility reduction to transfer results from standardised feed evaluation to ration planning for lactating dairy cows. The current work aimed at quantification of the difference between organic matter digestibility (OMD) estimated from standardised digestibility trials and actual OMD in lactating dairy cows.

Methods: From 2005 to 2016, digestibility trials with 17 TMR were conducted at the Experimental and Educational Centre for Agriculture 'Haus Riswick', Chamber of Agriculture of North Rhine-Westphalia, Kleve, Germany. All TMR were based on grass silage, maize silage and concentrate feed in varying proportions and crude protein concentrations ranged from 148 to 192 g / kg dry matter. Diets were fed to lactating German Holstein cows for ad libitum intake. Individual dry matter intake (DMI) was recorded. Based on individual animal data, mean body weight (BW) was 654 kg, ranging from 534 to 834 kg and mean DMI was 21.7 kg / d (14.8–28.8 kg / d). Each TMR was simultaneously fed to wethers in a standardised digestibility trial (3). Mean DMI for wethers was 970 g / d. Each TMR was fed to 3–4 or 4 animals for cows and wethers, respectively. In both cows and wethers, OMD was measured based on total collection of faeces. To evaluate the effect of feed intake on OMD, feed intake was expressed as feeding level (FL). For wethers, the FL was assumed to be 1. For cows, individual FL was calculated from DMI and BW, with FL=1 defined at DMI of 50 g per $kg^{0.75}$ (metabolic body size). Individual data on OMD were averaged per TMR and animal species. Linear regression of OMD on FL was calculated for each TMR. Slopes of resulting regression equations were tested for correlations with OMD at FL=1 and variables describing ration composition.

Results: In cows, the mean FL of the 17 TMR was 3.4 (2.9–3.9). Mean OMD of the 17 TMR was 80.7% (78.2–83.4%) for wethers and 75.1% (72.4–77.8%) for cows. Linear regressions performed for each TMR revealed slopes ranging from −3.7 to −0.6, with a mean slope of −2.4, describing the %-points decrease in OMD for each unit increase in FL. No correlation was found between slopes of OMD decrease and composition of the diets (both regarding ingredients and chemical composition). Slopes tended (p=0.08) to be correlated (r=−0.43) with OMD measured in wethers, that is, higher OMD at FL=1 causes stronger decreases in OMD with increasing FL.

Conclusions: The current results complement prior observations due to number and variability of tested TMR, inclusion of contemporary dairy cows and particular applicability for TMR typically fed in Western Europe.

Bibliographical references
[1] GfE (2004): Proc. Soc. Nutr. Physiol. 13: 195-198
[2] GfE (2001): Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt am Main, Germany
[3] GfE (1991): J. Anim. Physiol. Anim. Nutr. 65, 229-234

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18.

Endocrine and metabolic changes in dairy cows fed different diets at dry-off

Endokrine und metabolische Veränderungen bei Milchkühen mit unterschiedlichen Rationen zum Trockenstellen

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As nowadays milk yield in dairy cows is relatively high at the time of dry-off, concentrate withdrawal and feed restriction are common procedures to reduce milk production and to consequently facilitate dry-off. However, feed restriction prior to dry-off may lead to metabolic stress and is likely to transiently impair the immune system. The objective of this study was to investigate the effects of a differential supply of nutrients (i.e., carbohydrates, proteins, lipids) and feed restriction before dry-off on metabolic and endocrine adaptation of dairy cows around dry-off.

Methods: Holstein dairy cows (n=43) of similar body weight (BW; 712±11 kg; mean value±SEM) and milk yield (18.0±0.4 kg/d) were studied from d 12 before until d 6 after abrupt dry-off (56 d before expected calving). Cows were supplied with concentrate rich in either crude protein (aminogenic, n=14), glucogenic precursors (glucogenic, n=14), or lipids (lipogenic, n=15) from d 9 until d 4 before dry-off (3 kg/d from d 9 to d 7 before dry-off, 6 kg/d from d 6 before dry-off onwards). Beginning at d 3 before dry-off, feed allowance was restricted to 50% in half of the animals of each dietary group until the end of the study, whereas feed allowance remained unchanged in the other animals serving as controls. Dry matter intake (DMI), BW and milk yield were recorded daily. Daily blood samples were analyzed for concentrations of glucose, non-esterified fatty acids (NEFA), urea, insulin, glucagon, and insulin-like growth factor (IGF)-1. Statistical analysis (SAS, version 9.4) was carried out using a mixed model with dietary group, time, and diet × time interaction as fixed effects and cow as random subject. The Tukey-Kramer post hoc test was used for detection of significances between groups at $P<0.05$.

Results: Energy balance turned negative immediately after the start of feed restriction in all dietary groups, turned positive after dry-off, and was less positive compared to non-restricted cows until d 6 after dry-off ($P<0.05$). During feed restriction, milk yield decreased until dry-off ($P<0.05$). Glucose concentration in plasma was not altered by restriction or diet before dry-off, but increased in all groups after dry-off, and with the exception of the aminogenic group, glucose concentration was greater in non-restricted compared to restricted animals ($P<0.05$). Plasma NEFA concentrations were unaffected by the different dietary types, but increased in all restricted animals, peaked 1 d before dry-off, and remained higher compared to control cows until 3 d after dry-off ($P<0.05$). Plasma urea concentration increased regardless of restriction with increasing amounts of the aminogenic concentrate, and was higher in restricted glucogenic and lipogenic fed animals compared to non-restricted ones before dry-off ($P<0.05$). Insulin and IGF-1 concentrations slightly decreased in all restricted animals before dry-off, but significantly lower insulin concentrations were detected only in restricted cows receiving the lipogenic diet ($P<0.05$). Glucagon concentrations tended to be lower in restricted animals fed the aminogenic and lipogenic diet before dry-off ($P<0.10$), and were lower in all restricted animals after dry-off ($P<0.05$). The insulin:glucagon ratio was not affected by diet or restriction before dry-off, but was increased in all groups after dry-off with the restricted groups showing a lower ratio than the control groups ($P<0.05$).

Conclusions: Feed restriction before dry-off causes a sustained increase of plasma NEFA concentration until the first days after dry-off. Dry-off increases glucose, insulin and IGF-1 concentrations. Dietary type had a minor influence on tested metabolic and endocrine factors, with the exception of urea that increased with higher amounts of the aminogenic concentrate.

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19.

Short-term alterations of metabolic and endocrine parameters in mid lactation dairy cows exposed to dietary nutrient imbalances

Kurzfristige Veränderungen von metabolischen und endokrinen Parametern infolge Nährstoffimbalanzen bei Kühen in der Mitte der Laktation

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Short-term imbalances of dietary nutrients may occur during fluctuations in roughage quality or temporal shortages of supplementary feed components. In contrast to a deficiency, especially dairy cows with a moderate performance, in mid or late lactation, or in extensive farming systems may be exposed to imbalanced rations. The objectives of the present study were to induce transient changes in metabolism and insulin responsiveness through a differential supply of macronutrients by concentrate feeding. Endocrine alterations of the insulin and glucagon system were hypothesized to respond differently to increased levels of aminogenic, glucogenic and lipogenic concentrate.

Methods: Based on parity number, lactational stage, milk yield and body weight (BW), 9 mid lactating cows (211 ± 19 days in milk) were grouped into 3 blocks of 3 animals each. The experiment was carried out as a crossover study. Concentrate supplements (aminogenic: rich in crude protein and nitrogen sources; glucogenic: high content of carbohydrates and glucogenic precursors; lipogenic: high lipid concent) were fed in a factorial arrangement at 3 increasing levels (2.5, 5.0 and 7.5 kg / d) for 3 d each. Each block of cows (3 cows per block) underwent in total 9 experimental periods of 3 d each. The order of feeding the three concentrate types was randomized. A „washout“ phase of 5 d was applied between the changes of the concentrate types. Milk yield, BW and feed intake were recorded daily. Blood and milk were sampled every 3 d at the end of each concentrate level. Milk fat, protein, lactose and urea contents were determined. In blood, concentrations of various metabolites, endocrine factors and enzyme activities (e.g. glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate, urea, cholesterol, triglycerides, insulin, glucagon, ASAT, GGT, GLDH) were measured. Statistical analysis was carried out using SAS (version 9.4). The MIXED procedure was used to analyze data with blocks and block × treatment considered random effects. Within each treatment (aminogenic, glucogenic and lipogenic concentrate), a MIXED model with concentrate level (2.5, 5.0 and 7.5 kg / d) as fixed and cow as random effect was applied to investigate effects increasing supply of the different concentrates on animal performance, milk composition, and metabolic and endocrine factors in blood. Differences between supply levels of concentrates within each type were tested using the Tukey-Kramer procedure adjusting tests for multiple comparisons. Effects were considered significant at $P<0.05$.

Results: Milk yield, milk composition and BW were not affected by diets and concentrate levels ($P>0.05$). Feed intake increased in cows receiving the aminogenic and lipogenic diet ($P<0.05$). Milk und plasma urea concentrations were clearly elevated in the aminogenic and to a lesser extent in the lipogenic treatment. Glucose concentrations in plasma were not affected by treatments, whereas insulin and glucagon increased only in the aminogenic treatment with concomitantly reduced NEFA concentrations ($P<0.05$). Whereas plasma BHB concentration was not affected in cows fed the aminogenic and glucogenic concentrates, cows receiving a high level of lipogenic concentrate (7.5 kg / d) showed the lowest plasma BHB concentration ($P<0.05$). No alterations of the insulin:glucagon ratio were observed with increasing concentrate levels. Activities of ASAT, GGT and GLDH as well as the total antioxidant capacity were not affected by diets ($P>0.05$).

Conclusions: We observed immediate metabolic and endocrine responses due to the short-term dietary changes. Particularly a surplus of nitrogen supply via the aminogenic diet affected metabolic responses and stimulated insulin and glucagon secretion.

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20.

Are there breed-independent relationships between energy availability and fatty acids in hair from Simmental and Holstein cows in early lactation?

Gibt es rasseunabhängige Zusammenhänge zwischen der Energieversorgung und Fettsäuren im Haar von frühlaktierenden Fleckvieh- und Deutsche Holsein-Kühen?

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Previously, we have shown that primiparous cows with a short interval from calving to conception and a high milk protein yield [1] as well as cows with a high energy utilisation in lactation weeks 1 to 6 [2] had higher contents of lauric acid in hair in lactation week 8. Until now, it is unknown if there are relationships between energy availability and the hair fatty acid composition, independent of farm, breed and time point of hair sampling. Therefore, the current study investigates if a breed-independent relationship exists between energy intake, energy requirement for maintenance and milk production, and energy balance and the hair fatty acids across farms, and time points of hair sampling.

Methods: For the study, 121 Simmental (SIM) and 55 German Holstein (HOL) cows from three experimental farms as part of the optiKuh joint project were investigated. The lactation number varied from 1 to 9. Individual feed intake was measured daily. After calving, cows were fed four diets differing in energy concentration of roughage (6.1 or 6.5 MJ NEL / kg DM) and concentrate (150 or 250 g / kg ECM). Energy intake, energy requirement for maintenance and milk production, and energy balance were used as weekly means in lactation weeks 2 to 6. Energy balance was calculated as the difference between energy intake and energy requirements for maintenance and milk production. Hair samples were taken in lactation weeks 4 and 8. Hair lipids were extracted and analyzed from 200 mg cleaned and mill-ground hair using a fatty acid extraction kit [3]. After esterification fatty acid methyl esters were detected via gas chromatography. Within each farm, Pearson's correlation coefficients between parameters of energy availability and fatty acids in hair were calculated. Since hair mirrors the metabolism retrospectively, correlations between the hair fatty acids at 4 and 8 weeks and the corrected parameters of energy availability in lactation weeks 2, and 2 to 6 were examined, respectively. Beforehand, the parameters of energy availability were corrected for feeding group and lactation number using a linear model since these effects influence the parameters of energy availability. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for data analyses.

Results: Twenty-two fatty acids were reliably detected in hair across the three farms. Here, we only mention significant correlations which were consistent across at least two of the examined farms and two time points of hairs sampling. Positive correlations were found between the corrected energy intake normalized for metabolic body weight [MJ NEL / $BW^{0.75}$] in lactation weeks 2 to 6 and the essential fatty acids linoleic acid and α-linolenic acid across all three farms and both time points of hair samplings in lactation weeks 4 and 8. Higher energy intakes normalized for metabolic body weight in week 2 [MJ NEL / $BW^{0.75}$] correlated to higher linoleic acid in hair of lactation week 4 in farm 1 and 2 (both SIM) significantly ($0.34 \leq r \leq 0.37$; $P < 0.05$) and in farm 3 (HOL) in tendency ($r = 0.28$; $P = 0.10$). Higher energy intakes normalized by metabolic body weight in week 4, 5 and 6 [MJ ME / $BW^{0.75}$] were also associated with higher linoleic acid in hair of lactation week 8 in all farms ($0.27 \leq r \leq 0.37$; $P < 0.05$). In farm 1 and 2 (both SIM), α-linolenic acid in weeks 4 and 8 showed similar results to the energy intake normalized for metabolic body weight in lactation weeks 2, 4, 5 and 6 [MJ ME / $BW^{0.75}$], respectively.

Conclusions: In the early lactation, higher levels of energy intake were mirrored in higher levels of linoleic acid and α-linolenic acid in hair 2 to 4 weeks later. Even if the correlation coefficients were moderate, the findings were consistent across all three farms, both breeds and both time points of hair sampling.

Bibliographical references

[1] MÖLLER R., NÜRNBERG K., SCHMITT A.O., BROCKMANN G.A. (2013): J. Anim. Sci., 91: 1669-76

[2] MÖLLER R., DANNENBERGER D., NÜRNBERG G., STRUCKEN E.-M., BROCKMANN G.A. (2019): J. Dairy Res., 86: 77-85

[3] MÖLLER R., NÜRNBERG G., ALBRECHT E., RUTH W., BROCKMANN G.A., DANNENBERGER D. (2019): Eur. J. Lipid Sci. Technol., 121: 1900143

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21.

Effect of different forage proportions in diets of fattening bulls on performance and body composition

Einfluss von unterschiedlichen Grundfutteranteilen in der Ration von Mastbullen auf die Leistung und Körperzusammensetzung

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The current recommendations for energy and nutrient supply are not sufficient. Therefore, the aim of this study was to investigate the performance and body composition (BC) of fattening bulls with varying forage proportions in the diet in order generate data necessary for derivation of energy and nutrient requirement for modern male Holstein genetics.

Methods: Fifty-six fattening bulls of the German Holstein breed were slaughtered at different body weights (mean 494 kg, minimum 218 kg; maximum 787 kg). Eight animals were slaughtered at the start of the experiment and served as an initial group (IG). The remainder animals were divided into two feeding groups. Over the experimental period (forty-three weeks in total) the animals were slaughtered after different weeks distributed over the entire experimental period. One half of the animals received a ration with a high forage proportion (HF, 80% forage on dry matter (DM) basis) and the remainder animals a low forage proportion (LF, 40% forage on DM basis) in the ration. The ration was fed as a total mixed ration and contained corn silage as the forage component and concentrate in both feeding groups. The individual dry matter intake was recorded daily and the animals were weighed once a week. During the slaughter process bull's body parts were assigned to different fractions, which were homogenized, weighted and analyzed for DM, fat, protein and ash content for determination of BC. The empty body weight (EBW) was calculated as the whole body of the bull without claws off cut, ingesta and content of urinary and gall bladder. Performance data were analyzed by using the proc mixed procedure of SAS 9.4. BC data were analysed with linear or non-linear regression models by using the Statistica software version 13. The BC data of the IG were used as starting values for the HF and LF group. In order to describe the accuracy of the estimation, the coefficient of determination (r^2) and the residual standard deviation (RSD) were calculated.

Results: Average daily gain (ADG) increased in the weight ranges 250 - 360 kg, 360 - 480 kg, 480 – 600 kg and was not increased in the weight range from 600 – 760 kg in the LF group. However, values for energy utilization (MJ ME intake / kg ADG) decreased in all weight ranges of the HF groups. Regardless to the group allocation the EBW varied over a range from 162 kg to 633 kg with a mean of 393 kg and a standard deviation of ± 132 kg.The fat content as percentage of EBW varied over a range from 11.6 % to 33.2 % with a mean of 20.2 % and a standard deviation of ± 5.6 %. Body protein content varied over a range from 13.3 % to 18.5 % with a mean of 16.1 % and a standard deviation of ± 1.1 %. The results of the regression analysis of the empty body fat mass suggested that starting from a live weight of 400 kg the body fat mass of the LF group ($r^2 = 0.95$, RSD = 0.08) increased more per kg of live weight gain compared to the HF group ($r^2 = 0.95$, RSD = 0.09). The empty body crude protein mass increased equally in both feeding groups (HF group $r^2 = 0.95$, RSD = 0.23; LF group $r^2 = 0.97$, RSD = 0.30) per kg of live weight gain.

Conclusions: Under conditions of the present experiment the different forage proportions in the ration influenced the performance and body composition of bulls of the German Holstein breed. The body fat content increased in course of the experiment while the body protein content slightly decreased irrespective of feeding group. In the LF groups, an increased body fat accumulation at the end of the experiment lead to a less efficient energy utilization with an unaffected ADG.

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22.

Comparison of long-chain fatty acid contents in the colostrum of sows of different parities

Vergleich der Zusammensetzung von langkettigen Fettsäuren im Kolostrum von Sauen unterschiedlicher Paritäten

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Aside from supplying immunoglobulins, colostrum is the first source of energy and nutrients necessary for optimal development. Especially the supply of colostral fat is crucial to piglets due to their low body fat reserves at birth and as source for essential fatty acids (FA) [1]. The FA are also important precursors for bioactive lipid mediators, which modulate inflammatory signalling in the body. Although evidence exist that the colostral composition of long-chain FA (LCFA) can be influenced by the dietary fat source of the sow [1], little is known about the influence of sow parity on its composition. Therefore, our aim was to improve our knowledge about the effect of sow parity on the FA composition of colostrum. This was based on the hypothesis that body weight fluctuations and associated changes in body fat content with increasing parity may affect the ratio of unsaturated to saturated FA in colostrum of sows.

Methods: In total, 20 colostrum samples were collected from 20 sows (Large White) within 12 hours postpartum. Sows were clustered into three parity groups (PG; PG A: parity 1 and 2; PG B: parity 3 and 4; PG C: parity 5 and 6). Based on the similarity among samples using cluster analysis in R, parity 1 and 2 were classified together. Sows were fed with a commercial diet based on corn, wheat, barley and soybean meal providing 129g protein / kg diet, 51.5g crude fat / kg diet and 14.7 MJ / kg diet (2 meals of 1.2kg). Additionally 150g soaked linseeds / day were fed, providing 33.9g protein , 65.7g crude fat / day and 3.2 MJ per day, and the sows had free access to hay. LCFA were analysed using gas chromatography [2]. Data from single LCFA and grouped LCFA (saturated, unsaturated, n-3 and n-6 FA) were subjected to ANOVA using the Mixed procedure of SAS with the fixed effect of parity group and differences at $P < 0.05$ were considered significant. The pairwise comparisons among least-square means were performed using the Tukey–Kramer post hoc test.

Results: The main LCFA in colostrum were vaccenic acid (C18:1 cis-11; 33%), palmitic acid (C16:0; 26%) and linoleic acid (n-6 C18:2; 21%). In total, saturated FA accounted for 37% and unsaturated FA for 63%; whereby 38 and 25% of the FA were monounsaturated and polyunsaturated FA, respectively. Margaric acid (C17:0) decreased in colostrum with increasing parity by 22% ($P = 0.011$). Arachidic acid (C20:0) increased from PG A to PG B, but it was not detected in the colostrum of sows in PG C ($P = 0.001$). The content of unsaturated LCFA myristoleic (C14:1n-9) and heptadecenoic (C17:1n-8) acid in colostum decreased from PG A to PG B by 86 and 54%, respectively, while their content increased by 468 and 104%, respectively, in sows in PG C compared to PG B ($P < 0.05$). However, levels of myristoleic (C14:1n-9) and heptadecenoic (C17:1n-8) acid still remained lower in PG C compared to PG1 ($P < 0.05$). The a-linolenic acid (C18:3n-3) increased by 5% from PG A to PG B but it decreased by 45% in PG C compared to PG B ($P = 0.045$). This change of a-linoleic acid (C18:3n-3) was in parallel to the change in overall n-3 LCFA in colostrum, which increased by 4% from PG A to PG B and decreased by 37% from PG B to PG C.

Conclusions: Results showed that parity influences the contribution of n-3 LCFA, mainly a-linolenic acid (C18:3n-3), to the FA profile in colostrum of Large White sows, having similar levels between parity 1 to 4 (PG A and B) and decreasing greatly afterwards (PG C). Due to their anti-inflammatory effects in the intestinal tract and body as well as their essentiality for neonatal brain development, sows in parity 5 and higher should receive additional dietary n-3 LCFA supplementation to ensure adequate nutrition of newborn piglets with essential FA.

Bibliographical references

[1] METZLER-ZEBELI B.U. (2021) Animals 11:2781. https://doi.org/10.3390/ani11102781

[2] METZLER-ZEBELI B., KLINSODA J., VÖTTERL J., SHARMA S., KOGER S., SENER-AYDEMIR, A. (2021) J. Anim. Sci. 99:1-12.

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23.

Influences of glyphosate residues and different concentrate feed proportions in dairy cow rations during early gestation on offsprings' energy-metabolism and leukocytes' gene expression

Einflüsse von Glyphosatrückständen und variierenden Kraftfutteranteilen in Rationen von trächtigen Milchkühen auf Energiemetabolismus und Leukozytengenexpression der Nachkommen

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Since livestock is orally exposed to glyphosate (GLY), an active agent in non-selective herbicides, possible teratogenic and other adverse effects of GLY on maternally exposed offspring were discussed (1). Therefore, the present study investigated putative GLY effects of GLY-containing feed due to a pre-harvest treatment of plants according to German law in 2014. GLY-containing feedstuffs were fed to dairy cows during early gestation while energy-metabolism and leukocytes' gene expression of their offspring were analyzed.

Methods: In the present study, 39 lactating dairy cows originating from a feeding trial recently described (2) were fed low (30%, LC) or high (60%, HC) concentrate feed proportions (CFP) with (GLYLC, GLYHC) or without GLY-contaminations (CONLC, CONHC) in the rations for 16 weeks during early gestation (59±4 days at the beginning of GLY exposure; mean±standard error). After calving, blood samples of 38 newborn calves were collected before colostrum intake (5-345 min after birth) and serum was analyzed for energy-metabolism related clinical-chemical traits. Furthermore, expression of 10 genes of interest in blood leukocytes was analyzed by quantitative real-time PCR. Calves were clinically examined and weighed within 24h after birth. Depending on distribution of residues (SAS v9.4; proc univariate), statistical analyses (proc mixed) were conducted on untransformed or transformed (proc transreg or proc rank) data. Gene expression data were log10 transformed after normalization against three reference genes taking primer specific efficiencies into consideration.

Results: During the feeding trial, mean daily GLY exposures of dams were 1.2 (CONLC, CONHC), 112.5 (GLYLC) and 130.3 µg/kg body weight/d (GLYHC). The mean depletion period (time between termination of experimental feeding and parturition) amounted to 107±4 days, while gestation length of dams was not influenced by dietary treatment (GLY, CFP) of dams. Of 41 calves including two twin parturitions, 38 were clinically inconspicuous at parturition, while three calves were born dead (two from twin parturition; 1x CONLC, 2x GLYHC). Statistical analyses did not indicate an influence of dietary treatments of dams (GLY, CFP) on birth weight, blood glucose, cholesterol and β-hydroxybutyrate levels in calves. Lower levels of blood non-esterified fatty acids (NEFA) in calves of GLY groups compared to those of CON groups ($p_{GLY}<0.05$) were detected and likely resulted from coincidentally shorter timeframes between birth and sample collection in GLY groups than in CON groups. Strongly time-dependent responses of NEFA levels until colostrum intake were observed within the first 105 minutes after birth (Spearman rank correlation $R=0.76$, $p<0.001$). Likewise, triglycerides (TG) showed a time-dependent pattern ($R=0.7$, $p<0.001$) irrespective of treatment of the dams. In the calves, leukocytes' gene expression of NEFA-related genes such as free fatty acid receptors, e.g. FFAR2, genes downstream of NEFA-related receptors in signaling pathways, e.g. NFκB inhibitor alpha (NFκBIA), or genes related to oxidative status, e.g. superoxide dismutase 2 (SOD2), were not influenced by treatment of dams (GLY, CFP) during gestation. However, for Toll-like receptor 4 (TLR4) highest expression was observed in CONHC followed by GLYHC, GLYLC and CONLC (pCFP=0.035). Contrary to our expectations, no significant correlation between TLR4 expression and NEFA levels occurred.

Conclusions: Under applied experimental conditions, no teratogenic or other clear effects of GLY or CFP on energy-metabolism-related clinical-chemical traits and NEFA-related gene expression in leukocytes of newborn calves maternally exposed to GLY and varying CFP for 16 weeks during gestation were observed. In contrast, time between birth and colostrum intake had a strong influence on blood NEFA and TG levels in calves.

Bibliographical references

[1] KRÜGER M., SCHRÖDL W., PEDERSEN L.B., SHEHATA A.A. (2014): J. Environ. Anal. Toxicol. 4: 230

[2] SCHNABEL K., SCHMITZ R., VON SOOSTEN D., FRAHM J., KERSTEN S., MEYER U., BREVES G., HACKENBERG R., SPITZKE M., DÄNICKE S. (2017): Arch. Anim. Nutr. 71(6):413-427

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24.

Chronic exposure of dairy cows to pyrrolizidine alkaloids: Effects on health and performance

Chronische Exposition von Milchkühen gegenüber Pyrrolizidinalkaloiden: Auswirkungen auf Gesundheit und Leistung

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The increasing spread of ragwort *(Jacobaea spp., syn. Senecio)* is cause for concern. Ragwort, like tansy ragwort *(Jacobaea vulgaris)* or marsh ragwort *(Jacobaea aquatica)*, contains hepatotoxic and potentially carcinogenic pyrrolizidine alkaloids and their corresponding N-oxides [2] (collectively termed PA). Grazing animals avoid ragwort when pasture management is adequate. Preserved feed, such as silage and hay prepared from ragwort containing grassland might, however, pose a risk of PA exposure [3]. Literature regarding PA exposure critical for the health of dairy cows is lacking and earlier dose-response studies were mostly performed using not always clearly defined plant material [1, 3]. Therefore, the aim of the present experiment was to identify critical PA doses in a chronic scenario by using a well-defined PA-extract.

Methods: A total of 20 lactating Holstein cows (body weight (BW) 649 ± 51 kg, 2. lactation, days in milk 170 ± 30) were assigned to 5 groups (n=4) receiving a daily oral administration of an extract of Jacobaea vulgaris divided into 3 different PA doses (PA1: 0.47 mg PA / kg BW / day (d); PA2: 0.95 mg PA / kg BW / d; PA3: 1.91 mg PA / kg BW / d), water (CONWater) in equal quantities, or molasses (CONMolasses) to account for the sugar content of the PA-extract in a similar exposure scenario. The PA-content of the extract was characterised by HPLC-MS. Feed (Total mixed ration: 30% both maize and grass silage, 40% concentrate on dry matter (DM) basis) and water were offered ad libitum at computer-controlled self-feeding stations (Insentec, B.V., Marknesse, The Netherlands) for individual recording of feed intake. Cows were milked twice a day. Milk yield was determined automatically (Lemmer Fullwood GmbH, Lohmar, Germany), as well as BW. A detailed clinical examination was conducted weekly. The experiment started with one blood sample taken immediately before the first oral administration of the test substances (water, molasses, PA-extract). The administration was afterwards performed daily after the morning milking in the reticulo-rumen using a stomach tube. During the first 24 hours blood samples were taken frequently (after 0.5 hours (h), 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h, 24 h). Starting from the second day, blood samples were taken 90 min after supplementation (d 1, 2, 3, 5, 7, 10, 14, 21, 28 after the first administration of PA or from control) and subsequently prepared for serum and analysed for liver-related parameters such as albumin, bilirubin, urea, alkaline phosphatase creatinine, gamma-glutamyl transferase (GGT), total protein, alanine aminotransferase aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) using an automated system (IndikoTM Plus, Thermo Fisher Scientific, Hennigsdorf, Germany). All data were statistically analysed, either untransformed or transformed to take non-normally distributed model residues into account, using the MIXED procedure for repeated measurements of the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results: None of the PA doses resulted in an impairment of performance parameters. DM intake, milk yield and BW remained inconspicuous in all groups. Inspection of sclerae and vulva, as well as unaffected bilirubin values, showed no signs of hyperbilirubinaemia. Nevertheless, the clinical chemical parameters GGT and GLDH showed a significant increase in group PA3. The significant group x day (g*d) interactions detected for GGT ($p_{g*d} = 0.011$), GLDH ($p_{g*d} = 0.033$) and AST ($p_{g*d} < 0.001$) resulted from a time-dependent increase at higher dosages and may hint at chronic hepatotoxic effects of PA.

Conclusions: Based on the parameters evaluated so far, daily dosages of 0.95 to 1.91 mg PA / kg BW / d appear to be critical for lactating dairy cows with regard to liver integrity in a chronic scenario for 28 days. The relevance for practical feeding (pasture or conserves) needs to be evaluated based on PA occurrence data in feed and on pasture management practices.

Bibliographical references

[1] DICKINSON J.O., COOKE M.P., KING R.R., MOHAMED P.A. (1976): J. Am. Vet. Med. Assoc. 169(11):1192-1196

[2] EFSA (2016): EFSA Journal 14(8): e04572. doi:10.2903 / j.efsa.2016.4572

[3] HOOGENBOOM L.A.P., MULDER P.P.J., ZEILMAKER M.J., VAN DEN TOP H.J., REMMELINK G.J., BRANDON E.F.A., VAN EGMOND H.P. (2011): Food Additives & Contaminants, Part A 28(3):359-372

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25.

Investigations on the transfer of non-dioxin like polychlorinated biphenyls from feed into meat and liver of pigs

Untersuchungen zum Transfer von nicht-dioxinähnlichen polychlorierten Biphenylen aus dem Futter in das Fleisch und die Leber von Schweinen

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Non-dioxin-like polychlorinated biphenyls (ndl-PCBs) are ubiquitous environmental pollutants that pose a risk to humans and animals through their accumulation in fatty tissues. Due to their persistence and environmental transport, they may enter the food chain. So far, little data is available on the transfer behavior of individual ndl-PCB congeners. Therefore, we conducted a feeding study with fattening pigs using feed with known indicator ndl-PCB concentrations (PCBs 28, 52, 101, 138, 153 and 180).

Methods: A total of 15 pigs aged 8 weeks were divided into three groups and kept for a period of 12 weeks. During a 1-week adaptation period, all animals were fed a commercial compound diet with a very low background ndl-PCB concentration (0.04 +/- 0.004 µg/kg ndl-PCBs; 88 %DM) expressed as the sum of the 6 indicator ndl-PCBs (sum ind). The control group (A) consisted of 6 animals that received the commercial compound diet throughout the entire period. At the beginning and at the end of the experimental period, 3 animals of the control group were slaughtered to assess the background ndl-PCB level in muscle fat and liver. Group B, consisting of 6 animals, received a diet contaminated with ndl-PCBs (11.67 +/- 0.03 µg/kg sum ind. ndl-PCBs; 88% DM) for 21 days immediately after the adaptation period. At the end of this sub-period, 3 animals were slaughtered to determine the ndl-PCB content in muscle and liver. The remaining animals in this group were then again fed the commercial compound diet. Group C, consisting of 3 animals, received the commercial compound diet until 7 days before the end of the experimental period and was then switched to the diet contaminated with ndl-PCBs. At the end of the experimental period, all remaining animals were slaughtered and samples of muscle and liver were analyzed. The ndl-PCB concentration was analyzed by gas chromatography and high-resolution mass spectrometry and expressed for feed as µg per kg (88% DM), for muscle in ng/g fat and for liver in ng/g fresh weight.

Results: After only 21 days of feeding a contaminated diet, the ndl-PCB concentration in muscle in group B reached between 58.06 and 68.85 ng ndl-PCB/g fat, which exceeds the current maximum level of 40 ng ndl-PCB/g fat (1), even though the maximum levels for feed materials of plant origin of 10 µg ndl-PCB/kg (88% DM) (2) were only slightly exceeded in the contaminated diet. The concentrations of ndl-PCB decreased again as expected after feeding with the commercial compound diet and reached levels below the maximum level at the end of the experimental period for both groups B and C. The concentration of indicator ndl-PCBs in the liver after 21 days of feeding with the contaminated diet was 11.67 ng/g fresh weight, which exceeds the current maximum level of 3 ng/g ndl-PCB fresh weight (3). For the liver, even the short contaminated feeding at the end in group C was sufficient to exceed the current maximum level. The congener-specific analysis shows that the ndl-PCBs can be subdivided into two groups of congeners with similar kinetic properties: PCB 52 and PCB 101 as fast-eliminated congeners and PCB 28, PCB 138, PCB 153 and PCB 180 as slow-eliminated congeners, showing a similar pattern as laying hens and fattening chickens.

Conclusions: In summary, it can be shown that even after a short contaminated feeding period with a diet containing ndl-PCB only slightly above the maximum level, the concentrations of ndl-PCBs increase rapidly in both muscle and liver. The individual ndl-PCB congeners show different kinetic properties allowing the development of a congener-specific predictive toxicokinetic model.

Bibliographical references

[1] Commission Regulation (EU) No 1259/2011of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. Official Journal of the European Union L 320, 1-15

[2] Commission Regulation (EU) No 277/2012 of 28 March 2012 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls. Official Journal of the European Union L 91, 1-9

[3] Commission Regulation (EU) No 1067/2013 of 30 October 2013 amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminants dioxins, dioxin-like PCBs and non-dioxin-like PCBs in liver of terrestrial animals. Official Journal of the European Union, L 289/56–L 289/57

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26.

Investigations on the consumption of peat and disinfectant powder by fattening pigs and a possible transfer of therein contained toxic metals and trace elements into food of animal origin

Untersuchungen zur Aufnahme von Torf und Einstreupulver durch Mastschweine und eines möglichen Transfers darin enthaltener toxischer Metalle und Spurenelemente in Lebensmittel tierischer Herkunft

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European standards for animal housing include appropriate enrichment and bedding materials for pigs to perform species specific exploratory behavior [1]. As these exploration activities include biting and chewing, pigs consume bedding materials up to 14% of their daily ration [2]. A recent study could show considerable amounts of toxic metals and trace elements in bedding and enrichment materials and disinfectant powders for pigs [3]. Considering a transfer of these substances into the food chain, the materials might pose a risk for animal health and food safety. However, a risk assessment requires knowledge about the actual material intake by the animals. To quantify material intake by pigs, we investigated the suitability of toxic metals in provided materials and animal tissues as main effect variable. Based on the accumulation of arsenic, lead and cadmium in tissues of pigs fed a known material quantity, material intake of pigs with free access to the material was estimated.

Methods: A total of 28 male, castrated pigs (Yorkshire x Danish Landrace; 24.0 ± 1.83 kg) were used to investigate voluntary uptake of peat and disinfectant powder by pigs, respectively. These materials contained considerable amounts of arsenic, lead and cadmium. Pigs were divided into seven groups (n=4) receiving different material treatments (e.g. control, inclusion of material in the diet or material for voluntary intake): the negative control group received no material with the diet nor presented for voluntary intake; positive control groups received a diet with 10% and 20% peat, respectively, or a diet with 3% and 5% disinfectant powder, respectively; two test groups received a regular diet and peat or disinfectant powder were presented in a tub for voluntary uptake, respectively. Additionally, 5 g titanium dioxide / kg was added to peat and disinfectant powder presented in the tub as an additional indicator of consumption. The entire trial lasted 12 weeks. Pigs were slaughtered at a final body weight of 102 ± 8.97 kg and liver, kidney, muscle tissue and metacarpus IV sampled and stored at - 20°C until analysis of elements. The content of 11 elements (As, Cd, Pb, Hg, Fe, Cu, Zn, Ni, Cr, Mn, Se) in the respective sample material was determined by inductively coupled plasma mass-spectrometry. To assess the voluntary material intake of animals in the test groups, univariate linear regression analysis was performed. The relationship of material intake in control groups as predictive value of tissue element concentration was used to formulate the linear regression equation, which was then used to calculate assumed voluntary material intake based on the given organ element concentration in test groups.

Results: Feed intake was not affected by peat and disinfectant powder added to the diet in the positive control groups. Analyses of titanium dioxide in pig faeces of test groups clearly showed voluntary intake of peat and disinfectant powder presented in tubs. Furthermore, content of toxic elements in tissues of pigs receiving material treatment were higher than in tissues of pigs receiving no material (e.g. negative control). Based on the element concentration in liver and kidney of pigs, data point to a voluntary consumption of up to 12% peat and up to 3% for disinfectant powder of the daily ration, respectively.

Conclusions: The study demonstrated that pigs voluntary consume considerable amounts of provided peat and disinfectant powder and these materials contribute to the daily ration. A transfer of toxic metals and trace elements into animal tissues and thus the food chain occurs. Due to material consumption, element content in animal tissues might exceed European maximum levels for foodstuff, labelling guidelines for these materials can be a tool to limit the entry of toxic metals and trace elements from peat and disinfectant powder into the environment.

Bibliographical references

[1] EU (2008): OJEU L47:5-13

[2] VAN BARNEVELD R. (2012): Australian Pork® https://australianpork.infoservices.com.au/items/2005-2012-REPORT, accessed 11 June 2021

[3] KOCH F., KOWALCZYK J., WAGNER B., KLEVENHUSEN F., SCHENKEL H., LAHRSSEN-WIEDERHOLT M., PIEPER R. (2021) Animal 15: 100319

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27.

Investigations on a transfer of Hypogylcin A into milk using the isolated perfused bovine udder

Untersuchung eines Transfers von Hypoglycin A in die Milch unter Verwendung des isolierten perfundierten Rindereuters

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Hypoglycin A (HGA) is a secondary plant constituent produced by some members of the "soapberry" family Sapindaceae. Oral ingestion triggers various forms of intoxication, such as the so called "Jamaican vomiting sickness" and hypoglycemia in humans. HGA intoxications also occur in horses after ingestion of seeds and seedlings of the sycamore maple tree (Acer pseudoplatanus) (1). Clinical manifestations, also known as atypical myopathy or seasonal pasture myopathy have been increasingly reported in the past years (1). Clinical effects in cows are not known yet. However, HGA levels were recently detected in raw milk samples likely due to uptake of sycamore seeds or seedlings by the cows on the pasture (2). To investigate a possible transfer we used the isolated perfused bovine udder as an ex-vivo-model (3).

Methods: Five udders from slaughtered healthy dairy cows were used. The cows were selected according to defined criteria (lactating, somatic cell count/mL milk <150 000, multipar, Holstein-Friesian). Only one udder half was used for the study at a time. Right after each slaughter and bleeding, the udders were removed from the animal's body. For arterial supply, the Arteria pudenda externa dexter and sinister were catheterized and immediately flushed with Tyrode solution (7.99 g/L NaCl, 1.09 g/L $C_6H_{12}O_6•H_2O$, 1 g/L $NaHCO_3$, 0.2 g/L KCl, 0.065 g/L $NaH_2PO•2H_2O$, 0.21 g/L $MgC_{l2}•6H_2O$, 0.26 g/L $CaC_{l2}•2H_2O$) to avoid clot formation. The venous drainage was accomplished via the Vena epigastrica cranialis superficialis. The udder was fixed in a designated rack connected to the system and perfused with the Tyrode at a flux rate of 100 to 120 mL/min. The Tyrode solution was constantly tempered at 38.5°C and gased with carbogen (95% O_2, 5% CO_2). To check the vitality of the udders, glucose consumption was monitored hourly in venous outflow. Marbofloxacin (2.6 µg/mL) was added to the Tyrode solution as a positive control for blood to milk transfer. After an adaptation period of 30 minutes HGAsolution (1 µmol HGA/L Tyrode) was added to the Tyrode solution (Udder A + B, n=2). Due to measurements near the limit of detection the remaining udders (Udder C, D, E; n=3) were infused with Tyrode solution spiked with sycamore maple extract (9.3 µmol HGA/L Tyrode). Samples of the milk-like secretion, the perfused solution and the venous outflow were taken at 0, 5, 30, 60, 120 and 180 minutes and stored at -20°C until analysis. Samples were analyzed for HGA content using a LC/MS-MS method. The analysis of milk components was performed by infrared spectroscopy using MilcoSan. The D-glucose HK assay kit from Megazyme was used to quantify glucose consumptions.

Results: In each perfused udder, glucose consumption exceeded 0.6 g/h, confirming vitality of the udders. Marbofloxacin was detected in the milk with a mean value of 305.48 ± 5.75 ng/mL after 180 minutes. The milk samples had an average fat content of 0.7 ± 0.3%, protein content of 3.6 ± 0.2% and lactose content of 4.6 ± 0.3%. The concentration of HGA in milk for udder A and B was near or below detection limit. However, when using the maple extract (Udder C, D and E), levels of HGA in artificial milk increased over time and a transfer could be demonstrated. Nevertheless, the concentration in milk varied between the different udders for yet unknown reasons.

Conclusions: The study demonstrated that a transfer of HGA into the bovine milk is generally possible. The use of an alternative method to animal experiments provided a first insight into the HGA's transfer behavior. Despite the confirmation of the vitality of the udders and the detection of marbofloxacin in the artificial milk, the model shows limitations regarding possible metabolizations of HGA in ruminants, such as degradation mechanisms by the rumen microbes, which also occur with other secondary plant constituents. Further data are required to quantitatively evaluate the transfer and biotransformation of HGA.

Bibliographical references

[1] BOCHNIA M., ZIEGLER J., SANDER J., UHLIG A., SCHAEFER S., VOLLSTEDT D., GLATTER M., ABEL S., RECKNAGEL S., SCHUSSER G.F., WENSCH-DORENDORF M., ZEYNER A. (2015): PLoS One 10:9

[2] BOCHNIA M., ZIEGLER J., GLATTER M., ZEYNER A. (2021): Toxins (Basel). 13

[3] KIETZMANN M., LOESCHER W., ARENS D., MAAS P., LUBACH D. (1993): JPM 30(2): 75-84

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28.

Can the different toxicity of pyrrolizidine alkaloids in cows and pigs be explained by their intestinal permeability?

Kann die unterschiedliche Toxizität von Pyrrolizidinalkaloiden bei Kühen und Schweinen durch deren intestinale Permeabilität erklärt werden?

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Pyrrolizidine alkaloids (PAs) are secondary plant metabolites produced mainly by flowering plants of the families Asteraceae or Boraginaceae. In view of their occurrence in Europe, Senecio species are of particular interest, with senecionine being a representative PA. PAs cause hepatotoxic effects in humans and animals, and contaminated food or feed is the major source of exposure. In terms of susceptibility to PA toxicity, marked differences were observed between species, with comparatively high susceptibility in pigs, whereas lower susceptibility was observed in cattle (1). This can only in part be explained by differences in hepatic metabolCan the different toxicity of pyrrolizidine alkaloids in cows and pigs be explained by their intestinal permeability?ism (2), but it might be also related to other factors such as intestinal permeability and metabolism. In addition, porcine intestinal epithelia may be considered as model tissue for humans based on the similarity in physiology (3). The aim of our study was to determine differences in intestinal metabolism and intestinal permeability for the PA senecionine in cattle and pigs using the Ussing chamber technique.

Methods: Epithelia from four different intestinal regions (duodenum, jejunum, ileum colon) from four healthy pigs and five healthy, multiparous dairy cows (Holstein Friesian) in mid lactation were obtained and mucose stripped off L. muscularis and L. serosa. Epithelia were placed in Ussing chambers (surface 1cm2, buffer solution: pH 7.4, 288 mOsmol / L) and senecionine (final concentration: 15µmol / L) was administered to the mucosal compartment after 15 min equilibration period. Short circuit current and tissue conductance was monitored and samples were collected from the mucosal and serosal sides at different time points (0, 30, 60, 90, 120 min). Concentration of senecionine in each sample was detected using liquid chromatography coupled to mass spectrometry. Total recovery of senecionine was calculated for both species and each intestinal region using the summed concentration of the mucosal and respective serosal side relative to the administered concentration at t0. The flux was calculated as increase in senecionine concentration over time per tissue area (µmol / cm2 / s). Statistical analyses were conducted with one-way ANOVA in SPSS Version 26.0.0.1.

Results: Generally, flux of senecionine was low in both animal species. The data show a slightly increasing flux rate for senecionine from proximal to distal intestinal segments in both species ($p_{pigs} = 0.153$, $p_{cows} = 0.520$) and slightly higher flux rates in intestinal tissues from dairy cows as compared with pigs ($p_{duodenum} = 0.004$, Jejunum $p_{jejunum} = 0.021$, $p_{ileum} = 0.025$, $p_{colon} = 0.551$). The data furthermore suggest an apparent intestinal metabolism of senecionine in pigs, whereas almost no apparent metabolism could be observed in tissues from dairy cows. This trend was observed in all intestinal regions studied.

Conclusions: Measurement of permeability to senecionine in the intestine tissue of cattle and pigs revealed that the differences in susceptibility cannot be explained by a higher bioavailability due to a higher passage through the gut barrier.

Bibliographical references

[1] PETZINGER E. (2011): Tierärztl Prax; 39 (G): 363-372

[2] KOLREP F., NUMATA J., KNEUER C., PREISS-WEIGERT A., LAHRSSEN-WIEDERHOLT M., SCHRENK D., THESE A. (2018): Arch. Toxicol.; 92(3):1089-1097

[3] ROURA E., KOOPMANS J.S., LALLÈS J.-P., LE HUEROU-LURON I., DE JAGER N., SCHUURMAN T., VAL-LAILLET D. (2016): Nutr. Res. Rev. 29: 60-90

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29.

Influence of the mycotoxin Ochratoxin A on growth of juvenile carps *(Cyprinus carpio L.)*

Einfluss des Mykotoxins Ochratoxin A auf das Wachstum juveniler Karpfen (Cyprinus carpio L.)

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The mycotoxin ochratoxin A (OTA) has nephro-, hepato- and neurotoxic properties as well as mutagenic, teratogenic, cancerogenic, and immunosuppressive effects in vertebrates. Omnivorous fish, such as carp, which is one of the most widely used fish species in aquaculture [1], are especially exposed to the risk of being fed OTA-contaminated grain. We investigated possible effects of OTA on juvenile carps kept in aquaculture. In a first trial tissue retention of OTA was monitored after chronic intake of OTA over a period of 4 weeks. The second trial aimed to determine possible effects of chronic feeding of OTA (8 weeks) on growth performance and selected health parameters in growing carps.

Methods: Juvenile common carps *(Cyprinus carpio)* derived from Kortmann GbR Hohenweststedt, Germany (trial 1) and Thünen-Institute, Bremerhaven, Germany (trial 2) were fed at an amount of 2% of body weight with iso-energetic and isonitrogenous diets (19.56 MJ kg^{-1} and 373.2 g XP protein kg^{-1}; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) containing 0, 0.5 and 1.0 mg OTA kg^{-1} feed. As a source of OTA, wheat inoculated with Aspergillus sp. was used. Both experiments were performed in quadruplicate tanks with 24 (trial 1) and 25 (trial 2) fish, respectively. In trial 1 fish were fed once daily (16:00) and sampled (2 fish per tank) before and at days 1, 3, 7, 14, 21, and 28 of the feeding period. Serum, hepatopancreas, and kidney were completely taken, lyophilized and analysed for OTA by HPLC using a fluorescence detection method [2]. For the second trial, the fish were fed twice daily (9:00 and 16:00) and blood samples and blood smears were taken before and after 4 and 8 weeks. Leukocyte counts as well as percentage of monocytes, heterophiles and lymphocytes and erythrocyte counts were determined by microscopic counting (SYNLAB Holding, GmbH, Germany). Serum was analysed photometrically for various biochemical parameters (total protein, albumin, globulins, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bile acids, cholesterol, creatine kinase, globulines, glucose, calcium, phosphate, potassium, triglycerides, urea, and uric acid). To estimate growth performance, fish were weighed in 2-week intervals. In addition, 2 fish per tank were sampled for analysis of body composition (crude protein, crude fat, crude ash) as well as energy content was determined by Weender analysis before and at the end of the second trial. To avoid pseudo replicates, the data of fish per tank were pooled. Statistical evaluation of data was done by the statistical software R [3] based on a mixed model including dose and time, as well as their interaction as fixed factors. Following an analysis of variances (ANOVA) group means were compared by Tukey‘s multiple comparison test. Differences were considered as significant with $p < 0.05$. Values are given as means ± SD.

Results: No OTA or OTα was found in serum or tissues investigated. Haematological and biochemical parameters were not different between dietary treatments. Weight gain, feed conversion ratio (1.16 ± 0.02 dry matter per g weight gain), specific growth rate (1.51 ± 0.02 % weight gain day^{-1}), and body composition were also not different between groups. Protein efficiency ratio and the productive protein value were calculated as 2.00 ± 0.19 weight gain per g protein fed and 32.41 ± 2.13%, respectively.

Conclusions: At least at OTA doses of up to 1 mg kg^{-1} feed, growing carps do not accumulate OTA within the body. Moreover, OTA at doses used in our trials does not have a negative effect on growth performance, body composition and health of carps after 8 weeks of chronic OTA intake. Thus, common carp appear to better tolerate dietary OTA compared to most other vertebrates, presumably due to specific conditions within the gastrointestinal tract affecting OTA uptake.

Bibliographical references

[1] FAO – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (2020): The State of World Fisheries and Aquaculture 2020
[2] ABBAS Z., BLANK R., WEIN S., WOLFFRAM S. (2013): Food Addit. Contam. Part A: Chem. Anal. Control Expo Risk Assess. 30(5): 861-866
[3] R CORE TEAM (2020): R: A Language and Environment for Statistical Computing

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30.

Ensiling effects on cannabinoid contents in hemp

Siliereffekte auf den Gehalt an Cannabinoiden in Hanf

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In the EU cultivation of hemp varieties listed in the „Common catalogue of varieties of agricultural plant species“, so-called industrial hemp varieties containing less than 0.2% of the cannabinoid tetrahydrocannabinol (THC) in dried plant material, is allowed. Cannabinoids such as THC or cannabidiol (CBD) appear as inactive acids (THCA, CBDA) or active forms in hemp plants. Although it is known that factors like temperature or UV light lead to activation of the inactive acids, nothing is yet known about feed preservation processes like ensiling on cannabinoid profiles. Such knowledge is pivotal to assess the possible risks for animal health and safety of food of animal origin (1). Thus, we conducted an ensiling experiment with differently treated laboratory scale silages of a whole industrial hemp variety.

Methods: Silages of whole plants of Cannabis sativaL. var. Ivory were prepared in 1.5 L jars in a multifactorial design with five replicates for each treatment combination. Treatments per kg fresh plant material were: 1) untreated control, 2) addition of 10 mL homofermentative lactobacilli at 10^5 cfu / mL, 3) addition of 10 mL homofermentative lactobacilli at 10^5 cfu / mLplus 30 g molasses, and 4) addition of propionic acid (10 mL / kg). Each silage was individually prepared and thoroughly mixed before being filled at similar packing density into a glass jar, sealed and stored at ambient temperature for either 10 or 90 days. After 10 or 90 days weight loss was recorded and samples were taken for analyses of DM content, pH, volatile fatty acids (VFA) and concentration of cannabinoids. The VFA were detected by GC, Cannabinoids were analyzed in dried silage samples (drying at room temperature for 4 days) by LC-MS / MS. Data were analyzed by ANOVA in a multifactorial design considering treatment, storage duration and their interaction as fixed effects, followed by Tukey-Kramer post hoc test using SAS.

Results: DM content was lowest ($P<0.01$) in control silages with 32.4%, while DM of the other treatments averaged 33.9%. Ensiling of the whole hemp plant resulted in unexpectedly high pH-values for control (pH=8.5) and the propionic acid supplemented silages (pH=8.1), with only slightly lower values in lactobacilli inoculated silages (average pH=7.4). Ammonia levels averaged 0.22% in DM in lactobacilli treated silages , which was significantly higher ($P<0.01$) than in control silages (0.20% DM) and silages treated with propionic acid (0.13% DM). Lactic acid was highest ($P<0.05$) in silages inoculated with lactobacilli (3.4% in DM), followed by silages inoculated with lactobacilli and molasses (2.6% in DM). The other silages did not differ from one another with average contents of only 0.6% in DM. All silages were free of butyric acid. Ensiling affected individual cannabinoids differently, but the overall sum decreased from 13.3 g / kg to 10.6 g / kg on day 10 and 10.5 g / kg on day 90 (time effect $P<0.001$) with differences between treatments. The concentration of THC increased from 49 mg / kg in the dried pre-ensiling material to 128 mg / kg on day 90 (time effect $P<0.001$) with highest contents in control silages (157 mg / kg) and lowest contents in lactobacilli inoculated silages (111 mg / kg). On the other hand, tetrahydrocannabinolic acid (THCA) decreased from 320 mg / kg in the pre-ensiling material to 210 mg / kg on day 10 and 129 mg / kg on day 90. Similarly, the content of cannabidiolic acid (CBDA) decreased with time of ensiling from 10‘950 mg / kg in the pre-ensiling material to 6‘212 mg / kg at 90 days, while contents of cannabidiol (CBD) increased from 479 mg / kg in the pre-ensiling material to 2‘150 mg / kg.

Conclusions: Although pH-values were extremely high in hemp silages, contents of lactic acid and VFA indicated good silage qualities. Confirming our hypotheses ensiling increased the contents of the psychoactive THC up to 3-fold, likely by metabolisation of THCA. Accordingly, care must be taken when fermenting feedstuffs containing THCA.

Bibliographical references

[1] WAGNER B., GERLETTI P., SPOLDERS M., BERNSMANN T., FÜRST P., GUSOVIUS H.-J., IDLER C., LAHRSSEN-WIEDERHOLT M., PIEPER R. (2020): Proc. Soc. Nutr. Phys. 29:102

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31.

Cis-Inhibition of Amino Acid Uptake by L-Methionine in the Porcine Small Intestine

Cis-Inhibition der Aminosäureaufnahme durch L-Methionin im porzinen Dünndarm

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Methionine (Met), an essential amino acid (AA), is one of the limiting AA in pigs. The uptake of Met and other AA in the small intestine is highly complex. Most relevant apical AA transporters are the sodium dependent B^0AT1, ASCT2, IMINO and $AT^{B0,+}$ and the sodium independent $b^{0,+}AT$ [1], which all transport Met but do also accept various other AA. To design feed that guarantees good fattening performance, it is essential to understand the intestinal uptake of Met, other essential amino acids and their interactions. Therefore, the aim of the present study was to elucidate a possible functional interaction of luminal L-Met with the uptake of ten selected AA in presence and absence of sodium to conclude on the involved transporters.

Methods: Ten Danbred x Piétrain pigs with an initial age of 12 - 14 weeks received a basal diet conforming to the guidelines of the German Society of Nutrition Physiology (GfE; 18% CP, 10.3 MJ / kg NE), for at least 10 days. Uptakes of 14C-radiolabeled AA (L-Gly, L-Gln, L-Leu, L-Lys, L-Ser, L-Thr, L-Trp, L-Tyr, L-Val) were measured at a concentration of 50 µM in the Ussing chamber over 1 min. Each uptake was performed in presence (135 mM) or absence of mucosal sodium either without or with the co-presence of 5 mM L-Met. Bathing solutions (pH 7,4 ± 0.03, osmolarity 288 ± 5 mosmol / l) were gassed with O_2. Data were compared by two-way ANOVA.

Results: Uptakes of Trp, Val, Leu, Ser, Thr and Gln were significantly inhibited by the mucosal presence of L-Met ($P < 0.05$), whereas Gly and Lys uptakes were not influenced by L-Met presence. Most AA, including Trp, Val, Ser, Thr and Gln showed a significant “sodium x cis-inhibition” interaction effect ($P < 0.05$) or a trend thereof ($P < 0.10$), indicating that L-Met inhibition was only present in sodium-containing buffered solution. In contrast, the cis-inhibition of L-Met on uptakes of Leu and Tyr was not affected by the presence of mucosal sodium. The apical uptake of Gly and Lys were neither affected by L-Met nor by sodium presence in the mucosal buffered solution.

Conclusions: Of the tested AA, only Lys and Gly were not affected by sodium or L-Met presence, suggesting that the absorption pathway of these AA is different to that of L-Met. As Lys is a cationic AA, it is perceivable that it uses another transporter family than Met, e.g., cationic amino acid transporters (CAT) [2]. For Gly, it is known that large portions are absorbed across the intestine via a specific Gly transporter GLYT1 [3]. The other neutral AA tested potentially use the same transport systems as Met. Considering that excessively high concentrations of Met were used in the present study to achieve effective cis-inhibition, it is perceivable that the functional impact of Met on the absorption of these other AA will be much less *in vivo*.

Bibliographical references

[1] MASTROTOTARO L., SPONDER G., SAREMI B., ASCHENBACH J.R. (2016): IUBMB Life. 68 (12): 924-934

[2] FOTIADIS D., KANAI Y., PALACIN M. (2013): Mol. Aspects. Med. 34(2-3): 139-158

[3] HOWARD A., HIRST B.H. (2011): Biol. Pharm. Bull. 34(6): 784-788

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32.

Porcine organoids as an intestinal model for epithelial transport functions and pathogen-epithelial cells interactions

Porcine Organoide als intestinales Modell für physiologische Epithelfunktionen und Epithel-Pathogen-Interaktionen

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Since more than one decade, organoids showing tissue-specific properties grown from stem cells have been established [1]. Whereas most of these experiments have been done in laboratory species, substantial work is needed for respective research in livestock species. Therefore, we have established porcine organoids from the jejunum and colon and cultivated them in a 2D-manner [2]. Our aim was to investigate physiological epithelial transport properties in the small intestine and to gain knowledge on infection mechanisms in the large intestine.

Methods: Organoids were generated by isolating intestinal crypts from the large and small intestine of one pig and cultivation in Matrigel. After one week 3D-organoids, which had formed were further dissected and seeded on Snapwell™ membranes with a pore diameter of 0.4 µm. Transepithelial electrical resistance (TEER) was measured at every medium change and confluent layers of the jejunum were further characterized structurally by immunofluorescence (IF) and immunohistochemical (IHC) staining. Jejunum-based layers were further characterized functionally by Ussing chamber experiments. Colon-derived cell layers were inoculated with isolates of the intestinal pathogens Brachyspira hyodysenteriae or Brachyspira pilosicoli at a MOI of 100. After 2h, 4h and 6h incubation Snapwell™ membranes with 2D colon-derived cell layers were harvested, fixed and pathogen-cell interaction was visualized using scanning electron microscopy.

Results: Monolayers generated from jejunal and colonic organoids developed TEER values of 150 and 340 $\Omega^{*}cm^2$, respectively when used for experiments. These values were further supported by IF and IHC staining of the adherent junction protein e-cadherin and tight junction-associated protein zonula occludens-1. The abundance of different mucosal cell types like absorptive enterocytes, goblet cells as well as enteroendocrine cells could also be demonstrated by IF and IHC. The jejunum-derived 2D layers demonstrated characteristic epithelial transport functions such as glucose absorption and carbachol or forskolin stimulated chloride secretion as detected electrophysiologically after mucosal or serosal glucose or carbachol or forskolin application, respectively [3]. Inoculation of colon-derived cell layers with Brachyspira species showed a typical association between both pathogens and the mucus produced by the goblet cells.

Conclusions: Porcine organoids show physiological properties similar to the native tissues, with regard to cellular composition, transport properties and interaction with porcine typical pathogens. These results promote the use of organoid-based cultures as a successful *in vitro* model to address (patho)physiological questions related to the porcine intestine. This is a promising issue in order to reduce the number of animals used for scientific purposes that is in line with EU directive 2010 / 63 / EU.

Bibliographical references

[1] SATO T., VRIES R.G., SNIPPERT H.J., VAN DE WETERING M., BARKER N., STANGE D.E. (2009): Nature 459(7244):262-5

[2] VAN DER HEE B., LOONEN L.M.P., TAVERNE N., TAVERNE-THIELE J.J., SMIDT H., WELLS J.M. (2018): Stem. Cell Research 28:165-71

[3] HOFFMANN P., SCHNEPEL N., LANGEHEINE M., KÜNNEMANN K., GRASSL G.A., BREHM R. (2021): PLOS ONE 16(8):e0256143

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33.

Effect of phytogenic compounds on serotonin- and acetylcholine-induced contractions in the jejunum of pigs

Effekt phytogener Wirkstoffe auf Serotonin- und Acetylcholin-induzierte Kontraktionen im Jejunum von Schweinen

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The use of phytogenic agents in animal feeding is increasing, but the basic mechanisms of most agents are still largely unknown. Some of the phytogenic agents could mediate relevant effects on animal digestion via the regulation of serotonin-mediated contractions. Activation of TRPA1 in enterochromaffin cells could thereby mediate the secretion of serotonin in the intestine [1]. However, some agents that are also considered as activators of TRPA1 have also been shown to have an inhibitory effect on serotonin receptors [2,3]. Therefore, the aim of this study was to investigate the effect of phytogenic agents on smooth muscle contractions of the jejunum in pigs and the possible implementation of serotonin. The effect on acetylcholine-induced contractions was measured for comparison.

Methods: In organ baths with 80 ml Krebs-Henseleit buffer (37 °C), jejunal tissues from 4 pigs (approx. 2 cm) were connected to isometric force transducers. After equilibration for 30 min, 100 µM menthol, carvacrol, cinnamaldehyde or an equivalent amount of ethanol (control) was added. After an incubation period of 30 min, the concentration was increased to 300 µM for another 30 min. The response to 10 µM serotonin and, after another 15 min, the response to 2.5 µM acetylcholine were tested. After a washout, the response to 2.5 µM acetylcholine was tested again. The frequency, basal muscle tone, as well as the amplitudes or force changes, after addition of the substances were examined during the experiment. Statistics were performed using a two-way ANOVA with repeated measures and comparison against control with Bonferroni correction.

Results: The frequency of contractions was decreased after the addition of 100 µM and 300 µM carvacrol compared to the control ($p < 0.001$), but not in the menthol and cinnamaldehyde groups. Compared to control, basal muscle tone was decreased after the addition of 100 µM carvacrol ($p < 0.001$) and after the addition of 300 µM menthol or carvacrol ($p < 0.01$). The amplitudes of contractions were decreased after the addition of 100 µM carvacrol and after the addition of 300 µM menthol, carvacrol, or cinnamaldehyde compared with the control ($p < 0.001$, each). The increase in force after the addition of serotonin was decreased in the menthol ($p < 0.05$), carvacrol ($p < 0.001$), and cinnamaldehyde group ($p < 0.01$). Acetylcholine-induced increases in contractions were also decreased in the carvacrol ($p < 0.001$) and cinnamaldehyde ($p < 0.01$) group. The addition of acetylcholine after washout of the phytogenic substances elicited contractions in all groups; however, the contraction force was decreased in the cinnamaldehyde group compared to the control ($p < 0.01$).

Conclusions: The phytogenic agents menthol, carvacrol, and cinnamaldehyde inhibit spontaneous jejunal contractions in a concentration-dependent manner. High concentrations of these substances also inhibit serotonin-induced contractions. Acetylcholine-induced contractions were decreased by only carvacrol and cinnamaldehyde, the latter being inhibitory even after washout. This suggests different modes of action for the relaxing properties of the tested substances on intestinal smooth muscles.

Bibliographical references

[1] BELLONO N.W., BAYRER J.R., LEITCH D.B., CASTRO J., ZHANG C., O'DONNELL T.A., BRIERLEY S.M., INGRAHAM H.A., JULIUS D. (2017): Cell 170: 185-198

[2] HEIMES K., HAUK F., VERSPOHL E.J. (2011): Phytother. Res. 25: 702-708

[3] ZIEMBA P.M., SCHREINER B.S.P., FLEGEL C., HERBRECHTER R., STARK T.D., HOFMANN T., HATT H., WERNER M., GISSELMANN G. (2015): Biochem. Biophys. Res. Commun. 467: 1090-1096

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34.

Do turkeys and broiler chickens exhibit differential hepatic metabolite profiles during early development?

Zeigen Puten und Masthühner während der frühen Entwicklung unterschiedliche Lebermetabolitenprofile?

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The liver is the most prominent accessory gland in young poultry and a primary site for energy metabolism and poultry growth and development.Differences in hepatic profiles were shown to vary depending on poultry species (e.g., turkeys and chickens), developmental phase (e.g., growth phase), and nutritional interventions (e.g., phytase supplementation) [1,2]. Although it has been evidenced that changes in liver metabolism are crucial for optimal poultry development [3], this has not been researched sufficiently. This study aimed to identify the hepatic metabolite profile of young turkeys and broiler chickens.

Methods: Briefly, 480 B.U.T.6 turkeys and 480 Ross 308 broiler chicken hatchlings were reared in floor pens on wood shavings and fed diets that met each species‘ feeding recommendations. On d15 and d36, birds were allocated to 48 perforated floor pens. Two-hundred forty birds per species were provided with experimental diets between d15 and d22 of age (sampled at d22) and 240 birds between d36 and d43 of age (sampled at d43). Diets were identical for both species varying uniquely in calcium and phosphorus or phytase (1500 phytase units / kg feed). Metabolomics analyses were conducted from one bird per pen. On sampling days, birds were weighed, anesthetized, and euthanized. The medial section from livers was washed, cut, and collected in 5 ml cryotubes. Then 60 mg liver pieces were homogenized in 99% 2-propanol buffer. The targeted metabolomic profiling MxP® Quant 500 Kit was implemented following the manufacturer‘s recommendations. Statistics and chemometrics analyses were performed using JMP statistical software (SAS Institute) and MetaboAnalyst 5.0 (www.metaboanalyst.ca).

Results: No significant effects of the diets on metabolite profiles were observed. Therefore, stratification into the variables species and age was made. Turkeys and broilers revealed a differential hepatic metabolite profile, and it was also influenced by age. Broiler chickens showed higher concentrations of lipids (mostly triglycerides) (False Discovery Ratio (FDR) < 0.05). Carnitine, propionylcarnitine, and four triglycerides showed an interaction between species and age (FDR) < 0.05). Concerning the small molecules, homoarginine differed between species and along time whereas glycochenodeoxycholic acid, and cis-4-hydroxyproline differed along time in both species (FDR < 0.05). Differences were also observed in the correlation patterns of metabolites toward the average daily gain (ADG) as an indicator of metabolic performance. ADG correlated positively with triglycerides in both species, but only in turkeys; it also correlated significantly with two bile acids (Correlation coefficient > 0.5; FDR < 0.05). Respecting negative correlations, ADG correlated negatively with phosphatidylcholines in broilers while in turkeys it did with acylcarnitines (Correlation coefficient $< - 0.5$; FDR < 0.05).

Conclusions: Species differences were observed, in part, because turkeys and chickens are physiologically different at the same age. In addition, it appeared that liver pathways such as lipolysis and lipid accumulation were higher in broiler chickens at the same age along with early development, probably because broiler chickens developed faster than turkeys due to their higher growth potential. Changes in carnitine, propionylcarnitine, glycochenodeoxycholic acid may indicate increases in fatty acids oxidation and bile flow, respectively; likely in response to the increase of lipid accumulation. Regarding homoarginine, a positive marker of renal health and amino acids transporters activity, it may be possible that broilers activated more mechanisms to regulate metabolic functions given their tense metabolic conditions. Our results indicated that different metabolic statuses were attributed to species, age, and their interaction, suggesting specific interventions in handling should be considered along with the early phase development of poultry.

Bibliographical references

[1] KOUBA M., CATHELINE D., LECLERCQ B. (1992): Br. Poult. Sci. 33:1003-1014

[2] LIU N., RU Y., WANG J., XU T. (2010): Br. J. Nutr. 103:862-868

[3] ALSHAMY Z., RICHARDSON K.C., HARASH G., HÜNIGEN H., RÖHE I., HAFEZ H.M., PLENDL J., AL MASRI S. (2019): PLoS ONE 14: e0226903

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35.

The core microbiome of two high-yielding laying hen strains fed with different levels of calcium and phosphorus

Das Kernmikrobiom von zwei Hochleistungs- Legehennenrassen, die mit unterschiedlichen Calcium- und Phosporgehalten gefüttert wurden

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The needs of nutrients in the diet have to be specific for the breed due to essential functions in bone development and physiology (1). The calcium (Ca) concentration is directly correlated to phosphorus (P) level in laying hens, with the highest requirement during the laying period. Previous studies showed a Ca-related effect on the microbiome composition with special emphasis on ileal and caecal digesta samples disregarding the other sections of the gastrointestinal tract (GIT). Moreover, the impact of the essential supplements Ca and P in different concentration levels is not characterized. We hypothesized that changes in dietary P and Ca supplementation might affect the core microbiome along the GIT by modifying the distribution of bacteria and that these changes are influenced by the bird's genetic background.

Methods: Forty Lohmann-Classic Brown (LB) and 40 Lohmann LSL-Classic (LSL) were fed with adequate levels of all nutrients according to the recommendations of the Gesellschaft für Ernährungsphysiologie (GfE), except for Ca and P. Four different experimental diets were fed: P+Ca+; P+Ca-; P-Ca+; P-Ca-.The dry matter (DM) (available amount of nutrients in feed) for calcium was 39.6 g/kg in Ca+ or 33.9 g/kg in Ca- and for phosphorus 5.3 g/kg in P+ or 4.7 g/kg in P-. A total of 678 samples from digesta and mucosa of the crop, gizzard, duodenum, ileum, and caeca were obtained. Total nucleic acids were extracted using a commercial kit and then subjected to 16S rRNA gene target amplicon sequencing followed by bioinformatic analysis with Mothur (2). PERMANOVA routine was used to study the significant differences and interactions between groups and diets. The core microbiome was identified with the phyloseq & microbiome library in R.

Results: The microbiome composition showed no significant difference between the four diets. A strain-related effect was observed regarding the genera colonizing the GIT ($p<0.05$) and between the gastrointestinal sections ($p<0.05$). Lactobacillus, uncl. Lactobacillales, Megamonas and Bacteroides were more abundant in the LSL and uncl. Lachnospiraceae and Ruminococcus2 were prevalent in the LB strain. Significant differences between digesta and mucosa samples were observed across sections and between strains on genus level ($p<0.05$). Except for ileum samples of the LSL strain, digesta samples were more diverse than mucosa samples in all other sections based on the Shannon index. Digesta samples of gizzard and crop from the LSL strain had more than twice unique amplicon sequence variants (ASV´s) compared to the mucosa samples of the same strain, and the contrary was observed for duodenum and ileum samples. A core microbiome of five species was detected in more than 95% of all samples. The core microbiome is represented by an uncultured Ligilactobacillus (average relative abundance (avra) 12-71%), Megamonas funiformis (avra 7-52%), Ligilactus salivarius (avra 4.5-30%), Lactobacillus helveticus (avra 11- 46%) and an uncultured Fusicatenibacter (avra 1-11%).

Conclusions: Despite the lower values of Ca and P supplementation, no effect was observed in the GIT of the laying hens, however an effect of the breed was detected in the main bacterial groups. In this study, a core microbiome in laying hens was revealed for the first time.

Bibliographical references

[1] SELLE P.H., COWIESON A.J., RAVINDRAN V. (2009): Livest Sci 124: 126–141

[2] KOZICH J.J., WESTCOTT S.L., BAXTER N.T., HIGHLANDER S.K., SCHLOSS P.D. (2013): Appl Environ Microbiol 79(17):5112-20

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36.

Differences in performance and metabolic parameters between early lactation dairy cows with high and low hepatic FGF21 expression

Unterschiede in der Leistung und im Stoffwechsel zwischen frischlaktierenden Milchkühen mit hoher und niedriger hepatischer FGF21 Expression

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Fibroblast growth factor 21 (FGF21) is a liver-derived metabolic regulator being induced in response to multiple stress conditions. A dramatic induction of FGF21 in the liver and a marked increase of FGF21 concentration in plasma has been demonstrated in cows during early lactation suggesting that FGF21 plays a particular physiological role during this phase. While the function of FGF21 has been extensively studied in pathologic rodent models [1], knowledge about the physiologic role of FGF21 in dairy cows is scarce. In order to improve the understanding of FGF21 in dairy cows during early lactation, the present study aimed to explore metabolic differences between high-yielding dairy cows differing in their hepatic expression level of FGF21 during early lactation.

Methods: Liver biopsies and plasma samples of 30 multiparous Holstein cows were taken 1 week postpartum (p.p.). Two groups of cows with high (body weight at wk 1 p.p.: 649 ± 54 kg, parity no: 2.8 ± 1.2) and low (body weight at 1 wk p.p.: 615 ± 35 kg, average no: 2.3 ± 0.5) hepatic FGF21 expression (n = 8 / group) were selected based on their FGF21 mRNA level that was 4.23-fold higher in the group with high than in that with low hepatic FGF21 expression. Metabolic differences between the two groups were analyzed using liver transcriptomics, targeted plasma metabolomics and supplemental analysis of inflammatory and oxidative stress-related parameters. Data were statistically analyzed by Student‘s t-test using Minitab statistical software.

Results: Dry matter intake, net energy intake, energy balance, milk yield and energy-corrected milk yield did not differ between cows with high and low hepatic FGF21 expression. Plasma concentrations of NEFA, BHBA, TAG and cholesterol and hepatic concentrations of TAG and cholesterol were not different between groups. Liver transcriptomics revealed a total of 410 differentially expressed transcripts between groups. Bioinformatics analysis showed that significantly enriched biological processes of the upregulated transcripts included intrinsic apoptotic signalling pathway in response to endoplasmic reticulum (ER) stress, response to nutrient levels and positive regulation of proteasomal ubiquitin-dependent protein catabolic process ($P < 0.05$), whereas cellular amino acid catabolic process was amongst the most enriched biological processes of the downregulated transcripts. While screening of transcriptomics data for genes involved in fatty acid oxidation, ketogenesis and TCA cycle revealed almost no differentially expressed genes, screening for genes involved in inflammation, ER stress and Nuclear factor E2 related factor-2 (Nrf2)-dependent cytoprotection revealed 14 genes to be upregulated in the group with high compared with low hepatic FGF21 expression ($P < 0.05$). Targeted plasma metabolomics of > 200 metabolites revealed only seven plasma metabolites being different between groups ($P < 0.05$). The concentrations of important antioxidants (tocopherols, β-carotene, total glutathione) in liver and / or plasma and plasma concentrations of trolox equivalent antioxidant capacity, oxidative stress-related parameters (thiobarbituric acid-reactive substances, proteincarbonyles) and most acute-phase proteins did not differ between groups.

Conclusions: Despite clear indications of an induction of ER stress-dependent genes involved in ER stress in the liver of cows with high hepatic FGF21 expression and the known link between ER stress and oxidative stress and inflammation, the present data suggest that cows with high hepatic FGF21 expression at 1 wk p.p. do not exhibit a systemic inflammatory process and oxidative stress – likely, as a consequence of induction of the cytoprotective Nrf2-pathway. This would suggest that FGF21 plays an important role in the adaptation to cellular stress conditions in dairy cows during early lactation.

Bibliographical references

[1] EDER K., GESSNER D.K., RINGSEIS R. (2021): J. Anim. Sci. Biotechnol. 12:97

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37.

Bile acids in serum and subcutaneous adipose tissue of dairy cows with high versus normal body condition

Gallensäuren im Blut und im subkutanen Fettgewebe bei über- und normal-konditionierten Milchkühen

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Bile acids (BA) solubilize dietary fat to facilitate fat digestion. Being recognized as hormone-like signaling molecules, BA have been determined in several tissues, including adipose tissue (AT) [1], mediating their function via specific receptors [2]. Alterations in BA concentrations that depend on hepatic synthesis, intestinal bio-transformation, and tissue BA metabolism, were reported to affect glucose and lipid metabolism as well as energy homeostasis including body fat content [2]. The transition from late gestation to early lactation in dairy cows implies metabolic changes. After parturition, cows mobilize body fat reserves depending on their body condition before calving. Along with the mobilization of body fat reserves, we hypothesized that differently conditioned cows differ in BA profile in subcutaneous AT and serum during the periparturient period.

Methods: Multiparous German Holstein cows (n = 38) were divided into two groups according to their body condition score (BCS) and backfat thickness (BFT) before calving: high BCS cows (HBCS, n=19) and normal BCS cows (NBCS, n=19). Cows were fed differently from 15 weeks (wk) ante partum (ap) to 7 wk ap (HBCS: 7.2 NEL MJ/kg dry matter (DM)); NBCS: 6.8 NEL MJ/kg DM) to reach the targeted differences in BCS and BFT (NBCS: BCS < 3.5, BFT < 1.2 cm; HBCS: BCS > 3.75, BFT > 1.4 cm) until dry-off. From the time of dry-off (7 wk ap), the animals were fed the same total mixed ration for ad libitum intake. Until calving, the diet had an energy density of 5.4-5.8 MJ NEL/kg DM and after calving, 7.1 MJ NEL/kg DM. Biopsies of subcutaneous AT and serum samples were collected at wk -7, 1, 3, and 12 relative to parturition. Using LC-ESI-MS/MS with the BiocratesTM BA Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria), 20 primary and secondary BA, including their conjugates were analyzed in both, serum and AT. Statistical analyses of BA concentrations in serum and AT were performed using a linear mixed model with repeated measures (IBM SPSS version 21). Within this model, group, time, and their interaction were classified as fixed effects.

Results: In serum, a total of 14 BA [cholic acid (CA), chenodeoxycholic acid (CDCA), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), deoxycholic acid (DCA), taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), β-muricholic acid MCA(β), lithocholic acid (LCA), taurolithocholic acid (TLCA), glycolithocholic acid (GLCA) and tauromuricholic acid (α+β), (TMCA(α+β)], and 7 BA (CA, GCA, GCDCA, GDCA, TCA, TCDCA, TDCA) in AT were detected. Concentrations of BA were consistently higher (up to 424-fold) in serum than in AT. In both serum and AT, CA and GCA were the most abundant BA metabolites. In case of group differences,concentrations of CA, CDCA, TCDCA, TDCA, GDCA, and GCA in serum as well as concentrations of CA, GCA, GCDCA, GDCA, TCA, TCDCA, and TDCA in AT, were higher in NBCS cows than in HBCS cows.

Conclusions: Both primary and secondary BA were detected in bovine AT; however, it is yet unknown whether BA are metabolized and/or synthesized in this tissue. The primary BA CA as well as the conjugated primary BA GCA were the most abundant BA metabolites. Both were reported as ligands for the BA receptors in human AT, and have been suggested to impact the regulation of energy expenditure [3]. However, their importance in bovine AT in the context with the adaptive responses to the onset of lactation in dairy cows requires further research.

Bibliographical references

[1] JÄNTTI S.E., KIVILOMPOLO M., ÖHRNBERG L., PIETILÄNINEN K.H., NYGREN H., ORESIC M., HYÖTYLÄINEN T. (2014): Anal Bioanal Chem. 406:7799-7815

[2] SHAPIRO H., KOLODZIEJCZYK A.A., HALSTUCH D., ELINAV E. (2018): J Exp Med. 215(2): 383-96

[3] MOLINARO A., WAHLSTRÖM A., MARSCHALL H.U. (2018): Trend Endocrinol & Metabol. 29: 31-41

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38.

Impact of breed and concentrate level on efficiency traits in Fleckvieh and Holstein dairy cows

Einfluss von Rasse und Kraftfutterniveau auf die Effizienz von Milchkühen der Rassen Fleckvieh und Holstein

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The increase of milk yield in dairy cows was accompanied by increasing body weight (BW) and energy density in diet. Heavier cows must produce more milk to be as efficient as lighter cows to dilute their maintenance requirements, but feed intake adapted insufficiently. Milk yield, feed intake capacity, energy partitioning between milk yield and other life functions but also the extent of body tissue mobilisation and replenishment are main sources of genetic variability in efficiency on animal level[1]. This study examines the effect of genotype and diet quality on efficiency traits.

Methods: Data were derived from three German research institutes (Data N = 10,060; 4,498 Holstein [HO]; 5.562 Fleckvieh [FV]; 231 cows; data collection 2015-17) feeding two different forage qualities (6.1; 6.5 MJ NEL/kg dry matter [DM]) and two concentrates levels (L: 150 g; H: 250 g/kg ECM)[2]. Data provided information on diet composition, feed and nutrient intake, milk performance, BW and Body condition score (BCS). Efficiency traits were body weight efficiency (kg ECM/kg$^{0.75}$ BW), feed efficiency (kg ECM/kg DM]), energy efficiency (MJ energy in milk/MJ NEL intake). The mixed model (SAS) included the fixed effects breed, parity (1, 2, 3+4, ≥5), lactation month (1-11) and their interactions, forage FOR (6.1; 6.5), concentrates CON (L; H) and their interactions FOR×CON, FOR×breed, CON×breed. Changes within lactation were included as FOR×lactation month, CON×-lactation month, breed×FOR×lactation month and breed×CON×lactation month. Cow (1-231) was set as random effect.

Results: Influence of breed, parity, lactation month and their interactions were mostly significant (P<0.001). Impact of concentrate level was higher than of forage quality. The interactions FOR×CON, FOR×breed, CON×breed were not significant, while their interactions with lactation month were mostly significant (P<0.001). Main effects for FV, HO; L, H:

Feed intake = 18.6, 20.7; 18.8, 20.6 kg DM/d; Milk yield = 24.1, 27.7; 25.1, 26.8 kg ECM/d
Body weight = 767, 666; 704, 729 kg; BCS = 3.81, 3.05; 3.32, 3.54 (points 1-5)
BW efficiency = 0.167, 0.213; 0.186, 0.194 kg ECM/kg$^{0.75}$ BW
Feed efficiency = 1.307, 1.358; 1.347, 1.318 kg ECM/kg DM
Energy efficiency = 0.627, 0.642; 0.651, 0.681 MJ lactation energy/MJ NEL

Interaction breed×CON×lactation month: ECM, DMI, BW, BCS and BW efficiency were reduced at the low concentrate level. ECM decreased relatively less than DMI leading to an increased feed and energy efficiency based on mobilisation. The high concentrate level reduced feed and energy efficiency by less BCS loss and earlier BCS gain despite increased milk yield. While FV partitioned additional nutrients at high concentrate level in both higher milk yield and an earlier BCS gain, HO mobilized equally in both groups and regenerated BCS mainly in late lactation. Highest efficiency appeared in first third of lactation as milk production and DMI are decoupled.

Conclusions: The results show a diverse interplay of the main sources of efficiency depending on lactation stage, genotype and nutrient supply. The importance of considering time trends for interpretation of efficiency traits has to be pointed out. Insufficient adaption of milk yield on low or reduced DMI indicates a high metabolic priority of milk performance causing an energy deficit. Mobilisation “fakes” a high efficiency for milk production, regaining body reserves a too low one. Feeding according to requirements is necessary for meaningful efficiency calculations by avoiding additional under-supply and allowing physiological regeneration of body tissue. Efficiency requires a broad view on animal considering trade-offs between production and other life functions (e.g. health, fertility, longevity).

Bibliographical references
[1] VEERKAMP R.F., EMMANS G.C. (1995): Livest. Prod Sci. 44: 87-97
[2] SPIEKERS H., HERTEL-BÖHNKE P., MEYER U. (2018): Verbundprojekt optiKuh. Tagungsband. LfL Schriftenreihe 2/2018

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39.

A meta-analysis on estimates of efficiency of phosphorus utilization in lactating dairy cows

Meta-Analyse zu Schätzwerten für die Phosphorverwertbarkeit bei laktierenden Milchkühen

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Phosphorus (P) is essential for health, milk production, and reproduction of dairy cows. Thus, an adequate P supply is of utmost concern to meet the requirement of the animal while avoiding unnecessary excretion. For calculation of the P requirement, an estimate of the efficiency of P utilisation is needed. The value applied by the GfE is 70% and independent of the P source [1]. The objective of the present study was to evaluate whether this value can be confirmed or should be adjusted based on published studies.

Methods: The data set included 234 observations from 45 studies on lactating dairy cows obtained from literature (mean milk yield 31 kg / d, SD 9.2; mean dry matter intake (DMI) 21 kg / d, SD 3.9). Inclusion criteria were that at least data on P intake (PIN), P faecal excretion (PFE), DMI and milk yield were reported. Data on PIN and PFE were used to calculate the standardised P digestibility (sPD; corrected for faecal endogenous losses assuming 1 g P / kg DMI [1]). The net requirement was estimated as the sum of P secreted with milk and inevitable P loss (1 g P / kg DMI [1]). Other calculations and statistical evaluation followed an approach described for estimates made on calcium [2]. Firstly, the P supply (PS; P intake (g / d) - P net requirement (g / d)) and the PS ratio (PS (g / d) / P net requirement (g / d)) were calculated. A linear mixed model was used to describe the relation of PFE (Y) and PS (X) using the study intercept as random effect. To describe sPD (Y) in dependence of the PS ratio (X) the following exponential model was used: $Y_{ij} = a_i + (C_0 - P) \times e^{(-K \times Xij)} + P + e_{ij}$ where C_0 is the inter-study intercept of the exponential regression, P is the response of Y at an infinite value of X (plateau) and K is the rate constant of the regression as the fixed effects. The individual intercept of the experiment ai was used as random effect and eij is the residual error. Furthermore, a data subset was created for observations made at marginal P supply (PS ratio <0.35) including 68 observations (50 observations without supplementation of mineral P, 18 observations with supplementation of mineral P). Means of this data subset were compared using an ANOVA considering the experiment as random effect. Statistical analyses were performed using SAS (version 9.4 with Proc Mixed for the linear model and the ANOVA and Proc Nlmixed for the exponential function) and observations were weighted according to the number of replicates.

Results: PFE increased linearly with increasing PS (slope = 0.78, R^2=0.94). The exponential regression equation for sPD in dependence of PS was $Y = (87.6 - 33.3) \times e^{(-0.82 \times X)} + 33.3$, with a RMSE of 5.96 ($R^2 = 0.86$). The mean sPD for diets with and without mineral P did not differ significantly (76% vs. 81%, respectively; $p = 0.10$).

Conclusions: P exceeding the net requirement of cows is excreted almost completely via faeces. The estimated intercept of 87.6% indicates the maximal sPD at marginal P supply and can be interpreted as the efficiency of P utilisation. The currently applied value of 70% for the efficiency of P utilisation [1] underestimates the potential of P utilisation in lactating dairy cows. It was confirmed that a differentiation in P utilisation between P sources is not meaningful.

Bibliographical references

[1] GfE (2001): Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt am Main, Germany

[2] WILD K.J., SIEGERT W., WINDISCH W.M., SÜDEKUM K.-H., RODEHUTSCORD M. (2021): Animal 15: 100315

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40.

Phosphorus digestibility and metabolisable energy concentration of compound feeds for pigs based on rye or wheat and soybean meal or rapeseed meal

Phosphor-Verdaulichkeit und Umsetzbare Energie in Mischfuttermitteln für Schweine basierend auf Roggen oder Weizen und Soja- oder Rapsextraktionsschrot

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The public requests reducing environmental impacts of animal husbandry, mainly concerning nitrogen and phosphorus (P) emission, animal welfare and type of animal production. Moreover, climate change confronts agriculture with additional challenges. To meet demands and changing conditions, rye and rapeseed meal may significantly contribute to meet nutritional requirements of and minimise nutrient excretion by pigs. Due to its high drought tolerance rye is more climate-adapted than wheat. Rye is also considered to benefit intestinal health. Rapeseed meal as a home-grown protein source is an interesting climate-friendly alternative to imported soybean meal.

Methods: Eight different compound feeds (CPF), consisting of 70% cereal grains, either wheat (W) or rye (R), and 30% protein source, either soybean meal (SBM) or rapeseed meal (RSM), were tested with and without phytase (phyt) supplementation. The test ration (TR) were formulated by mixing a basal ration (BR) low in P [1] with the corresponding CPF to ensure a calculated total concentration of digestible P (dP) of no more than 2 g/kg dry matter (DM) assuring a suboptimal P supply. Twenty-four male castrated crossbred pigs, divided in two subgroups of twelve pigs each [initial body weight (SD): subgroup 1, 28.2 kg (6.0 kg); subgroup 2, 34.2 kg (5.8 kg)], were assigned to duplicate 3 x 3 Latin squares and randomly allotted to twelve metabolism crates. Within every duplicate Latin square two different TR and a BR (control) were tested on six animals. Feed was offered twice daily at approximately 2.5 times their metabolisable energy (ME) requirement for maintenance. After an adaption period of 7 days, faeces and urine were collected quantitatively over a 5-day period. Analyses to determine ME and digestible P were done in feed, faeces and urine.

Results: The ME [means (SD)] ranged from 14.2 (0.47) MJ ME/kg DM for (R+RSM) to 16.0 (0.27) MJ ME/kg DM for (W+SBM). There were no differences between the ME values of wheat- or rye-based CPF, but mixtures with RSM had significantly lower ME values than the SBM-containing CPF, regardless of the supplementation of phytase. The P digestibility ranged from 55.3% (R+RSM) to 70.2% (W+SBM+phyt). All CPF with supplemented phytase achieved a significantly higher P digestibility of about 10 percentage units, compared with feeds without a supplementation.

Conclusions: This study demonstrates that rye provides similar results as wheat regarding ME values and P digestibility. The high intrinsic phytase activity of rye did not show any additional advantage over wheat, yet the added commercial phytase improved digestibility and should be added in terms of 'good agricultural practice'.

Bibliographical references

[1] GfE (1994): Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen. DLG-Verlag, Frankfurt am Main, Germany

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41.

Effect of various insoluble dietary fibres on ileal morphology, caecal gene expression, performance and footpad health of broilers

Einfluss unterschiedlicher unlöslicher Faser auf die ileale Morphology, zäkale Genexpression, Fußballengesundheit und Masteistung von Broilern

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Depending on dietary fibre properties, the inclusion of even moderate levels of fibrous material has already shown to positively influence the functionality of the gastrointestinal tract (GIT) of broiler chicken. Especially insoluble dietary fibre affects gut motility and transit time of digesta, thereby influencing the morphological characteristic in GIT. In this context, lignocellulose as insoluble fibre source has recently gained the interests of nutritionist [1]. The aim of this study was to investigate the impact of various insoluble dietary fibres, differing in fibre properties on performance data, ileal morphology and caecal gene expression of immune related genes. We hypothesized that the insoluble dietary fibre positively effects the performance data and different fibre properties result in variations, especially concerning the gene expression.

Methods: A total of 5,040 one-day old Ross 308 (♂ and ♀) was allocated 36 pens and fed four different diets on a three-phase feeding program. The control diet (C) resembled a commercial broiler diet low in fibre based on maize, wheat and soybean meal. Experimental treatments were supplemented on top with either 0.8 % standard lignocellulose product (Li1), 0.8 % eubiotic, partially fermentable lignocellulose product (Li2) or 1.6 % soybean hulls. On day 20 of the trial intestinal sampling was done on two birds per pen (n=72): Ileal tissue samples from two centimetres to the ileocecal junction were taken for morphometric measurements, whereas for qPCR analysis caecal tissue samples were taken to quantify the expression of immune related pro-inflammatory marker genes (IL1 β, IL6, IL8).Body weight was recorded after each feeding phase (starter: pen wise, grower & finisher: individually), carcass analyses was performed on four birds per pen (n=144) on day 36. Additionally, excreta was collected for dry matter analyses as well as the feet of 30 animals per pen were scored. One-way ANOVA was performed using the mixed procedure of SAS 9.4 combined with Tukey Kramer post hoc test considering experimental treatment as fixed factor. For nonparametric parameter a Mann- Whitney-Wilcoxon test was performed.

Results: In the grower phase, birds fed SH and Li1 had higher body weights and daily weight gains compared to C ($p<0.05$), whereas Li2 showed a tendency towards heavier bodyweights. Feed intake, feed:gain ratio, excreta dry matter as well as the caecal gene expression remained unaffected by the fibre supplementations. Ileal morphometry showed higher villi when birds were fed either Li1 (+ 10.2 %), Li2 (+7.8 %) or SH (+12.2 %) compared to C. Furthermore, foot pad dermatitis score revealed significant improvement of feet health with additional Li1, Li2 and SH ($p<0.001$).

Conclusions: The research shows that even the low levels of additional insoluble dietary fibre sources, positively affected the intestinal morphology in the ileal section and significantly improved feet health, regardless of lignification, solubility and fermentability.

Bibliographical references

[1] MATEOS G., GUZMAN P., SALDANA B., BONILLA A., LAZAR R., JIMENEZ-MORENO E. (2013): Proc. ESPN 19: 1–11

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42.

Changes in apparent precaecal digestibility, microbial metabolites and intestinal histology under the influence of feeding *Hermetia illucens* protein meal and fat to broilers

Veränderungen der scheinbaren praecaecalen Verdaulichkeit, mikrobiellen Metabolite und Darmhistologie durch die Fütterung von Proteinmehl und Fett der Hermetia illucens an Masthühner

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Scientific interest regarding the use of insects as animal feed has yet mainly focused on defatted larvae meal as protein source. Protein meal from *Hermetia illucens* (HI) has been shown to be a suitable substitute for soybean meal (SBM) in broiler feeding when used in small quantities (15% SBM crude protein substitution) [1]. The larvae fat remaining during defattening may also be of interest, since it is characterised by a particularly high content of lauric acid, a medium-chain fatty acid, which is known for its antimicrobial properties [2]. Therefore, the present experiment aimed not only to investigate the apparent precaecal digestibility (AID) of HI larvae protein and fat, but also their effect on intestinal histology and caecal and colonic fermentation products. We hypothesized that the replacement of SBM and its oil with protein meal and fat from HI will not impair AID of the diet, but may favourably shift the production of microbial metabolites, e.g. biogenic amines and short-chain fatty acids (SCFA).

Methods: In a 2 × 3 factorial arrangement, 432 male Ross 308 1-day old broiler chickens were randomly assigned to one of six dietary treatments and allotted to 36 pens with 12 animals per pen, resulting in six replicates per treatment. The arrangement included two types of protein, i.e. SBM (S) or SBM and 15% of its crude protein (N × 6.25) replaced by HI larvae meal (L), and three levels of fat sources, namely 0% (0L), 50% (50L) or 100% HI larvae fat (100L). Thus, the following treatment groups were created: 1) S+0L, 2) S+50L, 3) S+100L, 4) L+0L), 5) L+50L) and 6) L+100L. Animals were slaughtered after 35 days and digesta was sampled from the ileum to determine AID, as well as caecum and colon to analyse the concentration of biogenic amines and SCFA. Moreover, intestinal tissue was taken from the jejunum and ileum in order to investigate intestinal histology. Statistics were performed using PROC MIXED of SAS v 9.4 with protein and fat as fixed effects, and pen as random effect.

Results: Analysis of AID revealed that HI larvae meal as protein source positively affected dry matter ($p<0.01$), organic matter ($p<0.01$) and fat digestibility ($p=0.02$). In addition, the feeding of larvae protein decreased the caecal concentration of biogenic amines as seen for agmatine ($p<0.01$), spermidine ($p<0.01$), spermine ($p<0.01$) as well as ammonia ($p<0.01$). The caecal concentration of butyric acid was higher ($p=0.03$) in S compared to L groups. Furthermore, larvae fat feeding increased the concentration of agmatine in 50L groups compared to 0L in the colon ($p=0.03$). Besides, an interaction was observed for the villus area measured in the jejunum, which increased in L+100L compared to S+100L ($p=0.01$). Moreover, villus width increased in L groups compared to S groups ($p=0.03$).

Conclusions: The results of the present study suggest the possibility of substituting low amounts of SBM and up to 100% of soy oil with HI larvae meal and fat respectively in broiler feeding. Feeding HI larvae meal as protein source even positively affected AID in the finisher phase. Moreover, with regard to gut health, the partially diminished concentration of possibly harmful microbial metabolites and an increased villus area can be considered positive.

Bibliographical references

[1] HARTINGER K., GREINIX J., THALER N., EBBING M.A., YACOUBI N., SCHEDLE K., GIERUS M. (2021): Animals 11: 1628

[2] ÇENESIZ A.A., ÇIFTCI İ. (2020). Worlds Poult. Sci. J. 76: 234-48

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43.

Feeding yellow mealworms (larvae of *Tenebrio molitor*) with various protein sources and their effects on growth performance

Zum Einsatz unterschiedlicher Proteinquellen im Futter des Gelben Mehlwurms (Larven von Tenebrio molitor) und den Auswirkungen auf die Wachstumsleistung

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In May 2021 the European Food Safety Agency (EFSA) approved mealworms, whole or processed as powder, as the first insect-based novel food in human nutrition. Since September 2021 processed insect proteins – including mealworms – are also allowed in the feeding of monogastric livestock as pigs and poultry. Therefore, knowledge about the nutrient requirements of this insect species becomes more and more important. The aim of the present study was to evaluate the effects of several protein feedstuffs (dried whey, wheat gluten and protein isolates from peas, soybeans, and potatos) varying in their amino acid (AA) patterns and contents on the growth performance of mealworms

Methods: Young growing mealworms (average body weight of 20 mg) were allocated randomly to 40 groups (35 g mealworm biomass per group = approx. 1700 animals) kept in plastic bowls at 21 °C and 50% relative humidity in a climate chamber. Ten experimental diets were formulated using either pea protein, wheat gluten, dried whey, soybean or potato protein as the major sources of AA. The protein sources were added in quantities between 8% and 19% depending on the particular protein concentration to a basal feed mixture consisting of wheat flour, oil, and a vitamin-mineral mixture without additional free amino acids (AA) to create diets with crude protein (CP) concentrations of either 16% or 20%. Each diet was offered to four mealworm groups. Feed was mixed with some water to create a tough dough, and was given every second day in several small pieces into each insect bowl. By this means, drinking water and feed were provided for ad libitum intake and feed residuals could be collected completely. Biomass (BM) of mealworms, feed supply (FS) and feed residuals (FR) per insect bowl were recorded weekly. After 21 days, mealworms were devitalized by freezing at -21 °C. Diets and mealworms were analysed for nutrients and AA concentrations. Weight gain (WG), final BM, FS, feed consumption (supply minus residuals) and expenditure of feed for growth were statistically analysed using ANOVA.

Results: Depending on the protein source, there were significant differences in growth performance of mealworms while dietary protein concentration had no marked effect, neither on feed consumption nor on growth performance. Mealworm BM increased during the experimental period by 50 % with pea, 100% with wheat gluten, 140% with whey, 190% with soybean and 200% with potato protein. Total feed consumption per bowl for the different protein diets was 38 g, 45 g, 53 g, 56 g, and 60 g, respectively. although FS was equal for all groups. Feed-to-gain conversion was best with potato and worst with pea protein (2.5 versus 5.3 g feed per g biomass gain, respectively). Independent of the protein source, dietary CP concentrations of 16% were sufficient for optimal mealworm growth, as higher CP concentrations did not improve growth performance or feed efficiency. The source of protein has a prevailing effect on growth performance. Potato protein with high concentrations of essential AA and a balanced AA pattern proved to be obviously not only a very suitable protein source for monogastric animals but also for mealworms. In contrast, pea protein with a relative lack of sulphur-containing AA (methionine, cysteine) and probably additional antinutritive factors impaired mealworm growth.

Conclusions: Growth performance, i.e. biomass increase and feed-to-gain efficiency of mealworms is highly depending on the protein source, i.e. the AA pattern in the diet. Deficiencies of single AA do not seem to be compensated by higher feed intake. In contrast, such AA imbalances impair feed consumption leading to lower growth rates. This correlation is well-known in common livestock feeding. Obviously, dietary protein and amino acid requirements of mealworms regarding quantities and patterns are quite similar to common monogastric livestock.

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44.

Effects of replacing soybean meal by regional co-products (DDGS, rapeseed meal, sunflower meal) and a phytonutrient on broiler performance and footpad health

Effekte von regionalen Nebenprodukten (DDGS, Raps- und Sonnenblumenextraktionsschrot) als Ersatz für Sojaextraktionsschrot sowie einem phytogenen Zusatz auf die Leistung und Fußballengesundheit von Broilern

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In the last years protein feed obtained from industrial co-products gained the interest of animal nutritionists. A replacement of soybean meal (SBM) with regional co-products might provide a reasonable option to counteract the dependency on imported soybean meal and to increase sustainability in meat production. Furthermore, the utilization of co-products in livestock feeding that are inappropriate for human consumption decreases feed-food competition [1]. In this study a mixture of commercial co-products was tested with the aim of total renunciation of SBM. Additionally, a phytonutrient obtained from wood, high in polyphenols was tested with the aim of promoting the zootechnical performance. We hypothesized, that co-products can replace SBM under consideration of a balanced available nutrient content without impairing performance. Furthermore, the supplemented phytonutrient might improve the performance.

Methods: In the present study 4,500 one-day-old chicks (Ross 308; ♂ and ♀) were equally distributed to 30 pens. Five diets were created: a control treatment (CON) based on maize (50 %), SBM (27 %) and wheat (9 %), whereas in treatment T1 the SBM level in the diet was reduced to 14.6 % as well as AMEN content (- 4.5 %) and substituted with a mixture of rapeseed-, and sunflower meal as well as dried distillers' grains with solubles (DDGS). Treatment T2 was formulated as SBM- free diet and with reduced AMEN content (- 6.5 %). Hence the alternative protein mixture was applied as major protein source. The phytonutrient (0.04 % in fresh matter, according to supplier´s recommendation) was tested in combination with CON (CON+) as well as T2 (T2+). In the finisher phase, 0.5% of titanium dioxide was added as undigestible marker to allow determination of apparent total tract digestibility (ATTD). Body weight was recorded after each feeding phase (starter: pen wise, grower & finisher: individually). On day 36 of the trial, carcass analyses were performed on 4 birds per pen (n=120), excreta was collected and feet of 30 animals per pen were scored. For performance and digestibility parameters pens were considered as experimental unit and animal for carcass characteristics. The statistical model applied a two-factorial approach with soybean level and phytogenic supplementation as fixed effects, as well as their interaction.

Results: Overall, the average daily weight gain showed significant differences between CON (61.5 g / d) and T2 (59.6 g / d), with T1 being intermediate. Birds fed SBM reduced or SBM free diets responded with increased daily feed intake (+ 2.5 % and + 4.9 % respectively) thus feed:gain ratio was impaired, whereby these effects where more dominant with SBM free diets. ATTD of dry matter, organic matter and gross energy was significantly decreased with T1 and T2 ($p<0.05$). Birds fed the phytonutrient significantly improved feed:gain ratio ($p<0.05$). The examination of the feet revealed that T2 had a significant lower foot pad dermatitis score compared to T1, with CON being intermediate. Significant interaction between SBM free diets and phytonutrient supplementation were observed, indicating a negative effect on feed health by phytonutrient.

Conclusions: Exact knowledge of available nutrient content can be considered as a precondition to avoid impairing effects on performance. Dietary inclusion of a phytonutrient component beneficially effects feed to gain ratio, however in diets including high fibrous protein sources the phytonutrient declined feet health.

Bibliographical references
[1] SCHEDLE K. (2016): Die Bodenkultur 67: 45-60

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45.

Creatine contents in feed components for monogastric animals: Are current feeding programs adequate for omnivores?

Kreatingehalte in Futterkomponenten für Monogastrier: Sind gängige Fütterungsstrategien Omnivoren gerecht?

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Creatine (Crea) plays a crucial role in the cellular energy metabolism of all vertebrates. Although Crea can be synthesized endogenously, omnivores such as poultry and swine are evolutionarily designed to consume this biomolecule via matter of animal origin in their natural diet. Crea is scarce in modern conventional, mostly plant based, feeds, as plants do not synthesize Crea. Animal derived, therefore Crea containing, feed ingredients were only just re-introduced in the EU [1] and could provide a source of Crea for monogastrics. However, as Crea itself is not heat stable, a supplemental source of Crea, namely Guanidinoacetic Acid (GAA), the direct metabolic precursor, is registered in the European Union since 2009 and could safely bring the lost nutrient Crea back into compound feeds for livestock. The present study aimed at examining Crea contents in typical animal derived feed components and compared the Crea-uptake between typical monogastric feeding programs to an exemplary Crea uptake in a wild habitat. This way, we tried to examine how adequate modern diets are regarding their Crea supply.

Methods: The Crea contents of 301 feed ingredient samples from various origins used in diets for poultry and swine were analysed via ion chromatography and compiled by feed type in a database. In a further step, the Crea supply achievable with practical diets for monogastric animals was calculated based on the determined Crea contents in feed components and their recommended inclusion rates. In addition, an attempt was made to reflect Crea uptake in the wild using published data on diet composition, especially the percentage of animal matter, of red junglefowl [2] and Crea levels in small prey animals [3] as an example for monogastric omnivores. The comparison based on the Crea levels in mg per kg feed as fed.

Results: In general, Crea levels within each feed component were highly variable. Highest Crea levels were found in fish derived feed ingredients with a median of 1174 mg/kg (min 144 mg/kg - max 27039 mg/kg) feed component, followed by dairy-based feedstuff, with 805 mg/kg (min 90 mg/kg - max 4990 mg/kg). Ingredients from meat ranged around 206 mg/kg (min 10 mg/kg - max 410 mg/kg), whereas plant derived products were all below the determination limit (<20 mg/kg). Based on the analyses of grains it was concluded that modern feeding programs, on a purely vegan basis, do not contain Crea. Red junglefowl's natural diets contain an estimated 289 mg Crea/kg. Nevertheless, typical fish meal would also only contribute 13% of what red junglefowl would receive through their natural feeding habits. Meat and bone meal would only provide 4% of the Crea supply in the wild and 31% of what broilers would consume with a diet including fish meal.

Conclusions: The diets of omnivores in their natural habitat contain significant amounts of Crea. However, present diets for monogastric animals provide only a fraction of this Crea supply expected from uptake in the wild, as they are mainly plant-based. Crea is provided only by animal derived ingredients or supplemental forms of Crea, such as GAA. Due to the low inclusion rates, highly variable Crea levels as well as lack of heat-stability, omnivore-appropriate Crea supply in current feeding programs can best be secured by supplementing alternative Crea sources.

Bibliographical references
[1] Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001
[2] ARSHAD M.I. et al. (2000): Pakistan J. Biol. Sci. 3(6):1024-1026
[3] DOBENECKER B., BRAUN U. (2015): J. Anim. Physiol. Anim. Nutr. 99(6):1017-1024

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46.

Meta-analysis on the estimation of ruminal amino acid degradability from crude protein degradability

Meta-Analyse zur Schätzung des ruminalen Abbaus von Aminosäuren aus dem Rohproteinabbau

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In ruminants, amino acid (AA) supply in the small intestine includes AA from microbial protein and ruminally undegraded AA, with increasing relevance of the latter with increasing performance level. Different feed evaluation systems for ruminants currently express requirement and supply of protein to the duodenum in terms of total metabolisable or utilisable protein, although the animal has a requirement for AA and not for protein per se. The ruminal AA degradability of rapeseed meal could be estimated with high accuracy from ruminal crude protein (CP) degradability determined in *in situ* studies [1]. Nevertheless, systematic studies on whether this is possible for other feedstuffs in order to differentiate between protein sources do not exist. Therefore, the objective of this meta-analysis was to estimate ruminal AA degradability from CP degradability for a broad range of feedstuffs and to investigate variation of ruminal AA degradability of different protein sources.

Methods: The data set included 329 observations compiled from 61 *in situ* studies with ruminants obtained from literature (52 studies with cattle, 9 with sheep) . Those studies reported AA concentrations of original feed and *in situ* residues, or CP and AA degradability. Data were not included in the data set when AA concentration of original feed or CP degradability were not available or when degradability was reported only via graphs. Data on feedstuffs of animal origin were not included in the data set. Considered data used ruminal incubation times between 12 and 24 h. Linear mixed models considering the CP degradability as independent variable and the AA degradability as dependent variable were applied using PROC Mixed of SAS (version 9.4) to estimate essential AA degradability including all feedstuffs. Study was considered as random effect and observations were weighted according to the respective number of replications. In addition, a data subset of protein-rich feedstuffs was created considering rapeseed meals (n = 48), soybean meals (n = 47), distiller's grains (n = 43) and other feedstuffs containing more than 10% ruminally undegraded CP in dry matter (n = 62). Linear mixed models were used to differentiate AA degradability between those four categories of protein-rich feedstuffs.

Results: When all feedstuffs were evaluated together, estimated intercepts of the linear regression equation for His, Ile, Leu, Lys, Met, Phe, Thr, and Val were 8.5, -0.1, -4.2, 5.3, 4.3, -3.3, 0.3, and -0.2, respectively and the respective estimated slopes were 0.91, 0.97, 1.03, 0.94, 0.93, 1.03, 0.98, and 1.00. Root mean square error varied between 5.4% (Val) and 7.5% (Lys) and coefficient of determination was highest for Thr and Val (0.94) and lowest for Met and Lys (0.89). Application of those equations assuming a ruminal CP degradability of 60% gave estimated AA degradability between 58% (Ile, Leu) and 63% (His). The differentiation between protein-rich feedstuffs delivered equations with similar root mean square error and coefficient of determination. Variation of estimated AA degradability of the four feed categories was low and within the range of the estimation error.

Conclusions: Variation of estimated degradability of different AA and feedstuffs was low and within the range of the estimation error. Therefore, CP degradability can be used as a reasonable approximation for individual AA degradability of all AA. A differentiation between protein sources does not appear to be necessary based on the current data set.

Bibliographical references

[1] STEINGASS H., KNEER G., WISCHER G., RODEHUTSCORD M. (2013): Animal 7, 1119-1127

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47.

Ruminal and post-ruminal phytate degradation in wethers fed rapeseed meal- or soybean meal-based diets

Ruminaler und post-ruminaler Phytatabbau von Hammeln bei Fütterung von Soja- oder Rapsextraktionsschrot in der Ration

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Although ruminants are known to overall efficiently degrade phytate ($InsP_6$), a variable extent of ruminal $InsP_6$ disappearance has been reported. *In situ* results have shown a lower ruminal effective degradation of $InsP_6$ (ED) for rapeseed meal (RSM) compared to soybean meal (SBM) [1]. Ruminally undegraded $InsP_6$ can be hydrolysed in the post-ruminal section of the digestive tract [2], yet the extent of post-ruminal $InsP_6$ degradation has been barely studied. The aim of this study was to investigate ruminal and post-ruminal $InsP_6$ degradation in wethers fed RSM- and SBM-based diets and to compare ruminal degradation with values determined *in situ*.

Methods: Firstly, the ED (calculated for passage rates of 2 and 5%/h) of RSM and SBM was evaluated using a standard *in situ* procedure with three rumen-fistulated lactating Jersey cows and incubation times of 2, 4, 6, 8, 16, 24, 48, and 72 h. The values of 0 h incubation were obtained by washing the feed samples without ruminal incubation. $InsP_6$ concentrations of the meals were 21.6 and 14.2 g/kg DM for RSM and SBM, respectively. Secondly, eight adult wethers were randomly assigned to two treatment groups that were fed two different diets (Diet RSM and Diet SBM). Each diet consisted of 60% meal (either RSM or SBM) and 40% maize silage on a fresh matter basis and was fed for 8 weeks (500 g per day, fresh matter basis) in two meals per day. Wethers were kept in two separate groups according to their treatment in a free-stall with straw bedding and had free access to hay and water. At the end of the adaptation period, TiO_2 (1.3 g per meal) was added to the diets for 5 days. Wethers were then stunned and exsanguinated. Contents from the reticulo-rumen were collected, pooled, and immediately separated into three phases: large particulate matter, small particulate matter, and fluid phase. Digesta from the omasum, abomasum, colon (middle of the ascending part), and rectum were totally collected. Digesta from a defined section of the jejunum was flushed out using ice-cold double-distilled water. Samples were analysed for concentrations of TiO_2, inositol phosphates, and myo-inositol. Data were statistically analysed in a one-factorial approach using SAS 9.4 with diet as fixed effect and animal as random effect. Statistical significance was declared at $p < 0.05$.

Results: ED of $InsP_6$ studied *in situ* was higher for SBM compared to RSM (ED2 = 93 vs. 83%; ED5 = 85 vs. 64%, respectively). Ruminal $InsP_6$ disappearance determined in wethers differed significantly between the diets (Diet SBM: 89%; Diet RSM: 76%). No $InsP_6$ was determined in the fluid phase of the rumen. Total tract $InsP_6$ disappearance measured at the rectum was also significantly lower for Diet RSM (83%) than Diet SBM (93%), while total post-ruminal $InsP_6$ disappearance did not differ between treatments (Diet RSM: 6%; Diet SBM: 4%). A significantly higher amount of ruminally and post-ruminally degraded $InsP_6$ was observed upon feeding Diet RSM (ruminally: 4.5 g for Diet RSM and 3.4 g for Diet SBM; total post-ruminally: 0.4 g for Diet RSM and 0.1 g for Diet SBM). InsP5 was detected only in very small concentrations in some sections, and InsP1-4 were not detected in any sample. Myo-inositol was not quantifiable in the majority of the digesta samples (rumen pool, colon, and rectum), concentrations were very low in omasum+abomasum (< 0.15 g/kg DM), and not significantly different in jejunum (Diet RSM 0.99 g/kg DM; Diet SBM: 0.72 g/kg DM).

Conclusions: Compared to ruminal $InsP_6$ degradation, post-ruminal degradation of $InsP_6$ was negligibly low. A low ruminal passage rate in wethers can be assumed as the feeding level was low and the determined $InsP_6$ disappearance was consistent with the *in situ* values calculated for ED2. This indicates that at low passage rates, other than at high passage rate, $InsP_6$ from the feed is degraded to large extent in the rumen, and $InsP_6$-P leaving the rumen is mainly unavailable to the animal.

Bibliographical references

[1] HAESE E., KRIEG J., GRUBJEŠIĆ G., FEYDER A., RODEHUTSCORD M. (2020): Animal 14: 1461-1471

[2] PARK W.-Y., MATSUI T., YANO H. (2002): Anim. Feed Sci. Technol. 101: 55-60

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48.

Effect of hay steaming on the estimated pre-caecal digestibility of crude protein and selected essential amino acids in horses

Einfluss des Bedampfens von Heu auf die geschätzte präzäkale Verdaulichkeit von Rohprotein und Aminosäuren beim Pferd

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Steaming of hay reduces the viability of adhering microorganism [1] and is therefore increasingly used to treat low quality hay. Preliminary results [2] however indicate that exposure to high temperatures and moisture associated with hay steaming may cause protein damage and thus reduce pre-caecal (pc) digestibility (D) of crude protein (CP) and amino acids (AA). The objective was to investigate the effect of steaming meadow hay on the content of CP and selected essential AA (EAA), their pcD and the resulting contents of pc digestible (pcd) CP and EAA. As proof of principle, treatment effect on selected Maillard products should furthermore be examined in an exemplary selection of hay samples.

Methods: Sixbatches of meadow hay were collected from practice and steamed with 4 replicates each at approximately 100 °C for 1 h (Haygain HG 2000, Farm & Stable, West Sussex, UK). The native and steamed hay samples were investigated for proximate nutrients, AA and neutral detergent soluble CP. From this, contents of pcdCP and pcdEAA as well as pcD CP (and thus EAA) were calculated. To estimate the soluble part of the crude protein (NDSCP) we used the difference between CP and neutral detergent insoluble CP (NDICP). From each batch of hay, one native and one corresponding steamed sub-sample were investigated for the Maillard products furosine and carboxymehtyllysine (CML) by LC–MS [3]. Statistical analysis was performed using a mixed model with batch as random effect and treatment as fixed effect. Concentrations of Maillard products were analysed by Wilcoxon rank sum test. The level of significance was $P < 0.05$.

Results: The native and steamed hay contained 960 ± 3.41 and 888 ± 3.41 g dry matter (DM) per kg ($P < 0.001$), respectively. Concentrations of EAA and pcdEAA (in g / kg DM) for native / steamed hay were Lys 3.31 ± 0.300 / 2.51 ± 0.300, pcdLys 1.78 ± 0.105 / 0.72 ± 0.105, Met 1.16 ± 0.130 / 1.08 ± 0.130, pcdMet 0.62 ± 0.043 / 0.31 ± 0.043, Cys 0.72 ± 0.054 / 0.62 ± 0.054, pcdCys 0.39 ± 0.016 / 0.18 ± 0.016, Thr 3.01 ± 0.344 / 2.91 ± 0.344 and pcdThr 1.62 ± 0.110 / 0.83 ± 0.110. Contents of Cys and Met in sum and pcdMet+Cys (in g / kg DM) were for native hay 1.87 ± 0.181 and 1.69 ± 0.181 and for the steamed one 1.01 ± 0.056 and 0.49 ± 0.056. The treatment effect was significant ($P < 0.01$) for all shown EAA and pcdEAA. The CP content was not affected by steaming (native 69.1 ± 7.72, steamed 67.3 ± 7.72 g / kg DM; $P > 0.05$). The contents of NDICP in native and steamed hay were 27.3 ± 5.37 and 45.7 ± 5.37 g / kg DM and of NDSCP 41.8 ± 2.52 and 21.5 ± 2.52 g / kg DM, respectively ($P < 0.01$). Steaming thus decreased the content of pcdCP from 37.6 ± 2.27 to 19.4 ± 2.27 g / kg DM ($P < 0.01$). The pcD CP of 55.6 ± 2.11 % in native hay was therefore substantially reduced by steaming down to 29.9 ± 2.11 % ($P < 0.01$). Steaming numerically elevated the contents (in mg / 100 g DM) of CML from 1.8 - 9.9 to 3.6 - 23.6 and of furosine from 4.8 - 39.1 to 17.0 - 57.7 (both $P > 0.05$).

Conclusions: Steaming of hay reduced the pcD CP and thus the content of pcdCP and pcdEAA substantially which needs to be balanced in the ration. The ascertained increase in Maillard products allows an initial explanation for the detected effect and is further explored in an ongoing study.

Bibliographical references

[1] MOORE-COLYER M.J., TAYLOR J.L., JAMES R. (2016): J. Equine Vet. Sci. 39: 62-68

[2] GLATTER M., BOCHNIA M., WENSCH-DORENDORF M., GREEF J.M., ZEYNER A. (2021): Animals 11: 2729

[3] HOFMANN T., ENGLING A.C., MARTENS S., STEINHÖFEL O., HENLE T. (2020): European Food Research and Technology 246: 253-256

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49.

Silage volatile organic compounds and ruminant feeding behaviour – a review

Flüchtige organische Verbindungen in Silagen und Futteraufnahmeverhalten von Wiederkäuern – eine Übersicht

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Silage is one of the important constituents of the ruminant diet, principally in the western hemisphere. Several volatile organic compounds (VOC) are formed during ensiling. While some of them influence silage preference and DMI, the others impact milk quality and human health [1]. Nonetheless, there is a lack of a comprehensive review on silage VOC, especially the effect of propionic acid (PA), butyric acid (BA), ethanol, esters, and other minor VOC on ruminant feeding behaviour.

Methods: We used Google Scholar, PubMed, ScienceDirect for our literature search. Studies that were published between 1960 and 2021 were included in this review. This study aims to highlight the different mechanisms through which silage VOC can alter feeding behaviour, and the comparison of individual VOC effects on preference and dry matter intake (DMI) when (1) observed from silage intake models, (2) present in the silage due to the addition of microbial inoculants, and (3) the VOC is added as a pure substance. The possible reasons for the differences in the results obtained between infusion studies, silage intake models, and feeding trials are discussed. A small attempt was also made to highlight the use of silage metabolomic studies in assessing silage intake in ruminants.

Results: The type of forage used for ensiling, animal species, physiological state of the animal, and diet composition showed a large variation between studies. The VOC differed in their magnitude of impact on preference and DMI. Data from silage intake models showed that, for each 1 g/kg DM increase in AA and PA, the reduction in DMI ranged from 30 to 130 g DM/d for AA and up to 168 g/d for PA. The effect of PA on intake was 3 to 9 times greater than that of BA. While esters and ethanol showed slight negative correlations in some studies, there was no relationship in other studies. Ruminal infusion of AA, PA, and BA resulted in a significant reduction in intake with PA having the greatest effect. Interestingly, no effect on DMI was observed when the concentrations of VOC were increased due to heterofermentative lactic acid bacteria. When the VOC were added as pure substances, only AA seems to have a negative effect on intake. However, in some studies, there was no effect of AA on feeding behaviour. The extent of reduction in DMI varied from 5 to 10% depending on the amount of AA added to the diet. Apart from AA, the other VOC such as PA, ethanol, 2,3-butanediol, propanol, methanol, and esters showed no significant effect on preference or DMI, and at low concentrations, BA and some esters seem to increase preference. When high concentrations of the substance were used, the reduced motility and the subsequent reduction in passage rate due to AA, PA, and BA accumulation, and oxidation of acetyl coenzyme A in the liver in case of PA, and palatability factors in case of AA are thought to be the major mechanisms responsible for reduced preference and DMI. Silage metabolomic studies revealed some VOC which may have some palatability effects on preference or DMI.

Conclusions: Due to the formation of several VOC simultaneously, it is difficult to assign the effect to a particular VOC from silage intake models. It is likely that the combination of different fermentation end products rather than a single compound may be responsible for lower silage feed intake when compared to non-fermented forage. Infusion studies used very high quantities of VOC which are not found in silage and were infused at high rates into the rumen. The studies that added PA in roughage are lacking. In some cases, the presence of VOC such as AA and PA may outweigh their disadvantages. This is because of the improved aerobic stability due to the presence of AA and PA. Silage metabolomic studies may help in identifying the VOC which are not routinely measured.

Bibliographical references

[1] KUNG L., SHAVER R.D., GRANT R.J., SCHMIDT R.J. (2018): J. Dairy Sci. 101:4020-4033

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50.

Application of rumen anaerobic fungi-derived enzymes as additives in straw and grass silages

Nutzung anaerober Pansenpilzenzyme als Additive in Stroh- und Grassilagen

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Straw constitutes a widely neglected by-product of grain production, particularly with regards to its use in livestock feeding. The intense lignocellulosic complexes present in straw limit the ruminal degradability and must therefore be macerated to improve its nutritional utilization. Although the ensiling process itself is known to improve the ruminal degradability of straw [1], additional treatments are necessary to sufficiently cleave recalcitrant fiber structures and therefore allow a higher exploitation of such substrates. Anaerobic fungi (AF) are potent fibrolytics in the rumen, possessing a diverse enzyme repertoire, and thus are crucial for the degradation of tough plant fiber [2]. Consequently, using AF enzymes during ensiling may support cleavage of lignocellulosic biomass in the silo, which then would result in an enhanced ruminal fiber degradability and finally improved energy exploitation by the ruminant. Therefore, our research aimed to develop a protocol for isolating anaerobic fungal enzymes and subsequently use them as a silage additive in both straw and grass silages to improve their quality characteristics.

Methods: For isolating the AF-derived enzymes, a mixture of fungal strains isolated from the ovine rumen, was cultivated on xylan for four days. Afterwards, the complete fungal culture was centrifuged and the supernatant was stored at -20 °C. Further refinements of the isolation protocol included ultrafiltration and lyophilization steps. For each protocol tested, activities of β-glucosidase and xylanase were determined according to [3]. Silages were produced in glass jars using either wheat straw or grass at the research dairy farm of the University of Veterinary Medicine Vienna (Pottenstein, Austria). Both wheat straw and grass were manually cut to 5 cm particle length before ensiling. All silages were prepared with approximately 35% dry matter (DM) concentration. Thus, grass was wilted in the sun, whereas tap water was added to wheat straw. Additionally, sucrose was added to wheat straw before ensiling (10% of DM). Regarding the applied treatments, both grass and straw were ensiled with either 16 mL of AF enzyme solution or 16 mL of heat-inactivated AF enzyme solution as control. Each treatment was prepared in triplicate and stored for 90 days. Subsequently, the fermentation patterns of all silages were determined and analyzed in an ANOVA using SAS v9.4 (SAS Institute Inc., USA). At present, silages are under analysis of proximate nutrient composition and ruminal fiber degradability.

Results: Compared to the original supernatant, activities of β-glucosidase and xylanase could be increased fivefold and eightfold, respectively, when the supernatant was further ultrafiltrated, lyophilized and finally re-diluted: 34 vs. 170 µg / ml / h for β-glucosidase and 508 vs. 4120 µg / ml / h for xylanase. Regarding the silages, the AF treatment decreased the pH by 0.31 units to pH 4.14 ($P=0.04$). Likewise, AF enzymes generally reduced the acetic acid concentration in the silos ($P<0.01$) and for straw silages, acetic acid was less abundant in AF treatment (16 g / kg DM) than in the control (26 g / kg DM; $P<0.01$). Moreover, we observed a tendency for lower DM losses in silages treated with AF enzymes compared to control silages ($P=0.07$), i.e. 1.6 % vs. 2.1 %.

Conclusions: Our pending analyses will show whether the beneficial effects of AF enzymes observed at silage level are reflected in ruminal degradability. However, the present fermentation data seem to already indicate that AF enzymes can improve silage quality, particularly when ensiling strawy biomass.

Bibliographical references
[1] ZHAO J., DONG Z., LI J., CHEN L., BAI Y., JIA Y., SHAO T. (2018): Bioresour. Technol. 266:158-165
[2] HESS M., PAUL S.S., PUNIYA A.K., VAN DER GIEZEN M., SHAW C., EDWARDS J.E., FLIEGEROVÁ K. (2020): Front. Microbiol. 11:2621
[3] LEVER M. (1977): Anal. Biochem. 81:21–27

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51.

Effects of ensiling and thermic treatment on protein solubiltiy and *in vitro* fermentation of faba bean partial crops

Einfluss von Silierung und thermischer Behandlung auf die Proteinlöslichkeit und in vitro Fermentation von Ackerbohnenschröpfschnitten

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Partial crop (PC) of faba bean (FB) can be used as a forage for ruminants, which combines relevant protein, fiber and energy concentrations. Ensiling of legume forage presumably increases protein solubility (PS) while thermic treatment is assumed to cause the opposite. We hypothesized that i) toasting PC with DM contents of around 500 g/kg and ii) later stage of maturity (up to 700 g DM/kg) reduce PS. Furthermore, iii) content of metabolizable energy (ME) and *in vitro* organic matter digestibility (IVOMD) are not affected by maturity or treatment.

Methods: FB were hand-harvested with actually 500 (PC500) and 708 (PC700) g DM/kg at approximately 20 cm height and ensiled without additives for 62 d. Both native and ensiled FB were toasted on laboratory scale at 160°C for 60 min. *In vitro* incubation was conducted in 1:2 (v/v) ruminal fluid-buffer solution (pH = 6.74; redox potential, -187 mV) at 39 °C with 80 rpm agitation for 48 h, 4 runs for each material [1]. Pressures of accumulated headspace gas in the fermenters were measured using the ANKOM RF Gas Production System. DM, crude protein (CP) and other nutrient concentrations were analyzed according to VDLUFA methods [1]. Concentrations of organic acids and ethanol in silages were determined by HPLC and aerobic stability using the method of Honig [2]. Protein fractions were determined as defined by the Cornell Net Carbohydrate and Protein System (1992). Based on 24 h-gas production (GP), IVOMD and ME concentrations were calculated according to GfE [3]. Statistical analysis was performed by SAS 9.4 using a model with fixed effects of maturity, treatment (ensiling, toasting, ensiling + toasting) and their interaction and a random effect of run. Differences of GP profiles were assessed using likelihood ratio 95% confidence intervals. Statistical significance was considered at $P < 0.05$.

Results: FB silages (PC500/PC700) had a final pH of 4.85/6.12 and per kg DM, 37.9/22.7 g lactic acid, 4.3/2.1 g acetic acid, and 11.8/4.3 g ethanol/kg DM. Propionic acid, i- and n-butyric acid, iso- and n-valeric acid, 1,2-propanediol, and 1-propanol were only present in traces. The silages were stable under aerobic conditions for at least 7 d and the pH remained at 4.91/6.09 (PC500/PC700). CP (g/kg DM) and PS (% of CP) were in PC500 195 and 56 in native, 202 and 58 in ensiled, 194 and 29 in toasted and 210 and 38 in ensiled plus toasted FB and in PC700 187 and 54 in native, 206 and 60 in ensiled, 163 and 40 in toasted and 201 and 34 in ensiled plus toasted FB. Ensiling of PC500 had no effect on PS. All other treatments both in PC500 and in PC700 had significant effects on PS ($P < 0.05$). GP profiles, IVOMD and ME did not notably differ between maturity stages or among treatments. IVOMD of all treatments ranged in PC500 between 75 and 76 % and in PC700 between 72 and 78 %; ME ranged between 10.4 and 10.5 MJ and 9.8 and 10.6 MJ/kg DM, respectively.

Conclusions: Toasting of native PC of FB significantly reduced PS and hence, it can be expected that FB protein becomes more protected against ruminal degradation. Ensiling is instead a practically relevant method for conservation and harvest at later maturity (700 g DM/kg) may overcome detrimental effects on PS. The combination of ensiling and thermic treatment is then successfully reducing PS.

Bibliographical references
[1] VDLUFA (2012): Methodenbuch Vol. 3. VDLUFA-Verlag, Darmstadt
[2] HONIG K. (1990): Proc. EUROBAC Conf., 73-78
[3] GfE (2017): Proc. Soc. Nutr. Physiol. 26: 194-202

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52.

Effect of nutritive quality and grass share of semi-natural pastures on the nitrogen utilisation of lactating dairy cows

Einfluss des Nährstoffgehalts und Grasanteils semi-natürlicher Weideflächen auf die Stickstoffnutzung laktierender Milchkühe

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Grazing-based milk production exhibits several benefits, such as the chance to turn unobtainable grassland protein into human edible protein. A diet exclusively based on forages from pasture or other grassland, however, can possibly cause harmful excesses of nitrogen (N) for the animal and environment. On organic dairy farms using semi-natural grassland for grazing, this challenge might be enforced by a lack of control over the high variability in crude protein (CP) content, e.g. owing to a diverse species abundancy. The objective of this study was, thus, to examine the influence of the nutritional and botanical composition of semi-natural pastures on the N utilisation of lactating dairy cows. It was hypothesised that the effect of pasture quality would differ between animals supplemented with or without concentrates.

Methods: In 2019 and 2020, on-farm trials were conducted on six organic dairy farms in Southwest Germany. Each farm was visited for 2 or 4 periods á 6 days. Per period, 8 to 28 cows were selected among the herd for daily sampling of milk and urine. Pasture forage was sampled once per period. The share of grass in the aboveground pasture biomass was visually determined, and used as proxy for botanical composition with three categories of grass share (high: >60 % of fresh matter (FM); medium: <60 and >45 % of FM; low: <45 % of FM). Milk was analysed for contents of urea N (MUN) and protein, urine analysed for N (UN), creatinine (C) and purine derivatives (PD), and pasture samples for N and metabolisable energy (ME). Animals were classed by daily concentrate supplementation: with either ≥0.6 (Con: n=144) or <0.6 kg dry matter concentrate (NoCon: n=116). The effects of concentrate group in interaction with pasture CP, ME or grass content on MUN, milk protein yield, UN, N to C, PD to C, and PD to N ratio were analysed using mixed models with cow as random effect. A p-value<0.05 was considered as statistically significant.

Results: Average (±standard deviation) milk and milk protein yield of groups Con and NoCon were 26.1 (±6) and 0.841 (±0.154) kg / d, and 21.4 (±5) and 0.683 (±0.124) kg / d, respectively. The milk protein yield was, thus, significantly greater in the Con group, and significantly affected by pasture CP, ME and grass content, however, without an interaction with concentrate group. A significantly greater MUN and N to C ratio was observed with increasing CP and decreasing ME content, and with a medium grass share. A significant interaction indicated that pasture quality had a lower effect on MUN and N to C ratio in the Con group. A greater ME was related to a lower PD:N ratio, i.e. to a lower N utilisation by rumen microbes, in the Con group. The NoCon group benefited from an increase in pasture ME content through an increase in PD:N ratio. The PD:C ration was not affected by concentrate group nor by the grass share, but there was a positive relation to pasture CP and negative correlation with pasture ME.

Conclusions: Significant effects of nutritional and botanical composition were observed on N utilisation parameters of lactating dairy cows grazing semi-natural grasslands. Excess in pasture CP and a lack of ME, for instance, likely resulted in increased N to C ratios, thus, in a potentially higher N excretion via urine. A medium share of grasses between 45 and 60 % of the fresh pasture biomass was related to a lower N utilisation than with a high or low grass fraction. The N utilisation of animals with concentrate supplementation was affected to a smaller extend by pasture quality. For more profound interpretation of these findings, the factors influencing the nutritional and botanical composition of semi-natural grassland should be investigated further.

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53.

Sensory evaluation of hygienic status of conserved forages and concentrate feeds in Austrian dairy farms

Sensorische Prüfung vom Hygienestatus der konservierten Grünfutter und Kraftfutter österreichischer Milchviehbetriebe

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Sensory evaluation of conserved forages (hay, silages, and straw), as well as concentrate feeds on-farm has multiple advantages for feeding management and the veterinary practice. It makes possible a fast and cheap obtainment of information concerning the hygienic status of the main dietary ingredients of rations. Thus, the sensory analysis is a complementary method to the commonly employed laboratory chemical analysis, which is representative for the time of sampling, small sample and can vary widely from spot to spot and depending on the delivery time. The sensory evaluation of feeds allows the identification of objective organoleptic characteristics related to the acceptance as well as and feed safety/health risks (e.g., presence of toxic plants and mould/mycotoxin contamination) of the dietary rations of dairy cattle.

Methods: 100 farms dairy farms located in Styria, Lower and Upper Austria were inspected by a trained veterinarian during April 2019 and January 2020. Per farm, hygienic status of conserved forages and concentrates included in the rations of the lactating cows were evaluated. For the evaluation were considered representative samples (of at least 10 subsamples, which were composited [around 2 Kg] and immediately assessed). The sensory evaluation was performed considering characteristics of the appearance (colour along with the presence of impurities), odour and texture based on the methodological approaches described by Kamphues et al. (2014) [1].

Results: The most recurrent dietary ingredients included in the rations of dairy cows in the surveyed farms were concentrates (100%), grass silages (96%), maize silage (82%), straw (66%) and hay (33%). Maize silages represented the feedstuff with the highest occurrences of deficiencies regarding hygienic status (minor: 45%, significant: 2% and vast: 7%). Grass silages showed minor, significant, and vast deviancies in the hygienic status-related characteristics in 30, 8 and 3% of the inspected samples, respectively. Straw, hay, and concentrates presented deficiencies in 32%, 9% and 3% of the samples.

Conclusions: Most of the evaluated feeds presented proper hygienic status-related characteristics. However, silages (the main ingredients of the rations of Austrian dairy cows [2]), in special maize silage, showed the highest grade of hygienic status deficiencies, which were primarily related to mould contamination, which could implicate mycological and mycotoxicological risks for the dairy herds.

Bibliographical references

[1] KAMPHUES J., WOLF P., COENEN M., KLAUS E., IBEN C., KIENZLE E., LIESEGANG A., MÄNNER K., ZEBELI Q., ZENTEK J. (2014): IV Beurteilung von den Futtermitteln. IN: Supplemente zur Tierernährung für Studium und Praxis. Schlütersche, Hanover, Germany, 181-191

[2] FAO, IDF, IFCN (2014): World mapping of animal feeding systems in the dairy sector. Rome. 34-36

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54.

Estimation of microbial protein flowing from the rumen in dairy cows

Schätzung des Flusses von mikrobiellem Protein aus dem Pansen bei Milchkühen

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Estimation of protein supply to ruminants requires knowledge on the amount of microbial crude protein (MCP) flowing from the rumen. The GfE [1] stated an average of 156 ± 24 g of MCP per kg of digested organic matter in the total tract (DOM). Relating MCP to organic matter truly fermented in the rumen (FOM) may better reflect energy availability in the rumen than DOM. Moreover, carbohydrate- and nitrogen-containing fractions may impact MCP flow. Therefore, validity of the GfE approach was evaluated with more recent data. It was also tested if FOM was a better predictor of MCP flow than DOM.

Methods: The evaluation was based on a data set build by Roman-Garcia et al. [2], which was completed by additional studies found in literature. Either DOM or FOM had to be reported and estimates of MCP were all based on samples from the gastrointestinal tract. Dietary treatments were restricted to a maximum concentrate proportion of 0.6 and 10% to 20% of crude protein (CP) in dietary dry matter (DM). Treatments with more than 1% animal by-products were excluded to obtain results representative for dairy farms in Central Europe. Each treatment had to be tested with at least three animals and feed intake and chemical composition of the diets were incorporated in the data set. The resulting data set had 408 data lines from 137 studies. Effects of different microbial markers (^{15}N, purines, DAPA, NIRS) and sampling sites on MCP flow were tested by analyses of variance using the GLM procedure (SAS version 9.4). Moreover, concentrations of rumen-degraded CP (RDP) and ruminal N balance (RNB) were calculated for each treatment when possible. Mixed models applying the MIXED procedure with study as random effect were used to test the relation between MCP and DOM or FOM, respectively. Then dietary characteristics including feed intake, digestibility of OM (OMD) and fermentability of OM (OMF), RNB as well as dietary concentrations of CP, RDP, neutral detergent fibre, starch and ether extract were offered as additional variables to improve prediction accuracy.

Results: There was no significant effect of sampling site on MCP / DOM or MCP / FOM, but results differed depending on microbial marker. However, because all markers bear disadvantages, the marker type was not added to the models. Estimation of MCP flow with DOM (n = 324) or FOM (n = 349) as the only predictor revealed that 140 g ± 60 g or 166 g ± 69 g of MCP may flow from the rumen for each kg of DOM or FOM, respectively ($p < 0.001$). Calculation of mixed models using further predictors resulted in improved goodness-of-fit criteria, resulting in the following equations:

$$\text{MCP/DOM (g/kg)} = 305 - 2.74 \times \text{OMD} - 0.175 \times \text{CP} + 0.486 \times \text{RDP}$$

n = 289, $R^2 = 0.929$, $p < 0.001$, RSME = 10.0, study effect -75.8 to 61.2 g / kg

$$\text{MCP/FOM (g/kg)} = 294 - 2.80 \times \text{OMF} + 0.401 \times \text{RDP}$$

n = 310, $R^2 = 0.927$, $p < 0.001$, RSME = 12.7, study effect -100 to 71.9 g / kg

with OMD and OMF in % and CP and RDP in g / kg DM.

Conclusions: Compared with the estimate of GfE [1], less MCP flows from the rumen per kg of DOM and variation is larger with the new data set. Estimation of MCP / DOM and MCP / FOM using mixed models resulted in equations also including OMD or OMF as well as RDP and CP as predictors. These equations not only account for energy availability but also for CP characteristics. Prediction based on DOM is more precise than prediction based on FOM.

Bibliographical references

[1] GfE (2001): Empfehlungen zur Energie- und Nährstoffversorgung von Milchkühen und Aufzuchtrindern. DLG-Verlag, Frankfurt am Main, Germany

[2] ROMAN-GARCIA Y., WHITE R.R., FIRKINS J.L. (2016): J. Dairy Sci. 99: 7918-7931

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55.

Growth hormone receptor-insulin-like growth factor I axis in the kidney of young goats fed a nitrogen-reduced diet

Wachstumshormonrezeptor-Insulin-ähnlicher Wachstumsfaktor I-Achse in der Niere bei jungen Ziegen mit stickstoffreduzierter Fütterung

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Excretion of nitrogen (N) is an important source of environmental pollution and contamination. Therefore, ruminant feeding protocols should be modified to reduce pollution. However, feeding young goats a low N diet resulted in decreased plasma concentrations of insulin-like growth factor I (IGF-1) and insulin with unchanged plasma levels of growth hormone (GH) [1]. Previous studies have shown that hepatic expression of IGF-1 mRNA was decreased, resulting in lower blood IGF-1 levels, whereas renal IGF-1 mRNA expression increased in goats fed a low N diet [1]. The aim of the present study was to determine the effects of a low N diet on the expression of the growth hormone receptor (GHR) and subsequent IGF-1 signaling pathway in the kidney cortex of young goats, assuming organ-specific synthesis of IGF-1 mRNA expression.

Methods: Seventeen young male colored German goats (two months of age) were divided into two groups, receiving either an adequate or a reduced N supply (20% crude protein versus 9% crude protein). Both diets were isoenergetic and were offered for eight weeks. Kidney cortex samples were obtained after slaughter and the expression of GHR and the following IGF-1 pathway was determined by qPCR and Western blot analyses. Data were analysed by unpaired Student's t-test.

Results: Dry matter and concentrate intake did not differ between the two feeding groups, while N intake was significantly lower in the N reduced animals. Body weight at the end of the experiment was also not affected by feeding. Kidney mRNA expression of GHR was reduced, whereas the corresponding GHR protein was not altered. A statistically significant increase in the expression of janus kinase 2 (JAK2), signal transducers and activators of transcription 3 (STAT3) and suppressor of cytokine signalling 3 (SOCS3) was detected during a reduced N diet in the kidney of young goats. No other statistically significant differences were found for STAT1, STAT5B, SOCS2, the IGF-1 receptor, or the insulin receptor. The increased expression of STAT3 could be a consequence of the increased amount of JAK2 which led to higher IGF-1 mRNA expression in the kidney.

Conclusions: These results suggest that this increase in renal IGF-1 mRNA expression in the kidneys is due to alternative pathways that are not stimulated by GH. Further investigation of these pathways is needed to understand how and why low dietary N stimulates these pathways differently in the kidney than in the liver.

Bibliographical references

[1] FIRMENICH C., SCHNEPEL N., HANSEN K., SCHMICKE M., MUSCHER-BANSE A. (2020): Br. J. Nutr. 123(6): 652-663

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56.

Impact of the dietary protein quality and concentration on the fecal microbiota of healthy adult cats

Einfluss der Proteinqualität und -konzentration im Futter auf die fäkale Mikrobiota von adulten, gesunden Katzen

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Cats are obligate carnivores with a high protein requirement [1]. Although diets for cats are therefore usually rich in protein, the quality and amount of protein provided differs. As an impact of dietary protein on the intestinal microbiota of cats can be assumed, the present study aimed to investigate this aspect in more detail. The hypothesis was that a variation in the protein quality, particularly in the collagen content of the diet, and the amount of protein would have an effect on the composition and metabolic activity of the intestinal microbiota of cats.

Methods: Ten healthy adult cats received 6 complete canned diets that differed in their protein quality and concentration, using a randomized cross-over design. The low protein quality diets contained a higher amount of collagen-rich ingredients and achieved crude protein concentrations of 367, 450 and 561 g / kg dry matter (DM) (hydroxyproline concentrations: 3.76, 8.45 and 9.44 g / kg DM). The high protein quality diets were formulated with higher meat percentages and a lower amount of collagen-rich ingredients and achieved crude protein concentrations of 362, 433 and 549 g / kg DM (hydroxyproline concentrations: 2.56, 3.76 and 4.45 g / kg DM) [2, 3]. Each diet was fed for 6 weeks. At the end of the feeding periods, fecal samples were collected to analyse the microbiota (16S rDNA sequencing) and microbial metabolites (standard laboratory techniques). For statistical data analysis, a repeated measures ANOVA was used, and polynomial contrasts were calculated, with $P < 0.05$ being statistically significant.

Results: A lower protein quality in the diet was accompanied by a generally higher relative abundance (%) of Fusobacterium in the feces of the cats ($P = 0.039$). Increasing dietary protein concentrations increased the relative abundance of Fusobacterium and Bacteroides as well as the concentrations of ammonium and i-valeric acid in the fecal samples (linear contrasts: $P < 0.05$).

Conclusions: Dietary protein affects the bacterial protein fermentation in the intestine of cats, with a stronger impact of the protein concentration than of the protein quality in the diet.

Bibliographical references
[1] PLANTINGA A., BOSCH G., HENDRIKS W.H. (2011): Br. J. Nutr. 106: S35-S48
[2] PASSLACK N., KOHN B., DOHERR M.G., ZENTEK J. (2017): PloS One 12: e0169822
[3] PASSLACK N., KOHN B., DOHERR M.G., ZENTEK J. (2018): BMC Vet. Res. 14: 225

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57.

Glutamine supplementation improves growth and influences plasma metabolite and free amino acid patterns in neonatal low birth weight piglets

Eine Glutaminsupplementierung verbessert das Wachstum und beeinflusst die Konzentration an Metaboliten und freien Aminosäuren im Plasma bei neugeborenen Ferkeln mit niedrigem Geburtsgewicht

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Low birthweight (L) impairs growth of neonatal individuals compared to normal birthweight (N) [1]. Supplementary glutamine (Gln) has been shown to benefit the growth of weaned piglets, whilst the effects in suckling neonatal piglets are unclear [2, 3]. This study investigated the effects of oral Gln supplementation to early neonatal piglets on growth, milk intake, plasma metabolite and free amino acid (AA) patterns.

Methods: At birth, pairs of new-born L (0.8-1.2 kg) and N (1.4-1.8 kg) male siblings born to gilts, were assigned to Gln (1 g · kg^{-1} body weight (BW)· day $(d)^{-1}$; Gln-L, Gln-N; n=24 / group) or isonitrogenous alanine (Ala, 1.22 g · kg^{-1} BW· d^{-1}; Ala-L; Ala-N, n=24 / group) and age-class (5 or 12 d, n=12 / group / age class) groups based on their birth weight. This was done to ensure there was no significant difference in the mean birth weight of the L (Gln-L, Ala-L) and N (Gln-L, Ala-L) groups. Litter size was standardized to 12 piglets within 24 h after farrowing. We measured piglet BW (daily), milk intake (d 1, 11-12), plasma metabolite, insulin, free AA and liver triglyceride (TG) concentrations (d 5, 12). Data was analysed using the MIXED procedure of SAS (9.4) with repeated measure where applicable.

Results: Gln-L had higher BW (+7.5%, d 10, P=0.07, d 11-12, P<0.05) and milk intake (d 11-12, +14.7%, P=0.02) than Ala-L. On d 5, plasma TG was higher (+34.7%, P < 0.1) and carnosine was lower (-22.5%, P<0.05) in Ala-L vs. Ala-N and Gln-L, while liver TG was higher (+66.9%, P=0.03) in Ala-L vs. Ala-N. On d 12 plasma urea was higher (+37.5%, P<0.05) in Gln vs. Ala groups, whilst several proteinogenic AA (e.g., histidine, leucine) in plasma were lower (P<0.05) in Ala-N vs. Gln-N and Ala-N vs. Ala-L. Plasma arginine was higher (P<0.05) in Gln-N vs. Ala-N on d 5 and 12.

Conclusions: In the early neonatal period, Gln supplementation moderately improved growth and milk intake, appeared to affect lipid metabolism in L and AA metabolism in N piglets, suggesting potential effects on intestinal and liver function.

Bibliographical references

[1] LI N., WANG W., WU G., WANG J. (2017): Br. J. Nutr. 117: 1390-1402

[2] JI F., WANG L., YANG H., HU A., YIN Y. (2019): Animal. 13: 2727-2735

[3] WU G., BAZER F.W., JOHNSON G.A., KNABE D.A., BURGHARDT R.C., SPENCER T.E., LI X., WANG J. (2011): J. Anim. Sci. 89: 2017-2030

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58.

Association of oral glutamine supplementation during the neonatal period, on growth and blood immune cell parameters in low birth weight piglets

Auswirkungen einer oralen Glutaminergänzung während der Neugeborenenperiode auf Wachstum und Immunparameter im Blut von Ferkeln mit niedrigem Geburtsgewicht

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Weaning is a period of increased stress that negatively impacts piglet growth and health, particularly low birth weight individuals (1). Glutamine (Gln) supplementation during weaning has been shown to improve piglet body weight and alter stress associated blood immune markers (lymphocytes and leukocytes) (2). We have previously reported that Gln supplementation from 1 – 11 days of life (d) increased the body weight of low birth weight (LBW) piglets (3). However, the effects beyond supplementation have yet to be examined. Therefore, the objective of this study was to investigate the effect of Gln supplementation from 1 – 11 d on pre- and early post-weaning body weight development, and the immunophysiological response to increasing weaning associated stress. We hypothesize that Gln supplementation of LBW piglets is associated with improved pre- and early post-weaning body weight development and blood lymphocytes and leukocyte profiles in response to weaning.

Methods: At birth (0 day (d)), 10 pairs of LBW (0.8–1.2 kg) and normal birth weight (NBW; 1.4–1.8 kg) male German Landrace littermates, born to first parity sows, were assigned to Gln (1 g/kg BW/d; n = 20) or Alanine (Ala, 1.22 g/kg BW/d; isonitrogenous to Gln; n = 20) supplementation groups. Assignment of each LBW or NBW sibling pair to a supplementation group was performed so that the mean birth weight of each experimental group (LBW-Gln vs. Ala) or (NBW-Gln vs. Ala) was not significantly different. At 24 hours (h) post farrowing, litter sizes were standardized to 12 piglets. Piglets were supplemented with 33% of their respective daily dose three times daily, until 11 d, and were suckled by their birth dam until weaning (28 d). At weaning, piglets were housed according to supplementation group (Gln or Ala). Body weight was recorded during the pre- (0 – 12, 14, 21 and 28 d) and early post-weaning (29, 30 and 32 d) phases. Post-weaning feed intake was recorded daily. Blood samples were collected via venipuncture pre- (27 d) and during early post-weaning (29, 30 and 32 d). Flow cytometry was performed to quantify total leucocyte count and Pappenheim stained blood smears were used to differentiate leucocytes (banded and segmented neutrophils, eosinophils, lymphocytes and monocytes). Data was analysed using the MIXED procedure of SAS, and where applicable, with repeated measures. Least square means were separated using the Tukey test ($P<0.05$).

Results: Throughout the study, LBW piglets were lighter ($P<0.05$) compared with NBW, and Gln supplementation had no effect on LBW or NBW body weight or average daily gain compared to Ala littermates. During weaning, no difference in BW was observed between LBW and NBW Gln piglets ($P<0.05$; day 28 – 30), and higher feed intake and feed intake per kg BW was observed in LBW Gln compared to LBW Ala offspring ($P<0.05$; day 29, 30). The number of monocytes ($P=0.05$; day 29) and eosinophils ($P=0.03$; day 30) were lower in NBW Gln compared to NBW Ala, and banded neutrophils ($P=0.05$; day 29) higher in NBW Gln compared to LBW Gln.

Conclusions: Oral Gln supplementation during the neonatal period appears to be associated with a transient improvement in LBW piglet feed intake, but no effect on body weight or blood immune cells. This suggests that neonatal Gln supplementation has a limited carryover effect on weaning growth performance.

Bibliographical references
[1] FIX J.S. et al. (2010): Livest. Sci. 132(1-3): 98-106
[2] JI Z. et al. (2019): Animal 13(11): 2727-2735
[3] LI Z. et al. (2019): EAAP Book of Abstracts 70th annual Meeting

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59.

Influence of oral glutamine supplementation on bacterial metabolites in stomach and jejunum in neonatal piglets

Einfluss einer oralen Glutaminergänzung auf bakterielle Stoffwechselprodukte im Magen und Jejunum von neonatalen Ferkeln

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The non-essential amino acid glutamine (GLN) has been shown to improve growth and immunological functions in pigs after weaning [1]. Low birthweight piglets (LBW) typically face a higher risk of mortality before weaning, often due to an impaired gastrointestinal function [2]. Bacterial metabolites, such as SCFA and lactate provide the host with energy, whereas biogenic amines have multiple metabolic effects. The aim of this study was to examine the concentration of bacterial metabolites in the stomach and jejunal digesta of low and normal (NBW) birth weight piglets under the influence of Gln or alanine (Ala) supplementation.

Methods: For the trialLBW (0.8 - 1.2 kg) and NBW (1.4 - 1.8 kg) male littermates born to gilts were paired. The piglets received an oral supplementation of either 1 g Gln / kg BW [3] or 1.22 g Ala / kg BW three times daily until d 12. Thus, four different groups were studied: LBW+Gln; NBW+Gln; LBW+Ala; NBW+Ala (n = 12 / group). Piglets suckled throughout the study. Animals were euthanized at 5 and 12 d and digesta of stomach and jejunum was snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis of SCFA, lactate and biogenic amines. Statistical analyses were performed using IBM® SPSS® Statistics (version 25, IBM, USA). Multivariate ANOVA procedure was used for testing the three fixed factors, birth weight, supplementation, age group and their interactions. Tukey test was conducted and differences were considered statistically significant at $p < 0.05$.

Results: L-lactate in the stomach was increased in 12 d old piglets supplemented with Gln ($p < 0.05$) compared to piglets supplemented with Ala. D-lactate in 5 d Ala compared to Gln supplemented animals was higher ($p < 0.05$) whereas at 12 d of age it was the opposite ($p < 0.05$). In jejunal digesta L-lactate decreased in all groups ($p < 0.001$) from 5 to 12 d. Acetic acid and total SCFA were higher in Gln supplemented animals at 5 ($p < 0.05$) and 12 d ($p < 0.01$). NBW Ala compared to NBW Gln supplemented piglets at 12 d of age had a significantly lower acetic acid ($p < 0.01$) and total SCFA ($p < 0.05$) concentration in the stomach content. Piglets supplemented with Ala showed increased i-butyric acid concentrations at 5 d of age. For i-butyric ($p < 0.001$) and i-valeric acid ($p < 0.05$) a decrease was observed from 5 to 12 d in all groups. Butyric acid was higher in all piglets supplemented with Gln ($p < 0.05$) and in all LBW compared to NBW piglets ($p < 0.001$). The Tukey test showed that butyric acid was significantly higher in jejunal digesta of LBW piglets supplemented with Gln compared to LBW piglets supplemented with Ala ($p < 0.05$) and to NBW piglets supplemented with Gln ($p < 0.01$). Butyric acid and total SCFA decreased in jejunum of all groups from 5 to 12 d ($p < 0.05$). Propylamine ($p < 0.01$) and spermidine ($p < 0.05$) concentrations were higher in the stomach of Gln supplemented piglets at 12 d. Age revealed a significant increase in histamine, spermidine, spermine , tyramine and total biogenic amines ($p < 0.05$) in stomach content of all groups from 5 to 12 d. In jejunal digesta spermidine was higher in 5 d old Ala compared to Gln supplemented piglets ($p < 0.01$). Birth weight positively affected spermine in 12 d old NBW compared to LBW piglets in jejunal digesta ($p < 0.05$). From 5 to 12 d propylamine decreased ($p < 0.05$) and spermine increased ($p < 0.05$) in jejunal digesta of all groups.

Conclusions: In the first two weeks after birth, concentrations of bacterial metabolites such as lactate and SCFA were higher in the stomach than in jejunal digesta, whereas for biogenic amines it is the other way around. Also, the biggest influence of Gln supplementation was seen in changes of metabolites in the stomach. This might indicate that early oral Gln supplementation might have an influence on pH and microbiota in the stomach and less changes in bacterial metabolites could be visible in jejunum because of a possible more proximal absorption.

Bibliographical references
[1] WANG et al. (2008): J. Nutr. 138:1025-1032
[2] HALES et al. (2013): J. Anim. Sci. 91:4991-5003
[3] HAYNES et al. (2009): Amino Acids 37:131-142

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60.

Use of low dosage amino acid mixtures to prevent stress-related piglet diarrhea

Einsatz niedrig dosierter Aminosäuremischungen zur Vorbeugung stressbedingter Ferkeldiarrhöe

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Weaning is a challenging period for piglets associated with reduced feed intake, impairment of gut integrity, and diarrhea. Previous studies demonstrate that supplementation with single functional amino acids promote piglets' performance due to the improvement of intestinal health. Thus, we hypothesized that a combination of amino acids provided beyond the postulated requirement for growth could facilitate the weaning transition. The amino acids used were chosen based on physiological actions as reviewed previously [1].

Methods: At the age of 28 days, 90 piglets (Danbred x Piétrain) derived from a commercial site were weaned and immediately transported over 100 min duration as additional stress factor. Thereafter, the piglets were allotted equally according to sex and body weight to three feeding groups and randomly distributed to pens in pairs of two animals. Piglets had ad libitum access to feed and water. Apart from the feed, all animals were kept under ceteris paribus conditions. The control feed (CTRL = basal diet) was calculated to meet the requirements recommended for weaned piglets [2]. The treatment groups were supplemented on top with 0.3 % amino acid mixture as follows: AAB-1 = 42 % L-arginine + 33 % BCAA + 25 % L-cystine or AAB-2 = 31 % L-arginine + 25 % BCAA + 19 % L-cystine, + 25 % L-tryptophan). The BCAA, L-leucine, L-valine, and L-isoleucine were premixed in a ratio of 2:1:1. The CTRL group did not receive a placebo. Faecal consistency was assessed daily according to a scoring system ranging from 1 (entirely liquid) to 3 (normal) to 5 (hard faecal pellets). The zootechnical performance was assessed weekly. After 28 days of experiment, a subset of 10 piglets per group were euthanized for collection of gut tissues (duodenum, jejunum, and ileum) to analyze gut morphology and relative mRNA expression of genes related to gut function. Performance and morphology data were analyzed by General Linear Model ANOVA, using SPSS Statistical Software (IBM SPSS Statistics Standard 25, Armonk, NY, USA). In case of significant effects ($p<0.05$), means of the groups were compared Tukey test. The ranked variable fecal score was analyzed by Kruskal-Wallis test. The gene expression data of gut tissues was analyzed by REST 2009 software (Qiagen GmbH, Munich, Germany).

Results: Weaner pigs supplemented on top with AAB-2 showed firmer feces after weaning and less days with diarrhea compared to CTRL. A recovery from the stress events of weaning and transport was observed after 14 days. The zootechnical performance was not affected by the experimental treatments. The jejunal expression of the MUC-2 gene was reduced ($P < 0.05$) by feeding AAB-2. Both amino acid mixtures increased crypt depth in the duodenum ($P < 0.05$).

Conclusions: Our results showed that, in our study, dietary supplementation with 0.3 % AAB had no significant effect on growth performance. However, AAB-2 markedly reduced the days with fecal scores ≤ 2 accompanied with a reduced gene expression of jejunal MUC2, thus indicating less stimulation of the intestinal immune system due to a less aggressive intestinal microbiota. The reduced incidence of diarrhea might indicate a lower stimulation of the intestinal immune system and / or an improvement of intestinal health due to synergistical effects of the single AA components even if provided in low dosage.

Bibliographical references

[1] LE FLOC'H N., WESSELS A.G., CORRENT E., WU G., BOSI P. (2018): Anim. Feed Sci. Technol. 245:104-116

[2] GLOAGUEN M., LE FLOC'H N., VAN MILGEN J. (2013): INRA Productions Animales 26:277-288

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61.

Estimating optimal amino acids intake of broiler chickens using Gaussian process regression and genetic algorithm

Schätzung der optimalen Aminosäureaufnahme bei Broilern mit Gaußscher Prozessregression und genetischem Algorithmus

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To take full advantage of the richness of experimental data, a principled framework for analysis and inference must be considered. Data analysis using modern mathematics and computer programming provides a basis to define proper prediction, optimization and information [1]. Two techniques, among others, used in the area of artificial intelligence (AI) are Gaussian process regression (GPR) [2] and genetic algorithm (GA). The GPR method is known to work well on small datasets (n<100). The evolving field of AI may be beneficial to understand complexities in animal nutrition. This study aimed to first-time establish GPR to model broiler performance based on daily intake of amino acids (AA). Optimizing nutrient intake based on developed models using GA was investigated to reach maximum performance.

Methods: A dataset consisting of 90 data lines from a study on 7–21-d-old broilers investigating varying intake of prececal digestible glycine equivalents (pcGlyequi), prececal digestible threonine (pcThr), and total choline (tCho) was used [3]. Two separate models were developed for average daily gain (ADG) and gain to feed ratio (G:F) based on the input variables pcGlyequi, pcThr, and tCho. A MATLAB (MathWorks, R2021b) program was developed to formulate GPR models using the fitrgp function. pureQuadratic and squaredexponential were chosen as basic and kernel functions, respectively. The hyperparameters of GPR models were obtained by minimizing the negative log-likelihood [2]. A k-fold cross-validation (k=10) method was used to monitor R^2 and root mean square error (RMSE) as goodness-of-fit criteria of the GPR models. The constructed models were subjected to optimization using GA to find the combination of input variables to maximize ADG and G:F of broilers. For the GA process, the mutationadaptfeasible and roulette wheel selection method with an initial population of 30, a generation number of 1000, and a crossover rate of 0.85 was set. Variable sensitivity ratio was calculated to rank the importance of the input variables for the outcomes.

Results: The GPR model resulted in R^2 values of the training sets of >0.99 (RMSE= 0.48 g / d) and 0.94 (RMSE=0.012 g / g) for ADG and G:F, respectively. For the validation sets, goodness-of-fit was R^2=0.96 and RMSE=1.49 g / d for the ADG model and R^2=0.87 and RMSE=0.017 g / g for the G:F model. Optimization showed that maximum ADG was achieved with intakes of 1397 mg pcGlyequi / d, 482 mg pcThr / d, and 83 mg tCho / d, while maximum G:F was achieved with 1038 mg Gly_{equi} / d, 421 mg pcThr / d, and 67 mg tCho / d. Converting the values to dietary concentrations revealed that the maximum ADG needed 2.63, 1.19, and 0.14% of $pcGly_{equi}$, pcThr, and tCho, respectively, in the feed while concentrations needed for maximum G:F was at 2.02, 1.15, and 0.12% of $pcGly_{equi}$, pcThr, and tCho, respectively. Ranking the input variables was the same for ADG and G:F: pcThr > $pcGly_{equi}$ > tCho. The combination of input variables for maximum ADG and G:F were similar to those obtained in previous study [3] where the same data were examined using artificial neural network modelling and optimization methods.

Conclusions: An AI based GPR method was successfully applied to model broiler performance based on their daily nutrient intake. The method offers an operator-independent optimization that performs well on small datasets. However, poor generalization may happen if the model does not learn with adequate informative data. This matter should be considered because animal nutrition data sets are often small.

Bibliographical references

[1] ROBERTS S., OSBORNE M., EBDEN M., REECE S., GIBSON N., AIGRIN S. (2013): Philos. Trans. Royal Soc. A. 371:20110550

[2] RASMUSSEN C.E., WILLIAMS C.K.I. (2006): Gaussian Processes for Machine Learning. MIT Press, Cambridge, MA

[3] SIEGERT W., AHMADI H., HELMBRECHT A., RODEHUTSCORD M. (2015): Poult. Sci. 94:1557-1568

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62.

Effect of the sex of broilers on the precaecal digestibility of amino acids from dehulled pea and lupin grains

Effekt des Geschlechtes von Broilern auf die praecaecale Verdaulichkeit von Aminosäuren aus geschälten Erbsen- und Lupinenkörnern

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The effect of the sex of broilers on the precaecal digestibility (pcD) of amino acids (AA) is not yet sufficiently clarified. Previous studies showed interactions between sex, age and genotype on the pcD of AA [1]. The aim of this study was to investigate the impact of the sex of broilers on the pcD of AA from dehulled pea and lupine grains.

Methods: Dehulled pea and lupine grains (crude protein, 187/382; crude fat, 16/78; crude fibre, 15/26 g/kg; as fed basis) of organic origin were used. The legume grains were included at levels of 0, 100 and 200 g/kg into a basal diet (0%, 10%, 20%) at the expense of maize starch and tested in 2 trials (1: peas; 2: lupines). All diets contained TiO_2 as indigestible marker (5 g/kg). During the first 4 weeks, the birds were fed a commercial starter feed. Afterwards the experimental diets were offered ad libitum for 5 or 6 days. Ten pens per sex with 6 to 8 birds each (Isa 757) were allocated to the individual treatment. In week 5, birds were sacrified by asphyxiation with CO_2, the content of the medial and terminal ileum was flushed out with distilled water, pooled for all birds of a pen and immediately frozen [2]. Total N, AA and TiO_2 were analysed in diets and freeze-dried ileal digesta. For measuring the pcD of AA, the feed intake of the last 24 h prior to digesta removal was recorded. The pcD of AA of the test ingredients were determined by linear regression analysis [3]. Differences in pcD of N and AA between male and female broilers were tested by using the procedures REG and MIXED (SAS 9.4, SAS Institute Inc., Cary, NC USA). The level of significance was preset at $P < 0.05$.

Results: The mean feed intake for diets with peas varied between 74 (basal diet, females) and 89 g/d (20%, males; $P = 0.021$) and 92 (20%, females) and 105 g/d (20%, males; $P = 0.077$) for diets including lupines. The mean pcD of essential AA (arginine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine) for dehulled pea grains ranged between 91 and 95 % for males and females. The level of mean pcD of essential AA from dehulled lupine grains was numerically lower ($P = 0.239$) in males (88 %) than females (91 %). The essential AA with the highest pcD was methionine for peas (males/females, 95/99 %) and arginine for lupines (males/females, 94/96 %). Contrary, tryptophan showed the lowest pcD for both, peas (males/females, 85/89 %) and lupines (males/females, 75/80 %). The pcD of none of the essential AA was significantly influenced by the sex ($P > 0.05$).

Conclusions: The pcD of essential AA of dehulled pea and lupine grains was not significantly affected by the sex of broilers under the conditions of the recent study. It seems however that female broilers have the potential to digest AA at a higher level until the end of the small intestine. This needs to be further studied with special attention to interactions between broilers' sex and the kind of feed. Continuing studies are required to examine whether this effect is applicable for further ingredients.

Bibliographical references
[1] KIM E.J., CORZO A. (2012): Poult. Sci. 91: 908-917
[2] KLUTH H., MEHLHORN K., RODEHUTSCORD M. (2005): Arch. Anim. Nutr. 59: 271-279
[3] RODEHUTSCORD M., KAPOCIUS M., TIMMLER R., DIECKMANN A. (2004): Br. Poult. Sci. 45: 85-92

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63.

Influence of an increasing dietary arginine content on performance parameters of starter broiler chickens

Einfluss steigender Arginin-Gehalte im Alleinfutter für Broiler auf Leistungsparameter

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Low crude protein (CP) diets are an important part in the discussion about resource-efficient animal production. To avoid over- or undersupply of essential amino acids, it is important to know the exact requirement. In corn-soybean-meal diets, L-Arginine (Arg) is regarded as the 5th limiting amino acid (Fernandez et al. 1994), however, the suggested Arg requirement may need to be adjusted with recent genetic improvements. Therefore, the present study investigated the Arg requirements in broilers.

Methods: A total of 990 male Ross 308 broiler chickens were randomly allocated to 7 dietary treatments. The animals were kept on floor pens (15 birds per pen), which were covered with wood shavings in a thickness of about 5 cm. Birds and feed were measured at pen level at day (d) 0 and d 10. The basal diet (T1) consisted mainly of corn, soybean meal, rapeseed meal and corn gluten meal (CP: 21.4%; AME: 2960 kcal/kg; digestible lysine: 1.28%; digestible methionine and cysteine: 0.95%) and had a deficient digestible Arg content of 1.02% of the diet. In treatments T2-T7, crystalline Arg was added to the basal diet in inclusion rates of 0.06%, 0.12 %, 0.18%, 0.30%, 0.45%, and 0.61%, respectively. Feed and water were provided ad libitum. Data were analysed with R (version 3.2.5.). Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were analysed using linear regression models (procedure lm of the core package). Residual plots were checked to evaluate model fit. Statistical significance was assessed at $P \leq 0.05$.

Results: The birds fed the basal diet (T1), had significantly lower weight gain, feed intake, and FCR than animals fed with Arg supplemented diets. Supplementation of Arg to a deficient basal diet improved the broiler's performance in a dose-response manner. Birds were reaching their maximum BW, ADG, ADFI and minimum FCR with 0.45, 0.33, 0.31, and 0.43% Arg supplementation, respectively. Therefore, 1.47, 1.35, 1.33 and 1.45% standardized ileal digestible Arg was estimated to be required to achieve the best BW, ADG, ADFI, and FCR results.

Conclusions: Adding Arg to a deficient diet led to gradually increased performance. It can be assumed that modern broilers benefit from a higher dietary Arg content as compared to Aviagen recommendations (Aviagen 2014).

Bibliographical references
[1] FERNANDEZ et al. (1994): Poult. Sci. 73(12): 1887-1896
[2] AVIGEN (2014): Ross 308 broiler nutrition specifications

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64.

Impact of varying trypsin inhibitor activities in differently processed soybean expellers on nitrogen utilization of male broiler chickens

Einfluss einer variierenden Trypsininhibitoraktivität in unterschiedlich aufbereiteten Sojakuchen auf die Stickstoffverwertung männlicher Mastbroiler

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Local and small-scale soybean cultivation has been steadily rising in Central Europe. Due to insufficient quality management, domestic soybean products often exceed the generally accepted threshold for trypsin inhibitor activity (TIA) of 4.0 mg / g in full-fat products. More recent investigations indicate that amino acid digestibility and thus performance and meat quality is affected even below that threshold (1,2). The present study examined the effect of finely graded differences in dietary TIA on nitrogen (N) utilization of male broiler chickens until the end of fattening.

Methods: Raw material for this study consisted of two homogenous batches of soybeans (Sultana and Merlin) with a native TIA of 37.3 mg / g and 40.5 mg / g. Four processing techniques were used (thermal, hydrothermal, pressure and kilning) to create thirty-four soybean expeller variants with gradual differences in TIA (0.3 mg / g - 23.6 mg / g). These soy variants as well as a control of solvent extracted soybean meal were included into a common grower and finisher diet for broiler chicks at fixed amounts (grower: 35%; finisher: 25%) and tested in a 35 d fattening experiment with 1,680 broiler chicks (grower phase: day 11 to 24; finisher phase day 25 to 35). At the end of the experiment, the birds were euthanized using electrical stunning and cervical dislocation. Two broilers per pen (420 in total) were randomly selected for determining feed N utilization. Feathers were removed from the bodies before freezing (-20°C) prior to homogenization in an experimental meat grinder. Subsequently, feathers and whole-body homogenates were analyzed for N according to standardized methods and total N intake (g / bird), total N retention (g / bird), total N retention efficiency (%) and total N excretion (g / bird) were calculated. Statistical analyses included linear regression models (R 3.6.1) with TIA as independent variable with an assumed threshold of significance at $P \leq 0.05$.

Results: The earlier reported linearly reduced feed intake with rising dietary TIA (1) consequently promoted a significantly and linearly reduced total N intake, with 43 g / bird and 68 g / bird being ingested by the groups with the highest and lowest dietary TIA (8.7 and 0.5 mg / g), respectively. Consequently, the reduced feed intake with rising dietary TIA levels led to a reduced N intake. The corresponding drop in N retention was even more pronounced and accounted for 1.82 g / bird ($P < 0.001$). The nitrogen retention efficiency declined by 1.68% with each unit further increase in dietary TIA. In contrast, N excretion remained unaffected by dietary TIA.

Conclusions: Rising levels of dietary TIA may linearly depress N retention due to progressive induction of protein deficit in diets composed according to standard recommendations. Earlier studies indicated a linear decrease in amino acid digestibility (1) as well as a linear reduction in breast muscle (2), and thus meat yield. Regarding these facts, it is indispensable to improve quality of domestic soybean products and consider TIA in official feeding recommendations.

Bibliographical references
[1] KUENZ S., THURNER S., HOFFMANN D., DAMME K., WINDISCH W., BRUGGER D. (2020): Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science, Wageningen Academic Publishers, ISBN: 978-90-8686-349-5, 434
[2] KUENZ S., HOFFMANN D., THURNER S., DAMME K., WINDISCH W., BRUGGER D. (2020): Proc. Soc. Nutr. Physiol. 29: 101
[3] HOFFMANN D., BRUGGER D., WINDISCH W., THURNER S. (2017): Chem. Eng. Trans, 58:379-384

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65.

Effect of a rumen-protected Zinc-methionine complex on distinct productive performance parameters of high lactating Holstein dairy cows during an environmental heat stress period

Auswirkungen eines pansengeschützten Zink-Methionin-Komplexes auf ausgewählte Leistungsparameter hochlaktierender Holstein-Milchkühe während natürlich auftretendem Hitzestressperiode

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Heat stress (HS) impairs the productivity of dairy cows either directly (e.g., lower milk production) or indirectly (e.g., decreased feed intake) [1]. In order to regulate core body temperature during HS, dairy cows decrease their feed intake. Consequently, the milk production as well as milk composition, specifically milk protein concentration, will be altered. Changes in productivity of the animals during this period cannot be simply justified with decreased feed intake. An imbalanced dietary amino acid (AA) supply might be more likely the reason as AA would be utilized for other pathways such as gluconeogenesis and heat shock protein synthesis during HS [2]. Dietary supplemental AA, particularly rate-limiting AA such as methionine, could be a critical step to overcome and alleviate the negative consequences. Besides, feeding dietary Zinc (Zn) seems to be beneficial for animals to maintain their core body function during HS. Recent studies have shown that addition of dietary organic Zn source could improve the mammary integrity in dairy cows under HS challenge [3]. The objective of this work was to determine the impact of a commercially available rumen-protected Zinc-methionine complex (RPZM) (Loprotin; Kaesler Nutrition GmbH, Cuxhaven, Germany) on performance of high producing Holstein dairy cows during a HS period.

Methods: Sixty-two multiparous lactating Holstein cows [balanced by days in milk (mean ± SD) = 28 ± 7 d; lactation number = 2.9 ± 0.6] were randomly assigned to one of 2 dietary treatments [TMR with RPZM (LP group) or without the RPZM inclusion (CON group)]. RPZM was included in the diet as 0.131% DM for a total period of 6 weeks. Air temperature (T) and relative humidity (RH) were monitored daily and temperature-humidity index (THI) was calculated based on $\mathrm{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$. Daily milk yield was recorded and weekly milk samples from individual cows were obtained at 3 consecutive milkings and analyzed for protein, fat, lactose, milk urea nitrogen, and total solids content. Fat corrected milk (FCM) standardized to 4 % fat was calculated as following: $\mathrm{FCM} = [0.4 \times \text{milk yield (kg)}] + [15 \times \text{milk fat (kg)}]$. Energy corrected milk (ECM) was calculated as following: $\mathrm{ECM} = [0.3246 \times \text{milk yield (kg)}] + [12.86 \times \text{fat yield (kg)}] + [7.04 \times \text{protein yield (kg)}]$. Weekly data of milk yield and milk composition was statistically analyzed using the Proc Mixed procedure of SAS (SAS Institute, 2003) for a completely randomized design with repeated measures. The model included the effects of group, week and the interaction between group and week.

Results: The average THI during the current experiment, i.e. whole period of 6 weeks, was 74 indicating a thermal stress for dairy cattle. Weekly feed intake between experimental groups was not significantly different ($p > 0.05$). The milk yield of the animals between the experimental groups was not significantly ($p > 0.05$) different (55.02 vs. 52.90 kg for LP and CON group, respectively). Both FCM and ECMwere significantly ($p < 0.01$) higher upon RPZM supplementationcompared with the control (48.80 vs. 42.52 and 52.85 vs. 46.70 kg, respectively).Both milk fat and protein concentrations of the animals in the LP group were clearly higher ($p < 0.0001$) compared with their counterparts in the CON group (32.78 vs. 27.30 and 31.03 vs. 30.14 g/kg, respectively).Milk fat yield was significantly higher ($p < 0.0001$) upon RPZM supplementation; however, this was merely a trend in milk protein yield ($p = 0.066$). Both concentrations of milk lactose and solid were evidently higher in the LP group in comparison with the control ($p < 0.05$).

Conclusions: Feeding RPZM was able to increase the milk fat and protein yield as well as the FCM and ECM in dairy cows under the environmental heat stress. The potent impact of RPZM supplementation on dairy cows metabolism, immune and oxidative status merits further investigation.

Bibliographical references

[1] BAUMGARD L.H., RHOADS R.P. (2012): J. Anim. Sci. 90:1855–1865

[2] RIUS A.G. (2019): Appl. Anim. Sci. 35: 39-48

[3] WENG X., MONTEIRO A.P.A., GUO J., LI C., ORELLANA R.M., MARINS T.N., BERNARD J.K., TOMLINSON D.J., DEFRAIN J.M., WOHLGEMUTH S.E., TAO S. (2018): J. Dairy Sci. 101:2617–2630

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66.

ProtiPig – Prediction of protein retention of growing pigs from blood metabolites

ProtiPig – Schätzung des Proteinansatzes wachsender Schweine anhand von Blutmetaboliten

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Previous studies have shown that the efficiency of nitrogen utilisation (NUE) varies among pigs of the same breed under standardized feeding conditions, indicating that a genetic influence on NUE may exist [1,2]. Large data sets are needed for genetic analyses. However, the effort of precise measurement of NUE in balance studies with a large number of pigs is very high. Therefore, easier recordable traits are necessary to determine the NUE of a larger population of animals. Thus, the objective of this study was to establish prediction equations based on nitrogen (N) balance data and blood metabolites.

Methods: As part of a larger experiment described in a companion abstract, 56 DL×Pi barrows with a defined family structure were individually housed in metabolism cages in two sampling periods (SP1 and SP2). Diets with a marginal level of lysine supply (90% of GfE recommendations) were provided for ad libitum intake. Following two days of adaption to the cages, faeces and urine were totally collected for four days to determine N retention and calculate NUE as N retention divided by N intake. For calculation of lysine utilisation efficiency (LUE), a lysine concentration of 7.2% in the retained body protein was assumed and the lysine retention was divided by lysine intake. Blood samples were taken on three consecutive days at about 13:00 h via puncture of the vena jugularis and analysed for blood urea nitrogen (BUN), serum cortisol (SC) and IGF-I concentrations. Data were analysed by one-way ANOVA, considering the fixed effect of SP and multiple linear regressions were performed to predict N retention and NUE based on N balance data and blood metabolites using the MIXED procedure in SAS. All possible combinations of variables were compared simultaneously and ranked according to their respective Akaike Information Criterion. The obtained models were validated using a Bootstrapping procedure. Goodness of fit of the derived prediction equations were evaluated using the adjusted R^2 and the root mean square error (RMSE).

Results: The pigs mean bodyweight (BW, 59.4 kg), average daily feed intake (ADFI, 2.21 kg dry matter) and average daily gain (ADG, 0.86 kg) were all significantly higher in SP2 than SP1 (40.0 kg, 1.69 kg and 0.77 kg, respectively), but varied widely among the individuals and SP (29.8 to 72.0 kg for BW, 1.33 to 2.88 kg for ADFI, and 0.50 to 1.17 kg for ADG). N retention was highly correlated with N intake and lysine intake (0.70 and 0.73, respectively) and the mean N retention did not differ between SP1 (27.9 g/d) and SP2 (26.7 g/d). The range in N retention was from 14.8 to 38.0 g/d. The mean NUE was significantly higher in SP1 (47.4%) than in SP2 (43.0%) and showed remarkable variation (34.5 to 56.5%). The calculated LUE ranged from 57.1 to 89.1% and was not significantly different between the SP (70.2% SP1 vs. 69.0% SP2). N retention (NR, g/d) could be predicted with a maximum adjusted R^2 of 0.74 and a minimum RMSE of 1.94, considering initial BW (IBW, kg), ADG (kg), ADFI (kg dry matter), N intake (NI, g/d), BUN (mmol/l), SC (ng/ml), and IGF-I (ng/ml) as predictors. The following equation was derived: NR = -32.65 - 38.86*ADG + 1.605*NI + 4.977*BUN + 1.267*IBW*ADG - 0.600*IBW*ADFI + 2.324*ADFI*BUN - 0.070*ADFI*SC - 0.163*NI*BUN + 0.001*SC*IGF-I. Predictions made for NUE had a lower maximum adjusted R^2 (0.42) and higher minimum RMSE (3.42) than the predictions made for N retention.

Conclusions: The data suggest that a prediction of N retention of growing pigs at marginal lysine supply level is possible based on body weight, N intake, and serum metabolite data. This reduces the experimental effort compared to N balance studies when a large number of animals needs to be phenotyped. The present data set did not allow for an independent validation of the predicted equations, which warrants further studies.

Bibliographical references
[1] RUIZ-ASCACIBAR I., STOLL P., KREUZER M., BOILLAT V., SPRING P., BEE G. (2017): Animal 11: 394-404
[2] PÉREZ DE NANCLARES M., TRUDEAU M.P., HANSEN J.Ø., MYDLAND L.T., URRIOLA P.E., SHURSON G.C., PIERCEY ÅKESSON C., KJOS N.P., ARNTZEN M.Ø., ØVERLAND M. (2017): Livest. Sci. 203: 1-9

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67.

ProtiPig – Variability of the efficiency of nitrogen utilisation in pigs fed at marginal level of lysine supply

ProtiPig – Variabilität in der Effizienz der Stickstoffverwertung von Schweinen bei knapper Versorgung mit Lysin

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Improvement of the efficiency of nitrogen utilisation (NUE) is crucial for sustainable pig production. While nutritional tools for affecting NUE are well known, the potential of breeding is less investigated. A prerequisite for successful selection is the existence of genetic variation in the target trait. The objective of this study was to evaluate NUE and related traits in a large F1 pig population with a defined family structure and to assess the perspectives of breeding towards improved NUE.

Methods: A total of 508 crossbred offspring of 20 Pietrain boars and DL sows (half gilts, half barrows) were raised under standard conditions over a period of 2.5 years. Starting at 77 d of age (±6 d; mean BW 29.5 kg), a two-phase fattening period was conducted using barley-wheat-soybean meal-based diets with marginal lysine concentration (90% of GfE recommendation). Pigs were kept in individual pens to record feed intake. Change from starter to grower feed was in the 4th week of the experiment at a mean BW of 47.1 kg. Feed was provided for ad libitum intake in mash form in two equal portions at 08:00 h and 16:00 h. Two sampling periods (SP) were conducted, one in the starter phase (SP1: 3rd week of experiment, mean BW: 40.5 kg) and one in the grower phase (SP2: 6th week of experiment, mean BW: 60.0 kg). In each SP, blood samples were taken on three consecutive days at about 13:00 h via vena jugularis puncture and analyzed for blood urea nitrogen, serum cortisol and IGF-I concentrations. Nitrogen retention and subsequently NUE were estimated using prediction equations derived from N balance data and blood metabolites of a subsample of pigs (reported in a companion abstract). Data were analyzed by two-way ANOVA using the MIXED procedure in SAS, considering the fixed effect of sex and SP and by one-way ANOVA, considering the boar as treatment. All pigs were genotyped with the porcine Illumina 60k SNP chip. Genetic parameters were estimated using a genomic mixed linear animal model in R (ASReml).

Results: The mean average daily gain was 0.90 kg in SP1 and no effect of sex existed. In SP2, barrows (1.01 kg/d) gained significantly more than gilts (0.93 kg/d). In both SP, barrows showed a higher dry matter intake than gilts (1.90 kg/d vs. 1.83 kg/d in SP1; 2.61 kg/d vs. 2.41 kg/d in SP2). The mean gain:feed ratio was 0.49 in SP1 and 0.38 in SP2 without sex difference. N retention ranged from 13.8 to 46.4 g/d, with differences between SP and sex. In SP1, no significant difference between gilts (28.3 g/d) and barrows (29.1 g/d) existed, whereas in SP2, barrows retained significantly more N (32.1 g/d) than gilts (30.6 g/d). Within the range of lysine intake (15-25 g/d), N retention linearly depended on lysine intake in both SP. Each one gram of additional lysine intake resulted in 9 g of additional protein accretion. Values of NUE were at a high level and ranged from 34.1 to 57.9%. The mean NUE decreased from 48.4% in SP1 to 44.2% in SP2. In both SP, gilts had significantly higher NUE than barrows (48.7% vs. 48.0% in SP1; 44.8% vs. 43.6% in SP2). The progeny of the boar with the highest mean NUE (49.1%) was significantly different from that of the boar with the lowest mean NUE (44.0%). The estimated heritabilities were 0.33 for N intake, 0.25 for NUE, and 0.18 for N retention.

Conclusions: The linear relationship between lysine intake an N retention indicates that lysine was the limiting factor and pigs could express their genetic potential for protein accretion. The high variation in NUE, the significant boar effect, and the estimated heritabilities show the potential of a selection for improved NUE in growing pigs.

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68.

ProtiPig- The role of microbiota on protein utilization efficiency in pigs

ProtiPig- Die Rolle der Mikrobiota in der Proteinnutzungseffizienz bei Schweinen

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The protein utilization efficiency of the pig is limited. About two-thirds of the protein consumed is excreted in feces and urine. This has widespread consequences for the environment and need for resources. Feed rations with varying protein content can influence the intestinal microbiota; however, the quantitative significance related to variation in protein utilization efficiency is unknown. This study aimed to characterize the fecal microbial and volatile fatty acid (VFA) composition of pigs, with a known family structure, in a two phase-feeding system based on diets with marginal lysine concentration (90% of GfE recommendation) and how it correlates with the efficiency of nitrogen utilization (NUE).

Methods: Offspring of boars were reared as described in Berghaus et al. abstract. In two sampling periods (SP1: mean BW of 40.5 kg and SP2: mean BW of 60.0 kg), one fecal sample of each pig (n= 458) was collected. DNA extractions were carried out with a commercial kit, and samples were submitted for Illumina amplicon sequencing. Bioinformatic analysis was conducted using QIIME 2 [1], followed by multivariate statistical analysis. Regarding the NUE values of the pigs, individual performance parameters were subdivided into three groups regarding the quantiles. Pigs belonging to the <25%- and >75%-quantile were used for further analysis to correlate NUE and microbiota. VFA in fecal samples were determined by gas chromatography.

Results: The microbial composition of the fecal samples at SP1 and SP2 differed significantly ($p < 0.05$). While Lactobacillus was the genus with the highest relative abundance in SP1 (16.9%), Unclassified Firmicutes was the most abundant group in SP2 (18.1%). The relative abundance of Prevotella 9 was significantly higher in SP1 (5.7%) compared to SP2 (4.4%) ($p < 0.05$). Clostridum sensu stricto was significantly less abundant ($p<0.05$) in SP1 (1.1%) compared to SP2 (2.5%). The concentrations of the VFA (acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, and valeric acid) significantly decreased from SP1 to SP2 ($p < 0.05$). In addition, a significant effect of the boars on the bacterial community of the pigs was revealed ($p < 0.05$). At the genus level, the relative abundance of Prevotella 9 and Megasphaera differed between fecal samples of pigs descended from different boars. It was observed a significant effect of the NUE on the microbial composition of pigs in SP1 ($p < 0.05$) and a trend for the SP2 ($p < 0.1$).

Conclusions: The study showed that microbial and VFA composition in the feces of pigs changed during phase feeding and was affected by the boars. Further analysis will be performed to analyze and visualize the correlation between the investigated pigs‘ microbial composition and function and other performance parameters.

Bibliographical references
[1] BOLYEN E., RIDEOUT J.R., DILLON M.R. et al. (2019): Nat Biotechnol 37: 852-857

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69.

ProtiPig – Protease activities and amino acid transporter expression along the porcine gastrointestinal tract

ProtiPig – Proteaseaktivitäten und Expression von Aminosäuretransportern entlang des porcinen Gastrointestinaltraktes

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To meet the animals‘ amino acid requirements, pigs depend on the regular intake of proteins and amino acids. However, pigs excrete around two thirds of the intake protein, and pork production is currently associated with high emissions of nitrogen (N) compounds. Thus, an optimized protein efficiency in pigs is still a key objective in order to reduce the use of valuable protein sources in the feed. The present study focuses on the characterization of the intestinal processes, including enzymatic activities and capacities of amino acid transporters, to identify possible correlations towards N efficiency.

Methods: Animals were raised as described in a companion abstract as part of a larger experiment and fed a diet with marginal lysine content. A total of 48 barrows (DL x Pi, average bodyweight 97.1 kg) were euthanised with ketamine and pentobarbital. Digesta and mucosa was sampled from stomach (sto), duodenum (duo), jejunum (jej), ileum (ile), caecum (cae) and colon (col). The proteolytic capacity of four pancreatic enzymes was determined in all digesta samples in three replicates using a microplate reader. Trypsin activities were measured using the Trypsin Activity Assay kit (ScienceCell) for 30 min in kinetic cycles. Chymotrypsin activities were measured using the Chymotrypsin Activity Assay Kit (BioVision) for 30 min with an excitation of 380 nm and an emission of 460 nm. Carboxypeptidase A activity was measured using N-(4-methoxyphenylazoformyl)-phenylalanine-OH potassium salt as substrate. The absorbance of the samples was measured at 350 nm for 10 min in kinetic cycles. Carboxypeptidase B activity was measured with N-(4-methoxyphenylazoformyl)-arginine-OH HCl as substrate using the same conditions as described before. The relative expression of the amino acid transporters was quantified in mucosal samples in three replicates of the duo, jej and ile. RNA was extracted and converted to cDNA. The expression of six amino acid transporters was measured in qPCR runs using β-actin as a reference gene. Pigs belonging to the <25%- and >75%-quantile were statistically analysed with JMP® Pro and correlated to performance parameters.

Results: Clear patterns of protease activities could be measured along the gastrointestinal tract. Average activities of trypsin increased from sto, duo, jej and ile (values in nmol/min/g) 0.4, 10.7, 13.0 and 11.4, respectively. A decline of 0.3 and 0.7 was shown in the cae and col, respectively. With chymotrypsin the values in the sto, cae and col with 11.8, 619 and 225 were lower than in the duo, jej and ile with 6,720, 7,756 and 7,303. The activities for carboxypeptidase A in the sto, cae and col were 853, 988 and 1357, while they increased in the duo, jej and ile to 40,988, 38,859 and 18,108. This trend was also found for carboxypeptidase B, with values in the sto, cae and col of 3,929, 9,301 and 4,786 and values in the duo, jej and ile of 96,431, 90,268 and 66,922. Quantitative PCRs to measure gene expression rates (normalized to ΔΔCq) of the transporter SLC1A1 revealed 23.2, 28.9 and 127.4 in the duo, jej and ile, respectively. The values for SLC1A5 were 34.2, 23.5 and 6.6. The expression for the transporter SLC5A1 was overall at a lower level with 9.2, 8.1 and 7.0. The transporter SLC6A19 showed values of 26.1, 29.5 and 25.5, while the transporter SLC7A1 showed values of 32.1, 10.7 and 17.3. The transporter SLC7A9 showed expressions of 46.7, 26.5 and 52.5.

Conclusions: The results showed that the enzymes are mainly active in the small intestine. No generalized statement can be drawn for the transporter expression rates. While SLC1A1 and SLC7A9 showed the highest levels of expression in the ile, gene expression of SLC5A1 and SLC7A1 were highest in the duo and SLC1A5 and SLC6A19 in the jej. Further data surveys will be conducted to identify possible correlations between the measured parameters and other animal data.

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70.

Can high quality hay replace concentrate-rich starter feeds in early calf nutrition without adverse effects on feed intake and daily gain?

Können in der Aufzuchtkälberfütterung kraftfutterreiche Starter durch hochqualitatives Heu ohne Einbußen in Futteraufnahme und Tageszunahme ersetzt werden?

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In the first months of life, calves are often fed starch-rich starter diets to promote early solid feed intake. However, a recent study showed that feeding starch-rich diets to calves can cause similar ruminal acidosis symptoms like in adult ruminants [1]. High quality forage could be an alternative in early calf nutrition. In a trial with dairy cows, feeding high quality hay reduced risk of ruminal acidosis and increased feed intake compared to a conventional hay-concentrate diet [2, 3]. Therefore, the hypothesis of the current trial was that feeding high quality hay as only feed source can replace concentrate-rich starter feeds in early calf nutrition without adverse effects on feed intake and daily gain.

Methods: This study consisted in a 2 × 2-factorial arrangement of diets with hay quality (medium quality hay vs. high quality hay) and concentrate inclusion (no concentrate inclusion vs. 70% concentrate in diet) as experimental factors. This resulted in four dietary treatments: 100% medium quality hay; 0% concentrate (MQH), 100% high quality hay; 0% concentrate (HQH), 30% medium quality hay, 70% concentrate (MQH+C), 30% high quality hay, 70% concentrate (HQH+C). 40 Holstein Friesian calves (20 male, 20 female) were randomly allocated to one of the four groups immediately after birth. In the first four weeks of life, calves had ad libitum access to acidified whole milk, and afterwards daily milk supply was continuously reduced until weaning at the end of week 12. Furthermore, calves were offered water and the experimental diet ad libitum from the first day of life until the end of the experiment on day 99. Milk, water and solid feed intake was recorded daily and live weight once a week. Samples of hays and concentrate were taken once a week and afterwards pooled samples of four consecutive weeks were used for analysis of nutrient composition. On the last seven days of the experiment, feces was collected to analyze apparent digestibility of diets. Furthermore, blood samples from each calf were collected on days 1, 3, 7, 21, 49, 77 and 91 to study the dietary effects on metabolic parameters.

Results: Compared to MQH, HQH was characterized by a higher CP (149 vs. 210 g / kg DM), WSC (124 vs. 205 g / kg DM) and ME concentration (9.4 vs. 11.2 MJ / kg DM) and a lower NDF content (522 vs. 455 g / kg DM). In the first 8 weeks of life, solid feed intake of calves was below 0.5 kg DM / day and did not differ between groups. From week 10 on, solid feed and total DM intake differed significantly between groups. The MQH group ingested less feed than the other groups, especially in weeks 13 and 14. Therefore, these calves also had lower CP and ME intake in this phase, while the other groups did not differ from each other. The difference in DM and nutrient intake between MQH and HQH fed calves was likely due to a significantly higher apparent OM, CP and NDF digestibility of HQH. Daily gain did not differ between diets until week 11 of life, but calves fed MQH and MQH+C gained less body weight and had higher feed to gain ratio than the two groups fed HQH after weaning (week 13 and 14). After weaning, calves fed only hay had higher beta-hydroxybutyrate (BHB) and cholesterol concentrations in the blood than those fed hay-concentrate mixtures.

Conclusions: The results of this study show that high quality hay can be used as an alternative to concentrate-rich starters in early calf nutrition without adverse effects on feed intake and daily gain. In contrast, feeding MQH as only solid feed reduced feed intake and daily gain. Furthermore, higher BHB and cholesterol contents in the blood of calves indicate that feeding hay-only diets promotes the development of rumen morphology and metabolism compared to hay-concentrate mixtures.

Bibliographical references

[1] GELSINGER S.L., COBLENTZ W.K., ZANTON G.I., OGDEN R.K., AKINS M.S. (2020): J. Dairy Sci. 103: 2762-2772

[2] KLEEFISCH M.-T., ZEBELI Q., HUMER E., KRÖGER I., ERTL P., KLEVENHUSEN F. (2017): Arch. Anim. Nutr. 71: 21-36

[3] KLEVENHUSEN F., KLEEFISCH M.-T., ZEBELI Q. (2019): J. Anim. Physiol. Anim. Nutr. 103: 466-476

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71.

Effect of replacing concentrates with a high and medium-quality hay in the starter feed of dairy calves on the development of chewing and gut fermentation

Einfluss des Ersatzes von Kraftfutter durch hoch- und mittelqualitativem Heu in der Fütterung von Aufzuchtkälbern auf die Entwicklung des Wiederkauens und der Pansen- und Darmfermentation

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Grain-rich starter feeds increase lactic acid concentration and decrease pH in the rumen, decrease the blood flow to the rumen wall and thereby rumen motility and these can lead to hyperkeratosis of rumen epithelium [1]. Palatable, high-energy hay, could be an alternative to concentrate-based starter diets in the feeding of dairy calves, because this hay might increase the structural effectiveness of the diet, enhancing chewing activity and likely rumen pH [2]. The aim of this study was to evaluate the effect of the composition of calf starter feed (hay-only vs. a hay-concentrate mixture) and hay quality on chewing and gut fermentation of dairy calves.

Methods: In this study, 40 Holstein-Friesian calves were randomly assigned to four different dietary treatments based on the birth weight (42.9±6.2 kg, n = 10 per group). The dietary treatments were: 100% high quality hay (HQH); 100% medium quality hay (MQH); 30% high quality hay + 70% concentrate (HQH+C) and 30% medium quality hay + 70% concentrate (MQH+C). The HQH had a higher crude protein (149 vs. 210 g / kg DM), water-soluble carbohydrates (124 vs. 205 g / kg DM) and metabolisable energy (9.4 vs. 11.2 MJ / kg DM) and lower neutral detergent fiber (522 vs. 455 g / kg DM) content compared to MQH. All calves received the same amount of milk and had ad libitum access to starter feed and water from day 1 to the end of the experiment (day 99). The hay was chopped and mixed together in the concentrate groups. Samples of ruminal fluid, feces, blood and rumen mucosal thickness (measured in the 11th intercostal space with an ultrasound scanner) were taken on several days of life according to a sampling plan. Chewing activity was measured on three consecutive days with RumiWatch halters in weeks 4, 6, 10 and 12 of life. Data were analyzed with SAS using PROC MIXED and a 2×2-factorial arrangement of diets with hay quality and concentrate inclusion as experimental factors.

Results: Calves fed only hay had higher daily peNDF>8mm intake compared to the concentrate groups from week 7 on ($P < 0.05$). The HQH group had the highest peNDF>8mm intake and showed the highest rumination time (min / d) and number of rumination boli per day in week 12 of life. Furthermore, eating time (min / d) was higher in calves fed only hay in week 10 and 12 compared to concentrate groups. The MQH group had the highest ruminal (from day 63 on) and fecal pH (from day 21 on). The ruminal and fecal pH of HQH fed calves was lower than in the MQH group but slightly higher than in calves fed hay and concentrates. Ruminal ammonia concentration tended to be higher in the HQH group from day 35 on. Calves fed only hay had higher proportions of acetate in ruminal and fecal short chain fatty acids (SCFA). In contrast, the concentrate fed groups had a higher propionate proportion in total SCFA. Furthermore, the HQH group had the highest butyrate proportion in ruminal SCFA from day 63 on. Interestingly, the fermentation capacity in the hindgut was already evident in the first fecal sample (meconium) of all calves with SCFA levels averaging 33 µmol / g (mainly acetate). The RMT and blood parameters showed no significant differences between the feeding groups.

Conclusions: Feeding only HQH resulted in significant increased peNDF$>_{8\ \mathrm{mm}}$ intake and rumination time, suggesting sufficient structural effectiveness of HQH, but this effect was not reflected in improved ruminal or fecal pH. Furthermore, feeding of HQH enhanced butyrate release in the rumen without affecting RMT as an indicator of the rumen development. Overall, feeding HQH instead of concentrate-based starters resulted in improved rumination and ruminal fermentation profile, without affecting ruminal pH and systemic and stress health variables. In contrast, feeding MQH resulted in the highest ruminal and fecal pH.

Bibliographical references

[1] KHAN M.A., BACH A., WEARY D.M., VON KEYSERLINGK M.A.G. (2016): J. Dairy Sci. 99(2):885-902

[2] KLEEFISCH M.-T., ZEBELI Q., HUMER E., KRÖGER I., ERTL P., KLEVENHUSEN F. (2017): Arch. Anim. Nutr. 71: 21-36

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72.

Feeding and rumination in goats and sheep – a closer look at rumination chewing and feeding pattern

Fressen und Wiederkauen bei Ziegen und Schafen – eine genauere Betrachtung des Wiederkauverhaltens und der Fressmuster

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Feeding and rumination are dominant activities for ruminants (1). The total duration and pattern of these behaviors seems to differ to some extent among domestic ruminants (sheep, cattle and goats; 1, 2). A better understanding of feed comminution behavior is helpful to understand fiber processing capacity (especially relevant in low input systems) or fiber requirements (especially relevant in more intensive systems) of ruminants. In a follow up study to (3), besides dry matter (DM) intake and feeding and rumination behavior (min/d, g/min), we took closer look at the feeding (daily distribution) and rumination behavior (e.g. rumination boli and chews).

Methods: In this experiment, sheep and goats were offered mixed grass hay cut to two different chop lengths in a 2 x 2 factorial, cross-over design. Each experimental period consisted of ten days of adaption and five days of data collection. Four non-lactating sheep (Blackface, ~83 kg body weight [BW]) and four non-lactating goats (Weiße Deutsche Edelziege, ~78 kg BW) were housed in two separate pens, fitted with the Calan Broadbent Feeding System, allowing individual feed intake measurements. Goats and sheep had ad libitum access to the hay (leftovers ~10 %) which was refilled twice a day (0700 and 1600 h). The mixed grass hay (nutrient composition, all per kg DM: 100 g crude protein, 636 g NDFom (neutral-detergent fiber corrected for residual ash), 337 g ADFom (acid detergent fiber corrected for residual ash) was chopped to two particle lengths: “long” (~35 mm; LH) and “short” (~7 mm; SH). Intake was recorded over five consecutive days. During these five days, feeding and ruminating was recorded using video cameras. Feeding was defined as each event during which the animals lowered their heads into feeder for more than 30 sec. In addition, chewing behavior during rumination was evaluated in more detail (for 15 min at daytime plus 15 min at night). Chews per bolus were counted and extrapolated over 24 h. Data were analyzed using the mixed procedure of SAS (Version 9.4). The model included the fixed effects of species, chop length and their interaction. Animal was considered as random effect. Treatment means were compared by Tukey-Kramer test. Significance was declared at $P<0.05$.

Results: Goats consumed more long hay than short hay (LH: 75.5 g/kg^{0,75}, SH: 57.2 g/kg^{0,75}; $P<0.001$), but there were no differences for sheep (SH: 70.8 g/kg^{0,75}, LH 61.5 g/kg^{0,75}). There were no differences in daily rumination time for goats (SH: 509 min, LH: 547 min) and sheep (SH: 572 min, LH: 570 min), but goats and sheep had a longer feeding time for the long hay than the short hay (goats SH: 202 min, LH: 245 min; sheep SH: 185 min, LH: 236 min; $P<0.001$). Feeding and rumination duration did not differ between sheep and goats. The through was more often visited by goats (SH: 44 times/d, LH: 40 times/d) than by sheep (SH: 22 times/d, LH: 21 times/d; $P<0.05$). Sheep ruminated more boli per day (SH: 600, LH: 604) compared to goats (short hay: 483, long hay: 544; $P<0.05$) but mean duration of rumination time per bolus was similar between species and chop lengths. Goats and sheep had a higher chewing frequency (chews/sec) for short hay compared to long hay (goats SH: 1.19, LH:1.26; sheep SH: 1.40; LH: 1.49; $P<0.001$), while sheep had a higher frequency compared to goats ($P<0.001$).

Conclusions: In this study, no differences in total feeding and rumination time were obvious for sheep and goats, in contrast to (3). Sheep had significant more rumination boli than goats but no differences in the duration and chews of one boli. However, the chewing frequency was higher for sheep than for goats. Daily feeding pattern seems to be distinctively different between sheep and goats, which fits a prediction of (2).

Bibliographical references

[1] BAUMONT R., DOREAU M., INGRAND S., VEISSIER I. (2006): In BELS V. Feeding in Domestic Vertebrates - From Structure to Behavior: 241-262

[2] HOFMANN R.R. (1989): Oecologia 78: 443-457

[3] KRONE B., HÜNERBERG M., HUMMEL J. (2021): Proc. Soc. Nutr. Physiol. 30: 87

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73.

Young grass-clover-silage in the diet of fattening pigs

Junge Rotkleegrassilage in der Ration von Mastschweinen

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Roughage feeding is obligatory in organic farming. In case of red-clover-silage, roughage can also contribute to the protein and amino acid supply of the diet [1]. There may be an effect on the consumption and the dietary value of silages, when pigs are habituated to it from birth or later in their development. The aim of our study was to evaluate the effect of feeding grass-clover silage beginning at a body mass (BM) of 50 kg or from birth on the fattening performance and carcass quality of pigs.

Methods: The experiment took place at the experimental farm of the Thünen Institute of Organic Farming in Northern Germany. Two identical trials were conducted in 2020-2021. The fattening pigs were born on the farm ((Norwegian land race*large white) / Pietrain) and raised according to the standard protocol of the farm (47d suckling period). Upon reaching 28 kg BM, a total of 80 pigs per trial was separated into 2 groups (Silage vs. Straw) with 4 replicates each. The group Straw had no access to silage until the mean BM reached 50 kg. The group Silage had access to grass-clover silage from birth. Both groups were fed with diets containing grass-clover silages from 50 kg BM until slaughter (final fattening = FF). Grass-clover silage for feeding trials was cut in May before pod formation and pressed at the same day. Feedstuffs were mixed and pelleted in the institute's milling and mixing installation. Trial diets were formulated to contain local feedstuffs. In the pre-fattening period (PF; 28-50 kg BM), the Silage group was fed 0.5 kg grass-clover-silage and a pelleted feedstuff lowered in protein and lysine content compared to the Straw group (Straw 166.3 g crude protein and 9.0 g Lys per kg feed with 88% DM; Silage 155.3 g crude protein and 8.7 g Lys per kg feed with 88% DM). Until 50 kg BM the pelleted feedstuffs were fed semi ad libitum. All groups were provided with a diet lowered in energy, protein, and lysine content from 50 – 122 kg BM (12.0 MJ ME, 120.9 g CP, 5.8 g Lys per kg feed with 88% DM). The pelleted feedstuff was restricted according to a feeding curve used on the farm starting with 2.2 kg / d up to 2.9 kg / d (fresh matter). Grass-clover-silage was added semi ad libitum (increase when rack was empty before 4 PM) starting with 1.5 kg per animal up to 3 kg fresh matter per animal. The pigs were weighed weekly and slaughtered upon reaching 119 kg BM in order to maintain a mean slaughter BM of 121 kg. Dressing percentage, lean meat content, and pH value after 60 minutes were recorded at the slaughter house. A general linear mixed effects model was used in R (package lmerTest, R stat version 4.0.2) to compare the feeding groups. Group was used as fixed factor, run, sex, pen and sow were used as random factors.

Results: There were no significant differences between the groups regarding fattening performance and carcass characteristics (Daily weight gain (DWG): Straw 792 g, Silage 802 g (SE 61.5, $p = 0.58$); DWG PF: Straw 723 g, Silage 732 g (SE 82.2, $p = 0.78$); DWG FF: Straw 822 g, Silage 833 g (SE 57.3, $p = 0.60$); age at slaughter: Straw 192 d, Silage 188 d (SE 9.5, $p = 0.45$); dressing percentage: Straw 78.5 %, Silage 77.9 % (SE 0.60, $p = 0.13$); lean meat content: Straw 57.8 %, Silage 58.4 % (SE 1.19, $p = 0.12$); Feed conversion PF (DM): 2.6 g (SE 0.25, $p = 0.88$); Feed conversion FF (DM): Straw 3.3 g, Silage 3.2 g (SE 0.26, $p = 0.34$)). In the FF, an average silage amount of 199 kg (Straw 193 ± 17.5 kg, Silage 205 ± 13.8 kg) was offered to each animal.

Conclusions: Although all pigs were fed a FF diet containing low amounts of crude protein, fattening performance and carcass quality were good. This might also be due to the good farm management. All piglets were born on a farm, where silage feeding is common practice. Thus, comparative studies would be needed to observe if pigs born and raised on a farm without silage feeding would have the same good fattening performance and carcass quality when fed silage during fattening.

Bibliographical references

[1] WITTEN S., MACHNER M.-T., BUSSEMAS R., AULRICH K. (2021): Proc. Soc. Nutr. Physiol. 30:46

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74.

Effect of a rye-based compound feed for lactating sows on performance of piglets

Effekte eines roggenbasierten Laktationsfutters für Sauen auf die Ferkelentwicklung

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Sow feeding in Western Europe has mainly focused on the cereal grains wheat and barley. Due to changing climate and agronomic practices, it may be of interest to the farmer to grow rye and use it for the sow. Rye is preceded by the bias of ergot contamination but breeding improvements (PollenPlus®) have reduced the risk of ergot in rye and therefore made it possible to feed it to breeding animals [1]. Additionally, rye is associated with advantages: Non-Starch-Polysaccharides (NSP) are present in rye to a greater extent than in other cereals. NSP are increasingly converted to butyrate in the animal‘s large intestine [2], which is said to exert positive effects on the animal’s organism, in this case might be mirrored in the sow’s milk production and hence in piglet weights.

Methods: Feeding trials were carried out in three different sow herds (h1-3). According to technical circumstances, parallel feeding was only possible in h1, h2+3 fed alternately for farrowing groups. The control diets (C) - the farm’s specific commercial lactation diets (h1: meal, h2: pellet, h3: liquid feed) contained the main components wheat, barley, cereal bran and soybean meal. The diets were converted isonitrogenously into the experimental diets (R) by reducing the proportion of wheat and barley allowing to add 30% of rye. No further feed additives were supplemented. Diets followed the general recommendations of the GfE for lactating sows [3].The sow’s individual feed allocation started one week before farrowing with increasing amounts until the end of lactation (3.5 ± 0.5 to 8.25 ± 0.25 kg/d). Individual piglet weights were determined on the first day of life (h1 n(C/R)=302/291; h2 n(C/R)=482/529; h3 n(C/R)=264/289) and on the day prior to weaning (h1 n(C/R)=264/262; h2 n(C/R)=419/459; h3 n(C/R)=232/232). Statistical evaluation was conducted with SAS Enterprise Guide® (t-test).

Results: Adding rye to the compound feed had no negative effect regarding sow’s feed intake. R groups in h3 were exposed to outdoor temperatures on average 10°C higher compared to C groups, which led to a lower feed intake in general. The investigations showed significant difference in mean individual body weight (BW, kg) on day one in h2+3 (h1 C 1.33 ± 0.31, R 1.37 ± 0.37// h2 C 1.31^{a} ± 0.34, R 1.23^{b} ± 0.33// h3 C 1.43^{a} ± 0.30, R 1.33^{b} ± 0.31) and the day prior to weaning, in h3 (h1 C 5.35 ± 1.38, R 5.53 ± 1.42// h2 C 6.45 ± 1.54, R 6.38 ± 1.49// h3 C 6.69^{a} ± 1.07, R 6.25b ± 0.92). Derived from the mean individual BW and days of lactation (h1 21d / h2+3 28^{d}), the average daily weight gain (g/day) showed no significant deviation (h1 C 201 ± 31, R 209 ± 31// h2 C 189 ± 40, R 187 ± 39// h3 C 194 ± 27, R 182 ± 22).

Conclusions: Results from h3 allow the assumption that there was temperature-related influence on feed intake and thus on piglet weights. The fact that individual piglet weights in h2 on day one were significantly lower in the R group, but not on the day prior to weaning, speaks in favor of the rye. To conclude, the feeding trial shows that rye can be reintroduced as a regional cereal component, besides wheat and barley, into sow’s diets in the peripartum period with shares of 30% without obvious disadvantage on either sow or piglets.

Bibliographical references

[1] KAMPHUES J., HARTUNG C., WILKE V., GRONE R. (2019): Übers. Tierernährg. 43, 107-163

[2] WILKE V. (2020): Effekte eines Mischfutters mit steigenden Anteilen von Roggen bzw. Roggen und Rapsextraktionsschrot auf die Verdaulichkeit und Leistung sowie Milieu- und Substratbedingungen im Magen-Darm-Inhalt junger Schweine, 134-139, DVG Verlag, Giessen

[3] GfE (2006): Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen, DLG-Verlag, Frankfurt am Main

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75.

Effects of dietary inclusion of rye for broilers on growth performance, litter quality and foot pad health: A field study

Auswirkungen eines Roggeneinsatzes im Mischfutter für Broiler auf Leistung, Einstreuqualität und Fußballengesundheit: eine Feldstudie

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Rye (Secale cereale) has advantages, namely tolerance for climate conditions, high resistance to moulds and its cultivation is relatively inexpensive. Thus, rye could be interesting in terms of the sustainability debate [1]. However, the use of rye for intensive poultry production has been limited to date. This study aimed to test the effect of upper inclusion levels of intact rye up to 20% in broiler chickens in a field study on growth performance, litter quality and foot pad health.

Methods: A total of 16214 (Trial 1) and 16120 (Trial 2) broilers were included in a field study and were divided into two equal groups in each trial. All birds were fed a commercial starter diet for the first week of life. Thereafter, the control group received commercial grower and finisher diets. Contrary to this, in the experimental group a pelleted supplementary feed (SF = supplementary feed to rye) was offered, to which increasing levels of intact rye were added of same batch in both trials (stepwise from 2% up to 20%). The SF-10% was mixed to a certain percentage with the commercial grower diet and rye from d 8 till d 18 of life, then only with rye from d 19 to d 21 of life. Likewise, the SF-20%, which was mixed to a certain amount with the commercial finisher diet and rye from d 22 to d 28, then only with rye from d 29 to d 33 of life. Final BW was obtained from slaughterhouse, while pure excreta and litter dry matter (DM) and foot pad scoring were done weekly in the farm. The $p < 0.05$ formed the basis of statistical significance.

Results: According to the farm data, the total feed intake was about 3039 g / bird vs 2863 g / bird for the experimental group in first trial, while in the second trial, the feed intake was about 2746 g / bird vs 2575 g / bird for the control and experimental groups, respectively. At d 33 of life, the birds fed control diets had about 2059 g BW vs 1947 g for those fed supplementary feeds with rye in the first trial (slaughterhouse data). For the second trial, the BW data of slaughterhouses at d 33 of life revealed that birds fed control diets had 1883 g vs 1747 g for birds fed SF with rye diets. The corrected FCR in the first trial was identical between the two groups (1.50), whereas in the second trial, the calculated corrected FCR was 1.49 for the control group vs 1.51 for the experimental group. In the first trial at d 32 of life, the excreta of birds fed the control diet had a significantly higher DM content compared to those fed SF with rye diets (22.6% vs 21.3%, respectively). Otherwise, no significant differences for the excreta DM content were observed between the two groups in the second trial (22.6% and 22.9% for control and experimental groups, respectively). Litter DM content in both trials from d 1 till d 28 was not differed significantly. At d 32 however, the DM content of the litter of broilers fed the control diet was significantly higher (66.7% and 66.3% for the first and second trials, respectively) compared to that of broilers fed SF with rye (63.8% and 62.4% for the first and second trials, respectively). At the end of the first trial (d 32), there were significant differences ($p < 0.05$) in foot pad lesions for the two feeding groups (2.70 vs 3.80 for control and experimental diets, respectively). Nevertheless, in second trial, no significant differences regards scores of foot pad were seen between the two groups.

Conclusions: The final BW was not differ between control and experimental groupsupon using two-factorial analyses (1890 g vs 1842 g, respectively).Including high proportions of intact rye with further adjustments in feed technology or the type / level of enzyme-addition, use of rye-based diet could get a valuable tool to increase sustainability in poultry. Further trials are running with additionally processing such as broken rye which may have a perspective promising results in broilers and turkeys.

Bibliographical references

[1] ABD EL-WAHAB A., LINGENS J.B., CHUPPAV B., AHMED M., OSMAN A, LANGEHEINE M., BREHM R, TAUBE V., GRONE R., VON FELDE A., KAMPHUES J., VISSCHER C. (2020): Sustainability 12(18): 7753

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76.

Effects of different levels of whole black soldier fly larvae in broiler rations on bone characteristics

Einfluss von unterschiedlichen Zugabemengen ganzer Schwarzer Soldatenfliegenlarven in Rationen für Broiler auf ausgewählte Knocheneigenschaften

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Black Soldier Fly larvae (BSFL) are known for their high Ca content [1]. They are one of only a few insect species to contain a wide calcium (Ca):phosphorus (P) ratio of up to 8.2:1 [2] [3]. We hypothesized that inclusion of up to 30% unprocessed BSFL in broiler diets have a positive influence on mineralization, in particular the ash, Ca and P content of the growing skeleton. Our objective was to determine which BSFL amount was the most beneficial.

Methods: Ross 308 chicks (n = 252) received daily either a control diet (CON) without access to BSFL or CON plus BSFL amounting to 10% (L-10), 20% (L-20) or 30% (L-30) of the feed intake (fresh matter) over a period of 6 weeks. Ash was determined according to proximate analysis; Ca and P after acid hydrolysis by atomic absorption spectrometry resp. photometer. Accordingly, CON and BSFL were composed as follows: Ash: 6.16, 4.83, and 4.48 for % of DM starter, grower, and finisher diets, respectively; 8.04 % of DM BSFL; Calcium: 1.21, 0.76, and 0.61 % of DM starter, grower, and finisher diets, respectively; 1.81 % of DM BSFL; Phosphorus: 0.60, 0.52, and 0.93 % of DM starter, grower and finisher diets, respectively; 0.93 % of DM BSFL. Feed intake of the CON birds was the basis for the calculation of BSFL amount given to the other 3 groups on the next day (d). On d 28 and 42 of the experiment, 12 animals/group were slaughtered and tibia and serum sampled. Size, weight, ash and mineral contents, and breaking strength of tibia were measured. Data were analyzed using the MIXED procedure of SAS followed by Tukey test to separate group means.

Results: At week 6, L-30 group had lower ($P<0.05$) body weight (BW) compared to L-20 group, but there was no difference to CON ($P>0.05$). On weeks 4 and 6 of the experiment, feeding up to 30% BSFL of the total feed intake to broilers did not affect serum Ca and P concentrations ($P>0.05$). On week 4, L-20 had a lower tibia diameter than CON ($P<0.05$), but there was no difference on week 6 among the groups ($P>0.05$). On week 4, there was no difference among the groups for tibia weight, however, on week 6 L-20 had a lower tibia weight than CON ($P<0.05$) but no difference was observed in L-30 group compared to CON ($P>0.05$). In addition, on week 6, L-10 tended to have a higher tibia length than L-20 ($P=0.099$). On week 4 there was no difference among the groups for tibia breaking strength ($P>0.05$), however, on week 6, L-20 tended to have lower tibia strength than L-30 ($P=0.092$). There was no difference among the groups for the tibia ash, Ca and P content ($P>0.05$).

Conclusions: Inclusion of 20% but not 30% BSFL was associated with negative effects on tibia condition. In addition, tibia characteristics of broilers fed 10% BSFL compared to birds fed CON diet did not differ. Our results suggest that there was no general negative effect of broiler rations containing whole BSFL for bone characteristics.

Bibliographical references
[1] BARRAGAN-FONSECA K.B., DICKE M., VAN LOON J.J. (2017): J. Insects as Food Feed 3(2):105-120
[2] FINKE M.D. (2013): Zoo Biol. 32(1):27-36
[3] SHUMO M., OSUGA I.M., KHAMIS F.M., TANGA C.M., FIABOE K.K., SUBRAMANIAN S., EKESI S., VAN HUIS A., BORGEMEITER C. (2019): Sci. Rep. 9(1):1-13

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77.

Reaction of ponies to increased sodium concentrations in their drinking water

Reaktion von Ponys auf erhöhte Natriumkonzentrationen im Tränkwasser

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Horses *(Equus caballus)* lose high amounts of Na during exercising through excessive sweating, their primary mechanism for heat dissipation [1]. Their sweat is hypertonic in relation to the plasma, and contains considerable amounts of Na. Fluid losses during endurance exercise can often not be replaced completely by voluntary water intake, resulting in dehydration. Hence, saline solutions have been used as rehydration therapy to regain post-exercise electrolyte balance. However, knowledge on voluntarily accepted saline water concentrations in horses is limited. The aim of the study was therefore to evaluate the sensitivity and tolerance of ponies towards different Na concentrations in their drinking water.

Methods: The study was performed under temperate conditions, involving six unexercised Shetland pony mares (average age of 8.2 ± 3.7 years, average body weight (BW) of 131.5 ± 11.6 kg), kept in individual pens. Hay, salt lick and water were offered for ad libitum intake. The experiment was conducted in three phases: (1) a control for 1 week with only fresh water provided in two buckets, (2) a pairwise preference test for 3 weeks offering in two buckets the choice between fresh water and a saline solution with stepwise increasing NaCl concentration (0.25, 0.5, 0.75, 1.0, 1.25 or 1.5%), and (3) a free-choice test for 3 weeks with six simultaneously provided buckets containing 0, 0.25, 0.5, 0.75, 1.0, or 1.25% NaCl, respectively. The BW, body condition score and intake of water, feed and Na were recorded. Statistical analyses were performed using R 4.0.5 with linear mixed-effects models and ANOVA. When assumptions for parametric tests were not met, non-parametric Friedman's tests were used.

Results: During the pairwise preference test, the ponies did not distinguish between fresh and saline water with 0.25% NaCl. However, they showed a clear preference for water with 0.5% NaCl, whereas solutions with more than 0.75% were avoided or rejected. Our results agree with pairwise preference tests, where weaning foals showed indifferent responses until 0.63% NaCl in their drinking water [2]. When the ponies were offered a free-choice system (phase 3), they exhibited a pronounced preference for fresh over saline water ($P<0.001$). The shares for the different concentrations from total drinking water intake were 55.5, 26.9, 9.1, 3.0, 2.7 and 2.8% for fresh water and NaCl concentrations of 0.25, 0.50, 0.75, 1.0 and 1.25%, respectively. Total daily Na intakes exceeded the recommended Na intake at maintenance of 0.9 at 100 kg BW and 1.4 g at 200 kg BW [3], averaging 0.15, 0.43 and 0.36 $g/kg^{0.75}$ during the control, pairwise preference and free-choice phase, respectively ($P = 0.009$). Interestingly, the Na intake via salt lick was not reduced as response to higher Na intakes via saline water, suggesting that not all voluntary Na consumption was related to nutritional requirement, but also to habit or taste preference.

Conclusions: The ponies exhibited a remarkable sensory discrimination capacity to detect different NaCl concentrations in their drinking water. The acceptance of solutions with low NaCl levels of 0.25 and 0.5% without adverse effects, shows their potential as rehydration solution for voluntary intake in exercising horses.

Bibliographical references

[1] MEYER H., HEILEMANN M., HIPP-QUARTON A., NORIEGA H.P., GOMDA Y. (1990): In: Contributions to Water and Mineral Metabolism of the Horse, 21-34. Parey, Hamburg

[2] RANDALL R.P., SCHURG W.A., CHURCH D.C. (1978): J. Anim. Sci. 47:51-55

[3] GfE (2014): Empfehlungen zur Energie- und Nährstoffversorgung von Pferden. DLG-Verlag, Frankfurt am Main, Germany

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78.

Content of major minerals in the empty body of growing Fleckvieh bulls fed rations with varying energy concentrations

Gehalt an Mengenelementen im Leerkörper wachsender Fleckviehbullen bei Fütterung von Rationen mit unterschiedlichen Energiegehalten

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The performance potential of Fleckvieh (German Simmental) fattening bulls has been improved by selective breeding during past decades. This might have affected the body composition and gain composition in growing bulls. Consequently, the animals‘ energy and nutrient requirements might have changed. A feeding experiment ending with a serial slaughter trial was conducted to reevaluate the deposition of energy and nutrients in growing Fleckvieh bulls, representing the current genetic level. This abstract presents data on the major minerals‘ content in bulls with different live weights after feeding diets with different energy concentrations.

Methods: 72 Fleckvieh bulls (age: 42 d, body weight (BW) 80 kg) were fed restricted amounts of milk replacer (120 g/l) and a concentrates/hay-based total mixed ration (TMR) until weaning at an average BW of 121 kg and subsequently on a TMR based on maize silage and concentrates for ad libitum intake. The fattening period began at an average BW of 225 kg. Bulls were randomly allocated to normal energy (NE) and high energy (HE) treatment groups fed 11.6 and 12.4 MJ ME/kg DM, respectively. Differences in the TMRs‘ energy concentrations were reached by varying the percentage of maize silage and concentrates. Bulls in the NE group were fed rations with 80 % maize silage and 20 % concentrates, while animals in the HE group were fed rations with 40 % maize silage and 60 % concentrates (basis DM). Individual feed intake was recorded daily and BW was determined at four-week intervals. The bulls were slaughtered in five final live weight groups of 120 (n=8), 200 (n=10), 400 (n=18), 600 (n=18), and 780 kg (n=18). During slaughter and carcass processing, the empty body weight was determined as the final live weight minus the contents of the urinary bladder and gastrointestinal tract (GIT) and the entire empty body was dissected to body tissue fractions: hide, blood, organs, empty GIT, body fat, muscle, bone and tendon. Body tissues were chemically analyzed for their mineral contents regarding calcium, phosphorus, sodium, potassium, sulfur, and magnesium per kg natural tissue. The mineral contents of the bulls‘ empty bodies (fresh matter basis) were calculated based on the mineral contents of the individual body tissues. Statistical analysis was performed using Proc Mixed of SAS (Version 9.4). The analysis included a two-way ANOVA with interaction (feed energy, weight group, feed energy x weight group). Results are shown in ranges and standard error and were compared by the PDIFF option with values of $p<0.05$ regarded as significant.

Results: The empty body weights of bulls in weight groups 120, 200, 400, 600, and 780 kg were 104, 176, 370, 553, and 734 kg, respectively. Since there were no significant effects of dietary energy concentration on mineral contents in normal and high energy treatment groups, the combined results of both animal groups are shown. The calcium content in the bulls‘ bodies did not vary between weight groups and averaged at 14.3 g/kg ±0.5. The contents of the other minerals decreased during growth ($p<0.05$; phosphorus content: 8.9-7.7 g/kg ±0.2; potassium content: 2.5-2.0 g/kg ±0.03; sulfur content: 1.7-1.5 g/kg ±0.02; sodium content: 1.6-1.2 g/kg ±0.02; magnesium content: 0.43-0.41 g/kg ±0.01).

Conclusions: Feeding different energy concentrations did not alter empty body mineral content in growing bulls. While the calcium content remained constant during growth, the content of the other minerals decreased with increasing live weight of the animals. Our results showed higher body mineral content compared to Fleckvieh bulls in previous studies (1), but the mineral content of current Fleckvieh bulls was comparable to data of growing Schwarzbunte bulls (2).

Bibliographical references

[1] SCHWARZ F.J., HEINDL U., KIRCHGESSNER M. (1995): Archives of Animal Nutrition, 48: 183-199

[2] SCHULZ E., OSLAGE H.J., DAENICKE R. (1974): Advances in Animal Physiology and Animal Nutrition, Volume 4: 1-70

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79.

Influence of dietary phosphorus concentration and breed on feed intake and growth performance of fattening bulls

Einfluss der Phosphorkonzentration der Ration und der Rasse auf Futteraufnahme und Leistung in der Bullenmast

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The officially valid standards for phosphorus (P) supply to fattening bulls in Germany were published 1995 [1]. Over the past years, however, the level of growth performance, as well as the final weight of fattening bulls has increased. This implies that P requirements may have changed and need to be adjusted. For this reason, a study was conducted to evaluate the effects of varying dietary P concentrations on performance of fattening bulls.

Methods: 36 Fleckvieh (FV, age: 157 d, body weight (BW): 226 kg) and 36 Braunvieh (BV, age: 152 d, BW: 211 kg) bulls were allocated by body weight and age at start of the trial to subgroups “P norm”, “P low”, and “P high”. Bulls were fed for ad libitum intake Total Mixed Rations (TMR) based on maize silage and concentrates. Pressed beet pulp silage and extracted soybean meal were included in diets to obtain low P concentrations. The diets of group “P low” contained a mineral mix without P, and was calculated to have concentrations of 2.8, 2.4, and 2.0 g P/kg DM for the starter, grower, and finisher period (25 % below recommendations [1]). For groups P norm and P high this diet was added by $Ca(H_2PO_4)2$ to obtain P supply according to recommendations and 25 % above recommendations, respectively. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. The bulls were slaughtered at a mean age of 466 d (SEM: 0.6). Data was evaluated by a two-factorial model with post hoc SNK comparison using SAS. Data of 68 bulls was used for analysis. Level of significance was set to $p<0.05$.

Results: There was no effect of dietary P concentration on DM intake, but BV bulls had lower ($p<0.05$) DM intake than FV bulls (8.8 vs 9.2 kg/d). Mean P intake was 30.6, 23.8, and 38.1 g/d ($p<0.05$) for groups P norm, low and high, respectively. This corresponds to 108, 86, and 136 % of recommendations for P supply as recalculated from actual requirement for maintenance (1 g P/kg DM) and gain (6.6 g/kg BW gain). There was no effect of dietary P concentration on final weight or daily gain, but final weight (685 kg) and daily gain (1.498 g) was lower ($p<0.05$) in BV compared to FV bulls (749 kg and 1.696 g). Most slaughter characteristics were unaffected by dietary treatment. Dressing percentage was, however, lower ($p<0.05$) in group P high (55.2 %) compared to group P norm (56.2 %), whereas group P low was comparable to other groups (55.7 %). Comparison of breeds revealed lower ($p < 0.05$) carcass weight, dressing percentage, eye muscle area, and haunch range in BV (374 kg, 54.7 %, 63.2 cm2, and 119 cm) than in FV bulls (424 kg, 56.7 %, 75.8 cm2, and 124 cm). Carcass classification was better ($p<0.05$) in FV than in BV bulls. P excretion, as calculated according to DLG [2] was calculated to be 6.2, 4.2, and 8.6 kg/animal in groups P norm, low, and high ($p<0.05$).

Conclusions: The results of the present study indicate that recommendations for dietary P supply exceed requirements for growth of fattening bulls even at a high level of zootechnical performance. A P supply above recommendations does not result in enhanced growth but leads to significantly increased P excretion. Therefore, it is concluded that for most practical diets, which are mostly based on maize silage as the sole roughage source and rape seed meal as the sole protein supplement, the use of a mineral mix with P is counterproductive. FV bulls revealed a considerable better fattening performance than BV bulls.

Bibliographical references

[1] GfE (1995): Empfehlungen zur Energie- und Nährstoffversorgung der Mastrinder. DLG-Verlag, Frankfurt a. Main

[2] DLG (2014): Bilanzierung der Nährstoffausscheidungen landwirtschaftlicher Nutztiere. DLG-Verlag, Frankfurt a. Main

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80.

Influence of monocalcium phosphate and phytase in the diet on phytate degradation in caecectomised laying hens

Einfluss von Monocalciumphosphat und Phytase im Futter auf den Phytatabbau bei caecectomierten Legehennen

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It is known from studies in broiler chickens, that mineral phosphorus supplementation decreases phytate ($InsP_6$) degradation when no phytase is supplemented to the feed and affects the efficacy of added phytase [1]. The only study on $InsP_6$ degradation in caecectomised laying hens [2] investigated effects of supplementation of 500 phytase units (FTU)/kg and mineral phosphorus was added as monocalcium phosphate (MCP), which also changed the calcium concentration of the feed. The present study investigated whether a higher dosage of phytase and the addition of MCP at unchanged calcium concentration of the feed influences $InsP_6$ degradation and concentrations of lower inositol phosphate isomers and myo-inositol in excreta of caecectomised laying hens.

Methods: Four diets mainly containing maize, soybean meal, rapeseed meal, grass meal, and a premix without mineral phosphorus were mixed. The diets were formulated without or with 6 g MCP/kg and without or with supplementation of 1,500 FTU/kg of an *E. coli*-derived 6-phytase (Quantum Blue). The calcium concentration was maintained equal by reducing limestone inclusion in MCP-supplemented diets. Each diet was tested in 5 replicates using a row-column design with ten 96–99-week-old caecectomised laying hens in two periods. In each period, the hens received 120 g of feed per day (equally distributed in two meals) while being housed individually in metabolism units for eight days. Total excreta were collected during the last four days of each period. The statistical model comprised MCP, phytase, and the interaction as fixed effects, and hen and period as random effects.

Results: The MCP × phytase interaction was not significant for $InsP_6$ degradation (P=0.054). Phytase increased $InsP_6$ disappearance from 13% to 83% (P<0.001) while MCP had no significant effect (P=0.100). Less $Ins(1,2,3,4,5)P_5$ and $Ins(1,2,4,5,6)P_5$ was determined in the excreta when phytase supplemented diets were fed (P<0.001) and MCP increased the concentration of $Ins(1,2,3,4,5)P_5$ (P=0.005). Higher and lower concentrations of $Ins(1,3,4,5,6)P_5$ and InsP(1,2,3,4,6)P5, respectively, were detected upon phytase supplementation with MCP having no significant effect. There was a significant interaction for excreta concentration of $Ins(1,2,5,6)P_4$ (P=0.031): In diets without phytase supplementation, it was low (≤0.5 µmol/g dry matter) and not significantly influenced by MCP. With 3.2 µmol/g dry matter, significantly more $Ins(1,2,5,6)P_4$ was found in the excreta upon phytase supplementation (P<0.001) and addition of MCP to phytase-supplemented diets further raised $Ins(1,2,5,6)P_4$ to 4.8 µmol/g dry matter (P<0.001). Supplementing phytase increased myo-inositol in the excreta from 0.2 to 0.6 µmol/g dry matter (P=0.002) while MCP had no effect.

Conclusions: The low $InsP_6$ disappearance without phytase and the big effect of phytase supplementation suggest that caecectomised hens can be used in phytase efficacy studies. The very low level of $InsP_6$ degradation without phytase supplementation may have caused, other than in broilers, that MCP did not reduce $InsP_6$ disappearance. The high concentrations of $Ins(1,2,5,6)P_4$ in phytase supplemented treatments indicate that dephosphorylation of this isomer was limited, as known from broiler chickens. MCP may enhance the limitation of dephosphorylation of this isomer in phytase-supplemented diets. The myo-inositol concentrations was on a lower level compared to ileal digesta of broiler chickens [1], which may be explained by microbial breakdown [3] or absorption in the rectum of caecectomised birds.

Bibliographical references

[1] SIEGERT W., ZUBER T., SOMMERFELD V., KRIEG J., FEUERSTEIN D., KURRLE U., RODEHUTSCORD M. (2019): Poult. Sci. 98:5700-5713

[2] AGBEDE J.O., KLUTH H., RODEHUTSCORD M. (2009): Br. Poult. Sci. 50:583-591

[3] YOSHIDA K.-I., AOYAMA D., ISHIO I., SHIBAYAMA T., FUJITA Y. (1997): J. Bacteriol. 179:4591-4598

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81.

A novel zinc oxide source for weaned piglets- Zinc reduction in feed and its influence on performance and fecal consistency

Eine neuartige Zinkoxidquelle für Absatzferkel – Zink-Reduktion im Futter und der Einfluss auf Leistung und Kotkonsistenz

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For many years, high amounts of zinc (up to 3000 mg/kg feed) have been used as a feed additive for weaning piglets to prevent or treat diarrhea as well as to improve their performance. However, several studies indicated that the use of high levels of zinc bears some serious disadvantages like the development of antimicrobial resistances, an undesirable impact on the environment. Hence, the European Union restricted maximum zinc inclusion levels in feed for pigs from 2022 to 150 mg zinc per kg dry matter and thus, efficient alternatives are of great interest. It was the aim of the study to investigate the effects of a novel, porous zinc oxide source and a standard zinc oxide source at different inclusion levels on weaned piglets' performance and faecal consistency.

Methods: For this study, 1440 weaned piglets, were randomly allotted to 12 treatment groups (144 pens, 12 replicates per treatment, 10 piglets per pen/replicate). Within those 12 groups, 6 received a standard ZnO product and 6 received a novel zinc source (HiZox). Both products were included in the diet that their zinc contents would be comparable. Inclusion levels were 150 mg, 300 mg, 600 mg, 900 mg 1500 mg and 3000 mg zinc product per kg of feed. The piglets were fed ad libitum according to their assigned group for two weeks after weaning, thereafter they were fed a diet containing only 150 mg of HiZox per kg of feed, regardless of their previous diet. Body weight (BW) and feed intake (FI) were obtained weekly and body weight gain (BWG), daily body weight gain (dBWG), daily dFI and feed conversion ratio (FCR) were calculated. Fecal scores were estimated per pen. At 3 different timepoints (day 1, 14 and 28 after weaning) pooled fecal samples were collected from all pens. SPSS (Version 26, IBM, USA) was used to perform an analysis of variance (ANOVA) and a post-hoc test (Tukey HSD). Differences were considered statistically significant at $p < 0.050$.

Results: BWG increased with increasing zinc concentrations. For groups fed a diet containing HiZox, the BWG was on average 3 % higher than in groups receiving the standard zinc source, however, the differences were without statistical significance. FI increased with increasing inclusion levels. Statistically significant differences could be shown between 300 to 600 mg/kg and 3000 mg/kg of standard ZnO. For HiZox however, the dose-dependent effect was somewhat lower and without statistical significance. For both zinc sources FCR values decreased numerically with increasing inclusion levels, as compared to 150 mg/kg of each zinc source. Supplementation of HiZox seemed to be more efficient. There were no signs of liquid diarrhea observed in this trial. However, pharmaceutical levels of standard ZnO (3000 mg/kg) could numerically improve the fecal consistency when compared to low levels of the same zinc source. 900 to 3000 mg/kg of HiZox showed similar effects of improving the consistency of feces. In general, the positive effects of increasing inclusion levels of the 14-day starter period were still present in the following 14-day grower period, where all piglets received a supplementation of 150 mg/kg.

Conclusions: Increasing levels of zinc oxide showed enhanced performance and an improved fecal score. HiZox at 900 mg/kg showed similar results as the standard ZnO at 3000 mg/kg. The continuing positive effects on performance and fecal consistency, even after the 14-day feeding trial with increasing inclusion levels of Zinc, indicate that the supplementation of Zinc can be reduced to 150 mg per kg of feed from the third week on after weaning.

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82.

Impaired glucose tolerance induced by low-carbohydrate, moderate fat diet in pigs

Reduzierte Glucosetoleranz bei Schweinen durch Futter mit niedrigem Kohlenhydrat- und moderatem Proteingehalt

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Pigs are used in translational medicine as a model for human diabetes mellitus. From other species it is known that a ketogenic diet (low carbohydrate and moderate protein content, LCMP) can cause insulin resistance and reduced glucose tolerance. The aim of the present study was to challenge pigs with a LCMP diet to monitor their glucose control and insulin sensitivity.

Methods: Six-months-old pigs were reared on commercial pig feed. At the beginning of the trial, they (n=6, single-housed) were fed the LCMP diet (14.4% CP, 62.1% EE; 15.9% CF) for a 4 wk "metabolic challenge". Then, they were abruptly switched to the control diet (CON; 18.2% CP, 4.7% EE, 5.5% CF, 53.7% NfE) that was also fed for 4 wks. Intravenous glucose tolerance tests (IVGTT) were conducted at the beginning (BASIS), after 4 wks on the LCMP diet (MC4) and after 4 wks on the CON diet (CON4) with a glucose bolus of 0.5g/kg body weight. Blood samples were taken at 14 time points relative to the glucose bolus (-10, 0, 1, 5, 7, 10, 15, 20, 30, 40, 50, 60, 90 min) and analysed for glucose (autoanalyzer) and insulin (automated sandwich-type chemiluminescence immunoassay, Liaison, Diasorin, Italy). The area under the curve (AUC) of both parameters was calculated and compared via ANOVA (Sigma Plot, $p<0.05$).

Results: The mean AUCGlucose was 534.5±31.5 mmol/L*t at BASIS, 830.13±71.5 mmol/L*t at MC4 and 557.8 ±57.7 mmol/L*t at CON4. The mean AUCInsulin was 2047.9 µU/L*t at BASIS, 3589.8 µU/L*t at MC4 and 2154.4 µU/L*t at CON4. For both glucose and insulin, the AUC at MC4 was significantly higher than at BASIS and CON4 ($p<0.001$), while there was no significant difference between BASIS and CON4 ($p=0.503$ for glucose, $p=0.356$ for insulin).

Conclusions: The ketogenic LCMP diet caused impaired intravenous glucose tolerance in pigs after 4 wks. This effect was reversible after 4 wks on the CON diet which contained a higher amount of carbohydrates. Analysis of further parameters (e.g. metabolomics, hormones) will give insights into further metabolic processes altered by the ketogenic diet.

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83.

Quantifying glucose supply and demand in high-producing dairy cows during early lactation from animal and feed data

Quantifizierung der Glukoseverfügbarkeit und des Glukosebedarfes von Milchkühen während der Frühlaktation anhand von Tier- und Futterdaten

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In early lactation of dairy cows, dry matter intake (DMI) and therewith, the supply of glucose and glucogenic precursors is limited. Simultaneously, large amounts of glucose are required for the synthesis of high milk yields as well as for immune processes associated with metabolic, infectious or environmental stressors [1]. In case of glucose shortage, metabolic trade-offs between milk production and self-preservation might emerge [2]. To assess the degree of glucose reserves in high-yielding dairy cows during early lactation, a herd data-based concept for the calculation of individual glucose supply and demand (glucose balance, GB) was developed.

Methods: Calculation of GB was performed for a total of 201 lactations (1 to 105 days in milk) of 157 cows kept in a research facility and fed isoenergetic total mixed rations (n=24; 11.5 ±0.2 MJ ME). Individual DMI, BW and milk yields were assessed on a daily basis. Daily fluxes of ruminal fermentable organic matter, ruminal propionate production as well as duodenal fluxes of protein and starch were estimated via the Systool Web application [3] from the composition of crude nutrients of feedstuffs, the percentage of concentrate in the diet and the levels of DMI and body weight (BW). Based on a body of previously published work in the field of dairy nutrition and physiology, net portal appearance of digestive precursors was predicted from digestive nutrient fluxes. The same applies for the amounts of mobilized alanine (protein tissue) and glycerol (adipose tissue), the amount of L-lactate derived from glucose catabolism in protein tissue, and glucose consumption of protein tissue, red blood cells, brain and udder, which were estimated from DMI, BW, the number of days in milk and milk yield (MY), respectively. GB was calculated as the sum of glucogenic carbon (C) derived from precursors minus the sum of glucogenic C demanded by major glucose consuming organs.

Results: Weekly averages of GB ranged from 3.2 ±13.5 mol C per day in the 1st week of lactation, followed by a steep increase during the 2nd and 3rd week of lactation, and a moderate but continuous increase up to 46.7 ±17.4 mol C per day in the 15th week of lactation. Mean contribution of dietary precursors to overall glucose supply averaged 54.2 ± 3.7 %, 30.7 ± 2.4 % and 5.2 ± 0.4 % for propionate, amino acids and L-lactate, respectively. Contributions of endogenous precursors decreased from 24.7 ± 7.5 % during the 1st week of lactation (13.9 ± 1.9 %; 8.0 ± 5.9 % and 1.0 ± 0.7 % for lactate, glycerol and alanine) to a basic level of 6.8 ± 1.4 % (6.2 ±0.8 %, 0.8 ±1.3 %, 0.0 ±0.0 %) in periods with few or no mobilization of adipose and protein tissue (week 7 to 14). Mean glucose demand of the mammary gland ranged from 56.4 ± 13.1 mol C per day in the 1st week of lactation to 81.8 ± 14.4 mol C per day in the 7th week.

Conclusions: GB is a promising new tool to estimate glucose reserves in dairy cows, both for scientific and practical aspects of dairy nutrition. The results indicate an exhausted glucose pool in the intermediate metabolism at the onset of lactation for a considerable number of high-producing dairy cows. Given the priority of transferring glucose to mammary cells during this period, glucose may be in short supply for challenges by immunoactivation. To assess the meaningfulness of GB data, external validation with regard to the associated risk of disease would be required.

Bibliographical references

[1] KVIDERA S.K., HORST E.A., ABUAJAMIEH M., MAYORGA E.J., FERNANDEZ M.V.S., BAUMGARD L.H. (2017): J. Dairy Sci. 100 (3):2360-2374

[2] HABEL J., SUNDRUM A. (2020): Animals 10(6):1028

[3] CHAPOUTOT P. (2015): Proceedings of the 66th Annual Meeting of the European Society of Animal Science, 31 August to 4 September, Wageningen Academic Publishers, Warsaw, Poland, 265

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84.

Effects of a *Cryptosporidium parvum* infection in the neonatal period on the carbohydrate, protein and fat metabolism in calves

Einfluss einer Cryptosporidium parvum Infektion in der neonatalen Phase auf den Glucose-, Protein- und Fettstoffwechsel von Kälbern

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Cryptosporidium parvum is a major pathogen found in calves suffering from diarrhoea, which is one of the most important reasons for animal and economic losses in the neonatal period [1, 2]. Less is known about the metabolic impact of C. parvum infection in young calves. The present study aimed to investigate the influences of a C. parvum infection on the protein, carbohydrate and fat metabolism in calves in the first week of life.

Methods: Male Holstein calves (n=15; birth weight = 43.3 ± 1.1 kg (mean ± SE)) were either treated orally with 2x107C. parvum oocysts (n=5) or with water (control, n=10) on day 1 after birth. Five of the control animals were spontaneously infected by C. parvum. Animals received 3 l of a colostrum pool after birth. Thereafter, 6 l milk replacer per day (145 g powder / l) were fed provided in three meals. From day 5 on, 2 l of an electrolyte supplement were fed in addition. Blood samples were collected before feeding on day 1, 5, 7 and 8 after birth to determine blood metabolites. On day 7 after birth, postprandial blood samples were collected hourly. Additional blood samples were taken to evaluate glucose turnover rates by oral [13C6]-glucose and intravenous [6,6-2H2]-glucose bolus injection on day 7 after birth. Metabolites and glucose turnover rate were statistically analysed by the MIXED as well as the GLM procedure of SAS including the fixed effects group (control [Con], experimentally [EIn] and spontaneously [SIn] infected) and time, if relevant. One animal from the SIn group had to be excluded from statistical analyses because of poor health status.

Results: Plasma total protein concentration was reduced by experimental C. parvum infection on day 7 ($p < 0.05$) and tended to be reduced on day 8 ($p < 0.1$). Plasma urea concentration was higher in EIn than in Con from day 7 on ($p < 0.05$).ExperimentalC. parvum infection reduced plasma triglyceride and total cholesterol concentrations compared to Con from day 5 on ($p < 0.05$). Plasma non-esterified fatty acid (NEFA) concentration was higher in EIn than in Con on day 8 ($p <0.05$). The postprandial increase of plasma glucose and xylose concentrations were lower in EIn compared to Con on day 7. The glucose turnover rate calculated from oral [13C6]-glucose tended to be decreased in EIn ($p = 0.056$). Plasma concentrations of protein, urea, xylose, triglyceride and cholesterol and glucose turnover rate in SIn did not differ from Con. Plasma glucose concentration of SIn was lower compared to Con on day 5 and 120 min after feeding on day 7 ($p < 0.05$). Plasma albumin and NEFA concentrations were higher in SIn compared to EIn and Con 8 h after feed intake on day 7 ($p < 0.05$).

Conclusions: An experimental infection with the currently used dose of C. parvum affects the energy and protein metabolism in calves in the neonatal period as indicated by the inhibition of the carbohydrate and fat absorption and glucose turnover as well as increased urea concentration in plasma. The spontaneous infection has only marginal effects on plasma metabolites in calves in the present study.

Bibliographical references

[1] BARTELS C.J.M., HOLZHAUER M., JORRITSMA R., SWART W.A.J.M., LAM T.G.J.M. (2010): Prev. Vet. Med. 93: 162-169

[2] SVENSSON C., LINDER A., OLSSON S.-O. (2006): J. Dairy Sci. 89: 4769-4777

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85.

Hepatic mRNA expression of genes regulating urea, creatinine and uric acid metabolism of dairy cows with divergent milk urea concentrations

Die mRNA Expression hepatischer Gene des Harnstoff-, Creatinin- und Harnsäurestoffwechsels bei Milchkühen mit unterschiedlicher Milchharnstoffkonzentration

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Milk urea concentration is used as an indicator for monitoring optimal dietary crude protein supply and assessing urinary nitrogen (N) excretions. Dairy cows with an intrinsically high (HMU) compared to low milk urea (LMU) concentration, however, do not differ in urinary urea and N excretion when feeding the same diet [1]. Rather HMU cows have reduced urea but also creatinine and uric acid renal clearance rates, which may at least partially account for the higher concentration of these N-metabolites in plasma or milk [1]. Whether the hepatic metabolism contributes to the concentration differences between phenotypes has not been studied. Therefore, the aim of this study was to investigate the mRNA expression of genes related to urea and non-urea N-metabolite synthesis in the liver of HMU and LMU dairy cows.

Methods: Thirty six German Holstein cows with high (276 ± 4 mg/L; n=18) and low (186 ± 4 mg/L; n=18) milk urea concentration during 5 consecutive monthly test recordings were purchased from commercial farms. Cows were at the end of 2nd to 4th lactation and fed two isocaloric diets (10.1 ± 0.1 MJ metabolizable energy/kg dry matter) with a low (LP; 13.8 ± 0.2%) and normal (NP; 15.9 ± 0.1%) crude protein level for 4 weeks. In the last week on each diet, animals of the 4 groups (HMU-NP, HMU-LP, LMU-NP, LMU-LP, each n=9) were blood sampled to obtain plasma and slaughtered to obtain liver tissue. Plasma samples were analyzed for amino acids, urea, creatinine and uric acid using HPLC. Liver tissue was extracted for total RNA, which was reverse transcribed and subjected to quantitative RT-PCR. Statistical analysis was carried out using the SAS software for Windows, version 9.4 (Copyright, SAS Institute Inc., Cary, NC, USA). Due to technical problems with climate control resulting in altered food and water intake, data of two cows fed the NP diet were excluded from statistical analysis. Data was analyzed by ANOVA including the factors milk urea concentration, diet and their interaction.

Results: Plasma urea and uric acid concentrations were higher in HMU than LMU animals, but plasma creatine and creatinine concentrations were not different between groups and diets. Plasma arginine concentrations increased in HMU but not LMU cows with increasing CP content ($P<0.05$) and were not different between groups. However, plasma citrulline concentrations were higher in HMU than in LMU cows, while plasma ornithine concentrations were not different between groups or diets. The mRNA expression of arginase 1 (ARG1), the rate-limiting enzyme of the urea cycle, tended to be higher in LMU cows on the NP compared to LP diet ($P<0.1$); however, no differences were observed between groups. Furthermore, the mRNA expression of nitric oxide synthase (NOS2), catalyzing the by-pass from arginine to citrulline in the urea cycle, was comparable between groups and diets. In addition, arginine:glycine aminotransferase (GATM) and guanidinoacetate N-methyltransferase (GAMT) mRNA expression, regulating creatine and creatinine synthesis, tended to be lower expressed in HMU compared to LMU cows on the NP diet ($P<0.1$). Xanthine dehydrogenase (XDH) forming uric acid from xanthine was lower expressed in HMU than LMU cows when fed the NP diet ($P<0.05$).

Conclusions: Higher plasma urea and arginine concentrations in HMU cows were not reflected by differences in hepatic ARG1 or NOS2 mRNA expression. Vice versa, lower GATM, GAMT and XDH expression in HMU cows were not paralleled by lower plasma creatine, creatinine and uric acid concentrations, respectively. These results suggest that the hepatic urea, creatinine and uric acid metabolism does not account for the different concentrations of these metabolites formerly observed in milk and plasma of HMU and LMU cows [1].

Bibliographical references

[1] MÜLLER C.B.M., GÖRS S., DERNO M., TUCHSCHERER A., WIMMERS K., ZEYNER A., KUHLA B. (2021): Sci. Total Environ. 755:143198

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86.

Intracerebroventricular N-arachidonylethanolamide administration increases feed intake and reduces milk yield in late lactating dairy cows – results from a pilot study

Die intracerebroventrikuläre Injektion von N-Arachidonylethanolamid steigert die Futteraufnahme und senkt die Milchleistung spätlaktierender Milchkühe – Resultate einer Pilotstudie

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The endocannabinoid system plays a key role in regulating energy homeostasis and energy intake. Recent outcomes of a study suggest the existence of a peripheral mechanism by which the endocannabinoid N-arachidonylethanolamide (AEA) increases short-term feed intake and modulates whole-body energy metabolism in dairy cows [1]. In the present pilot study we aimed to test the hypothesis if AEA has a long-lasting central effect in increasing feed intake that leads to an increase in milk yield of dairy cows.

Methods: Three non-pregnant German Holstein dairy cows in late lactation were equipped with an intracerebroventricular (i.c.v.) cannula guide directed to the lateral brain ventricle. After surgery, animals were housed in tie-stalls and fed a total mixed ration according to their requirements for ad libitum intake. After recovery and 7 d after surgery, feed residuals were removed from the feeding bin at 07:00 and cows had no access to feed until 09:00. Cows were i.c.v. injected 100 µl of either dimethyl sulfoxide (CON) or 12 µg AEA dissolved in DMSO. Immediately after injection, cows were given access to feed for ad libitum intake. Feed intake was measured as feed disappearance from the bin by an electronic registration device every 15 min. Feed samples were taken to analyze dry matter (DM) content and to calculate DM intake (DMI). Cows were milked at 06:30 and 17:00 and milk yield was calculated as the sum of the evening and subsequent morning milking. Due to the low number of animals and the high variation in body weight and milk yield, no statistical evaluation was performed, thus effects are reported for individuals.

Results: The i.c.v. AEA injection increased cumulative feed intake and DMI during the 10 h post injection period in all 3 cows. The magnitude of increase relative to CON injection 1 h post treatment was 1.13, 1.91 and 2.06 kg, respectively, which corresponds to a factor ranging between 1.1 and 1.3. Relative to CON injection, AEA administration reduced daily milk yield by 0.3 to 1.4 L/d. However, 22 h post injection, DMI was not markedly different between AEA and CON treatments. The efficiency measure milk yield/10-h DMI ratio was 0.23 to 0.45 L/kg, and the milk yield/22-h DMI ratio equal to or 0.7 L/kg lower relative to CON administrations.

Conclusions: The present pilot study demonstrates that i.c.v. administration of 12 µg AEA increased feed intake in short- but not long-term. The milk yield/ DMI ratios were lowest with AEA compared to CON treatment suggesting that dairy cows could not take advantage from increased energy intake to increase milk production. Further cows will be studied to confirm this data statistically.

Bibliographical references
[1] VAN ACKERN et al., unpublished data

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87.

MitoCow – Microbiome clusters correlate with health and performance of L-carnitine fed dairy cows

MitoCow – Mikrobiomcluster korrelieren mit der Gesundheit und Leistung von L-Carnitin gefütterten Milchkühen

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Transitioning from late gravidity to early lactation is associated with an increased vulnerability to infectious and metabolic diseases and consequently poses outstanding challenges for dairy cows. The individual variability and long-term changes of the fecal microbiome and metabolome were studied here.

Methods: Fecal samples were derived during the collaborative project "Mitochondrial Functionality of Dairy Cows" (MitoCow) [1, 2]. Cows were fed with rumen-protected L-carnitine (CAR) supplemented diet compared to control animals (CON) in a randomized experiment (n=54) around parturition and a lipopolysaccharide (LPS)-induced systemic inflammation. The trial started at day 42 antepartum and lasted until day 126 postpartum (pp). Animals were LPS-stimulated on day 111 pp, applying 0.5 µg LPS / kg body weight intravenously. Fecal samples were collected at 13 time points around both challenges (partus and LPS challenge). Extracted DNA was used for 16S rRNA Illumina sequencing (V1-2 region) of bacteria. A subset from seven time points was selected for targeted metabolomics measurements using AbsoluteIDQ®p180Kit (Biocrates). Data were analyzed by multivariate statistical analyses.

Results: Both challenges had clear impacts on total bacterial community and accompanied metabolite profiles, whereas CAR supply remained ineffective. Applying the "enterotype" algorithm known from human microbiome studies, individual animals were classified into three distinct microbiome clusters based on the relative abundances of bacterial members and the rate of dispersion. First cluster, called C-Bifi, was defined by the key taxa Bifidobacterium and showed a dynamic pattern of the fecal bacterial community composition among all time points, whereas cluster C-Spiro, defined by unclassified Spirochaetaceae, showed a static pattern. Animals belonging to C-Bifi showed lower bacterial diversity and highest residual energy intake than C-Spiro and C-Clos, which was defined by the key taxa unclassified Clostridiales. Animals assigned to the three clusters varied significantly in terms of illnesses, body weight, microbiome composition, and milk and blood parameters. C-Bifi animals were healthier and leaner than others. C-Spiro animals were heavier than C-Bifi animals and had a higher number of illness symptoms, but the diversity of the static microbiome was higher. Metabolomic analyses showed significant differences in respect to amino acids and biogenic amines between the animals of the respective microbiome clusters.

Conclusions: In summary, cow microbiome clusters vary in composition, respond differently to physiologic stress events of the host, and seem to be associated with animals' health and performance. These findings provide novel ideas to use the animal's microbiome as a functional trait for future breeding strategies.

Bibliographical references

[1] MEYER J., DANIELS S.U., GRINDLER S., TRÖSCHER-MUßOTTER J., ALAEDIN M., FRAHM J., HÜTHER L., KLUESS J., KERSTEN S., VON SOOSTEN D., MEYER U., MOST E., EDER K., SAUERWEIN H., SEIFERT J., HUBER K., REHAGE J., DAENICKE S. (2020): Animals 10(2):342

[2] MEYER J., KONONOV S.U., GRINDLER S., TRÖSCHER-MUßOTTER J., ALAEDIN M., FRAHM J., HÜTHER L., KLUESS J., KERSTEN S., VON SOOSTEN D., MEYER U., MOST E., EDER K., SAUERWEIN H., SEIFERT J., HUBER K., WEGERICH A., REHAGE J., DAENICKE S. (2021): Animals 11(1):136

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88.

MitoCow – New ways to identify metabolic types in carnitine supplemented dairy cows one hour after calving

MitoCow – Neue Wege zur Identifizierung von Stoffwechseltypen in Carnitin-supplementierten Milchkühen eine Stunde nach der Kalbung

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Calving represents a natural inflammatory challenge in dairy cow's productive life span and results in an individual adaptation to onset of [1]. Carnitine is an important factor for energy metabolism and might influence this adaptive process. Within a very short time, the metabolism must be adapted to the current situation. The metabolic adaptation is dependent on many factors including health status, lactation number, birth pattern, fat mobilization and feed intake. Metabolite profiling provides the opportunity to gain a better insight into metabolic processes, taking into account a wide range of influencing factors.

Methods: A total of 59 multiparous German Holstein-Friesian cows were assigned to a control (CON; n = 30) and a L-Carnitine supplemented group (25 g rumen-protected carnitine / d / animal; CAR; n=29). The dietary carnitine supplementation started on day 42 ante partum. Quantitative metabolite profiles were determined 1 h post partum in EDTA plasma samples by liquid chromatography and mass spectrometry using the AbsoluteIDQ p180 Kit (Biocrates Life Science AG, Innsbruck, Austria). Data were analyzed by using the Software JMP Pro and the online tool MetaboAnalyst. (https://www.metaboanalyst.ca).

Results: In the acute metabolic adaptation one hour after calving, the metabolite profile shows no clear differences between CON and CAR. Irrespectively of carnitine supplementation, three distinct metabolite profile patterns (metabotypes) could be identified by the K Means cluster analysis. The score plot of the principal component analysis showed a clear separation of the 3 metabotypes. The biogenic amines alpha aminoadipic acid ($p < 0.0001$; from high to low concentration: cluster 1, cluster 3, cluster 2), carnosine ($p = 0.0297$; from high to low concentration: cluster 3, cluster 2, cluster 1), kynurenine ($p = 0.0188$; from high to low concentration: cluster 3, cluster 2, cluster 1) and sarcosine ($p = 0.0015$; from high to low concentration: cluster 1, cluster 2, cluster 3) as well as the acylcarnitines C16:2 ($p = 0.0001$; from high to low concentration: cluster 1, cluster 2, cluster 3) and C 18:0 ($p = 0.0007$; from high to low concentration: cluster 1, cluster 2, cluster 3) showed significant differences between the metabotypes but not between CON and CAR. Furthermore, there was a significant difference in dry matter intake during the first 3 weeks of lactation ($p = 0.005$), with cluster 1 having the lowest dry matter intake.

Conclusions: In the acute adaptation to parturition, feed intake and milk production is still low. However, in this early time, there is a modulation of metabolism that is most likely based on individual genetic traits.

Bibliographical references

[1] HUBER K., DÄNICKE S., REHAGE J., SAUERWEIN H., OTTO W., ROLLE-KAMPCZYK U., VON BERGEN M. (2016): Scientific Reports 6:24642

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89.

Regulation of glucose transporters and glucose metabolism genes of rumen epithelium in cows transitioned from a forage-based to a high-grain diet

Die Regulation von Glukosetransporter-Genen, sowie Gene des Glukose Metabolismus im Pansenepithel von Kühen welche von einer Grundfutterration auf eine konzentratreiche Fütterung umgestellt wurden

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The bovine rumen epithelium has a crucial role in the uptake of the end products of fermentation and their metabolism. However, there is little knowledge available on the uptake of glucose / hexoses and their metabolism. Therefore, we examined the transcriptome of rumen papillae from cows which were transitioned from a forage diet to a high-grain diet.

Methods: Rumen biopsies were collected from nine ruminally-cannulated non-lactating Holstein-Friesian cows fed a forage diet (HF; 0% concentrate, n = 9) as baseline or a high-grain diet (HG; 65% concentrate, n = 9). Animals were transitioned in seven days from the forage diet to the high-grain diet with a daily increase of 10% and 5% concentrate. Total RNA was extracted and sequencing was performed on a NovaSeq 6000 (Illumina) platform using a 2 x 100 bp paired-end approach. Demultiplexed reads were processed and differential gene expression analysis was conducted with DESeq2 (v1.30.0) in R. Differences in expression were considered significant at a Benjamini and Hochberg corrected p-value of < 0.05.

Results: Transcriptome analysis identified 9,481 differentially expressed genes (DEGs). Within the top 5 most significant DEGs, we detected Solute Carrier Family 7 Member 8 (SLC7A8), which is mainly related to the transport of glucose and other sugars. Therefore we performed a deeper analysis for known glucose transporters among the 9,481 DEGs and found that GLUT2 / SLC2A2, GLUT3 / SLC2A3, GLUT4 / SLC2A4, GLUT9 / SLC2A9, GLUT10 / SLC2A10, SGLT2 / SLC5A2, and SGLT3 / SLC5A4 were all up-regulated when cows were transitioned to the high-grain diet, suggesting an increased uptake of glucose, which might be used for the increased energy need of cells to proliferate to enlarge papillae or being used for storage. Indeed, another interesting finding was that genes encoding key enzymes involved in the gluconeogenesis such as PC (pyruvate carboxylase) and PCK1 (phosphoenolpyruvate carboxykinase) as well as PKLR (pyruvate kinase, liver and RBC) were up-regulated due to grain feeding, suggesting a contribution of the rumen epithelium to the glucose metabolism similar to what is known for the liver. Further experiments are essential to evaluate and deepen the understanding of glucose metabolism within the rumen epithelium.

Conclusions: We observed that several glucose transporters were expressed within the rumen papillae and secondly that these transporters are regulated by a diet change. Furthermore, we found evidence of the up-regulation of genes involved in gluconeogenesis within rumen papilla in response to high-grain diets.

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90.

Assessment of plasma and leucocyte derived miRNAs as potential biomarkers for rumen health in cattle fed forage and transitioned to a high-grain diet

Evaluierung von miRNAs aus Plasma und Leukozyten als potenzielle Biomarker für die Pansengesundheit in Kühen, welche von einer Grundfutterration auf eine konzentratreiche Fütterung umgestellt wurden

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MicroRNAs (miRNAs) are a class of small non-coding RNAs with a base pair length of 22, which regulate post-transcriptional gene expression. Sub-acute ruminal acidosis (SARA), a metabolic disease characterized by low ruminal pH and accumulation of lactate is linked to the feeding of high-grain diets. We hypothesize that circulating miRNAs in blood could serve as potential biomarkers to detect cattle with SARA. To test it, a comprehensive identification of miRNA profiles in plasma and leucocytes was conducted and miRNAs were evaluated as potential biomarkers in cattle in which SARA was induced through high grain feeding.

Methods: Four rumen-cannulated dry Holstein cows were fed a control diet of 100 % forage and transitioned over one week to a 65 % grain diet (HG). The occurrence of SARA was verified after diet transition using a ruminal pH threshold of < 5.8 for 320 min / d. Blood samples were taken during forage feeding and after one week high grain feeding to assess miRNA expression profiles. Blood plasma was used to analyze free circulating miRNAs, whereas the buffy coat was used to investigate cellular miRNA expression in leucocytes. Total RNA was isolated and small RNA libraries were prepared with the NEXTflex Small RNA-Seq Kit and sequenced on an Illumina NovaSeq 6000 platform. sRNAbench was used to process the raw reads and high-quality (PHRED> 20) read counts were further filtered (>10 reads). Unique and shared miRNAs between diets were identified using tidyr in R (version 4.0.1) and Venny. DESeq2 (version 1.30.1) was used to perform differential expression analysis with an FDR threshold < 0.05.

Results: In plasma and leucocytes, a total of 520 and 730 miRNAs were found, respectively. From these, 498 miRNAs were found to be shared by plasma and leucocytes, with 22 being expressed solely in plasma and 232 being exclusively found in leucocytes. The differential expression analysis identified 10 upregulated and 2 downregulated miRNAs. Sixty-three circulating miRNAs were found only in the plasma of cows fed with high-grain diet, showing that these animals had a higher circulating miRNA count and diversity. Potential biomarkers for further analyses were chosen based on total read counts of filtered and sorted miRNAs expressed in all cows fed a high-grain diet, as well as differentially expressed miRNAs in terms of their read count and log2 fold change. These miRNAs were then queried on the available literature, as well as the annotated functions on miRBase 22. The best candidates were validated by qPCR to confirm the RNA-Seq results.

Conclusions: MiRNAs bta-miR-11982, bta-miR-1388-5p, bta-miR-12034, bta-miR-2285u, and bta-miR-30b-3p are auspicious candidates as potential biomarkers of SARA in cattle. Further analyses with larger sample sizes will be necessary to determine their potential applications. Interestingly, bta-miR-11982, although being a not well-known miRNA in cattle and based on its expression levels is a target for further investigations. Our findings suggest that dietary changes influence the release and expression of miRNAs in systemic circulation which potentially regulate post-transcriptional gene expression in cattle under SARA.

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91.

Effects of dietary phosphorus and calcium supply on insulin signaling in laying hens

Effekte der Versorgung mit Phosphor und Calcium auf die Insulinsignalwege bei Legehennen

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Chickens are special in their insulin-glucose homeostasis compared to mammals. They express less sensitivity to exogenous insulin which can potentially increase the risk of insulin resistance [1]. Two strains of laying hens (Lohmann Brown-Classic, Lohmann LSL-Classic) exerted differences in various traits regarding to the phosphorus (P) and calcium (Ca) metabolism along the digestive tract [2]. Additionally, according to the earlier studies, the brown hens expressed more sodium/phosphate co-transporters in the small intestine than white ones. This expression could also be affected by dietary mineral supplements [2]. P is essential for energy metabolism in various cellular pathways. Also in chicken, endocrine regulation of energy metabolism is dependent on insulin. Thus, a close association between P homeostasis and insulin metabolism can be assumed. Aim of the study was to identify potential relationships between these components of energy metabolism in chicken. It was hypothesized that low dietary P supply may result in reduced insulin sensitivity and this might be reflected by downregulating these insulin signaling proteins.

Methods: In this experiment, 40 brown and 40 white Leghorn laying hens were chosen, respectively, and details of the study were described before [2]. In brief, each of the strains was subdivided into four groups, standard dietary Ca concentration (39.6 g/kg dry matter; Ca+), reduced Ca concentration (33.9 g/kg DM; Ca-), standard dietary P concentration (5.3 g/kg DM; P+), reduced P concentration (4.7 g/kg DM; P-). Low P concentrations were 20% below the levels recommended by the GfE (1999). At the age of approximately 30 weeks, hens were moved to metabolism cages in order to conduct the 3-week-feeding program according to the experimental dietary regimen. After slaughtering (33 week of life), liver tissue was collected and immediately stored at -80 for further *in vitro* studies. The tissue was homogenized and protein concentration was determined by using Bradford reagent (Serva). Protein expression and extent of phosphorylation of insulin receptor ß (IRß;at Tyr1150), phosphatidylinositol 3-kinase (PI3K), serine/threonine protein kinase (Akt;at Ser 473), mammalian target of rapamycin (mTOR; at Ser 2448), and AMP-activated protein kinase (AMPK; at Thr 172) were semiquantified by Western blot. After antibody detection, bands intensities were evaluated by using Image Lab software (version 5.2.1). Expression of each protein was normalized over β-actin. The data analysis was performed by mixed model SAS. P value was set at $p<0.05$.

Results: As major results, strain has no effect on insulin signaling cascade with the exception of pAKT ($p<0.01$), AKT ($p<0.05$) and IRß ($p<0.05$). P treatment demonstrated a clear impact on expression of AMPK ($p<0.05$), phosphorylated AMPK ($p<0.01$) and PI3K ($p<0.01$). The mixed model of Ca as well as the interaction between strain and P demonstrated significant impacts on insulin signaling protein expression except pAKT.

Conclusions: In laying hens, improvement in insulin sensitivity might reduce predisposition to metabolic disorders and optimize ovary function. Ca and P dietary supplement have an important effect on insulin sensitivity in the liver. Brown hens respond significantly stronger to lower P supply and thereby, reduce insulin sensitivity.

Bibliographical references

[1] DUPONT J., TESSERAUD S., SIMON J. (2009): General and Comparative Endocrinology 163: 52-57

[2] SOMMERFELD V., OMOTOSO A.O., OSTER M., REYER H., CAMARINHA-SILVA A., HASSELMANN M., HUBER K., PONSUKSILI S., SEIFERT J., STEFANSKI V., WIMMERS K., RODEHUTSCORD M. (2020): Animals 10: 1736

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92.

Hygiene status of piglet feeding equipment and occurrence of resistant bacteria after cleaning and disinfection in different stable environments

Hygienestatus von Futtereinrichtungen für Saugferkel und Vorkommen resistenter Bakterien nach der Reinigung und Desinfektion in verschiedenen Stallumgebungen

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By consequently following the all-in all-out principle as well as applying thorough cleaning and disinfection measures with suitable agents, the bacterial load in the stable should be minimized in order to reduce the occurrence of diarrheal diseases in particular. Aim of this study was therefore to assess the hygiene status of farrowing units on different piglet producing farms after the farm-respective cleaning and disinfection measures were performed. Hereby, special attention was paid toward feeding and drinking equipment of sows and piglets.

Methods: Samplings took place on 18 piglet producing farms (13 conventional and 5 organic farms) in North Rhine-Westphalia and Rhineland-Palatinate, Germany (one sampling per farm). With a questionnaire, information on the farms' performance indicators, animal stock, health status and hygiene management including cleaning and disinfection measures was gathered. The samplings were performed after sows and piglets were rehoused and farrowing units were cleaned and disinfected according to farm-respective protocols. For the samplings, three farrowing pens were randomly chosen in the empty stable compartment and visually inspected for cleanliness. In each pen, samples were taken of a maximum of 15 sampling sites (e.g., trough and drinker of the sow, nipple drinker of the piglets and (if present) milk or pre-starter feeding equipment) and processed as described previously [1] to investigate the total viable count, coliform count and *E. coli*, as well as the growth of staphylococci, enterococci, salmonella, MRSA (Methicillin-resistant Staphylococcus aureus) and ESBL (Extended spectrum beta-lactamases) colonies. All microbiological data were log transformed. A preliminary statistical analysis was performed with Excel 2016 and SAS 9.4 by using general linear and mixed models and calculating Spearman rank correlations.

Results: The undertaken hygiene measures as well as the preliminary results differed significantly between the farms ($P < 0.05$). As found in a recent study investigating the hygiene status of fattening stables [1], the preliminary analysis indicated that feeding and drinking equipment in the farrowing units showed a high bacterial load after cleaning and disinfection measures have been performed. Especially nipple drinkers for piglets and permanently installed supplementary milk feeders were more contaminated than floor and walls of the piglet nest and other sampling sites ($P < 0.05$). Resistant bacteria were detected in samples of 16 farms (MRSA: $n = 6$, 33.3%; ESBL: $n = 16$, 88.9%). With regard to the piglet feeding and drinking equipment, drinkers contained ESBL on 75.0% of the visited farms ($n = 12$), while MRSA was detected in nipple drinkers and troughs of only one farm each (5.6%, respectively). ESBL was found in milk and pre-starter troughs and bowls of 7 farms (43,8%). Salmonella were not detected on any farm.

Conclusions: Feeding and drinking equipment in farrowing stables, especially the nipple drinkers and supplementary milk feeding equipment for piglets, can be regarded as critical points in hygiene management. They need to be paid close attention during cleaning and disinfection to ensure a reduction of the bacterial load and avoid a transmission of pathogens from one group of piglets to the next.

Bibliographical references

[1] HEINEMANN C., MEYER I., BÖGEL F.T., SCHMID S.M., HAYER J.J., STEINHOFF-WAGNER J. (2020): J. Anim. Sci. 98(1):1-10

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93.

Fecal excretion of ESBL-*E. coli* of calves fed with pooled colostrum

Fäkale ESBL-E.coli-Ausscheidung von Kälbern nach Fütterung mit gepooltem Kolostrum

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Prevalence of *E. coli* carrying extended spectrum beta-lactamase (ESBL) in young calves is higher than in older cattle. Feeding of waste milk could be identified as a risk factor for higher prevalence of this resistant bacterial species in young calves (1). In a German study an association between dry-off management with antibiotics and ESBL-*E. coli* prevalence could also be detected (2). Moreover, no association was found between fecal excretion of ESBL-*E. coli* of young calves and their dams (1). For other study purposes calves were fed with pooled colostrum. In this context, the objective of the present study was to determine if and to what extent the calves show fecal ESBL-*E. coli* excretion.

Methods: Directly after birthfifteen male HF-calves were fed with 3L of pooled colostrum on a dairy farm. Thereafter, they were transported individually to an experimental barn and fed with milk replacer (3 x 2L a day). The calves were strictly separated from each other. At day 1, 3 and 7 of life fecal swaps were taken and preserved with Amies-medium, followed by an examination for ESBL-*E. coli* (CHROM ID agar supplemented with 2 µg/ml Cefotaxim). After the results of fecal ESBL-*E. coli* excretion were known, a sample of the colostrum pool was also cultivated with the same technique after enrichment (LB-broth supplemented with 2 µg/ml Cefotaxim). Moreover, the dairy farm was visited and the equipment for harvesting and feeding colostrum (milking equipment, milking pot and nipple) was sampled and cultivated on CHROM ID agar with Cefotaxim. All ESBL-*E. coli* isolates were investigated via RAPD (Random Amplified Polymorphic DNA)-PCR and ESBL-positve isolates were subjected to sequence analysis.

Results: Already on the first day of life, 14 out of 15 calves were tested positive for ESBL-*E. coli*, although they had neither spatial nor temporal contact with each other. At day 7 all calves showed ESBL-*E. coli* excretion. It is assumed that the feeding of colostrum contaminated with ESBL-*E. coli* can be responsible for the intestinal colonization with ESBL-*E. coli*, because the same bacterial resistome was found in colostrum and in the feces of neonatal calves (3). Therefore, we decided to test the colostrum and the farm equipment of harvesting and feeding colostrum, additionally. In the colostrum sample ESBL-*E. coli* (5.5 x 10e1 cfu/ml) and ESBL-Enterobacter cloacae (8.1 x 10e2 cfu/ml) and in the milking pot ESBL-*E.coli* and in the nipple ESBL-Raoultella ornithinolytica were found. Five of the fecal ESBL-*E.coli* isolates had the same RAPD-pattern compared to the ESBL-*E. coli* detected in colostrum. This finding could be confirmed by whole genome sequencing suggesting a direct transmission of ESBL-*E. coli* via colostrum.

Conclusions: With the present study, we were able to demonstrate the direct transmission of viable ESBL-*E. coli* via contaminated colostrum for the first time. ESBL-*E. coli* originating from colostrum are, therefore, able to colonize the intestine of neonatal calves leading to release of resistant bacteria in the environment. How ESBL-*E. coli* contamination of the colostrum occurred is speculative, but the occurrence of ESBL-*E.coli* in the milking pot and ESBL-*Raoultella ornithinolytica* in the nipple at the farm suggests that excellent sanitation procedures of the colostrum equipment might be crucial for harvesting and feeding colostrum with good hygienic quality.

Bibliographical references

[1] WEBER L. (2021): Prävalenz und Risikofaktoren für das Vorkommen von Extended-Spektrum-Betalaktamase *Escherichia coli* bei Kälbern und deren Müttern aus großen deutschen Milchviehbeständen, Vetmed Diss, Hanover

[2] TETENS J.L., BILLERBECK S, SCHWENKER J.A., HÖLZEL C.S. (2019). Dairy Sci. 102(12):11449-11452

[3] LIU J., TAFT D.H., MALDONADO-GOMEZ M.X., JOHNSON D., TREIBER M.L., LEMAY D.G., DEPETERS E.J., MILLS D.A. (2019): Nature Communications 10:4406

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94.

Investigations of milk feeding practices and the prevalence of antibiotic-resistant bacteria in unweaned calves on Western German dairy farms

Untersuchungen zu Milchfütterungspraktiken und der Prävalenz von antibiotikaresistenten Bakterien bei nicht abgesetzten Kälbern auf milchviehhaltenden Betrieben in Westdeutschland

Hayer J.J., Heinemann C., Leubner C.D., Sib E., *Steinhoff-Wagner J. – Bonn

Although antibiotic-resistant bacteria have been recognized as a major concern for animal and human health, limited data is available on their prevalence in young livestock species and the factors leading to their development. Milk-fed dairy calves are of special interest in this regard. It is still common to feed them the colostrum of cows dried with antibiotics or waste milk containing antibiotic residues, which might enhance the development of antibiotic-resistant bacteria in calves and on their feeding equipment. In addition, a previous study has shown that milk buckets are often not well cleaned and can contain antibiotic-resistant bacteria. Hence, this study aimed to assess the prevalence of antibiotic-resistant bacteria in unweaned dairy calves in Western Germany and evaluate their relationship to milk feeding practices and the hygiene of teat buckets.

Methods: In total, 23 dairy farms in Rhineland-Palatinate and North-Rhine-Westphalia (55–700 lactating cows) were visited once. On-farm, farmers were questioned on their milk feeding practices, teat buckets were evaluated by protein and ATP rapid tests, and nasal and rectal swab samples were taken from 484 unweaned calves. Samples were processed within 24 h and analyzed on methicillin-resistant Staphylococcus aureus (MRSA) and Enterobacteriaceae resistant to 3rd generation cephalosporins (RE). Samples were pre-enriched for 24 h at 37°C in Mueller Hinton broth with 6% NaCl for MRSA and Mossel broth for RE, respectively. Afterward, samples were plated on selective plates, incubated, and grown colonies were identified using MALDI-TOF mass spectrometry. Results were analyzed descriptively, and Spearman's rank correlations were calculated with SAS® 9.4 to evaluate the farm's prevalence of MRSA and RE with feeding management practices.

Results: Most farms (65.2%) stated to feed waste milk to their calves regularly, and all 23 farms use antibiotics for drying off cows, with two farms stating to have a shorter dry-off period than recommended by dry-off manufacturers. MRSA was only found on two farms (six calves in total), whereas RE was found in samples of 19 farms (83%) and 47.3% of all calves. Resistant Enterobacteriaceae were mainly *Escherichia coli* (92.1%), Pseudomonas spp. (13.5%), or other coliforms (4.4%). No significant correlation of feeding waste milk or dry-off management with the prevalence of MRSA or RE was found. Furthermore, two farms stating to feed waste milk had no calf carrying MRSA or RE. Contrary to our expectations, the ATP values of the outside of the feeding teats correlated negatively with the prevalence of resistant coliforms ($r = 0.5$, $P < 0.01$).

Conclusions: This study indicates that antibiotic-resistant bacteria are prevalent in unweaned dairy calves. However, more detailed research with a greater sample size is needed to understand drivers for developing and spreading antibiotic-resistant bacteria in dairy calves. In our relatively small farm sample size, three different dry-off antibiotics were used, and antibiotics used in cows might also have an influence. Besides feeding management, possible drivers for antibiotic-resistant bacteria development in unweaned calves will be the subject of future investigations.

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95.

Meta analysis of the impact of antibiotic use in German pig farms on the fecal microbiota

Meta-Analyse zur Auswirkung des Antibiotikaeinsatzes in deutschen Schweinebetrieben auf die fäkale Mikrobiota

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The intestinal microbiota of pigs is of high interest in order to develop new health strategies against typical problems of pig production like newborn and post weaning diarrhea, especially to limit the use of antibiotics. Since there are different routines of antibiotic application in different farms there might be different impacts on the animal's microbiota. The aim of the study was therefore to find impacts of different antibiotic use in German sow farms on fecal microbiota of sows and piglets.

Methods: Eight hundred fecal samples were collected from 20 sow farms in Germany. Specifically, samples from 10 sows ante- and postpartum and from 10 piglets in the suckling and post-weaning periods were obtained. DNA was extracted with a commercial kit (QIAamp PowerFecal Pro DNA Kit, Qiagen, Hilden, Germany) and sequenced by Illumina MiSeq 16S DNA Sequencing. Bioinformatic analysis was performed using the pipeline DADA2. DNA was also subjected to quantitative Real-Time PCR with primers specific for *E. coli* fimbriae, *Clostridioides difficile* and *Clostridium perfringens*. Additionally, data about used antibiotic agents in the observed period were collected. Farms were clustered by their antibiotic use by nonmetric multidimensional scaling (NMDS) (R package "VEGAN") and hierarchical clustering. In every animal group differences in the fecal microbiota (genera abundant in at least 10% of samples) between the two clusters were statistically analyzed by the Welch t-test. Impacts of antibiotic use on the two clusters was also analyzed by principal component analysis.

Results: The cluster formation of antibiotic use for sows resulted in 2 clusters. Cluster 2 (5 farms) was mainly formed by the use of amoxicillin, enrofloxacin and lincomycin, which were not in use in cluster 1. The principal component analysis shows difference only for antepartum sows. The relative abundance of dominant genera like *Lactobacillus, Prevotella* and *Agathobacter* was significantly lower for antepartum sows in cluster 2 as well as bacterial diversity indices. Thus, other dominant genera become more dominant including *Escherichia, Terrisporobacter* and *Romboutsia. Postpartum* sows showed drastic increase in F4 fimbriae in cluster 2 and also higher *Enterococcus* and *Bifidobacteria*. Similar to antepartum *Lactobacillus* abundance was lower in cluster 2 and dominant genera were significantly higher abundant. Additionally increased genera in cluster 2 compared to cluster 1 have higher changes than decreased genera. This includes *Enterococcus, Collinsella, Terrisporobacter* and *Clostridium sensu stricto* 1. Genera belonging to *Ruminococcaceae* decreased significantly in cluster 2. In weaned piglets cluster 2 showed higher quantitative *C. difficile* and *Escherichia* counts, but lower *C. perfringens* and F18 fimbriae. In contrast to sows and suckling piglets there was no difference in dominant genera like *Clostridium sensu stricto* 1 and *Turicibacter* between the two clusters. Suckling and weaned piglets were generally less influenced by antibiotics.

Conclusions: Several markers for a balanced microbiota were affected by the use of Amoxicillin, Enrofloxacin and Lincomycin. Overall changes in the fecal microbiota were more pronounced in ante partum sows than in postpartum sows or piglets. There may be specific combinations of antibiotics that profoundly perturb the bacterial composition in sows and piglets during the production cycle.

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96.

Effect of dietary *Tenebrio molitor* larvae cuticles on the gut microbiome and lipid metabolism in obese Zucker rats

Wirkung der Haut von Tenebrio molitor Larven auf das intestinale Mikrobiom und den Lipidstoffwechsel von fettleibigen Zuckerratten

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Insect larvae meal has gained considerable attention as an alternative, sustainably produced source of protein for feeding of monogastric farm animals. A byproduct obtained from mass rearing of insect larvae are the old cuticles - the most external components of insects - which are periodically eliminated during ecdysis. Insect cuticles are a relevant source of indigestible fiber containing up to 50% of chitin, a polysaccharide composed of β-(1-4)-N-acetyl-D-glucosamine monomers. While several studies have shown that chitin modulates the gut microbiota and ameliorates lipid metabolic disorders in obese rodent models, feeding studies dealing with isolated insect cuticles are completely lacking. Thus, the present study tested the hypothesis that dietary insect cuticles modulate the gut microbiome and improve hepatic lipid metabolism in the obese Zucker rat.

Methods: A feeding trial with male, 10-week (wk)-old, obese Zucker rats (N = 30) was performed, which were randomly assigned to three groups [obese control (O), obese 1.5% cuticles (O1.5), obese 3.0% cuticles (O3.0)] of 10 rats each. In addition, one group of male, 10-wk-old, lean Zucker rats (group L, n = 10) with a healthy gut microbiota and normal metabolism served as a control to group O. Rats of all groups were fed the same nutrient-adequate, semisynthetic basal diet which was supplemented with either 0% (groups L and O), 1.5% (group O1.5) and 3.0% (group O3.0) *Tenebrio molitor* cuticles at the expense of cellulose. After 4 wk, rats were decapitated under CO_2 anesthesia and blood plasma, liver and cecal digesta were collected. The cecal digesta was used to analyze the microbial community by 16S rRNA sequencing and to determine the concentrations of short-chain fatty acids (SCFA). In the liver, lipid accumulation was evaluated by histological analysis and by determination of lipid concentrations. Normally and not-normally distributed data were analyzed by one-way ANOVA and Kruskal-Wallis test, respectively.

Results: Body weights (BW) at wk 4, BW gain and daily feed intake were higher in group O than in group L ($P<0.05$), but did not differ across the obese groups. Stained liver sections in all obese groups revealed a pronounced lipid accumulation compared to group L, but lipid accumulation was clearly less in group O3.0 than in groups O and O1.5. Hepatic lipid concentrations were markedly higher in group O than in group L ($P<0.05$), but were 30% lower in group O3.0 than in group O ($P<0.05$). Analysis of microbial community at the family level revealed that the relative abundances (RA) of 19 families were different between groups; regarding the obese groups, RA of Bifidobacteriaceae, Erysipelotrichaceae, Lactobacillaceae, Prevotellaceae and Sutterellaceae were higher and those of Anaeroplasmataceae, Eubacteriaceae and Ruminococcaceae were lower in group O3.0 compared to group O ($P<0.05$). RA of Coriobacteriaceae and unknown Clostridiales were higher in group O than in group L ($P<0.05$); while RA of Coriobacteriaceae was increased in group O3.0 compared to group O, that of unknown Clostridiales was decreased in group O3.0 compared to group O ($P<0.05$). The RA of Desulfovibrionaceae, Peptococcaceae, Saccharibacteria, unknown Deltaproteobacteria and unknown Firmicutes were lower in group O than in group L, whereas RA of Desulfovibrionaceae and Saccharibacteria were lower and those of Peptococcaceae, unknown Deltaproteobacteria and unknown Firmicutes were higher in group O3.0 than in group O ($P<0.05$). Cecal digesta concentrations of total SCFA, acetic acid and butyric acid were higher in group O3.0 than in group O ($P<0.05$), but did not differ between groups O and L.

Conclusions: Dietary supplementation with 3% of insect cuticles increases the abundance of gut bacterial families, such as Bifidobacteriaceae, Coriobacteriaceae and Lactobacillaceae, with known antisteatotic and hepatoprotective effects and exerts pronounced antisteatotic effects in the cecum of obese Zucker rats.

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97.

Preference for fibre rich feedstuffs in a cafeteria trial with sows

Präferenzen zwischen unterschiedlichen fasereichen Futtermitteln in einem Wahlversuch mit Sauen

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Fibre is a feed component, which is interesting as it may improve animal health and welfare by influencing not only satiety, but also the gut microbiome and immune system. While various fibre rich feedstuffs are available on the market, little is known about the preferences in pigs regarding the different options. The aim off the present study was to determine preferences for fibre rich by-products and roughage in sows.

Methods: Sixteen pluriparous, non-gestating German Landrace sows were kept individually in outdoor cages and fed either three fibre rich by-products or three roughage options. Fibre supplements were offered 45 min after feeding a complete diet for sows for 30 minutes per day. The offered by-products were apple pomace, sugar beet pulp and brewer's spent gain, while hay, hemp (Finola) and straw served as roughage choices. After 2 weeks, the feeding groups were changed according to a cross over design and the trial was continued for further 2 weeks. Feed intake was recorded and feed preferences observed. Faecal scoring was performed after each feeding period and the bodyweight was recorded before and after the trial. Statistical analyses were performed using IBM® SPSS® Statistics (version 25, IBM, USA). The Kruskal-Wallis test was used to determine differences, the Bonferroni post-hoc test was applied to form subgroups and differences were considered statistically significant at $p < 0.05$.

Results: The by-product frequently chosen as the first option to consume was apple pomace, while hay and hemp were preferred from the roughage choices. This was also mirrored by the by-product consumption, which was significant higher for apple pomace compared to sugar beet pulp and brewer's spent gain ($p < 0.001$). Straw commonly stayed untouched. Hence, while hay and hemp were consumed to equal amounts, straw consumption was significantly lower ($p<0.001$). The faeces from sows fed by-products was more compact, while faeces from sows fed roughage was formed spherically and had a stickier condition. The sows gained 6.2 ± 7.7 kg bodyweight (mean ± standard deviation) throughout the trial period.

Conclusions: Among the fibre rich by-products, sows preferred apple pomace, while hemp and hay were the preferred roughage choices. Regionally produced fibre rich by-products and roughages can offer a sustainable feedstuff for pigs, and the results from this trial might help to increase acceptance diets rich in these components in sows in the future.

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98.

Differences in feed intake duration, faecal consistency and short chain fatty acids in faeces from sows fed dried hemp plants *(Cannabis spp.)* either intact or grinded

Unterschiede der Futteraufnahmedauer, der Kotkonsistenz und kurzkettige Fettsäuren im Kot von Sauen, welche getrocknete Hanfpflanzen (Cannabis spp.) in ganzer oder vermahlener Form erhielten

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Chemical characteristics are commonly considered when comparing dietary fibres in swine nutrition. As it is known from other species, for instance cattle, physical characteristics such as the structure and particle size of roughage, also have a huge impact on digestive physiology, animal health, and performance [1]. However, this was only sparsely studied in pigs [2]. It was the aim of the study to compare the impact of grinded and intact, dried hemp plants on feed intake duration, faecal short chain fatty acid (SCFA) concentrations, and pH in the faeces, as well as the faeces consistency in adult sows.

Methods: Sixteen pluriparous, non-gestating German Landrace sows were kept individually in outdoor cages and fed a standard diet for gestating sows. The upper 20 cm of hemp plants (Finola) were harvested and subjected to hot-air drying. Dried hemp was added to the morning ration, either intact or grinded, at an inclusion level of 200 g/d. After 2 weeks, the feeding groups were changed according to a cross over design and the trial was continued for further 2 weeks. Feed intake and faecal scoring were recorded continuously. The duration (min) of feed intake was measured at three consecutive days in the second week of each feeding period. Bodyweight (BW) and back fat thickness were measured and faecal samples collected at the end of each feeding period. Dry matter (DM) content, pH, and nitrogen, SCFA, and lactate concentrations were obtained from the faecal samples. Statistical analyses were performed using IBM® SPSS® Statistics (version 25, IBM, USA). One-way ANOVA was applied for data with normal distribution, while the Mann-Whitney-U test was used to determine differences in non-parametric data. Differences were considered statistically significant at $p < 0.05$.

Results: Faecal scoring (4, both groups), BW (277 vs 271 kg) and back fat thickness were not affected by the particle size in the diet. Also, faecal pH, and faecal DM and nitrogen content did not differ between feeding groups. The concentration of SCFA, and lactate in the faeces was not affected by particle size. However, the duration of feed intake was significantly higher (15 ± 4 min/kg feed) in sows fed the unprocessed hemp compared to the group fed grinded hemp (11 ± 4 min/kg feed, $p < 0.001$).

Conclusions: Fibre rich feedstuff may increase the feed intake duration and thereby the chewing activity, if offered as larger particles. Further research is warranted to investigate how this influences behaviour and intestinal physiology, pH, and enzyme activity.

Bibliographical references

[1] MERTENS D.R. (1997): J. Dairy. Sci. 80:1463

[2] MOLIST F., MANZANILLA E.G., PEREZ J.F., NYACHOTI C.M. (2012): Brit. J. Nutr. 108:9

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99.

Effects of dietary rye and rapeseed on microbiota and electrophysiological parameters of the jejunum in weaner pigs

Effekte der Fütterung von Roggen und Raps auf Mikrobiota und elektrophysiologische Parameter im Jejunum von Absetzferkeln

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Rye and rapeseed meal (RSM) are alternatives to wheat and soybean meal (SBM) in pig nutrition. Rye can grow on poor soils and under dry climate conditions. Rapeseed is next to soybean the second most important protein source and can be cultivated in Europe. The fibre composition of feed components can affect intestinal microbiota and nutrient absorption via the gut wall [1]. Rye contains more soluble fibre than wheat and RSM more insoluble fibre than SBM [2]. Studies demonstrated that the feeding of high levels of rye and RSM did not reduce growth performance of pigs [3]. The aim of this study was to investigate the effect of dietary rye and RSM instead of wheat and SBM on the diversity and relative abundance of microbiota and electrophysiological parameters of the gut wall in the jejunum of weaner pigs.

Methods: A total of 88 weaner piglets was allocated to 44 pens, 4 isonitrogenous diets were fed over five weeks (n=11). Diets were based on either wheat/SBM (W-SBM), wheat/RSM (W-RSM), rye/SBM (R-SBM) or rye/RSM (R-RSM). Rye and wheat were included into the diets at 48%, RSM at 30% and SBM at 25%. Potato protein was added up to 5% to balance crude protein content. Diets were analysed for insoluble (IDF) and soluble dietary fibre (SDF). After the feeding phase, one piglet per pen was euthanized. For the measurement of parameters of the intestinal microbiome DNA was extracted from jejunal digesta and subjected to 16S rRNA sequencing. Jejunal tissue of 6 pigs per treatment (n=6) was mounted in Ussing chambers to assess basal tissue conductance (G_t) and the change of basal short-circuit current (ΔIsc) for lysine, glucose and carbachol. Statistics were carried out using a 2-factorial ANOVA with cereal (CER) and protein meal (PM) as fixed factors ($p<0.05$).

Results: Rye based diets had more SDF than wheat (R-SBM 37.1 and R-RSM 42.4 g/kg as-fed versus W-SBM 27.6 and W-RSM 27.1 g/kg). RSM based diets contained more IDF than SBM (W-RSM 137 and R-RSM 146 g/kg as-fed versus W-SBM 108 and R-SBM 115 g/kg). Ecological index of microbiota Richness was reduced by rye ($p = 0.047$), but Shannon Index and Evenness were not affected by the diets. Rye increased relative abundance of Firmicutes ($p = 0.039$) and decreased Proteobacteria ($p = 0.002$). RSM increased relative abundance of Proteobacteria ($p = 0.019$) and Actinobacteria ($p = 0.019$) and decreased Firmicutes ($p = 0.004$). Electrophysiological parameters of the gut wall were not affected by the diets.

Conclusions: This study shows that rye containing higher amounts of SDF may shift microbiota towards plant-cell wall degrading Firmicutes and a lower abundance of Proteobacteria, a phylum containing putative pathogens such as Salmonella. RSM resulting in less Firmicutes might be explained by high IDF content in RSM-based diets. However, alterations of resident microbiota seemed not related to transport physiology of the gut wall. More research is needed to further investigate the effect of dietary fibre from rye and RSM on host-microbiota interactions and gut health.

Bibliographical references

[1] AGYEKUM A.K., NYACHOTI C.M. (2017): Engineering 3: 716-725

[2] BACH KNUDSEN K.E. (2014): Poult. Sci. 93: 2380-2393

[3] WILKE V. (2020): Effects of increasing rye or rye and rapeseed meal (extracted) levels in diets for young pigs regarding digestibility and performance as well as diverse properties (milieu/substrate) of digesta in the alimentary tract, 230, DVG Service GmbH, Giessen

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100.

Impact of a maternal high-fat or high-protein diet supplemented by 10% inulin / oligofructose on milk oligosaccharide concentrations in mice

Einfluss einer maternalen Hochfett- oder Hochproteindiät mit 10 % Inulin / Oligofructose-Supplementierung auf die Milcholigosaccharid-Konzentrationen von Mäusen

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The composition of breast milk is influenced by maternal nutrition and plays a pivotal role in offspring development. Among others, milk oligosaccharides (OS) promote the growth of beneficial intestinal bacteria, modulate host-epithelial immune response, and prevent pathogen adhesion. Prebiotics are indigestible dietary fiber to the host, serve as fuel for the intestinal microbiota and have a positive impact on host microbiota composition. Little is known about the effects prebiotics may have on lactation and milk oligosaccharide composition. Our objective was to investigate the impact of supplementing high-fat (HF) and high-protein (HP) maternal diets with 10% inulin / oligofructose (FOS) on milk OS in mice.

Methods: Weaned offspring of five-week-old female C57BL / 6NCrl mice (n = 64) fed a control (C) diet, were randomly assigned to 6 feeding groups: C (n = 10), C with 10% inulin / FOS (1:1) (C+I; Orafti®HP inulin, Orafti®L95; n = 10), HF (40% gross energy from fat; n = 10), HF with 10% inulin / FOS (HF+I; n = 11), HP (40% gross energy from protein; n = 11) or HP with 10% inulin / FOS (HP+I; n = 12). At 8 weeks of age, female mice were mated (1:1) with males fed the same experimental diet. Litters were standardized randomly to 5 pups (n = 20-27 females and 23-33 males in either of the 6 feeding groups). On d10, dams and pups were separated for 3 h to perform milking. Dams were anesthetized and given oxytocin to induce lactation (2 IU, i.p.). After 15 min, milk was collected and 2-3 samples per dietary group were pooled. Lactose concentration was quantified by HPLC using an HPX-87C column. The OS concentrations of 3-fucosyllactose (3´FL), lacto-N-tetraose concentration (LNT), lacto-N-neotetraose (LNnT), lacto-N-hexaose (LNH), lacto-N-neo-hexaose (LNnH), sialic acid (SLA), 3´-sialyllactose (3´SL), 6´-sialyllactose (6´SL), disialyllactose (DSL), disialyllactose-N-tetraose (DSLNT), and sialyllactose-N-neotetraose (LSTc) were quantified by High pH Anion Exchange Chromatography with Pulsed Amperometric Detection using CarboPac PA. Data were analyzed using the MIXED procedure of SAS with the fixed effects diet, inulin / FOS, and their interactions. Multiple comparisons were done by Tukey-Kramer test.

Results: Diet effects were found for 3´FL, LNnH, LNT, LNH, SL, LSTc, 3´SL, DSL, and DSLNT ($P<0.05$), whilst an inulin / FOS effect was apparent for 3´FL, LNH, SL, 6´SL, and DSL ($P<0.05$). The diet by inulin / FOS interaction was significant for 3´FL, LNnH, LNT, 6´SL, and DSL ($P<0.05$). Milk 3´FL concentration was lower in C than HF and HP mice ($P<0.001$), whereas it was higher in HF+I than HF mice ($P<0.01$). Milk LNnH concentration was higher in HP than C and HF mice ($P<0.05$), whilst it was lower in C than HP+I and HF+I mice ($P<0.01$). The LNT concentration was higher in HP than C mice ($P<0.05$), whilst a higher LNT concentration was found in HF+I than HF mice ($P<0.05$). The LNH concentration was higher in C than HP and HF mice ($P<0.01$). The concentrations of 3´SL and 6´SL were higher in HP than HF mice ($P<0.05$), whereas the 6´SL concentration was lower in HP+I than HP mice ($P<0.05$). Lower LSTc and DSL concentrations were found in C than HP mice ($P<0.05$), whilst DSL was lower in C than HF mice ($P<0.01$). A higher DSL concentration was found in HF+I than HF mice ($P<0.05$). Further, DSLNT concentration was lower in HP than HF and C mice ($P<0.001$).

Conclusions: Higher milk LNT, DSL, and 3´FL concentrations in dams fed HF+I relative to HF and lower 6´SL levels in dams fed HP+I relative to HP indicate that the influence of inulin / FOS supplementation is dependent on the dietary background leading to a differential OS pattern in murine milk. The next steps will be the investigation of proximate composition of milk and long-term effects on offspring development and their gut microbiome.

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101.

Weaning age influences the host microbiome, metabolome and the host-microbe interactions in calves

Das Absetzalter beeinflusst das Mikrobiom, Metabolom und die Interaktion zwischen Mikroorganismen und dem Wirt in Kälbern

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Calves undergo extreme nutritional, metabolic and behavioural changes from birth to the complete weaning period, which influences their growth, health and productivity in the later stages. Negative effects can be minimized by carefully selecting the age at which the animal should be weaned. This study examined the impact of early vs. late weaning (7 vs. 17 weeks of age) on fecal microbiome and serum metabolome to understand the weaning-dependent shifts in host-microbe interactions.

Methods: This study was done by random allocation of 59 female German Holstein calves (8 ± 1.9 days of age) into an early-weaning (earlyC) and late-weaning (lateC) group. The experimental design is reported in [1]. Calves received 3 L of colostrum within 2 h after birth, followed by 3 L of pooled herd milk fed twice a day. From third day of life until the start of experimental period, pooled herd milk was mixed with milk replacer (MR). The MR amount increased from 0.3 kg / d (day 3) to 0.9 kg / d (day 5). MR was gradually increased from 0.9 kg / d to 1.35 kg / d (days 6-10) and remained constant until the start of weaning. During weaning, MR was reduced to 0.3 kg / d in a 14 days step down approach during days 28–42 for earlyC and days 98–112 for the lateC group, followed by ad libitum access to hay and a total mixed ration comprising of grass silage (48 %), maize silage (32 %), and concentrates (20 %) [1]. Blood and fecal samples were collected from each calf during days 1, 28, 42, 70, 98, 112, and 140. DNA was extracted from fecal samples using FastDNA SPIN Kit for Soil (MP Biomedical) and bacterial communities were analyzed based on Illumina sequencing targeting V1- V2 region of 16S rRNA gene. Blood samples were processed for targeted serum metabolomics using AbsoluteIDQ®p180Kit (Biocrates Life Science, Austria). Bioinformatic analysis of fecal microbiome dataset was done using QIIME 2 (2019.10), and the resulting amplicon sequence variants (ASVs) were assigned taxonomy based on RDP and BLAST databases. The multivariate analysis of serum metabolites was done using MetaboAnalyst 5.0. The microbiome and metabolome datasets were correlated using Spearman's rank correlations. For statistical analyses, Primer-E, R v4.0.3, and Calypso v8.84 were used [2].

Results: A total of 4,229 unique bacterial ASVs were observed in the fecal samples of all 7 time points. Weaning age clearly impacted the fecal microbiome (days 42–98) and serum metabolome (days 42–112), where earlyC group showed high dominance of unclassified members of fiber-degrading bacteria, while, the same-day-old lateC group had higher lactose- and starch-degrading bacteria. During days 42–112, the serum samples of earlyC group showed lower concentrations of most of the amino acids (AA), biogenic amines (BA) and sphingomyelins (SM) as compared to the same-day-old lateC group. Strong host-microbe interactions were observed during days 42–98, where the abundances of lactose- and starch degrading bacteria were strongly positively correlated with the serum concentrations of AA, BA and SM ($r > 0.5$, $p < 0.05$).

Conclusions: Weaning calves at an early age (7 weeks) clearly impacted their fecal microbiome, serum metabolome as well as host-microbe interactions. Late weaning at 17 weeks was beneficial as the fecal bacterial communities of lateC group showed quick adaptation to weaning-related dietary shifts at day 112, indicating their greater GIT maturation at weaning as compared to the earlyC group.

Bibliographical references

[1] SCHWARZKOPF S., KINOSHITA A., KLUESS J., KERSTEN S., MEYER U., HUBER K., DÄNICKE S., FRAHM J. (2019): Animals 9: 1132

[2] AMIN N., SCHWARZKOPF S., KINOSHITA A., TRÖSCHER-MUßOTTER J., DÄNICKE S., CAMARINHA-SILVA A., HUBER K., FRAHM J., SEIFERT J. (2021): Animal Microbiome 3: 31

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102.

The effect of age, breed, sex, and dietary factors on metabolite concentration and immunological traits in the caecum of broilers

Der Einfluss von Alter, Rasse, Geschlecht und Ernährungsfaktoren auf die Konzentration von Metaboliten und immunologische Merkmale im Zäkum von Broilern

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Bacterial metabolites play an important role in regulating immune responses, including cytokine production, in the gut. Age and genetic background as well as dietary factors may affect bacterial activity and subsequently the mucosa-associated immune system in the caecum. This study investigated the effect of age, breed, sex, and probiotic or phytobiotic supplementation as well as their interactions on bacterial activity (metabolites) and expression of genes related to epithelial barrier and inflammation in the caecum of broilers.

Methods: A total of 2,880 one-day-old male (M) and female (F) broiler chicks from two breeds, Ross-308 (RS) and Cobb-500 (CB), were randomly allocated to 72 pens (one breed and one sex per pen). Broilers were offered 3 experimental diets including a standard wheat-soybean based diet without (CO) or with supplementation of either a probiotic (PO; 2.4x 10^9 CFU / kg diet; Bacillus subtilis DSM32324 and DSM32325 and Bacillus amyloliquefaciens DSM25840) or a phytobiotic (PY; 165 ppm procyanidins and 585 ppm polyphenols) product. At day 7, 21, and 35 of age, one chicken per pen was sacrificed to dissect the caecum. The right caecal content was used for short-chain fatty acids (SCFA) and lactic acid (LA) analysis and the left caecal tissue was used for gene expression analysis. Data were subjected to ANOVA using GLM procedure with a 3 (age) × 2 (breed) × 2 (sex) × 3 (dietary treatment) factorial arrangement of the main factors.

Results: Overall, the concentration of SCFA including acetic, propionic, i- and n-butyric, i- and n-valeric acid increased with age ($p < 0.05$). Both L- and D-forms of LA decreased from day 7 to 21 ($p < 0.05$) and stayed constant until day 35 of age. These metabolites were not affected by breed, sex, dietary treatment, and interactions between main factors ($p > 0.05$), except for D-LA which showed a higher concentration in CB than RS ($p < 0.05$). The absolute gene expression (log10 copies number / ng RNA) of interleukin (IL)-2, 4, 6, 8, 10, 12, 17 and 18, as well as IFN-γ and TGF-β increased with age, whereas IL-1 and TNF-α increased from day 7 to 21 and then decreased at day 35 ($p < 0.05$). The expression of MUC-2 decreased from day 7 to 21, and then increased at day 35, while CLDN-5 only increased from day 21 to 35 ($p < 0.05$). Birds fed PY diet showed an increase in IL-10 expression compared with those fed CO diet ($p < 0.05$). CB showed a higher expression of IL-1, IL-6, TNF-α and CLND-5 compared with RS ($p < 0.05$). Sex and interactions had no effect on the variables measured ($p > 0.05$). Several metabolites, especially propionate, were positively correlated with expression of the investigated genes ($r = 0.160$ to 0.548), except for TNF-α which showed a negative correlation with all the metabolites ($r = -0.154$ to -0.285) but not acetate and n-butyrate. In contrast, D- and total LA concentration was negatively correlated with IL-1, IL-4, TNF-α and IFN-γ ($r = -0.324, -0.179, -0.209, -0.227$ and $r = -0.279, -0.175, -0.198, -0.170$, respectively), while total LA was positively correlated with MUC-2 ($r = 0.166$). L-LA concentration showed only a positive correlation with IL-12 and 17 as well as TGF-β, MUC-2 and CLDN-5 ($r = 0.136, 0.118, 0.140, 0.167$, and 0.183, respectively).

Conclusions: Age had significant impacts on almost all the variables measured in the caecum, while the nutritional treatments had almost no effect on them. A few observed significant impacts of breed on the variables measured did not show any systematic biological pattern. Furthermore, sex and interactions between main factors had no impact on any variables measured. The alterations in the evaluated bacterial metabolites by age affected gut immune response in the caecum of broilers. However, the mechanisms behind the effects need to be studied further.

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103.

Investigation of a specific prevention strategy against an *Escherichia coli* infection using a pre- / probiotic combination and parent stock vaccination in broilers

Untersuchung einer spezifischen Präventionsstrategie gegen eine Escherichia coli-Infektion unter Einsatz einer Prä- / Probiotikum-Kombination und einer Elterntierimpfung bei Masthähnchen

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Globally, antibiotic resistance poses a risk for human and animal health, which leads to a growing demand for effective alternatives [1]. Different effects of individual alternatives are described. Combining several methods to improve health could be an approach to achieve safer and more effective protection against bacterial infections in livestock [2]. Thus, the aim of the study was to investigate if a preventive treatment strategy, involving nutritional tools and specific parent stock vaccination, can enhance performance and intestinal bacteria metabolism in broilers, when challenged with *Escherichia coli (E. coli)*.

Methods: In an infection trial, 225 one-day-old Ross 308 chicks were randomly assigned to five treatment groups with nine birds per pen and five replicates per treatment. Feed and water were offered ad libitum throughout the entire trial period. The experimental diet was based on corn, wheat and soybean meal (control diet) and was fed to groups Cn (negative control), Cp (positive control) and VAC (parent stock vaccination). Moreover, the diet for groups FA (feed additives) and FA-VAC (feed additives and parent stock vaccination) were supplemented with a combination of both Enterococcus faecium (109 cfu / kg feed) and fructooligosaccharides (FOS, 10 g / kg feed). Chicks in groups VAC and FA-VAC derived from parent stock, which had been vaccinated with an inactivated *E. coli* strain of serotype O1 / O18 at 12 and 17 weeks of age. At eight days of age, broilers in group Cp and treatment groups were orally challenged with an avian pathogenic *E. coli* O1 / O18 field isolate (1,92 × 10^7 cfu / animal). Group Cn was not infected. Bodyweight (BW), bodyweight gain (BWG), Feed intake (FI) and feed conversion ratio (FCR) were obtained weekly. After four weeks, two chickens per pen (ten per treatment) were sacrificed by cervical dislocation and crop and caecal contents were analysed for pH and bacterial metabolites. Statistical analyses were calculated using ANOVA and post hoc Tukey-HSD for normally distributed data (performance, pH), whereas the non-parametric Kruskal-Wallis test with Bonferroni correction was performed for non-normally distributed data (metabolites). Differences were rated as significant at $p \leq 0.05$.

Results: Animals, which received both treatments (feed additives and parent stock vaccination), achieved the highest body weights, significantly higher than the negative and positive control groups throughout the experiment. The immune protected group without supplements also achieved significantly higher weights than the negative control at weeks two, three and four. Compared to the controls (pH 7.10 – 7.24), the pH-levels in the caecum were significantly reduced in the groups receiving the feed additives (pH 6.75 – 6.77). In the crop, however, a trend toward decreased pH compared to control groups (pH 5.16 – 5.17) was only observed in group FA-VAC (pH 4.74). Contrary to caecum samples, crop samples from groups receiving feed additives showed significantly higher L-lactate values (49.63 – 52.48 µmol / g) compared to the control groups (20.35 – 21.99 µmol / g). Concentrations of total fatty acids in the caecum were significantly increased in the groups with parent stock vaccination (121.60 – 126.02 µmol / g) compared to negative control (83.26 µmol / g).

Conclusions: Under conditions of *E. coli* challenge, prophylactic administration of Enterococcus faecium and FOS in combination with parent stock vaccination can improve broiler performance. In addition, pH-levels were decreased and lactate increased in the crop of animals receiving the feed additives and maternal vaccination.

Bibliographical references

[1] INTERAGENCY COORDINATION GROUP ON ANTIMICROBIAL RESISTENCE (2019): Report to the Secretary-General of the United Nation

[2] MEHDI Y., LÉTOURNEAU-MONTMINY M.P., GAUCHER M.L., CHORFI Y., SURESH G., ROUISSI T, BRAR S.K., CÔTÉ C., RAMIREZ A.A., GODBOUNT S. (2018): Animal nutrition 4: 170-178

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104.

Fecal microbiota of vaccinated or non-vaccinated clinically inconspicuous and conspicuous piglets under *Lawsonia intracellularis* infection

Fäkale Mikrobiota von geimpften und nicht-geimpften klinisch unauffälligen bzw. auffälligen Ferkeln unter Lawsonia intracellularis-Infektion

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In the present study, clinically moderate *Lawsonia intracellularis (L. i.)* infections resulted in significantly lower average daily weight gain and impaired apparent total tract nitrogen digestibility, while the total length of the small intestine increased [1]. However, pathogen exposure does not necessarily always lead to clinical disease. Gnotobiotic pigs lacking a normal gut microbiota are not colonized by the organism and fail to develop lesions [2]. Based on this observation, we hypothesize that colonization with *L. i.* and development of the disease might require the presence of certain anaerobic intestinal bacteria. Therefore, the aim of the present study was to test this assumption with a microbiota that developed in a farm environment.

Methods: In three consecutive trials, 27 *L. i.*-infected pigs (19.0 ±1.50 kg body weight) of one farm were divided into three groups at an age of seven to eight weeks (9 pigs / group): vaccinated via oral drenching on their 21st day of life (Enterisol®Ileitis, attenuated live vaccine (*L. i.*)) = Vac; non-vaccinated, without clinical findings = Non-vac / cs-; and non-vaccinated with clinical findings (healthy pigs with moderate to soft feces consistency) = Non-vac / cs+. Pigs were housed individually and fed a conventional diet ad libitum (XP: 176 g, XF: 23.5 g, XL: 33.6 g, ME: 13.8 MJ / kg diet) for 7 days. A bulk sample was formed from feces of the last five days (collection period), from which, subsequent to homogenization, an aliquot was taken for 16S rRNA analysis. Data visualization were done in R (version 4.1.0) with the R-package “phyloseq” (version 1.36.0).

Results: Permutational multivariate analysis of variance using Bray-Curtis distancerevealed significant differences in fecal sample’s bacterial composition between the three groups ($p = 0.001$, $R^2=0.1432$, R-package “vegan” (version 2.5.7)). Fecal bacterial diversity was not significantly different between the groups. However, all measured richness estimators of Non-vac / cs+ pigs were numerically lower compared to the other two groups. Corresponding to the selected criterion (Benjamini-Hochberg (BH)-adjusted p-values <0.05 and absolute log2 fold change >2) significantly different abundances were found for 5 of 124 OTUs (operational taxonomic units) only between the groups Vac vs. Non-vac / cs+ (compared using the R-package “DESeq2” (version 1.28.1) which uses tests based on the negative binomial distribution). Enriched sequences of OTUs in Non-vac / cs+ piglets compared to Vac belonged to two bacterial members of the family Veillonellaceae and to one bacterial member of the genus Collinsella, family Coriobacteriaceae. Additionally, relative abundances of the family Coriobacteriaceae increased from Vac (0.698% ±0.526) to Non-vac / cs- (1.62% ±0.764) and Non-vac / cs+ (2.37% ±2.06). Sequences of one OTU belonging to the genus Prevotella and one OTU belonging to the order Clostridiales were enriched in Vac compared to Non-vac / cs+. This OTU closely matched the species Prevotella copri. Gut inflammation leads to a nitrate-rich environment with increased oxygen concentration [3], an environment that does not confer a growth advantage to Prevotella copri, which is why its lower abundance within the microbiota of Non-vac / cs+ could rather be a consequence from gut inflammation and dysbiosis, not a triggering factor. Also higher abundance of bacterial members of the family Veillonellaceae in Non-vac / cs+ could be a response to the metabolites formed by Collinsella (lactate) that are used as substrate, and not primarily causative for alterations of fecal consistency.

Conclusions: The higher abundance of the genus Collinsella within the intestinal microbiota of pigs (lowest in vaccinated pigs) could be key factor for the outcome of a clinically obvious *L. i.* infection, a suspicion that should be evaluated further.

Bibliographical references

[1] VISSCHER C., MISCHOK J., SANDER S., SCHMICKE M., PEITZMEIER E.-U., VON DEM BUSCHE I., ROHN K., KAMPHUES J. (2018): BMC Vet. Res. 14: 323

[2] MCORIST S., JASNI S., MACKIE R., MACINTYRE N., NEEF N., LAWSON G. (1993): Infect. Immun. 61(10):4286

[3] GRESSE R., CHAUCHEYRAS-DURAND F., FLEURY M.A., VAN DE WIELE T., FORANO E., BLANQUET-DOIT S. (2017): Trends Microbiol. 25(10):851-73

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105.

Impact of colostrum from sows fed different fibers against Clostridioides difficile toxin-induced effects on IPEC-J2 cells

Die Auswirkungen von Kolostrum von Sauen mit unterschiedlicher Faserversorgung gegen Clostridioides-difficile-Toxin-induzierte Effekte auf IPEC-J2-Zellen

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Toxin A (TcdA) and toxin B (TcdB) are the primary agents causing intestinal epithelial damage being a main factor in the etiology of Clostridioides difficile-infection in neonatal piglets [1]. Porcine colostrum is rich in nutrients and bioactive components and has been shown to strengthen the epithelial barrier function in porcine gut and protect from clostridial toxin-induced effects [2]. Dietary fiber has been shown to modify colostrum and milk composition in sows [3]. Therefore, we hypothesized that colostrum from sows fed high-fermentable or low-fermentable fibers can differentially exert protective effects against clostridial toxins on intestinal porcine epithelial cell line (IPEC-J2).

Methods: Twenty sows were fed experimental gestation and lactation diets enriched with either high-fermentable sugar beet pulp (SBP; inclusion rate: 15 % sugar beet pulp, 3 % lignocellulose) or low-fermentable lignocellulose (LNC; inclusion rate: 15 % lignocellulose, 3 % sugar beet pulp) fibers. The diets were iso-caloric and iso-nitrogenic and were based on barley, wheat and soybean meal. The sows were fed restrictively during gestation, while ad libitum during lactation. Colostrum from randomly chosen teats was collected within 10 hours after beginning of the farrowing and once the afterbirth was excreted and was assessed for IgG-anti-TcdA- and TcdB-antibodies (ELISA). Further, IPEC-J2 cells were treated with the colostrum from individual sows or with toxins or with the combination of toxins and individual colostrum samples. The integrity of IPEC-J2 was analyzed hourly (0-5 h) using trans-epithelial electrical resistance (TEER) method. Data were analyzed by Kruskall-Wallis or Mann-Whitney U test (significance at $p \leq 0.05$) (SPSS version 27). The animal trial was approved by the Regional Office for Health and Social Affairs (LAGeSo Reg. G0112/19).

Results: Colostrum from sows fed SBP had slightly higher IgG-anti-TcdA-antibody titers, as compared to sows fed LNC ($p=0.968$). This same was noticed for colostrum IgG-anti-TcdB-antibody titers in SBP vs. LNC fed sows ($p=0.739$). Independent of the dietary treatment, IgG-anti-TcdA- or IgG-anti-TcdB-antibodies from sow colostrum showed strong positive correlation ($p=0.005$). Toxins decreased the integrity of IPEC-J2 cells in a time-dependent manner. Colostrum from sows fed either SBP or LNC exerted a protective effect against toxins as indicated by TEER in IPEC-J2 cells. The integrity of IPEC-J2 after 2 h of incubation was 72 % in control medium, 50 % in colostrum from SBP-fed sows, 47 % in colostrum from LNC-fed sows, 8 % in toxins, 37 % in colostrum from SBP-fed sows + toxins and 18 % in colostrum from LNC-fed sows + toxins of the TEER before any treatment was applied to the cells ($p=0.043$).

Conclusions: Colostrum of the sows fed either highly-fermentable (SBP) or low-fermentable (LNC) fibers contains antibodies against C. difficile toxins, which in addition to other present bioactive components, may have a potential to protect IPEC-J2 cells from intoxication. This may have important implications in protection from C. difficile-infection development in neonatal piglets.

Bibliographical references

[1] VOTH D., BALLARD J. (2005): Clin. Microbiol. Rev. 18:247-63

[2] GRZEŚKOWIAK Ł., PIEPER R., KRÖGER S., MARTÍNEZ-VALLESPÍN B., HAUSER A.E., NIESNER R., VAHJEN W., ZENTEK J. (2020): Microorganisms. 8:142

[3] KROGH U., BRUUN T.S., AMDI C., FLUMMER C., POULSEN J., THEIL P.K. (2015): Can. J. Anim. Sci. 95:211-23

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106.

Faecal characteristics of old vs younger adult horses under similar housing and feeding conditions

Kotparameter alter vs. jüngerer Pferde unter vergleichbaren Haltungs- und Fütterungsbedingungen

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Even healthy old horses (age > 20 yrs.) show remarkable anatomical and physiological changes which also include the digestive tract. Diverse faecal parameters such as pH value and contents of dry matter (DM), short chain fatty acids (SCFA), lactate and ammonia are suitable to characterize microbial activity and milieu conditions in the posterior part of the large intestine. It is questionable how clinically normal old and younger adult horses differ in this regard. The objective of the study was to compare faecal parameters of mobile horses of both groups under similar housing and feeding conditions in practice.

Methods: From a riding stable, 18 old (21 - 32 years; 6 mares, 12 geldings; OH; age 25.56 ± 3.55) and 17 younger (6 - 16 years; 7 mares, 10 geldings; YH; age 11.65 ± 3.26) warmblood type horses were subjected to the study. The horses' withers height was OH 164.39 ± 9.22 cm / YH 166.94 ± 6.94 cm, the body weight was OH 549.11 ± 78.73 kg / YH 587.41 ± 70.72 kg, the body condition score was OH 4.87 ± 0.91 / 5.20 ± 0.25 (scale 1 - 9) [1], and the cresty neck score was OH 1.64 ± 0.80 / 1.76 ± 0.44 (scale 0 – 5) [2]. The horses did not have any health problems that would had restricted mobility or received medication for the last four weeks prior to the study. All horses were regularly dewormed and vaccinated, had paddock turnout for 6 hours per day and were additionally moved for leisure. The boxes were equipped with wheat straw for bedding (2 horse wood shavings), automatic waterers and a salt lick. Three times per day 3 kg of meadow hay and 0.5 - 1.5 kg of concentrates (mostly oats:barley = 1:1) were fed. Two old horses got additionally a minor amount of hay cobs and one old horse hay cobs as solely fibre source. At the test day, fresh morning faeces were sampled, pH measured, and samples prepared for later detection of DM as well as SCFA, ammonia and L- / D-lactate in the faecal innate water [3]. Results were investigated by analysis of variance and regression analysis with the age within group as independent variable (SAS). Results are given as least square means ± standard error. The level of significance was pre-set at $P<.05$.

Results: FaecalDM content (g/kg: YH 199 ± 7.83, OH 202 ± 7.77) and the faecal waters pH (YH 7.0 ± .094, OH 6.9 ± .093) and content of ammonia (mmol/L: YH 8.4 ± 1.1, OH 7.3 ± 1.0), total SCFA (mmol/L: YH 36.8 ± 3.59, OH 36.7 ± 3.57), and proportion of propionate (Mol%: YH 17.7 ± .924, OH 19.3 ± .918) did not differ between groups ($P>.05$). The amount of acetate was lower (Mol%: YH 75.0 ± 1.18, OH 70.6 ± 1.17; $P<.012$) and that of n-butyrate (Mol%: YH 4.4 ± .23, OH 5.2 ± .23; $P<.018$), iso-butyrate (Mol%: YH 1.3 ± .18, OH 1.8 ±.18; $P<.048$) and iso-valerate (Mol%: YH 1.1 ± .18, OH 1.9 ± .198; $P<.004$) higher in OH vs YH. Within OH, molar proportion of n-butyrate increased (Bc=.500; P=.0006) with age. There was no group effect on the acetate-propionate quotient (YH 4.3±.24, OH 3.9 ± .24; $P<.171$). Lactate isomers (mmol/l) were higher in the group of old than young horses (OH L-lactate 3.13 ± 2.53; D-lactate 1.66 ± 1.06, YH L-lactate 3.04 ± 1.69; D-lactate 0.86 ± 0.39).

Conclusions: The results of the present study, except lower acetate%,did not reveal restricted fermentation of large intestine microbes in clinically healthy old horses under the conditions of the recent study. The profile of SCFA however shows notable age-related alterations between age groups and within the group of old horses, too. Beside lower acetate%, the higher proportions of n-butyrate and iso-SCFA in the old horses are of interest as n-butyrate has a trophic function for the gut wall and iso-SCFA indicate elevated microbial protein metabolism, respectively. Due to dynamic changes within the group of old horses, it is questionable whether it is justified to define these uniformly being > 20 yrs. of age.

Bibliographical references

[1] SCHRAMME S.C., KIENZLE E. (2004): Pferdeheilkunde 20: 517-524

[2] CARTER R.A., GEOR R.J., BURTON STANIAR W., CUBITT T.A., HARRIS P.A. (2009): Vet. J. 179: 204-210

[3] GLATTER M., WIEDNER K., HIRCHE F., MIELENZ N., HILLEGEIST D., BOCHNIA M., CEHAK A., BACHMANN M., GREEF J.M., GLASER B., WOLF P., BREVES G., ZEYNER A. (2016): J. Anim. Physiol. Anim. Nutr. 1: 16

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107.

Whole black soldier fly larvae in broiler rations: Impact on carcass characteristics, blood metabolites and fatty acids profiles in plasma, muscle and fat tissues

Einfluss von ganzen Schwarzen Soldatenfliegenlarven als Rationsbestandteil auf die Eigenschaften von Schlachtkörper, Blutmetabolite und Fettsäurenprofile im Plasma, Muskel- und Fettgewebe

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Soybean meal in poultry diets can be replaced by defatted black soldier fly larvae (BSFL) meal without affecting performance [1]. However, whole BSFL could be fed to chickens directly without processing [2]. We investigated the effects of different inclusion levels of unprocessed BSFL in broiler diets on metabolism, immune status, carcass characteristics, and fatty acids (FA) profiles in different tissues of broilers.

Methods: Ross 308 chicks (N = 252; 63 animals / group) received daily either a control diet (CON) without access to BSFL or CON plus BSFL amounting to 10% (L-10), 20% (L-20) or 30% (L-30) of the feed intake (fresh matter) over a period of 6 weeks. Feed intake of the CON birds was the basis for the calculation of BSFL amount given to the other three groups on the next day (d). On d 28 and 42 of the experiment, 12 chicks / group were slaughtered for blood sampling and determination of breast, leg, wing and abdominal fat mass. Also, pH, color, and drip loss in breast muscle were measured. Selected metabolites were measured in plasma and serum. Fatty acids were measured in plasma, abdominal fat and muscle tissue using gas chromatography. Data were analyzed using the MIXED procedure of SAS followed by Tukey test to separate group means.

Results: Until week 5, there was no difference for body weight (BW) among the groups. However, at week 6, L-30 group had lower BW compared to L-20 group. Feeding up to 30% BSFL of the total feed intake to broilers did not affect carcass characteristics, and serum calcium and phosphorus concentrations ($P>0.05$). Polynomial contrasts analyses showed a positive quadratic effect of increasing amount of BSFL on serum IgA concentration ($P=0.020$). Plasma triglyceride concentration was higher in L-30 than in L-10 and CON ($P<0.05$). Plasma glucose levels tended to be higher in L-30 than L-10 ($P=0.072$). Plasma non-esterified fatty acids (NEFA) concentration were higher in L-30 than in L-10 and the CON group ($P<0.05$). Also, NEFA was higher in L-20 than CON ($P<0.05$). In addition, L-30 had higher serum alkaline phosphatase (ALP) activity than CON ($P= 0.013$). Inclusion of 30% BSFL increased plasma and abdominal fat saturated FA (SFA) contents. In addition, polyunsaturated, omega-3 and omega-6 FA concentrations in plasma, breast and abdominal fat were lower in L-30 than in CON ($P<0.05$). Conjugated linoleic acids (CLA) content in plasma, muscle and abdominal fat increased in response to increasing level of larvae ($P<0.05$). Birds supplemented with BSFL had a higher concentration of C10:0 and C12:0 FA in plasma, abdominal fat and muscle than those in CON ($P<0.05$).

Conclusions: Up to 20% BSFL in broiler diet had no negative effect on growth performance. Inclusion of BSFL was associated with increased SFA and medium chain FA concentrations in plasma and abdominal fat while muscle was not affected. Increased plasma IgA concentration in response to feeding larvae suggest a stimulation of the immune system. However, altered blood metabolites and higher serum ALP activity in L-30 indicates a shift in lipid metabolism, and negative effects on the liver.

Bibliographical references

[1] SCHIAVONE A., DE MARCO M., MARTINEZ S., DABBOU S., RENNA M., MADRID J., HERNANDEZ F., ROTOLO L., COSTA P., GAI F., GASCO L. (2017): J. Anim. Sci. Biotechnol. 8: 1-9

[2] IPEMA A.F., GERRITS W.J., BOKKERS E.A., KEMP B., BOLHUIS J.E. (2020): Appl. Anim. Behav. Sci. 230:105082

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108.

Effects of the probiotic *Bacillus amyloliquefaciens* CETC 5490on primary cultured chicken immune cells

Effekte des Probiotikums Bacillus amyloliquefaciens CECT 5490 auf primär kultivierte Immunzellen vom Huhn

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Probiotics are commonly accepted and used in animal health. Feeding *Bacillus amyloliquefaciens (B. amyloliquefaciens)* CECT 5490, a widely used gram positive, sporulating bacterium and a licensed probiotic for chicken has been shown to possess positive effects on performance and feed conversion in chickens for fattening via modulating gut microflora and improving nutrient uptake. Furthermore, an effect on the immune system was hypothesized. Here, we evaluate direct immunomodulatory effects of *B. amyloliquefaciens* CECT 5490 on primary cultured chicken immune cells.

Methods: Cell culture experiments with primary cultured peripheral blood mononuclear cells (PBMCs) from chicken were conducted to explore direct interactions of lymphocytes (1×10^6 / ml) with vital B. amyloliquefaciens CECT 5490 in a ratio of 1:3, 1:5 or 1:10 (PBMCs : *B. amyloliquefaciens*) for 24 hours. For co-culture experiments, cells were cultured in RPMI-1640 medium supplemented with 10 % chicken serum at 41 °C with 5 % CO_2. Concanavalin A (conA, 10 µg / ml) was used as a positive control. We followed changes in the composition and activation status of lymphocytes via flow cytometry (FACS Canto II, BD Life Sciences) using monoclonal antibodies labeling different immune cell populations: T-helper cells (CD4-SPRD, CD25-FITC), cytotoxic T-cells (CD8-APC, CD25-FITC), and B-cells (Bu-1a-FITC). Analysis was performed using FlowJo™ v10.5.0 Software (BD Life Sciences). Due to high variation, the Δ relative cell count was calculated representing the difference between the treatment and the control for every replicate. Statistical significance of the Δ relative cell count was calculated using a one-sided Student's t-test against a hypothetical value "0".

Results: After treatment with *B. amyloliquefaciens* CECT 5490 in a ratio of 1:3, we found a significant increase of the Δ relative cell count for T-helper cells ($p < 0.05$). Treatment with conA, which served as a positive control within the cell culture assay to control for the capacity to react to stimuli, resulted in a significant increase of the Δ relative cell count for T-helper cells ($p < 0.01$), activated T-helper cells ($p < 0.001$), activated cytotoxic T-cells ($p < 0.001$), and B-cells ($p < 0.01$). If we treated the cells with more bacteria in a ratio of 1:5 or 1:10, we were not able to detect significant differences of the Δ relative cell count of T-helper cells or B-cells. However, we observed that the Δ relative cell count of cytotoxic T-cells decreased ($p < 0.05$) after treatment with *B. amyloliquefaciens* CECT 5490 in a ratio of 1:5 compared to the control, whereas conA treatment increased the Δ relative cell count of activated cytotoxic T-cells ($p < 0.01$), T-helper ($p < 0.01$) and activated T-helper cells ($p < 0.001$).

Conclusions: This study provides evidence of a direct immunomodulatory effect of *B. amyloliquefaciens* CECT 5490 on chicken immune cells *in vitro*. We found an immune stimulating effect on T-helper cells, which are more associated with the defense of bacterial infections, whereas cytotoxic T-cells, which kill primarily virus infected cells, seemed to be reduced.

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109.

Effect of a grape extract on performance, digestibility and microbial metabolites of weaning piglets in comparison to a negative and positive control

Einfluss eines Traubenextrakts auf zootechnische Leistung, Nährstoffverdaulichkeit und mikrobielle Metabolite bei Absetzferkel in einem Modell mit Negativ- und Positivkontrolle

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Enteric diseases like post-weaning diarrhoea often require antibiotic treatment of the entire litter to prevent the spread of disease, poor health and performance. Polyphenols, showing antimicrobial and anti-inflammatory properties (1), might be used as alternative to antibiotics to overcome post-weaning diarrhoea. Grape polyphenols have a positive impact on gut health, however, results are not consistent, e.g. regarding inclusion level (2, 3). In an 8-week *in vivo* trial the impact of a grape extract (GpE) in the diet of weaning piglets on performance, apparent total tract digestibility (ATTD), and microbial metabolites of the ileum and colon was investigated. It is hypothesized that GpE inclusion can positively affect performance of piglets at least at the same level as an initial therapeutic antibiotic treatment (positive control; PC). The comparison between the effects of GpE and PC on ATTD and concentration of microbial metabolites on two sampling points (T1: 4 weeks post weaning; T2: 8 weeks post weaning) allows to demonstrate where GpE acts in a similar manner as PC and where the mode of action may differ.

Methods: At weaning, 180 piglets (6.9±0.1 kg body weight (BW)) were assigned to three treatment groups (6 male and 6 female pens each, 5 piglets / pen). Piglets received a corn-based diet without supplementation (negative control; NC), in-feed antibiotic in a therapeutic dosage (PC, 20 mg amoxicillin / kg BW every 12h; Amoxicillin-Trihydrate 100, 1000 mg / g) from day 1 to day 5 post weaning or GpE (150g / t, dried extract from dried grapes (Vitis vinifera); ~ 40% total polyphenol content) diet supplementation for the whole trial duration. Diets were provided ad libitum as starter (d1-d13; 14.3 MJ / kg, 192 g CP / kg) and grower (d14-d56; 14.0 MJ / kg, 176 g CP / kg, 0.3% TiO_2 as inert marker) diet. To calculate ATTD, pooled samples of faeces were collected per pen twice a day on two consecutive days before euthanasia (1 piglet / pen) on T1 and T2. Upon euthanasia, ileal and colonic digesta was collected for determination of microbial metabolites (short chain fatty acids (SCFA), lactic acid, biogenic amines, ammonia). Statistical analyses were performed using MIXED procedure of SAS 9.4. Statistical model included treatment, sex and feeding phase / sampling day as well as the interactions of these factors. Least square means were compared by Tukey–Kramer Test and differences were considered significant at $p < 0.05$ or as trends for p-values between 0.05 and 0.10.

Results: Regarding total piglet performance (d1-d56), neither GpE nor PC showed an effect compared to NC. However, animals from PC showed higher BW on day 13 compared with NC and GpE, and a tendency for higher final BW ($p = 0.08$) compared to NC. Additionally, PC increased the average daily feed intake (ADFI) in the starter phase and the average daily gain in the early grower phase (d14-d24). Grape inclusion did not increase palatability (ADFI $p > 0.05$). Overall, in comparison to NC, GpE improved the ATTD of nutrients ($p < 0.05$) at the same level as PC (ash, acid-hydrolysed ether extract), or at a higher level than PC (dry matter, organic matter, gross energy, crude protein, P). There was no effect on SCFA and biogenic amine concentrations in the digesta of ileum and colon. However, both PC and GpE showed the tendency ($p =0.059$) to increase the lactic acid concentration in colonic digesta at T1. Additionally, GpE tended ($p = 0.084$) to decrease ammonia concentrations compared to NC in T1.

Conclusions: The GpE inclusion markedly enhanced ATTD of nutrients compared to NC, with minor effects compared to PC and showed beneficial trend on microbial metabolites in the first four weeks post weaning. Dietary inclusion of GpE may have beneficial effects on gut health compared to application of therapeutic antibiotic dosage, as frequently used at weaning.

Bibliographical references
[1] ZHANG H., TSAO R. (2016): Curr. Opin. Food Sci. 8: 33-42
[2] BRENES A., VIVEROS A., GOÑI I., CENTENO C., SAURA-CALIXTO F., ARIJA I. (2010): Span. J Agric. Res. 8: 326-333
[3] LAU D.W., KING A.J. (2003): J Agric. Food Chem. 51: 1602-1607

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110.

Effectiveness of combined feed additives for inactivation of deoxynivalenol and zearalenone in diets for weaned piglets

Inaktivierung von Deoxynivalenol und Zearalenon in Mischfutter für Absetzferkel

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Deoxynivalenol (DON) and zearalenone (ZEN) are mycotoxins frequently occurring in feed which may cause performance losses and reproductive disorders when critical concentrations in feed for pigs are exceeded. Therefore, effective strategies to inactivate these toxins are required. The use of sodium metabisulfite (SBS, $Na_2S_2O_5$) as an additive in wet preservation of feedstuff was demonstrated to be effective in inactivating DON [1,2]. However, this technique is not suitable for compound feed in agricultural practice. Therefore, the use of SBS in contaminated and dry compound pig feed should be evaluated. As SBS does not inactivate ZEN, the simultaneous decontamination of ZEN as a co-contaminant has been tested using ZENzyme®, a zearalenone hydrolase.

Methods: In order to evaluate the effectiveness of these combined inactivation strategies, 80 female weaned piglets weighing 6.02 ± 0.88 kg were assigned to four groups in a 2 x 2 factorial design and fed with the experimental diets for 5 weeks. The piglets were group-housed in 5 slatted floor pens per experimental diet (n = 4) with ad libitum access to feed and water. Experimental feeds included 50 % control (CON) or 50 % Fusarium-toxin contaminated (FUS; containing 2.57 ± 0.23 mg/kg DON and 0.24 ± 0.05 mg/kg ZEN) maize in the absence (-) or presence (+) of 2.5 g/kg SBS and 0.62 mg/kg feed of a ZENzyme® - maltodextrin formulation resulting in four feeding groups (CON-, CON+, FUS-, FUS+). Feed intake per pen (n = 4 pigs) and individual live weight were recorded weekly. At the end of the trial piglets were slaughtered, organs weights recorded and urine and blood samples were collected for analysis of mycotoxins and their metabolites. Statistical analysis was performed by ANOVA with contaminants (FUS), supplements (SUP), and their interaction (FUS x SUP) as categorical factors followed by post-hoc test (tukey) in case of normally distributed values (Statistica 13.0, StatSoft, Inc. 2014) or following transformation (SAS v9.4; proc rank) in case of non-normal distribution.

Results: The combined use of SBS and ZENzyme® improved the mean live weight gain (pSUP < 0.01) from 285 g/d (FUS-) to 349 g/d (FUS+) and the feed intake (pSUP < 0.01) from 474 g/d (FUS-) to 533 g/d (FUS+). These levels are in accordance with mean live weight gain of 323.71 g/d and feed intake of 538 g/d in the control group without feed additives. The mean relative ovaries and uterus weights were significantly reduced (pSUP = 0.02; pSUP < 0.01) from 29.32 mg/kg BW and 576.23 mg/kg BW in the FUS- group to 11.75 mg/kg BW and 399.81 mg/kg BW in the FUS+ group. These values were similar to those of control pigs with relative ovaries weights of 12.83 mg/kg BW (CON-) and 12.42 mg/kg BW (CON+), respectively. The relative uterus weights were 402.92 mg/kg BW in the CON- group and 316.98 mg/kg BW in the CON+ group. Analysis of biomarkers in plasma samples showed a reduction by 90 % α-zearalenol (α-ZEL), 77 % ZEN, and 17 % DON in the FUS+ group compared to the FUS- group, whereas urine concentrations in the FUS+ groups were reduced by 67 % (α-ZEL), 65 % ZEN, and 16 % DON compared to the FUS- group. The reduction of ZEN in the FUS+ group was accompanied by an increase of the non-estrogenic metabolites hydrolyzed zearalenone (HZEN).

Conclusions: These results indicate a successful inactivation of ZEN by its conversion to HZEN by ZENzyme®, which is confirmed by reduced organ weights as biological control. However, the application of SBS as dry feed additive was not effective enough in reducing the biomarker concentrations of DON substantially. Therefore, a further refinement of the SBS-inactivation technology is needed to decontaminate DON in mixed feed.

Bibliographical references

[1] DÄNICKE S., HEGEWALD A.K., KAHLERT S., KLUESS J., ROTHKÖTTER H.J., BREVES G., DÖLL S. (2010): Food Chem. Toxicol. 48: 2154-62

[2] DÄNICKE S., PAHLOW G., GOYARTS T., ROHWEDER D., WILKERLING K., BREVES G., VALENTA H., DÖLL S. (2009): Mycotoxin Res. 25: 215-23

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111.

Impact of a combination of fructooligosaccharides and a Bacillus multi-strain probiotic on performance parameters of piglets challenged with *Escherichia coli*

Wirkung einer Kombination aus Fructooligosacchariden und einem mehrstämmigen Bacillus-Probiotikum auf die Leistungsparameter von mit Escherichia coli infizierten Ferkeln

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Probiotics and prebiotics are considered promising alternatives to the use of antibiotics for improving post-weaning piglet health. However, the use of probiotics provides conflicting results, as some studies describe beneficial effects while others show no effect at all [1]. Previously, we have developed a novel ex vivo assay to determine the potential of specific combinations of pre- and probiotics to inhibit growth of pathogens in pigs [2]. Using this method, we demonstrated the highest inhibitory effect against an enterotoxigenic *Escherichia coli (E. coli)* strain with a combination of fructo-oligosaccharides (FOS) and a Bacillus probiotic. To test this result, we commenced with a challenge trial in weaned piglets with the same *E. coli* strain. We hypothesized that the combination of prebiotics and probiotics would provide beneficial effects on the performance and feces of piglets compared to piglets only receiving a basal diet.

Method: Twenty-four piglets were weaned at 28 d of age and randomly assigned to one of three experimental treatment groups: T1: basal diet (negative control group); T2: basal diet + challenge (positive control group); T3: basal diet supplemented with a dual-strain probiotic (*B. subtilis* and *B. licheniformis*; BioPlus) and a fructo-oligosaccharides + challenge (pre-/probiotic group). Piglets were challenged five days after weaning with the *E. coli* “Abbotstown” strain (serotype O149:K91).The experimental period of 29 days was divided into 15 days of challenge period and 14 days post-challenge observation. Efficacy of dietary treatment against challenge was assessed by evaluation of growth performance, feed conversion and fecal score.

Results: Feed conversion improved numerically in the pre-/probiotic group compared to the positive control in the first week after infection, but significant differences (p=0.038) for feed conversion were only observed between positive and negative control groups. There were high numerical differences in body weight gain between groups during the first week post-infection, but these were not significant due to the high heterogeneity of the data. The average fecal score of the pre-/probiotic group was also not significantly different from the positive control group. Effects of the pre- and probiotic combination on performance and fecal score were visible only in the first week after challenge.

Conclusions: The combination of FOS and the Bacillus probiotic showed slight impact on performance and fecal score during the critical phase of weaning but could not achieve lasting beneficial effects in piglets challenged with *E. coli*.

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Bibliographical references

[1] BARBA-VIDAL E., MARTÍN-ORÚE S.M., CASTILLEJOS L. (2018): Animal 12: 2489-2498

[2] ZEILINGER K., HELLMICH J., ZENTEK J., VAHJEN W. (2021): Beneficial Microbes 12: 567-581

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112.

Effects of sewage sludge recyclate addition to substrate on selected mineral contents of Black Soldier Fly larvae

Effekte einer Substrat-Supplementierung mit Klärschlamm-Rezyklaten auf ausgewählte Mineraliengehalte von Larven der Schwarzen Soldatenfliege

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The world population continues to grow. In order to satisfy the increasing demands for dietary proteins and minerals, in the future new sources of nutrients for animal feed have to be developed. Phosphorous (P) sources from phosphate rock are limited and meat and bone meal are banned in the EU. Black Soldier Fly *(Hermetia illucens)* larvae (BSFL) are an approved livestock species in the EU, and allowed as feed for aquaculture and since recently also for pig and chicken. The BSFL have a high protein content (ca. 35%), depending on the diet [1]. They can produce protein from low-quality nutrient sources. The German sewage sludge regulation emphasizes the need for future recycling of P, e.g. by pyrolysis (PY) or the Ash2Phos process (Ash2), providing a P recyclate from sewage sludge ash. Larvae produced with these recyclates as mineral rich supplements might be interesting to reintegrate these minerals into the nutrient cycle.

Methods: Newly hatched BSFL were fed with chicken starter feed (CF; 20.9 % crude protein, 12.5 MJ ME/kg, 9.0 g Ca and 7.9 g P / kg dry matter (DM) for 5 days (d) before they were separated from their substrate and counted. Per 40 x 60 cm crates 8000 5-d-old larvae were put on a total of 10 kg wet feed (water to feed ratio = 70:30 (w/w). Experimental diets were provided consecutively in three portions during the rearing period. The larvae were grown at 27.5 °C and 70 % humidity. The diets were based on a modified standard fly diet (Gainesville diet, GD) [2] containing wheat bran, corn meal, alfalfa hay and dried sugar beet pulp (15.4 % crude protein, 7.9 MJ ME/kg DM, 7.9 g Ca and 4.8 g P / kg DM). The diets were supplemented by 4% recycled sewage sludge (Ash2: 208.4 g Ca and 88.8 g P / kg DM; PY: 52.4 g Ca and 65. 9 g P / kg DM) at the expense of 2% wheat bran and corn meal (Ash2 and PY diets: 14.7 % crude protein, 7.4 MJ ME/kg DM). The growth performance of BSFL on P recyclate supplemented diets was compared to those on GD and CF control diets (4 diets, 6 replicates each). After the first BSFL reached the prepupal stage (i.e. 18 to 20 d after hatching), the larvae were harvested and analysed for Ca and P contents. Data were analysed using the GLM procedure of SAS ($P < 0.05$), multiple comparisons of groups were done by the Tukey test.

Results: Larvae fed with CF had the highest body weight (231.9 mg). Larvae supplemented with Ash2 had the lowest body weight (89.7 mg), which was also lower compared to GD (105.8 mg) but not relative to PY (99.5 mg). The Ca content was highest in the BSFL receiving the Ash2 supplement (64.6 mg / kg DM). In the CF group the Ca content was lowest (32.0 mg / kg DM), followed by GD (45.9 mg / kg DM) and PY (53.2 mg / kg DM). Highest P values were observed in the Ash2 supplemented group (10.9 mg / kg DM), which was higher compared to all other groups (CF: 6.7, GD: 7.0, PY: 7.2 mg / kg DM).

Conclusions: Using the Ash2 recyclate Ca and P can be enriched in BSFL, compared to both control diets. The lower growth in the Ash2 compared to the GD group could be a result of the lower energy and protein content but an effect of the high amounts of P alone or in combination with other minerals such as iron or zinc cannot be excluded. Depending on the applied technical process, sewage sludge ash recycling can reintegrate Ca and P in the nutrient cycle by supplementing BSFL.

Bibliographical references

[1] LILAND N.S., BIANCAROSA I., ARAUJO P., BIEMANS D., BRUCKNER C.G., WAAGBO R., TORENTSEN B.E., LOCK, E.-J. (2017): PlosOne 12: e0183188

[2] HOGSETTE J.A (1992): J. Econ. Entomol. 85: 2291-2294

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Effect of increasing doses of phytase on performance, apparent praecaecal nutrient digestibility and intestinal electrophysiological properties of the jejunum in 21-day-old broilers

Effekte von ansteigenden Phytasedosierungen auf die Leistung, scheinbare präzäkale Nährstoffverdaulichkeit und elektrophysiologische Eigenschaften des Jejunums bei 21 Tage alten Broiler

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Phytic acid ($InsP_6$) and its salts (phytates) are the main source of organic phosphorus (P) in broiler diets but for being utilized by the animal, phosphate groups need to be cleaved from the inositol ring (1). The supplementation of feed with dietary phytases enables broilers to hydrolyse the $InsP_6$. It is known that the use of dietary phytase increases nutrient digestibility and often improves performance parameters. It is speculated if that later positive effect of the use of phytase in broiler feed could be mediated by further impact on intestinal epithelial nutrient transport systems. In this line, high doses of phytase, beyond the usual recommendations, would be interesting. Thus, the aim of this study was to identify changes in glucose transport in small intestine epithelium induced by the addition of high doses of phytase that could be related with changes in performance or apparent ileal digestibility (AID) of 21-day-old broilers.

Methods: During the 21-day study, 384 broiler chickens were assigned to six dietary treatments and allocated in floor pens containing 8 animals (8 replicates / group). Two groups received a "phytate-free" diet based on corn starch, Hermetia meal, potato protein and fish meal without (T1) or with addition of Natuphos® E (T2, 3,000 FTU / kg feed) while the others (T3-T6) received a phytate-containing diet with corn, soybean meal and sunflower meal. The phytate-containing diets were prepared without (T3) or with supplementation of Natuphos® E at levels of 500 FTU / kg (T4), 1,500 FTU / kg (T5) and 3,000 FTU / kg (T6). The diets were formulated to meet or exceed the nutrient requirements for broiler chickens recommended by GfE (1999). T1 and T2 contained on average 14.4 MJ / kg of metabolizable energy (ME), 232 g / kg of crude protein (CP), 10 g / kg of calcium (Ca) and 6.8 g / kg of P. T3 to T6 were reduced by nutrients and energy and contained on average 12.4 MJ / kg of ME, 215 g / kg of CP, 8.2 g / kg of Ca and 4.9 g / kg of P. Body weight (BW) and feed intake (FI) were weekly registered. At day 21, three animals per pen were slaughtered for collection of ileal digesta (pool). In addition, one animal per pen from groups T1, T2, T3 and T6 was also slaughtered for the removal of the jejunum for electrophysiological measurements using the Ussing chamber technique. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey test, and linear and quadratic regressions were also used for testing the effects of increasing levels of phytase on performance parameters and ileal digestibility (T3-T6).

Results: There was a linear effect of increasing levels of phytase (T3-T6) on BW and FI during the whole experimental period. No impact by supplementing the "phytate-free" diet (T1) with phytase was found on performance or AID parameters. AID of crude ash and P increased by supplementing T3 with graded levels of phytase while AID of Ca decreased. The short-circuit current in jejunal mucosa after glucose addition showed a trend to increase in group T6 in comparison with T3 ($P = 0.079$). In that aspect no difference was seen in T2 compared to T1.

Conclusions: The current results underline the antinutritional effect of the phytate, observed in contrast to a phytate-free diet, and how the addition of dietary phytase helps to overcome them. Epithelial glucose transport in small intestine may be also influenced by the addition of Natuphos® E but further studies on the effect of phytase on epithelial passage of nutrients via active or passive transport, as well as mucus related measurements, would help to elucidate more physiological mechanisms involved in the positive effects of the dietary phytase utilization.

Bibliographical references

[1] RODEHUTSCORD M., ROSENFELDER P. (2016): Phytate destruction. Consequences for precision animal nutrition, 15-32. Wageningen Academic Publishers, Wageningen, The Netherlands

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Investigations into taste preference of dairy cows and its modulation by N-arachidonoylethanolamide

Untersuchungen zur Geschmackspräferenz von Kühen und deren Modulation durch N-Arachidonylethanolamid

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In the early postpartum (p.p.) period, dairy cows reveal insufficient feed intake to meet the energy requirements for milk production. One of the causes might be the taste of the presented feed. The endocannabinoid N-arachidonoylethanolamide (AEA) is known to enhance the consumption of sweetened water [1] and increase feed intake in mice [2]. The aim of this study was to investigate the taste preference of cows and test whether AEA can modify taste preference in the early p.p. period.

Methods: A feed preference test (FPT) involving 16 half-sib Holstein dairy cows in 2nd to 3rd pregnancy was performed. Prior to the FPT, feed formulated as total mixed ration was dried at 50-60°C to remove volatile organic acid compounds known to influence taste. Directly before use, feed was rehydrated with water to its former organic matter (OM) and supplemented with either 30 g sucrose / kg OM (sweet) or 5.1 g monosodium glutamate / kg OM (umami). Before the FPT, cows were familiarised with the different flavours by forced feeding and habituated to tie stall and the experimental procedure. The FPT was performed on 3 subsequent days 2 weeks antepartum (a.p.) as well as 23 days p.p. from 08:00 to 16:00. During the FPT, feed was presented for ad libitum intake. Three feeding bins were simultaneously placed in front of the cows. The bin in the middle was reserved for non-flavoured feed; the bin on the right and on the left were randomly assigned either to sweet- or umami-flavoured feed on a day-to-day basis. The parameter total duration (TD; time spent feeding per bin left, middle, and right) was determined by video analysis (Observer XT14) and the dry matter intake (DMI) was determined manually. The AEA and control treatment started directly after parturition. Half-sibs treated with AEA received 3 µg / kg body weight AEA (n=8) diluted in 0.9% NaCl intra-peritoneally, the counterpart (n=8) received the same volume of 0.9 % NaCl (CON). The statistical analysis was performed in R (version 3.6.2). The 3-day means of DMI and TD as well as the percentage of the TD and DMI were calculated and analysed with a linear model. Pairwise differences of least squares means were tested by using the Tukey Kramer test. Mean differences were considered significant at $P<0.05$ and a trend at $P<0.1$.

Results: Total feed intake was not significantly different between AEA and CON cows during the a.p. and p.p. FPT. Both groups showed a higher DMI and TD for sweet-flavoured feed than non-flavoured or umami-flavoured feed in the a.p. period ($P<0.05$). Similarly, the percentage of TD and DMI of sweet-flavoured feed was higher compared to non-flavoured or umami-flavoured feed ($P<0.05$). In the p.p. period, AEA-treated cows revealed higher DMI of sweet-flavoured feed than non-flavoured or umami-flavoured feed ($P<0.05$). The TD of AEA-treated cows spend on sweet-flavoured feed was significantly longer compared to non-flavoured feed ($P<0.05$) and tended to be longer than on umami–flavoured feed ($P<0.1$). In CON cows, DMI of the 3 diets and the TD spent on each diet did not differ. However, CON cows tended to ingest a greater relative portion of sweet- than non-flavour feed ($P<0.1$).

Conclusions: The results show that cows preferred feed with sweet taste in contrast to umami- flavoured and non-flavoured feed a.p. After parturition, CON cows showed no clear taste preference, whereas AEA-treated cows preferably consumed the sweet-flavoured feed. These results indicate that AEA promotes the preference for sweet-flavoured feed in early lactating dairy cows.

Bibliographical references

[1] YOSHIDA R., OHKURI T., JYOTAKI M., YASUO T., HORIOA N., YASUMASU K., SANEMATSU K., SHIGEMURA N., YAMAMOTO T., MARGOLSKEE R., NINOMIYA Y. (2010): Proc. Natl. Acad. Sci. U S A 107(2):935-939

[2] HAO S., AVRAHAM Y., MECHOULAM R., BERRY E.M. (2000): Eur. J. Pharmacol. 392:147-156

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Effects of a blend of essential oils on zootechnical performance of growing bulls

Effekte einer Mischung ätherischer Öle auf zootechnische Leistungsdaten wachsender Bullen

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Earlier studies reported positive effects on dairy cow performance when applying a certain blend of essential oils (Agolin Ruminant, Agolin S.A., Switzerland) on top of the total mixed ration. The increase in performance was explained by decreased ruminal methanogenesis in the presence of the product [1, 2]. So far, no published data is available for growing cattle. This study tested the effects of said product in growing bulls under Swiss rearing conditions.

Methods: 30 bull calves (AA hybrids: beef breed x milk breed) weaned at 56d of age were purchased from a local producer at an average life weight of 84 ±6.7 kg. One bull calf had to be euthanized, leaving 29 bulls. For the subsequent experimental period (trial d1-d267), animals were distributed by life weight over three pens on d0 of the trial (average life weight over all pens 171 ±30.8 kg). Within each pen, animals were randomly assigned to two feeding groups (Control (n=14), Treatment (n=15)). Each bull had access to its individual feeding place by a transponder-controlled system, allowing animal individual feeding and recording of feed intake. The basal diet consisted of 80% maize silage and 20% grass silage including concentrate (2.5 kg / d during the first and 1 kg / d during the 2nd half of the trial). The test substance was mixed with ground wheat to reach a final concentration of 102 mg / kg DM when adding 30 g / kg DM of this mixture on top of the basal diet. The control group received pure wheat at 30 g / kg DM as placebo. Feed was supplied ad libitum and feed intake was recorded over two consecutive days per week from d55 onwards. Life weight was recorded on days 31, 44, 54, 71, 100, 139, 170, 205, 240 and 267. From the obtained data, daily dry matter intake (DDMI), daily weight gain (DWG) and feed:gain (F:G) were estimated for each consecutive 3 week interval during d55-d267. Bulls were sent to the slaughterhouse with an average life weight of 490 ±35.6 kg and 309 ±13d of age. Data analysis comprised initial life weight at experimental start by 2-way ANOVA (diet, pen and interaction) as well as life weight development over time and changes in DDMI, DWG and F:G over time via repeated measures mixed model analysis (diet, pen and interactions) including the individual animal as fixed effect at $P \leq 0.05$. Data was presented as least square means including the standard error of the respective statistical estimation.

Results: Life weight at experimental start was not different between feeding groups (P=0.54, 83.4 ±1.65 vs. 84.9 ±1.72 kg for Treatment vs. Control) and was therefore not considered as further variable for the analysis of performance parameters over time. Life weight in both groups changed significantly over time ($P<0.0001$) from 151 ±2.35 kg to 491 ±4.87 kg during the observation period at an average rate of 1.62 kg / d. However, no effect was observed for the treatment group (P=0.60). The same was evident for the analysis of DDMI, DWG and F:G, with no significant effect for the treatment group (P=0.46, 0.17 and 0.24, respectively) but significant changes over time ($P<0.0001$) from 3.03 ±0.04 kg DM / d, 1.69 ±0.03 kg / d and 1.81 ±0.06 during the first 3 week observation interval to 4.29 ±0.08 kg DM day, 1.73 ±0.06 kg / d and 2.5 ±0.07 in the end of the study.

Conclusions: All animals showed good performance throughout the trial period, which was not changed by the addition of the test substance to half of the animals. Earlier studies reporting positive effects on dairy cow performance were conducted under conditions of different NDF:starch levels in the diet and at a higher daily DM intake than in the present study [1, 2]. Therefore, we conclude that the present dietary conditions did not cause a high enough pro-methanogenic potential to promote zootechnical performance in the presence of the test substance.

Bibliographical references

[1] HART K.J., JONES H.G., WADDAMS K.E., WORGAN H.J., ZWEIFEL B., NEWBOLD C.J. (2019): Open. J. Anim. Sci. 9: 259-267

[2] ELCOSO G.B., ZWEIFEL B., BACH A. (2019): Appl. Anim. Sci. 35:304-311

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Inhibitory agents controlling *Clostridium tyrobutyricum* as fermentation varmint in silages tested via cultivation methods

Untersuchungen zur Identifizierung von Wirkstoffen zur Hemmung von Clostridium tyrobutyricum als Gärschädling in Silagen mittels Kulturmethoden

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The bacterium *Clostridium tyrobutyricum* is known to interfere with fermentation in silages. Unfavourable conditions for lactic acid fermentation such as low dry matter or sugar content stimulate this butyric acid-producing bacterium and reduce the nutritive value of silage [1]. Different organic and inorganic compounds are known to inhibit the formation and sporulation of Clostridia [2, 3]. In this study, we sought inhibitory agents controlling C. tyrobuytricum and tested them using microbial plate growth inhibition measurement.

Methods: Bacteria were grown in Hungate-tubes in an anaerobic liquid medium (PY+X) at 37 °C as recommended (DSMZ, Braunschweig, Germany). Beside one negative (distilled water) and one positive (formic acid) control, ten different substances and/or compounds were tested: lauric acid, sodium nitrite, benzoic acid, glycerin monolaurate (GML), nisin, subtilin, carvacrol, sodium nitrite + carvacrol, pelargonic acid, and carboxylic acids mix. The substances were diluted in hexane, except for GML (acetone), nisin and subtilin (PBS buffer [phosphate buffered saline]) and applied at 5 different concentrations. In a first step, the inhibition zone was determined by plating the bacteria suspension onto solid medium (Reinforced-Clostridial agar; plate diameter 5 cm) with a cellulose filter disc (Ø 6mm) soaked with 10 µl of the specific agents placed in the center of the plate. Care was taken to maintain anaerobic conditions under constant nitrogen flow. Afterwards, the plates (in 3 replicates) were incubated at 37 °C for 3 d. The diameter of the inhibition zone was measured using ImageJ (University of Wisconsin, USA). In a second step, the pH in the liquid media (inoculated with the bacteria) was determined at 0, 1, 18, 24, and 27 h after adding the test agents to the liquid medium. Statistical analysis (one-way ANOVA with post-hoc Tukey-Kramer) was performed with SAS 9.4 using the fixed factor 'concentration per agent' with $P < 0.05$ as level of significance.

Results: Benzoic acid and subtilin showed no inhibition zone up to a concentration of 20 % ($P > 0.05$). Formic acid, as the positive control (not diluted), evoked a complete inhibition (5 cm). The commercial carboxylic acid mix showed the smallest inhibition zone (25 %: 1.7 cm vs. 100 % [non-diluted]: 3.7 cm; $P < 0,05$). In contrast, lauric acid and nisin revealed the least zone of inhibition, even at a dilution of 10 % (lauric acid, 100 %: 2.1 cm vs. 10 %: 1.2 cm; nisin, 100 %: 2.4 cm vs. 10 %: 0 cm; $P < 0,05$). GML, pelargonic acid, sodium nitrite, and the carvacrol + sodium nitrite mixture revealed a minimal inhibition zone at 5 % dilution (GML, 100 %: 4.1 cm vs. 5 %: 0.5 cm; pelargonic acid, 100 %: 3.9 cm vs. 5 %: 1.3 cm; sodium nitrite, 100 %: 4.1 cm vs. 5 %: 0 cm; carvacrol + sodium nitrite, 100 %: 5 cm vs. 5 %: 0 cm; $P < 0.05$). The most efficient substance was carvacrol, which evoked an inhibition zone as a 1 % dilution (100 %: 5 cm vs. 1 %: 0.6 cm; $P < 0.05$). The initial pH of the inoculated liquid medium was 6.7. Adding carvacrol (25 % dilution) or sodium nitrite (100 %) had no effect on the pH immediately (0 h: carvacrol, 6.8; sodium nitrite, 6.7), whereas the combination of both increased the pH (0 h, 8.0). Over time, the pH decreased slightly in the medium inoculated with carvacrol (27 h, medium: 6.7; carvacrol: 6.4). Adding sodium nitrite or carvacrol + sodium nitrite increased the pH slightly over time (27 h, sodium nitrite: 6.9; carvacrol + sodium nitrite: 7.9). Formic acid reduced the pH to 2.2 immediately and to 2.1 after 27 h.

Conclusions: The tested inhibitory agents did not cause relevant pH effect on the growth of the bacteria, except for the acid components. Herbal essential oils like carvacrol limit bacterial growth most efficiently. Further studies will evaluate, to which extent carvacrol has an impact on germination capacity of Clostridia tested in liquid media.

Bibliographical references
[1] RAMMER C. (1991): Grass For a. Sc. 51:88-95
[2] LINGVALL P., LTTEME P. (1999): J. Sc. F. Agri. 79 (2): 257-264
[3] PREUSS H.G., ECHARD B., ENIG M., BROOK I., ELLIOT T.B. (2005): Mol. cell. bioch. 272(1-2): 29-34

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Workshop

Rearing dairy calves with focus on health and welfare

Importance of colostrum supply for the development of the neonatal immune system

Bedeutung der Kolostrumversorgung für die Entwicklung des neonatalen Immunsystems

Schuberth H.J. – Hanover

Colostrum supply of calves is mandatory to ensure their health. Since 1922 it has become obvious that calves lacking colostrum supply die within a short period (1), mainly due to infections. The discovery of the protecting role of antibodies against pathogens together with the high concentrations of antibodies in colostral secretions moved the focus of attention towards colostral antibodies. A colostrum with high antibody content –is- currently the sole definition of a high-quality colostrum. This concept argues for a nearly mere protective role of colostrum, which contrasts with the developmental needs of a newborn calf's immune system. This short overview will focus on the educatory role of colostrum for the immune system of a newborn and will put the programming features of colostral ingredients into the forefront.

One major thesis is, that maternal colostral antibodies (if pathogen-specific!) act like an umbrella for the calf, under which other colostral ingredients can act in an educating, programming and guiding way. The immune system of a newborn is nearly complete. Especially the machinery to mount adaptive immune responses (requiring complex interactions between adaptive and innate immune cells) is already present in utero. Certain cell functions, the speed and magnitude of immune mechanisms, the concentrations of soluble immune components (e.g. complement factors) still differ from those of adult animals. In principle, the immune system of a newborn is ready to deal with threads. The best example are successful vaccinations of calves within days after birth. The real fundamental difference to adult animals is the still not developed gut immune system. This appears a major issue, since the gut and the gut microbiota will and must govern the response towards pathogens and vaccinations, and the balance between inflammatory and anti-inflammatory responses - not only in the gut but also in the periphery. Hence, the rapid and proper development of the gut immune system is mandatory for neonatal health. This development and cell programming is governed by a long list of colostral ingredients. Growth factors, cytokines (2), chemokines, soluble pattern recognition receptors (3, 4), exosomes (5, 6), a diverse family of oligosaccharides (7, 8), and immune cells (9-11) form a complex interactive network that program the neonatal immune system and tune its homeostatic regulation (12). The transfer of viable maternal immune to the calf, for instance, may have long-term consequences for their response towards pathogens and vaccines, extending far beyond weaning (13). Whether some of these cells may even lead to a life-long training and education of the calf is unknown. A phenomenon described as maternal-foetal chimerism in humans (14) has not been investigated in ruminants. Whereas the importance of viable maternal colostral cells is documented in the bovine, their number or their composition has not been addressed further with regard to the definition of a high-quality colostrum.

Other colostral ingredients act more directly in the gut. From here on it is worth mentioning that most of the developmental processes in the gut immune system in the calf are still obscure. In other mammals, notably in humans, the development shows two major waves of significant changes. One wave affects mainly the composition and density of gut microbiota as well as reprogramming events in gut cells in the very early post-partum period. The other wave occurs around weaning (15) and affects mainly cells of the adaptive and innate immune system. The first period is guided not solely but to a significant part by colostral ingredients. Whether the second period of significant changes depends on successfully colostrum-triggered events is not fully clear but very likely. Thus, colostrum-triggered maturation and reprogramming events of epithelial cells and other immune cells together with the developing gut microbiome seem to dictate the responsiveness and adequate responses of the system at later life stages. Colostral oligosaccharides, for instance, both favor the development of the gut microbiome due to their prebiotic nature and are able to reprogram resident epithelial cells (in addition to other immunoregulatory functions) (16). Cows can differ substantially in numbers of different colostral oligosaccharides and the ratio between members of this group (17). Whether this is decisive for their programming role or their microbiota-shaping function is unknown.

The impact of distinct colostral mediators or soluble components have been studied only partially in calves or with bovine cells. Most studies addressing the biological activity of bovine colostrum (apart from antibody concentrations) were performed in heterologous species (e.g. swine) or with heterologous cells. Recent attempts to characterize and to measure the biologic activity of bovine colostrum and its preparations utilized the growth and viability-enhancing properties for human epithelial tumor cells (18). Such studies clearly demonstrate that colostrum preparations can markedly differ in their biologic activity and suggest that also fresh colostrum of different cows may differ in their programming and educating potential. Whether the aspect "biologic activity" should be included in the definition of a high-quality colostrum needs a broader discussion. Now there seems to be no real need because the early feeding of enough colostrum with a high immunoglobulin content is commonly regarded as "sufficient" when looking at the first weeks of life. Since immunoglobulins alone cannot promote the developmental processes within the gut and its immune system, there is at least a chance that antibody concentrations positively correlate with the concentrations of bioactive ingredients. Trials investigating the relationship between these compound worlds are still lacking. The higher incidences of infectious diseases between birth and weaning (window of susceptibility (19)) and the higher susceptibility towards infectious pathogens around weaning (20, 21) may, however, be the consequence of an improper education and programming of the gut and the immune system via colostrum.

Bibliographical references

[1] SMITH T., LITTLE R.B. (1922): J Exp Med. 36: 181

[2] HAGIWARA K., KATAOKA S., YAMANAKA H., KIRISAWA R., IWAI H. (2000): Vet. Immunol. Immunopathol. 76: 183

[3] LABETA M.O., VIDAL K., NORES J.E., ARIAS M., VITA N., MORGAN B.P., GUILLEMOT J.C., LOYAUX D., FERRARA P., SCHMID D., AFFOLTER M., BORYSIEWICZ L.K., DONNET-HUGHES A., SCHIFFRIN E.J. (2000): J. Exp. Med. 191: 1807

[4] FILIPP D., ALIZADEH-KHIAVI K., RICHARDSON C., PALMA A., PAREDES N., TAKEUCHI O., AKIRA S., JULIUS M. (2001): Proc. Natl. Acad. Sci. U S A. 98: 603

[5] ROSS M., ATALLA H., KARROW N., MALLARD B.A. (2021): J. Dairy Sci. 104: 2499

[6] KIM H., KIM D.E., HAN G., LIM N.R., KIM E.H., JANG Y., CHO H., JANG H., KIM K.H., KIM S.H., YANG Y. (2021): Adv. Healthc. Mater. e2102027

[7] SISCHO W.M., SHORT D.M., GEISSLER M., BUNYATRATCHATA A., BARILE D. (2017): J Dairy Sci. 100: 3883

[8] LIU Z., WANG T., PRYCE J.E., MACLEOD I.M., HAYES B.J., CHAMBERLAIN A.J., JAGT C.V., REICH C.M., MASON B.A., ROCHFORT S., COCKS B.G. (2019): Sci. Rep. 9: 2137

[9] DONOVAN D.C., REBER A.J., GABBARD J.D., ACEVES-AVILA M., GALLAND K.L., HOLBERT K.A., ELY L.O., HURLEY D.J. (2007): Am. J. Vet. Res. 68: 778

[10] NOVO S.M.F., COSTA J., BACCILI C.C., SOBREIRA N.M., SILVA B.T., DE OLIVEIRA P.L., HURLEY D.J., GOMES V. (2017): Res. Vet. Sci. 112: 97

[11] REBER A.J., DONOVAN D.C., GABBARD J., GALLAND K., ACEVES-AVILA M., HOLBERT K.A., MARSHALL L., HURLEY D.J. (2008): Vet. Immunol. Immunopathol. 123: 305

[12] JENNEWEIN M.F., ABU-RAYA B., JIANG Y., ALTER G., MARCHANT A. (2017): Seminars in Immunopathology. 39: 605

[13] LANGEL S.N., WARK W.A., GARST S.N., JAMES R.E., MCGILLIARD M.L., PETERSSON-WOLFE C.S., KANEVSKY-MULLARKY I. (2016): J. Dairy Sci. 99: 3979

[14] OPSTELTEN R., SLOT M.C., LARDY N.M., LANKESTER A.C., MULDER A., CLAAS F.H.J., VAN ROOD J.J., AMSEN D. (2019): Sci. Rep. 9: 5247

[15] HORNEF M.W., TOROW N. (2020): Immunology 159: 15

[16] BODE L. (2012): Glycobiology 22: 1147

[17] BARILE D., MAROTTA M., CHU C., MEHRA R., GRIMM R., LEBRILLA C.B., and GERMAN J.B. (2010): J. Dairy Sci. 93: 3940

[18] PLAYFORD R.J., CATTELL M., and MARCHBANK T. (2020): PLoS One. 15: e0234719

[19] CHASE C.C., HURLEY D.J., REBER A.J. (2008): Vet. Clin. North Am. Food. Anim. Pract. 24: 87

[20] LUCAS A.S., SWECKER W.S., LINDSAY D.S., SCAGLIA G., ELVINGER F.C., ZAJAC A.M. (2007): Vet. Parasitol. 145: 228

[21] WILSON B.K., RICHARDS C.J., STEP D.L., KREHBIEL C.R. (2017): J. Anim. Sci. 95: 2170

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Implementation of adequate milk feeding until weaning

Umsetzung einer adäquaten Milchfütterung bis zum Absetzen

Koch C. – Münchweiler an der Alsenz

Introduction: Mortality and morbidity rates are still unacceptably high during early calf rearing. The mortality rate in the perinatal period, defined as the duration from birth to 48 h after birth, ranges in herds worldwide between 3% and 9% (1). A recent survey on mortality rates in Germany revealed up to 17% calf losses (calf losses after birth up to 6 months of age) in dairy farms (2). In US dairy herds, current mortality rates of 5% and morbidity rates of 34% were published for preweaning calves (3). The mortality rates have decreased since 1991, but morbidity rates have not changed over the same time period (4). High incidences of morbidity rates were confirmed by a recent survey in Germany (5) which is line with the results from USA (4, 3). It is obvious that high mortality and morbidity rates contradict the aim of increased animal welfare for farm animals and compromise the breeding of robust animals (6). Feeding management during the neonatal and preweaning period has a great impact on the success of calf rearing and, in addition, affects health, resilience and performance in later life (7, 8, 9). Beside respiratory diseases severe diarrhea is a main reason for neonatal calf losses, the management of milk feeding and especially colostrum supply in the first days of life is of particular importance for the success of calf rearing (2, 3). In dairy farming, calves usually do not grow up in a herd and are immediately separated from their dams after birth. Thus, farmers are highly responsible for the postnatal feeding management. Since colostrum and milk feeding management affects the health and well-being as well as growth, development and maturation of the calves (10, 11), farmers have a great influence on a successful rearing of dairy calves and can significantly contribute to the reduction of calf losses during the postnatal period. With this in mind, it is worth questioning the current colostrum and milk supply and contributing to an improving feeding management that results in a successful calf rearing.

Colostrum and transition milk supply: Because calves are born without significant amounts of immunoglobulins, colostrum supply is important for establishing passive immunity in calves, and the amount of colostrum fed to newborn calves directly correlates with the prevention of illness and calf losses (12, 13, 4). Furthermore, there is increasing evidence that an enhanced colostrum and transition feeding schedule during the first days of life not only affects growth and passive immunity but also promotes organ development and maturation of the calves (10, 14). Bovine colostrum provides newborn calves with high amounts of nutrient and non-nutrient biologically active substances (15, 16). In addition to the great importance of colostral immunoglobulins for the passive immunity (17,12), colostrum contains a large number of immunomodulatory peptides that may also affect neonatal immune response (18, 19, 16, 20, 21). Some of these factors are provided by colostral immune cells that are involved in the establishment of local and systemic neonatal immunity (22, 19, 23). In addition, potential effects of colostrum on the neonatal microbiome in the gut are likely and become more important in the research of early calf nutrition (24). Studies in humans, investigating the effects of breastmilk feeding on neonatal intestinal development, illustrate the significance of colostral immune cells and the intestinal microbiome on the maturation of the neonatal immune response in the gut (25). A comparable function of bovine colostrum in the neonatal intestine of the calf is conceivable but requires more investigations.

Bovine colostrum has an overall importance for the postnatal development of the gut (26). The high concentrations of hormones, growth factors and cell-modulating factors in colostrum (15, 16) stimulate villus growth of the small intestinal mucosa in calves (26, 27). Colostrum feeding promotes mucosal cell growth and protein synthesis in the enterocytes of neonatal mammals (28, 29, 30). The amount of overall ingested colostrum corresponds to the villus size in the intestinal mucosa, leading to a greater villus size in repeatedly colostrum-fed calves (26). In summary, the postnatal development and maturation of the neonatal intestine is enhanced due to colostrum and transition milk intake, and the improved intestinal maturation results in a greater nutrient absorption and stimulation of anabolic processes that are a prerequisite for accelerated postnatal growth (10).

From the mentioned studies above, it becomes obvious that calves should digest sufficient amounts of high-quality colostrum immediately after birth. Even though, colostrum contains many other non-nutritive factors in addition to immunoglobulins, colostrum quality is still defined by its IgG concentration, since the other factors are not easy to measure and a high concentration of IgG in colostrum suggests that the other factors are also present in elevated concentrations. Previous recommendations of 10 g IgG/l blood serum 24 to 48 h after birth are now considered the lowest limit for an adequate supply of colostrum in neonatal calves (4). Since there is a wide variation in the immunoglobulin concentration in colostrum and the efficiency of immunoglobulin absorption also varies among calves, it is reasonable to determine the immunoglobulin content in colostrum and to feed sufficient amounts of first colostrum (first milking after calving; 31, 32). Recent studies indicate that an improved immunoglobulin supply in calves leads to a reduction in diseases and deaths during rearing (31, 4). Thus, a minimum of 3 liters (or better 7,5 to 10 % of birth weight) of high quality colostrum (≥ 50 g IgG / l and low content of pathogens) should be provided to neonatal calves as first meal immediately after birth. The colostrum should be fed ad libitum and if calves drink more than 3 to 4 litre this is highly desirable. Delaying colostrum feeding by 6 or 12 h after birth decreased the passive transfer of IgG and the time to maximum blood serum IgG concentration compared with feeding colostrum immediately after birth (33).There is also clear evidence that continued feeding of first colostrum or feeding of transition milk (2nd to 6th milking after calving with a 12 h milking interval) has an additional positive effect on the immunoglobulin concentration in blood and health status of the calves in addition to the positive influence on intestinal development, even if the immunoglobulins can no longer be absorbed after 24 h of birth (34, 35).

Enhanced milk feeding and appropriate weaning strategies: To define enhanced milk feeding, that means that the calf can decide oneself when and which amount of milk the calf would like to drink. Enhanced milk feeding provides whole milk or MR in unrestricted amounts all day long for 24h. High intake of whole milk or high-quality MR to provide high nutrient supply is a prerequisite for appropriate calf growth and development. Besides providing an appropriate nutrient supply to the calves to achieve biological adequate growth rates, feeding high milk levels are highly appreciated to fulfill important demands in animal welfare, well-being and physiological normal feeding, drinking and suckling behavior (36). Furthermore, intensive milk feeding leads to more resilient calves (34) and indicate a faster resolution from diarrhea (37) and results in higher resistance against Salmonella typhimurium (38). The World Organization for Animal Health (OIE) defines animal welfare in the Terrestrial Animal Health Code as a state where the animal is healthy, comfortable, well nourished, safe, able to express natural behavior and not suffering from hunger, pain, fear and distress (39). Feeding calves limited amounts of liquid feed (i.e. 4 to 6 kg or liters of milk / day) during the first weeks of life results in a lack of expression in natural suckling behavior (40, 36) followed by hunger and stress for the calves (41, 42, 43, 44). Allowing calves to drink unlimited amounts of whole milk or MR (so called ad libitum milk feeding) for several weeks during the preweaning period more than doubles liquid feed intake compared with restricted amounts of 4 to 6 kg / day of MR or whole milk (45, 46, 47, 48). Therefore, an enhanced milk-feeding programme contributes to the overall well-being and reduces stress of preweaning calves.

In contrast to the described enhanced milk feeding strategies for calves during the preweaning period the limitation of milk intake was an accepted milk feeding strategy over the last 30-40 years to increase solid feed intake as soon as possible in the preweaning period (49, 50, 51). Independent from the intake of whole milk or MR solid feed intake during the first 3-4 weeks of life is low, and the digestibility of solid feed is impaired due to an immature forestomach especially an immature and not fully developed rumen (52, 53). Due to restricted milk feeding to foster solid feed intake in early life is challenging for an immature and underdeveloped rumen especially regarding an adequate rumen fermentation of proteins and carbohydrates (starch) originated from plants. If rumen fermentation is overwhelmed but the absorption capacity of the short-chain fatty acids (SCFA) is still impaired the risk of a fluctuating rumen pH is increasing with the consequence of leaky rumen and gut development (54). Low and unphysiological rumen pH-values due to restricted milk feeding and increased solid feed intake induced inflammation and SARA in calves over the pre- and postweaning period with detrimental effects of health and lower growth rates in calves (54, 55, 56). Despite low solid feed intake in the first weeks of life the calves suffer from SARA because of reduced absorption capacity of the SCFA through the immature rumen papillae. Accumulation of SCFA in the rumen increased the risk of an invasion of starch in the lower gut and in the hindgut with the risk of hindgut acidosis. Parsons et al. (2021, 57) compared different feeding regimes (elevated milk feeding: 15 L MR / day with 150 g MR / L; restrictive milk feeding: 6 L MR / day with 150 g MR / L) within the same weaning plan over 2 weeks starting on day 42 of life until day 63. Interestingly, the calves in the intensive feeding protocol showed physiological normal rumen pH-values of 6.3 at day 35 of life before weaning starts in comparison to low rumen pH-values in the restricted fed group (pH = 5.8) which indicates SARA conditions in the rumen. At the end of weaning at day 63, the high milk-fed group showed a lower rumen pH-value in comparison to the restricted fed group due to an immature and not fully developed rumen. The reason for inducing SARA conditions in the high milk fed group at the end of the study is due to a too fast weaning procedure because calves which received high amounts of milk need much more time to adopt rumen fermentation capacity to high amounts of solid feed. Therefore, calves with an enhanced milk feeding protocol have to be weaned carefully, i. e. they need sufficient time to adapt their rumen fermentation of elevated solid feeds during weaning. Huber (49) stated that the weaning process depends on age and development of the young ruminant and weaning is one of the biggest challenges in whole life of a ruminant if the weaning process is not well prepared. At the end Huber (49) recommended that the weaning process should not be finished between week 12 and 14 of life, but better at the age of 16 weeks of life. To overcome concerns that weaning later in life has delayed or detrimental effects on the maturation and development of the rumen, we need convincing research that convince of the opposite and we should encourage farmers to test the new feeding and weaning strategies for calves. No impairment of rumen development postweaning was observed due to ad libitum MR feeding for the first 5 or 8 weeks of life (58, 59). However, we have to face the challenge to introduce a smooth and long-lasting weaning period (up to 6 weeks) depending on age and maturation of the rumen to achieve appropriate growth rates and a well-being (avoidance of hunger) of the animal during the weaning process. The aim of the preweaning feeding strategy should be to encourage calves to eat solid feed in addition to consuming high volumes of milk. Calves that showed a greater MR intake per meal during ad libitum MR feeding had a greater concentrate intake during weaning (60). This finding confirms observations of milk and solid feed intake in beef calves, when elevated milk and solid feed intake occurs at the same time. Calves benefit from the high milk intake as evidenced by enhanced body growth (40) without impaired forestomach development during weaning.

Practical recommendations: For practical use it is highly recommended to feed the calf within 1 h after birth with fresh colostrum from their mother ad libitum. Over the first 5 days after birth it is recommended to feed the calf with transition milk from their dams ad libitum. Thereafter, calves should be fed with whole milk or MR ad libitum for 5-8 weeks of life. Weaning depends on appropriate growth rates and age and should starts not before week 5 of age. Because the increase of solid feed intake will be delayed in calves with enhanced milk feeding, the weaning process should be smooth and slow over 6-8 weeks and should be finished not before the age of 12 respective 16 weeks of life. Over the whole milk feeding period calves should receive fresh water, hay, concentrate or dry-TMR at free disposal to achieve a smooth and well adopted transition from liquid to solid feed.

Bibliographical references

[1] COMPTON C.W.R., HEUER C., THOMSEN P.T., CARPENTER T.E., PHYN C.V.C., MCDOUGALL S. (2017): J. Dairy Sci. 100: 1-16

[2] TAUTENHAHN A. (2017): Risikofaktoren für eine erhöhte Kälbersterblichkeit und geringe Tageszunahmen von Aufzuchtkälbern in norddeutschen Milchkuhhaltungen. Diss. Free University of Berlin, Berlin, Germany

[3] URIE N.J., LOMBARD J.E., SHIVLEY C.B., KOPRAL C.A., ADAMS A.E., EARLEYWINE T.J., OLSON J.D., GARRY F.B. (2018): J. Dairy Sci. 101: 9229-9244

[4] LOMBARD J., URIE N., GARRY F., GODDEN S., QUIGLEY J., EARLEYWINE T., MCGUIRK S., MOORE D., BRANAN M., CHAMORRO M., SMITH, G., SHIVLEY C., CATHERMAN D., HAINES D., HEINRICHS A.J., JAMES R., MAAS J., STERNER K. (2020): J. Dairy Sci. 103: 7611-7624

[5] BLE (Bundesanstalt für Landwirtschaft und Ernährung) (2020): Abschlussbericht: Tiergesundheit, Hygiene und Biosicherheit in deutschen Milchkuhbetrieben – eine Prävalenzstudie (PraeRi). Bundesministerium für Ernährung und Landwirtschaft (BMEL)

[6] HUBER K. (2018): Animal 12: 528-536

[7] KHAN M.A., WEARY D.M., VON KEYSERLINGK M.A.G. (2011): J. Dairy Sci. 94: 1071-1081

[8] BALLOU M.A. (2012): J. Dairy Sci. 95: 7319-7330

[9] VAN AMBURGH M.E., SOBERON F. (2013): Proc. Cow Longevity Conference, 28–29 August 2013, Hamra Farm, Tumba, Sweden: 178-197

[10] HAMMON H.M., LIERMANN W., FRIETEN D., KOCH C. (2020): Animal 14(S1): s133-s143

[11] VON KEYSERLINGK M.A.G., RUSHEN J., DE PASSILLE A.M., WEARY D.M. (2009): J. Dairy Sci. 92: 4101-4111

[12] GODDEN S. (2008): Vet. Clin. North Am. Food Anim. Pract. 24: 19-39

[13] MEE J.F. (2008): Vet. Clin. North Am. Food Anim. Pract. 24: 1-17

[14] KARGAR S., ROSHAN M., GHOREISHI S.M., AKHLAGHI A., KANANI M., ABEDI SHAMS-ABADI A.R., GHAFFARI. M.H. (2020): J. Dairy Sci. 103: 8130-8142

[15] BLUM J.W., BAUMRUCKER C.R. (2008): Adv. Exp. Med. Biol. 606: 397-422

[16] NISSEN A., ANDERSEN P.H., BENDIXEN E., INGVARTSEN K.L., RONTVED C.M. (2017): J. Dairy Sci. 100: 2711-2728

[17] BARRINGTON G.M., PARISH S.M. (2001): Vet. Clin. North Am. Food Anim. Pract. 17: 463-476

[18] CHASE C.C.L., HURLEY D.J., REBER A.J. (2008): Vet. Clin. North Am. Food Anim. Pract. 24: 87-104

[19] STELWAGEN K., CARPENTER E., HAIGH B., HODGKINSON A., WHEELER T.T. (2009): J. Anim. Sci. 87: 3-9

[20] PLAYFORD R.J., WEISER M.J. (2021): Nutrients 13(1): 265
[21] CARTER H.S.M., RENAUD D.L., STEELE M.A., FISCHER-TLUSTOS A.J., COSTA J.H.C. (2021): Animals 11(8): 2221
[22] LIEBLER-TENORIO E.M., RIEDEL-CASPARI G., POHLENZ J.F. (2002): Vet. Immunol. Immunopathol. 85: 33-40
[23] LANGEL S.N., WARK W.A., GARST S.N., JAMES R.E., MCGILLIARD M.L., PETERSSON-WOLFE C.S., KANEVSKY-MULLARKY I. (2015): J. Dairy Sci. 98: 3729-3740
[24] MALMUTHUGE N., GUAN L.L. (2017): J. Dairy Sci. 100: 5996-6005
[25] MOLÈS J.P., TUAILLON E., KANKASA C., BEDIN A.S., NAGOT N., MARCHANT A., MCDERMID J.M., VAN DE PERRE P. (2018): Pediatr. Allergy Immunol. 29: 133-143
[26] BLUM J.W. (2006): J. Anim. Physiol. Anim. Nutr. 90: 1-11
[27] STEINHOFF-WAGNER J., ZITNAN R., SCHÖNHUSEN U., PFANNKUCHE H., HUDAKOVA M., METGES C.C., HAMMON H.M. (2014): J. Dairy Sci. 97: 6358-6369
[28] DONOVAN S.M., ODLE J. (1994): Annu. Rev. Nutr. 14: 147-167
[29] BURRIN D.G., DAVIS T.A., EBNER S., SCHOKNECHT P.A., FIOROTTO M.L., REEDS P.J., MCAVOY S. (1995): Pediatr. Res. 37: 593-599
[30] XU R.J. (1996): Reprod. Fertil. Dev. 8: 35-48
[31] GODDEN S.M., LOMBARD J.E., WOOLUMS A.R. (2019): Vet. Clin. North Am. Food Anim. Pract. 35(3): 535-556
[32] GEIGER A.J. (2020): J. Anim. Sci. 98(Suppl 1): S126-S132
[33] FISCHER A.J., SONG Y., HE Z., HAINES D.M., GUAN L.L., STEELE M.A. (2018): J. Dairy Sci. 101: 3099-3109
[34] LORENZ, I. (2021): Ir. Vet. J. 74: 5
[35] SCHALICH, K.M., REIFF O.M., NGUYEN B.T., LAMB C.L., MONDOZA C.R., SELVARAJ V. (2021): J. Anim. Sci. 99(5): 1-12
[36] MILLER-CUSHON E.K., DEVRIES T.J. (2015): Can. J. Anim. Sci. 95: 341-350
[37] OLLIVETT T.L., NYDAM D.V., LINDEN T.C., BOWMAN D.D., VAN AMBURGH M.E. (2012): J. Am. Vet. Med. Assoc. 241: 1514-1520
[38] BALLOU M.A., HANSON D.L., COBB C.J., OBEIDAT B.S., SELLERS M.D., PEPPER-YOWELL A.R., CARROLL J.A., EARLEYWINE T.J., LAWHON S.D. (2015): J. Dairy Sci. 98: 1972-1982
[39] OIE (2017): Terrestrial Animal Health Code. Section 7. Animal Welfare. Retrieved on 3 July 2019 from http://oie.int/international-standard-setting/terrestrial-code
[40] SCHIESSLER G., NUSSBAUM A., HAMMON H.M., BLUM J.W. (2002): Anim. Sci. 74: 431-444
[41] JENSEN M.B., HOLM L. (2003): Appl. Anim. Behav. Sci. 82: 87-100
[42] DE PAULA VIEIRA A., GUESDON V., DE PASSILLE A.M., VON KEYSERLINGK M.A.G., WEARY D.M. (2008). Appl. Anim. Behav. Sci. 109: 180-189
[43] BORDERAS T.F., DE PASSILLE A.M.B., RUSHEN J. (2009): J. Dairy Sci. 92: 2843-2852
[44] GERBERT C., FRIETEN D., KOCH C., DUSEL G., EDER K., STEFANIAK T., BAJZERT J., JAWOR P., TUCHSCHERER A., HAMMON H.M. (2018): J. Dairy Sci. 101: 7348-7360
[45] HAMMON H.M., SCHIESSLER G., NUSSBAUM A., BLUM J.W. (2002): J. Dairy Sci. 85: 3352-3362
[46] MACCARI P., WIEDEMANN S., KUNZ H.J., PIECHOTTA M., SANFTLEBEN P., KASKE M. (2015): J. Anim. Physiol. Anim. Nutr. 99: 737-746
[47] SCHÄFF C.T., GRUSE J., MACIEJ J., MIELENZ M., WIRTHGEN E., HOEFLICH A., SCHMICKE M., PFUHL R., JAWOR P., STEFANIAK T., HAMMON H.M. (2016): PLoS ONE 11: e0168974
[48] FRIETEN D., GERBERT C., KOCH C., DUSEL G., EDER K., KANITZ E., WEITZEL J.M., HAMMON H.M. (2017): J. Dairy Sci. 100: 6648-6661
[49] HUBER J.T. (1969): J. Dairy Sci. 52: 1303-1315
[50] GELSINGER S.L., HEINRICHS A.J., JONES C.M. (2016): J. Dairy Sci. 99: 6206-6214
[51] KERTZ A.F., HILL T.M, QUIGLEY J.D. 3rd, HEINRICHS A.J., LINN J.G., DRACKLEY J.K. (2017): J. Dairy Sci. 100: 10151-10172
[52] DRACKLEY J.K. (2008): Vet. Clin. North Am. Food Anim. Pract. 24: 55-86
[53] KHAN M.A., BACH A., WEARY D.M., VON KEYSERLINGK M.A.G. (2016): J. Dairy Sci. 99: 885-902
[54] ZEBELI Q., METZLER-ZEBELI B.U. (2012): Vet. Sci. Res. J. 93: 1099-1108
[55] VAN NIEKERK J.K., MIDDELDORP M., GUAN L.L., STEELE M.A. (2020): J. Dairy Sci. 104: 3632-3645
[56] GELSINGER S.L., COBLENTZ W.K., ZANTON G.I., OGDEN R.K., AKINS M.S. (2020): J. Dairy Sci. 103: 2762-2772
[57] PARSONS S.D., STEELE M.A., LESLIE K.E., RENAUD D.L., REEDMAN C.N., WINDER C.B., DEVRIES T.J. (2021): J. Dairy Sci. 105: 281-300
[58] SCHÄFF C.T., GRUSE J., MACIEJ J., PFUHL R., ZITNAN R., RAJSKY M., HAMMON H.M. (2018): J. Dairy Sci. 101: 783-793
[59] KOCH C., GERBERT C., FRIETEN D., DUSEL G., EDER K., ZITNAN R., HAMMON H.M. (2019): J. Dairy Sci. 102: 8513-8526
[60] GHAFFARI M.H., HAMMON H.M., FRIETEN D., GERBERT C., DUSEL G., KOCH C. (2021): J. Dairy Sci. 104: 4650-46644

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Influence of milk supply on calf health

Einfluss der Milchversorgung auf die Kälbergesundheit

Lorenz I. – Poing

Introduction: Calf morbidity and mortality are still unacceptably high in intensive calf rearing systems around the world. The major health problems are multifactorial diseases like calf diarrhea and bovine respiratory disease. This means that besides - generally ubiquitous - infectious agents, environmental as well as management factors contribute to the outbreak of disease. During the last two decades it became increasingly clear that the way we feed our calves could have a major impact on health in artificially reared calves. The scientific advances have been comprehensively reviewed (1, 2, 3) and will be summarized here.

The role of colostrum intake in calf health Good colostrum management is still recognized as the single most important factor to preventing calf morbidity and mortality, however, it is now known that immunoglobulins are only one of many components of colostrum that are vital for the calf's development. Other non-nutrient factors like leucocytes, hormones and growth factors, oligosaccharides as well as microRNAs have significant effects on the development and maturation of the intestinal and systemic immune functions. They also promote the maturation and function of the intestine, thus enabling the calf to digest and absorb the nutrients provided with colostrum and milk. The improved energetic status of colostrum-fed neonates is reflected by an accelerated maturation of the somatotropic axis, which stimulates body growth and organ development. Colostrum oligosaccharides are presumed to play a major role in the development of a healthy intestinal flora. Colostrum also contains an abundance of antimicrobial components, like lactoferrin, lactoperoxidase, or lysozyme. Extended feeding of colostrum or transition milk further promotes small intestinal growth and maturation and is preventive of calf diarrhea.

Further nutrition of the dairy calf Traditionally, dairy calves have been fed milk or milk replacer to an amount of approximately 10% of the calf's body weight (BW) per day. This level of nutrition ('restricted feeding') allows only for maintenance requirements and minimal weight gain under thermo-neutral conditions. Restricted feeding was introduced to encourage calves to eat concentrates as early as possible and thus to minimize costs for relatively expensive liquid feeds. After the first 3 weeks of life, starter concentrate intake increases and the calves start to grow rapidly. It has been known for a long time that calves can grow a lot faster if they are supplied with more nutrients. However, worldwide interest in early calf nutrition has only been heightened in the past two decades. Calves suckling their dam or otherwise fed ad libitum ingest about 20% of body weight (BW) per day and reach up to 1 kg of daily weight gain. An intensive (biologically normal) milk-feeding program is now known to be necessary for optimal body growth, organ development and resistance to infectious diseases. Ad-libitum or close to ad-libitum feeding in the first three to four weeks of life also leaves calves less hungry thus improving calf welfare. Only calves fed intensively with colostrum and milk are able to reach their full potential for performance throughout their life.

Current milk feeding practices in Germany A recent representative study (4) investigating management factors and animal health on German dairy farms revealed that – depending on the region – 38 to 52 % of dairy calves were fed with 6 liters or less milk per day in their first 2 weeks of life. Only 9.3 to 17.7 % of calves received milk ad libitum in that age.

Conclusions: Enhanced colostrum intake and a subsequent intensive (biologically normal) milk feeding program support body growth and organ development in dairy calves. Only providing traditional restricted feeding is detrimental to resistance to disease, life-time performance and leaves calves hungry for long periods of time. This system is still widely practiced in Germany. Calf health could be improved considerably by changing these practices.

Bibliographical references
[1] HAMMON H.M., LIERMANN W., FRIETEN D., KOCH C. (2020): Animal 14: 133-43
[2] LORENZ I. (2021): Ir. Vet. J. 74: 5
[3] CARTER H.S.M., RENAUD D.L., STEELE M.A., FISCHER-TLUSTOS A.J., COSTA J.H.C. (2021): Animals 11(8): 2221
[4] PRAERI (2020): Final Report, June 30, 2020, https://ibei.tiho-hannover.de/praeri/pages/69#_AB

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Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology

(Mitteilungen des Ausschusses für Bedarfsnormen)

Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology

Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie

Recommended protocol for the determination of nutrient disappearance *in situ* for estimation of ruminal degradation

Empfohlenes Protokoll zur Durchführung der in-situ-Methode zur Schätzung des ruminalen Nährstoffabbaus

Suggested citation:

GfE [Gesellschaft für Ernährungsphysiologie] (2022): Recommended protocol for the determination of nutrient disappearance *in situ* for estimation of ruminal degradation. Proc. Soc. Nutr. Physiol. 31: 177-189.

1. Introduction

The idea of using the *in situ* method to measure ruminal nutrient degradation was first introduced by Quin et al. (1938), who suggested placing feedstuffs in cylindrical silk bags in the rumen to investigate digestion processes in the forestomaches of sheep. Since then, the *in situ* method has been developed into a standard procedure to measure ruminal degradation processes. As such, it is implemented in several systems for protein evaluation (e.g. NRC 2001; Volden 2011; INRA 2018) for the estimation of ruminal crude protein (CP) degradation. A standardisation of assay conditions and data processing is therefore necessary to ensure the comparability and reproducibility of results (Madsen and Hvelplund 1994; Vanzant et al. 1998; Südekum 2005). Nevertheless, the suggested standard protocols are not consistent. This statement provides a standard protocol for the conduction of *in situ* studies and subsequent data processing, with emphasis on ruminal CP degradation. It will be the basis of revised feed evaluation systems recommended by the Committee for Requirement Standards (Ausschuss für Bedarfsnormen, AfBN) of the Society of Nutrition Physiology. An overview of the recommended standard procedure is provided in Table 1, and explanations and background description are subject of the following text.

2. Sample preparation

2.1 Drying of feedstuffs

Drying of moist feedstuffs (e.g. wet forages or wet cereal grains) for sample preparation can lead to considerable changes in ruminal degradation characteristics, even when applying moderate temperatures of about 40°C (López et al. 1995; Dulphy et al. 1999; Matthé et al. 1999). The diminishing effects of temperature on ruminal degradation are especially relevant for the degradation of CP (López et al. 1995; Dulphy et al. 1999; Matthé et al. 1999), but have also been observed for starch (Ali et al. 2014) and increased for both nutrients with an increased drying intensity (Matthé et al. 1999). Therefore, the AfBN recommends drying feedstuffs for *in situ* studies by freeze-drying. Air drying with low temperatures (≈ 40°C or lower) is also discouraged because of the high risk of spoilage of feedstuffs.

Abbreviations used:

BW = body weight; CP = crude protein; DM = dry matter; DMI = dry matter intake; NDF = neutral detergent fibre.

Table 1 Overview of the recommended *in situ* procedures for the estimation of ruminal nutrient degradation.

	Recommendation
Sample preparation	
Drying of feedstuffs	Freeze drying
Screen size	2 mm for concentrates 3 mm for forages
Determination of particle size distribution	Recommended as an additional descriptive characteristic of the studied feedstuffs
Bag characteristics	
Ratio of sample size to bag surface area	≈ 15 mg dry matter/cm²
Bag pore size	50 µm
Bag material	Polyester
Animals and feeding	
Animals	Cattle and small ruminants
Feeding level	Not specified
Feeding scheme	≥ 2 times daily
Forage : concentrate ratio	≈ 2/3 : 1/3
Diet composition	According to supply recommendations Adequate representation of all nutrients
Adaptation	14 days
Ruminal incubation of bags	
Number of animals and replications	≥ 3 animals
Pre-soaking of bags	Warm water (39°C) for 10 minutes with gentle movement and smoothing of bags
Location of bag in the rumen	Base of the ventral rumen
Incubation times	At least 2, 4, 6, 8, 16, 24 and 48 hours Additional incubation times depending on the expected extent and rate of degradation
Sequence of incubation	Simultaneous beginning of incubation before morning feeding
Ending of incubation	Immersion in ice water
Sample processing and analyses after ruminal incubation	
Rinsing procedure	Removal of coarse attachments of ruminal ingesta
	Cold washing without detergents and spinning
Drying	48 hours at 45°C
Determination of water-soluble fraction	Yes
0-h bags	≥ 3 (according to number of animals)
Data evaluation	
Correction for losses of small particles	Yes, after plausibility test According to Weisbjerg et al. (1990)
Correction for microbial attachments	Yes According to Parand and Spek (2021)
Model	Exponential models after examination of goodness-of-fit
Lag phase	Yes

2.2 Grinding of feedstuffs

After drying, feedstuffs under study must be mechanically treated to mimic the reduction of particle size that would be achieved by mastication and rumination. Barrell et al. (2000) and Cohen and Doyle (2001) reported that mincing of forages with a meat chopper can better reflect the particle size reduction *in vivo* than the grinding of feedstuffs. Nevertheless, most *in situ* studies and suggested standard protocols (e.g. Vanzant et al. 1998; NRC 2001; Volden 2011; INRA 2018) recommend grinding of feedstuffs, which might be related to the difficulties in achieving homogenous sample material after mincing. However, the suggestions for screen sizes chosen for grinding are not consistent among standard protocols and range between 0.8 mm (INRA 2018) and 2 mm (Vanzant et al. 1998; NRC 2001). Vanzant et al. (1998) reviewed the results of 54 *in situ* studies where the applied screen sizes varied between < 1 and 6 mm. Udén and van Soest (1984) highlighted that screen sizes below 2 mm increase the risk of losing small, water-insoluble particles from the bags and should therefore not be used. Consistently, Michalet-Doreau and Cerneau (1991) reported a significantly increased wash-out fraction with a screen size of 0.8 mm compared to 3 and 6 mm. However, the homogeneity of feed samples is reduced and, thus, the variability of estimated ruminal degradation characteristics increased when larger screen sizes were used (Michalet-Doreau and Ould-Bah 1992). Against this background, the AfBN recommends that dried samples are ground using a centrifugal mill with a screen size of 2 mm for concentrates and 3 mm for forages.

For the investigation of ruminal starch degradation, Peyrat et al. (2014) and Fernandes et al. (2018) recommended using larger screen sizes (4–6 mm) to reduce wash-out losses of starch granules, although ruminal degradation is decreased with larger screen sizes. Nevertheless, this is an example that the suggested screen sizes of 2 or 3 mm may need to be adapted for specific research questions.

Because the particle size distribution after grinding can be different among feedstuffs, although ground through the same sieve, particle size distribution of the incubated material should be determined and reported in *in situ* studies.

3. Bag characteristics

3.1 Dimension of bags and ratio of sample size to bag surface area

Vanzant et al. (1998) and Diao et al. (2020) compiled data of several studies in which sample size to bag surface area ratios between 10 and 20 mg/cm² were used consistently; with increasing sample size to bag surface area ratios, ruminal degradation determined *in situ* decreased. The results of Nocek (1985) indicate that *in situ* measurements are in accordance with *in vivo* measurements when using a sample size to bag surface area ratio of 12.6 mg/cm², which was supported by the results of Diao et al. (2020), who used a sample size to bag surface area ratio of 12.5 mg/cm². Huntington and Givens (1995) recommended using a sample size to bag surface area ratio of 16 mg/cm² and pointed out that for high-moisture forages, a wider ratio might be necessary. Also, Diao et al. (2020) highlighted this aspect and consequently suggested using a sample size to bag surface area ratio of about 20 or 25 mg/cm² for concentrates and forages, respectively. Nevertheless, lower sample size to bag surface area ratios appear preferable to the AfBN because some of the estimated ruminal degradation characteristics were affected with ratios equal or greater than 20 mg/cm² in the study of Diao et al. (2020). To conclude, the AfBN suggests using a ratio of sample size to bag surface area of about 15 mg/cm² as compromise to achieve sufficient sample amounts on the one hand and to avoid impairment of ruminal degradation on the other. This ratio can be achieved with sample amounts of approximately 1.5 or 6 g, using commercially available bags with internal dimensions of 5 × 10 cm or 10 × 20 cm, respectively. Note that with increasing bag dimensions, the risk of clumping is enhanced, especially when incubating concentrates. Bags should therefore be moved and gently smoothed out during pre-soaking (cf. 5.2).

3.2 Bag pore size

Available standard protocols consistently recommend using bags with pore sizes between 40 and 60 µm for the evaluation of ruminal CP degradation (NRC 2001; Volden 2011; INRA 2018). A fair degree of standardisation regarding the pore sizes used for the investigation of ruminal CP degradation is also indicated by the results of Liebe et al. (2018), who reported a mean pore size of 49 µm with a standard deviation of ±8 µm in a meta-analysis including 756 observations. Consistently, the AfBN recommends using a pore size of 50 µm as compromise between particle losses on the one hand and clogging of bag pores on the other.

3.3 Bag material

In accordance with other protocols (Vanzant et al. 1998; NRC 2001; Volden 2011), the AfBN recommends using monofilament bags made from polyester. Using nylon bags has the disadvantage that they cannot be subjected to N-analysis as nylon contains nitrogen, requiring the time-consuming and error-prone emptying of bags prior to analysis. Reuse of bags of any material is discouraged as with reused bags, wash-out losses are affected (Kaswari and Lebzien 2001), likely due to the alteration of pore size and structure during ruminal incubation and subsequent processing steps (washing, drying) (Südekum 2005).

3.4 Closing of bags

The method of closing the bags appears not to be relevant for standardisation of the *in situ* method as long as the internal bag dimension and, thus, the ratio of sample size to bag surface area can be maintained so that any method of closing the bags may be used (Volden 2011).

4. Animals and feeding

4.1 Animals

Huntington and Givens (1997) and Prigge et al. (1984) consistently reported no differences in the ruminal degradation characteristics determined *in situ* between dry cows and wethers and between steers and wethers. Although Siddons and Paradine (1983) observed differences in the level of ruminal degradation characteristics between sheep and steers, the ranking of feedstuffs was the same. Klevenhusen et al. (2021) pointed out that differences between ruminant species were, to a large part, related to differences in the ruminal passage rate. They concluded that systems in which reticulo-ruminal retention times are controlled independently from the ruminant species are less prone to be affected by species differences, which also applies to *in situ* studies by the choice of fixed incubation times. Additionally, for *in vitro* studies, CP degradation does not differ when sheep or cattle are used as donor animals, but the effect of diet composition is more important to consider (Boguhn et al. 2013). The higher impact of the diet on ruminal degradation was also highlighted by Henderson et al. (2015), who found that microbial community composition was predominantly associated with the diet, whereas the host was less influential. Based on this, the AfBN suggests that cattle and small ruminants (sheep and goats) with a fully developed forestomach system can be used for *in situ* studies.

4.2 Feeding level

Some studies suggest an effect of the feeding level on *in situ* ruminal nutrient degradation characteristics (Volden 1999; Rodríguez et al. 2008; Zanton and Heinrichs 2008; Schadt et al. 2014), but the results are not consistent. Volden (1999) observed a higher degradation rate of CP and a lower percentage of potentially degradable CP for a concentrate mixture and grass silage in cows at a high feeding level (dry matter intake (DMI) 3.3% of body weight (BW)) compared to an intermediate feeding level (DMI 1.7% of BW). In contrast, Schadt et al. (2014) reported the highest ruminal degradation rate and effective degradation of CP with a DMI of 1.5% of BW and a significant reduction at a DMI of 3.2 and 4.5% of BW when incubating several feedstuffs. In a study with wethers, Rodríguez et al. (2008) observed no significant effect of feeding level on potentially degradable CP and ruminal degradation rate of CP, but effective CP degradation was lower at the higher feeding level (DMI 2.8% of BW) compared to a lower feeding level (DMI 1.4 % of BW). Zanton and Heinrichs (2008) reported no significant effects of feeding level on ruminal *in situ* degradation characteristics of CP in heifers fed a high-forage diet in different amounts (DMI 1.25, 1.5, 1.75 and 2% of BW). The results of Volden (1999) and Schadt et al. (2014) might be biased by the use of animals in different stages of lactation and unequal forage to concentrate ratios in the study of Schadt et al. (2014). Therefore, the available literature results are too inconsistent to recommend a certain feeding level for *in situ* studies. Nevertheless, this does not appear as a restriction for the eligibility of the method, as the feeding level is considered by modelling of the passage rate during data evaluation. The feeding level should therefore be adapted to the nutritional requirements of the animals to avoid over- and undersupply of energy and nutrients and to ensure a constant feeding level during an experiment.

4.3 Feeding scheme

Feed should be provided at least two times daily. A higher frequency appears not to cause problems. Care should be taken to provide a consistent and homogenous diet composition (complete diets should be provided instead of separate provision of forages and concentrates) and that meals are distributed equally over the day.

4.4 Diet composition

The experimental diet fed to the fistulated animals should consist of two-thirds of forages and one-third of concentrates, which is in accordance with other recommendations (Volden 2011; INRA 2018). Animals should be fed with a versatilely composited experimental diet in which all nutrient fractions are adequately represented.

4.5 Adaptation

An adaptation phase of 14 days is recommended to adapt the animals to the experimental diet. When a series of several incubations is conducted with the same experimental diet, a repeated adaptation between the incubations is not necessary.

4.6 Miscellaneous data

Beyond the data concerning the ruminal degradation of nutrients, *in situ* studies should report nutrient composition and, if applicable, details on pre-treatments of the investigated feedstuffs and of the experimental diet (ingredient composition and nutrients such as dry matter (DM), crude ash, CP, ether extract, and acid and neutral (NDF) detergent fibre). Additionally, data on feed intake and body weight of the animals should be reported.

5. Ruminal incubation of feedstuffs

5.1 Number of animals and replications

Variations of *in situ* degradation characteristics can be large among individual animals (Mehrez and Ørskov 1977; Hoffman et al. 1999) and among different days within the same animal (Mehrez and Ørskov 1977; van der Koelen et al. 1992). Therefore, a sufficient number of independent replicates is necessary in several animals and, where appropriate, on different days, although suggestions for the appropriate number of replications are diverse.

Relating the importance of number of animals and replications on different days, Vanzant et al. (1998) found that most of the variance was attributed to variations among animals, wherefore using several animals should be preferred compared to replications on different days. To achieve a fair number of replicates for statistical evaluations, the AfBN recommends a minimum number of three animals for *in situ* studies, which is in accordance with the recommendation of the Norfor system (Volden 2011). Deviating from this suggestion, other protocols for the *in situ* method recommend using at least two animals and replicating incubations on two or more days (NRC 2001). In this context, Vanzant et al. (1998) highlighted the importance of replications on different days, as most of the day-to-day-variation was consistent across animals. Based on that, it might also be possible to use only two animals and conduct replications on two or more days when three animals are not available. Nevertheless, it has to be kept in mind that replications are not independent in this case, and therefore, the use of at least three animals should be preferred.

The necessary number of bags per animal and incubation time is usually between three and six and has to be chosen considering the expected residual sample amount, depending on the type of the incubated feedstuff and the respective incubation time. Note that the use of several bags per animal and incubation time is necessary to achieve a sufficient amount of sample material for analysis, but these bags cannot serve as experimental replicates.

5.2 Pre-soaking of bags

Pre-soaking of bags is recommended in the *in situ* standard protocols by NRC (2001) and Volden (2011) and should support instant accessibility of microorganisms and their secreted enzymes to the substrate after placement in the rumen (Nocek 1985; Michalet-Doreau and Ould-Bah 1992). The AfBN suggests to pre-soak bags in warm water (39°C) for 10 minutes before placement in the rumen to ensure moisturisation of feed and adjustment of the feed temperature to the conditions in the rumen. Bags should be moved and smoothed out gently to avoid clumping.

5.3 Location of bags in the rumen

The location of bags in the ventral sac of the rumen is preferred because of the higher mobility and purging of bags in the more aqueous phase of the rumen compared to other sections (Südekum 2005). Furthermore, Stewart (1979) proposed that microorganisms present in the ventral phase of the rumen could more effectively colonise feedstuffs in the bags and degrade their nutrients because they are more liquid-associated and can move more freely compared with microorganisms of the more solid phase of the dorsal rumen, which are mostly adhered to feed particles. Consequently, incubation of bags in the ventral rumen is consistently suggested by all standard protocols for the *in situ* method (e.g. Vanzant et al. 1998; NRC 2001; Volden 2011; INRA 2018). Steady flushing of bags with ruminal fluid and location in the ventral rumen must be ensured by the fixation of bags to an anchor weight attached to the rumen fistula with a sufficiently long nylon rope. For instance, anchor weights can be steel rings or cylindrical plastic weights with perforations for the attachment of bags, as described by Südekum et al. (1996) or Terler et al. (2017), respectively, with a weight of approximately 0.8 to 1 kg.

5.4 Incubation times

The conduction of incubation for 2, 4, 6, 8, 16, 24 and 48 h is well established in standard protocols (NRC 2001; Volden 2011; INRA 2018). Nevertheless, protocols disagree regarding the desired end-points especially for forages, with suggestions varying between 72 h (NRC 2001; INRA 2018) and more than 240 h (Steingaß and Südekum 2013). End-points should be chosen related to the expected degradation rate of the incubated feedstuffs. Therefore, the AfBN suggests using the aforementioned incubation periods between 2 and 48 h as a general standard for all feedstuffs and additional incubation periods according to the expected degradation rate. For instance, this could mean 72 h as end-point for concentrates and 96 h for forages.

Olaisen et al. (2003) tested a bilinear approach to estimate the effective protein degradability of concentrates using only two or three incubation periods. They estimated the effective degradation with high accuracy compared with using seven incubation periods. The use of one specific incubation period might allow the investigation of a large number of feedstuffs in comparative feed evaluation at a given passage rate. Such an approach might be useful when the estimation of only effective degradation is of interest, but the kinetics of ruminal nutrient degradation cannot be characterised and the results cannot be applied in a flexible manner that considers the passage rate.

5.5 Sequence, start and end of incubation

Two different incubation sequences are widely used. The first includes the placement of all bags in the rumen simultaneously and the removal at different time points. Alternatively, bags are placed at different time points and removed from the rumen simultaneously (Michalet-Doreau and Ould-Bah 1992). Nocek (1985) found slower degradation rates for DM and CP of soybean meal when the bags were placed simultaneously compared with simultaneous removal. In agreement with this, Dong et al. (2017) reported slower degradation rates and longer lag phases with the simultaneous placement when incubating maize silage, lucerne haylage, soybean meal and maize grain. Nocek (1985) hypothesised that this was related to an interruption of incubation when bags intended for longer incubation times were retrieved and reinserted into the rumen upon removal of the shorter incubated bags and, consequently, recommended to apply simultaneous removal. Nevertheless, Michalet-Doreau and Ould-Bah (1992) argued that the conditions in the rumen (e.g. pH value, osmolarity, dilution, temperature) underlie diurnal changes. Simultaneous placement appears to be advantageous compared to simultaneous removal as ruminal conditions will be the same for all bags during overlapping incubation periods (Michalet-Doreau and Ould-Bah 1992; Südekum

2005). Vanzant et al. (1998) concluded that simultaneous placement is advantageous compared with simultaneous removal as long as rapid removal is ensured to minimise interruption of incubation. Based on that, the AfBN recommends simultaneous placement and staggered removal of the bags from the rumen.

Incubation is recommended to start before the morning feeding because of simpler handling and logistic reasons: when the start of incubation is before the morning feeding, the bags can be placed when the rumen is relatively empty, and removal of almost all bags can be done during daytime (except 16 h incubations). At the end of incubation, immediate immersion in ice water is necessary to instantly minimise further microbial activity.

6. Sample processing and chemical analyses following ruminal incubation

6.1 Rinsing procedure

The lack of standardisation of the rinsing procedure appears to be a major source of variability of results of *in situ* studies (Vanzant et al. 1998). Nevertheless, standardisation is difficult because different types of washing machines and programs are being used. Programs used must be cold-wash programs without spinning and a frequent change of water (three to four times) and should not use any detergents. To achieve this, rinsing programs of common washing machines can be repeated four times. Bags for the determination of the wash-out losses should be rinsed together with the bags incubated in the rumen to ensure consistent conditions during rinsing.

6.2 Drying and analysis of bag residues

Drying of the rinsed bags should be conducted for 48 h at 45°C, as higher temperatures can affect the determination of fibre fractions and starch in the residues (Südekum 2005). Due to low sample amounts, the risk of spoilage appears to be negligible, and changes in solubility, discussed for sample preparation, are irrelevant as the soluble fraction has, by definition, disappeared during ruminal incubation of the bags. Analysis of bag residues should be conducted according to the respective official methods for feed analysis, applying the same methods as for the original feed samples.

7. Considerations concerning data evaluation

7.1 Determination of the water-soluble fraction and correction for losses of small particles

The wash-out losses (0 h value, determined by rinsing bags without preceding incubation in the rumen) consist of a water-soluble fraction and small water-insoluble particles. These small particles are assigned to the water-soluble fraction so that they are assumed to be degraded immediately, although they might not necessarily be instantly degraded, which can lead to severe misjudgement of ruminal CP degradation (Hvelplund and Weisbjerg 2000). Weisbjerg et al. (1990) suggested correcting for these particles by applying the degradation kinetics of the particles remaining in the bag to the small, water-insoluble particles (i.e. small, water-insoluble particles are added mathematically to fraction b). Nevertheless, Hvelplund and Weisbjerg (2000) highlighted that for some feedstuffs (e.g. oat grains), this correction will lead to unreliable results because of strongly differing characteristics of small particles and contents of bag residues. Furthermore, for specific nutrients, the correction suggested by Weisbjerg et al. (1990) leads to implausible results (phosphorus, phytate; unpublished data) and is not applicable for starch because of methodical reasons. Based on this, the AfBN suggests the application of the correction suggested by Weisbjerg et al. (1990) only after critical evaluation of the plausibility of the obtained results. This includes the examination of the reasonability of the level and the relation of the wash-out fraction and the water-soluble fraction for the respective nutrients and their documentation, even when the use of the correction for losses of small particles is omitted.

7.2 Correction for microbial attachments

During incubation of the bags, the rumen microbiota tightly attaches to the incubated feed and cannot be completely removed during rinsing of the bags. The contribution of microbial N can make up more than 90% of residual N in bags (Olubobokun et al. 1990) or initially incubated N (Krawielitzki et al. 2006). This can lead to a considerable underestimation of ruminal CP degradation (Olubobokun et al. 1990; Rodríguez et al. 2008) as the remaining microbial CP is assigned to the feed residue. This is especially relevant for feedstuffs that are rich in fibre but low in CP concentration (e.g. forages, hulls, sugar beet pulp; Rodríguez and González 2006) because they are intensively colonised by rumen microbes and the contribution of microbial N to total residual N in bags is high (Südekum 2005). Vanzant et al. (1998) already noticed that the issue of microbial attachment is rarely considered in the suggested standard protocols (e.g. Volden 2011; INRA 2018) and in *in situ* studies, despite of its high quantitative relevance.

Microbial attachment has been estimated using marker methods (15N, purine bases), separation of microbes with a stomacher or the amount of NDF-bound N in incubation residues. Because of the high effort, the estimation with marker methods is not feasible for routine use, and the treatment with a stomacher does not completely remove microbiota from bag residues (Schmidt et al. 2005). Estimation of microbial attachment based on the NDF-bound N assumes that all dietary CP that is not NDF-bound is degraded after 16 h of incubation. Therefore, microbial CP attachment can be calculated as the difference between CP and NDF-bound CP in bag residues incubated for 16 h or longer. This approach has been validated with the estimation of microbial attachment with purine bases (Mass et al. 1999), but it requires the additional determination of NDF-bound N in all bag residues incubated for 16 h or longer. Therefore, this approach does also not appear feasible for routine use because of the high effort.

Michalet-Doreau and Ould-Bah (1989) and Krawielitzki et al. (2006) found a positive relationship of microbial colonisation with NDF concentration on the one hand and a negative relationship with the CP concentration of feedstuffs on the other. Based on this relationship, both working groups developed similar regression equations for the estimation of microbial attachments from NDF and CP concentration of feeds. In a recent meta-analysis, Parand and Spek (2021) extended the equations suggested by Krawielitzki et al. (2006) and developed differentiating equations for roughages and concentrates, estimating microbial attachments with high accuracy. The AfBN suggests using the following equations of Parand and Spek (2021) for all feedstuffs, as this will considerably increase the accuracy of the determination of CP degradation for feedstuffs with a low CP concentration without affecting the determination for feedstuffs with a high CP concentration:

Forages:

$$N_{MA} = (89.0 - 0.209 \cdot CP) \cdot (1 - e^{(-0.117 \times t)}) \quad (1)$$

Concentrates with a CP concentration below 300 g/kg DM:

$$N_{MA} = (53.0 - 0.188 \cdot CP + 0.031 \cdot NDF) \times (1 - e^{(-0.072 \cdot t)}) \quad (2)$$

Concentrates with a CP concentration above 300 g/kg DM:

$$N_{MA} = (43.8 - 0.07 \cdot CP + 0.015 \cdot NDF) \times (1 - e^{(-0.068 \cdot t)}) \quad (3)$$

where NMA is the *in situ* microbial N attachment (% of residual N) after t hours of incubation and CP and NDF are the concentrations of CP and NDF in the incubated feedstuff (g/kg DM).

7.3 Lag phase

Usually, the degradation of incubated feedstuffs will not start immediately with the placement of the bags in the rumen, as the rumen microbiota needs time to adhere to the feed. This phase, in which no or only slow degradation occurs, is generally referred to as the lag phase (McDonald 1981). When a model is fit to the degradation data, inclusion of a lag phase in the model affects the estimated degradation parameters (Denham et al. 1989). Therefore, the occurrence of a lag phase needs to be tested before the estimation of the degradation parameters. In case of a non-significant lag phase, it should be excluded from the model, and ruminal degradation parameters should be re-estimated without consideration of a lag phase.

7.4 Model

For most feedstuffs, the monoexponential model suggested by Ørskov and McDonald (1979) or the model extended by the lag phase (McDonald 1981) provide a good estimation of ruminal degradation characteristics:

$$\text{Deg} = a + b \cdot (1 - e^{-c \cdot (t - \text{lag})}), \text{ when lag} > 0; \qquad (4)$$

$$\text{Deg} = a + b \cdot (1 - e^{-c \cdot t}), \text{ when lag} = 0, \qquad (5)$$

where Deg (%) is the degradation after t hours, a (%) is the rapidly disappearing fraction or the wash-out fraction, b (%) is the potentially degradable fraction with the rate constant of degradation c (%/h), t is the incubation time (h) and lag is the lag phase (h). The definition of a as rapidly disappearing or as a wash-out fraction has to be examined depending on the specific feedstuff and nutrient.

The effective degradability (ED) of nutrients depending on the ruminal passage rate can be calculated using the following equations:

$$\text{ED} = a + \frac{b \cdot c}{c + k} \cdot (e^{-k \cdot \text{lag}}), \text{ when lag} > 0; \qquad (6)$$

$$\text{ED} = a + \frac{b \cdot c}{c + k}, \text{ when lag} > 0; \qquad (7)$$

where k is the estimated passage rate from the rumen and a, b, c and lag are the same parameters as described for equations 4 and 5. Equation 6 is a modification of the equation suggested by McDonald (1981), where the term e^{-k} · lag” reads “$e^{-(c + k) \cdot \text{lag}}$”, suggesting that during the lag phase, both degradation and passage occur, which is not correct as it is assumed that only the passage of undegraded particles from the rumen but no microbial degradation occurs during the lag phase (Südekum 2005). The AfBN recommends reporting the estimated parameters a, b, c and lag phase instead of the effective degradability to enable a flexible further use of the data, especially for the compilation of feed databases. Additional data recommended to be reported for *in situ* studies are indicated in Table 2.

For specific feedstuffs, the recommended exponential model may not fit the course of ruminal degradation, and another type of model has to be applied (e.g. sigmoidal function for S-shape curves). In case of the application of another model, the estimated ruminal degradation parameters might have to be interpreted differently than those based on exponential functions.

7.5 Applicability of the method and limitations

The applicability of the *in situ* method for the evaluation of ruminal nutrient degradation has to be critically examined during the conduction of the method and data evaluation, as the obtained ruminal degradation characteristics can severely misjudge ruminal degradation in some cases. For instance, this applies to feedstuffs with either high concentrations of soluble constituents or ruminally undegradable small particles that can escape from the bags without being degraded (Dewhurst et al. 1995). This may apply to the feedstuff per se when it is very finely ground or has an intrinsically small particle size (e.g. microalgae; Wild 2019) or to specific nutrients of a certain feedstuff (e.g. starch granules of oats; Seifried et al. 2015). Furthermore, several authors described accumulation of gas in the bags, probably related to clogging of the pores, indicating an obstructed outflow of fermentation end-products (Nocek et al. 1979; Udén and van Soest 1984; Seifried et al. 2015), which likely leads to a misjudgement of ruminal degradation processes. Consequently, the applicability of the method has to be evaluated critically, considering the specific feedstuff and nutrients under study.

Table 2 Overview of information recommended for inclusion in reports of *in situ* studies.

	Recommendation
Sample preparation and analyses of incubated feedstuffs	Details on the drying method, including temperature and duration of drying
	Screen size and mill type Details on the processing of feedstuffs Particle size distribution Water-soluble fraction and wash-out fraction
	Proximate nutrient composition and concentration of all substances for which ruminal degradation was investigated
Bag characteristics	Bag dimensions, bag pore size and bag material
	Ratio of sample size to bag surface area
Animals and feeding	Animal species and number of animals
	Feed intake, body weight and performance level
	Diet composition (ingredients, proximate nutrient composition and forage: concentrate ratio)
Ruminal incubation of bags	Number of replications and incubation times
	Details on pre-soaking of bags, location of bags in the rumen and sequence of incubation
Sample processing and analyses after ruminal incubation	Details on rinsing procedure and sample processing
Ruminal degradation characteristics	Applied correction models (loss of small particles and microbial attachments). In case correction is omitted, reasons for omission. Applied model and estimated values for all parameters (e.g. a, b, c and lag phase for exponential models)

8. References

Ali M., Cone J.W., Hendriks W.H., Struik P.C. (2014): Oven-drying reduces ruminal starch degradation in maize kernels. Anim. Feed Sci. Technol. 193:44-50

Barrell L.G., Burke J.L., Waghorn G.C., Attwood G.T., Brookes I.M. (2000): Preparation of fresh forages for incubation and prediction of nutritive value. Proc. N. Z. Soc. Anim. Prod. 60:5-8

Boguhn J., Zuber T., Rodehutscord M. (2013): Effect of donor animals and their diet on in vitro nutrient degradation and microbial protein synthesis using grass and corn silages. J. Anim. Physiol. Anim. Nutr. 97:547-557

Cohen D.C., Doyle P.T. (2001): Effect of sample preparation on in situ estimates of protein degradability for white clover herbages. Aust. J. Exp. Agric. 41:619

Denham S.C., Morantes G.A., Bates D.B., Moore J.E. (1989): Comparison of two models used to estimate in situ nitrogen disappearance. J. Dairy Sci. 72:708-714

Dewhurst R.J., Hepper D., Webster A.J.F. (1995): Comparison of in sacco and in vitro techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. Anim. Feed Sci. Technol. 51:211-229

Diao X., Dang S., Liu S., Jing L., Wang Y., Zhang W. (2020): Determination of the appropriate ratio of sample size to nylon bag area for in situ nylon bag technique evaluation of rumen digestibility of feedstuffs in sheep. Livest. Sci. 241:104254

Dong S.-Z., Azarfar A., Zou Y., Li S.-L., Wang Y.-J., Cao Z.-J. (2017): Effects of sequence of nylon bags rumen incubation on kinetics of degradation in some commonly used feedstuffs in dairy rations. Journal of Integrative Agriculture 16:162-168

Dulphy J.-P., Demarquilly C., Baumont R., Jailler M., L'Hotelier L., Dragomir C. (1999): Study of modes of preparation of fresh and conserved forage samples for measurement of their dry matter and nitrogen degradations in the rumen. Ann. Zootech. 48:275-288

Fernandes T., Ávila C.L.S., Pereira M.N., Ferraretto L.F. (2018): Short communication: Effect of washing method, grinding size, and the determination of an indigestible fraction on in situ degradation of starch in mature corn grain. J. Dairy Sci. 101:9052-9057

Henderson G., Cox F., Ganesh S., Jonker A., Young W., Janssen P.H. (2015): Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep. 5:14567

Hoffman P.C., Brehm N.M., Hasler J.J., Bauman L.M., Peters J.B., Combs D.K., Shaver R.D., Undersander D.J. (1999): Development of a novel system to estimate protein degradability in legume and grass silages. J. Dairy Sci. 82:771-779

Huntington J.A., Givens D.I. (1995): The in situ technique for studying the rumen degradation of feeds: a review of the procedure. Nutr. Res. Rev. Ser. B 65:63-93

Huntington J.A., Givens D.I. (1997): Studies on in situ degradation of feeds in the rumen: 1. Effect of species, bag mobility and incubation sequence on dry matter disappearance. Anim. Feed Sci. Technol. 64:227-241

Hvelplund T., Weisbjerg M.R. (2000): In situ techniques for the estimation of protein degradability and postrumen availability. In Forage evaluation in ruminant nutrition (ed. D.I. Givens), CABI Publishing, Wallingford, UK, 233-258.

Institut national de la recherche agronomique [INRA] (2018): INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, Netherlands

Kaswari T., Lebzien P. (2001): Untersuchungen zum Einfluss der Trocknungstemperatur von Nylonbeuteln auf die Höhe der Auswaschverluste bei wiederholter Nutzung. 113. VDLUFA-Kongress, Berlin, Kurzfassungen der Referate. VDLUFA-Schriftenr. 57:137

Klevenhusen F., Südekum K.-H., Breves G., Kolrep F., Kietzmann M., Gerletti P., Numata J., Spolders M., Pieper R., Kowalczyk J. (2021): Predicting the transfer of contaminants in ruminants by models - potentials and challenges. ALTEX

Krawielitzki K., Schmidt T., Voigt J., Kowalczyk J., Gabel M. (2006): Dynamics of microbial contamination of protein during ruminal in situ incubation of feedstuffs. J. Anim. Feed Sci. 15:313-328

Liebe D.M., Firkins J.L., Tran H., Kononoff P.J., White R.R. (2018): Technical note: Methodological and feed factors affecting
measurement of protein A, B, and C fractions, degradation rate, and intestinal digestibility of rumen-undegraded protein. J. Dairy Sci. 101:8046-8053

López S., Hovell F.D.D., Manyuchi B., Smart R.I. (1995): Comparison of sample preparation methods for the determination of the rumen degradation characteristics of fresh and ensiled forages by the nylon bag technique. Anim. Sci. 60:439-450

Madsen J., Hvelplund T. (1994): Prediction of in situ protein degradability in the rumen. Results of a European ringtest. Livest. Prod. Sci. 39:201-212

Mass R.A., Lardy G.P., Grant R.J., Klopfenstein T.J. (1999): In situ neutral detergent insoluble nitrogen as a method for measuring forage protein degradability. J. Anim. Sci. 77:1565-1571

Matthé A., Lebzien P., Flachowsky G. (1999): Influence of maize grain drying process on its in situ degradability in dairy cows. J. Anim. Feed Sci. 8:379-386

McDonald I. (1981): A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 96:251-252

Mehrez A.Z., Ørskov E.R. (1977): A study of artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. 88:645-650

Michalet-Doreau B., Cerneau P. (1991): Influence of foodstuff particle size on in situ degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35:69-81

Michalet-Doreau B., Ould-Bah M.Y. (1989): Estimation of the extent of bacterial contamination in bag residues and its influence on in sacco measurements of forage nitrogen degradation in the rumen. In 16th International Grassland Congress, Nice, Vol. II:909-910

Michalet-Doreau B., Ould-Bah M.Y. (1992): In vitro and in sacco methods for the estimation of dietary nitrogen degradability in the rumen: a review. Anim. Feed Sci. Technol. 40:57-86

National Research Council [NRC] (2001): Nutrient Requirements of dairy cattle, Seventh Revised Edition. National Academy Press, Washington D.C., USA

Nocek J.E. (1985): Evaluation of specific variables affecting in situ estimates of ruminal dry matter and protein digestion. J. Anim. Sci. 60:1347-1358

Nocek J.E., Cummins K.A., Polan C.E. (1979): Ruminal disappearance of crude protein and dry matter in feeds and combined effects in formulated rations. J. Dairy Sci. 62:1587-1598

Olaisen V., Mejdell T., Volden H., Nesse N. (2003): Simplified in situ method for estimating ruminal dry matter and protein degradability of concentrates. J. Anim. Sci. 81:520-528

Olubobokun J.A., Craig W.M., Pond K.R. (1990): Effects of mastication and microbial contamination on ruminal in situ forage disappearance. J. Anim. Sci. 68:3371-3381

Ørskov E.R., McDonald I. (1979): The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503

Parand E., Spek J.W. (2021): Development of equations to estimate microbial nitrogen contamination in rumen incubation residues using 15N data and chemical composition of feedstuffs. Anim. Feed Sci. Technol. 273:114798

Peyrat J., Nozière P., Le Morvan A., Férard A., Protin P.V., Baumont R. (2014): Effects of ensiling maize and sample conditioning on in situ rumen degradation of dry matter, starch and fibre. Anim. Feed Sci. Technol. 196:12-21

Prigge E.C., Baker M.J., Varga G.A. (1984): Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers. J. Anim. Sci. 59:237-245

Quin J.I., van der Wath J.G., Myburgh S., Du Toit P.J. (1938): Studies on the alimentary tract of Merino sheep in South Africa. IV. Description of experimental technique. Onderstepoort J. Vet. Sci. Anim. Ind. 11:341-360

Rodríguez C.A., González J. (2006): In situ study of the relevance of bacterial adherence to feed particles for the contamination and accuracy of rumen degradability estimates for feeds of vegetable origin. Br. J. Nutr. 96:316-325

Rodríguez C.A., González J., Alvir M.R., Caballero R. (2008): Effects of feed intake on in situ rumen microbial contamination and degradation of feeds. Livest. Sci. 116:108-117

Schadt I., Mertens D.R., van Soest P.J., Azzaro G., Licitra G. (2014): Stage of lactation and corresponding diets affect in situ protein degradation by dairy cows. J. Dairy Sci. 97:7995-8007

Schmidt T., Krawielitzki K., Voigt J., Gabel M. (2005): Methodische Aspekte der in situ-Technik zur Schätzung des Nährstoffabbaus im Pansen - Mikrobielle Kontamination und Verzögerung des messbaren mikrobiellen Abbaus. Übers. Tierernährg. 33:87-100

Seifried N., Steingaß, H., Rodehutscord M. (2015): In vitro and in situ evaluation of secondary starch particle losses from nylon bags during the incubation of different cereal grains. Anim. Feed Sci. Technol. 210:26-36

Siddons R.C., Paradine J. (1983): Protein degradation in the rumen of sheep and cattle. J. Sci. Food Agric. 34:701-708

Steingaß H., Südekum K.-H. (2013): Proteinbewertung beim Wiederkäuer - Grundlagen, analytische Entwicklungen und Perspektiven. Übers. Tierernährg. 41:51-73

Stewart C.S. (1979): Problems in the assessment of fibre digestion in the rumen. In Straw decay and its effect on disposal and utilization. Proc. Symposium on Straw Decay and Workshop on Assessment Techniques, held at Hatfield Polytechnic, April 10-11th 1979 (ed E Grossbard), pp. 315–319. John Wiley & Sons, Chichester, New York, Brisbane, Toronto

Südekum K.-H., Thordsen J.P., Stangassinger M. (1996): Effect of wheat silage maturity on in situ degradation of organic matter, crude protein, starch and fibre. J. Appl. Anim. Res. 10:1-14

Südekum K.-H. (2005): Möglichkeiten und Grenzen einer Standardisierung der in situ-Methodik zur Schätzung des ruminalen Nährstoffabbaus. Übers. Tierernährg. 33:71-86

Terler G., Gruber L., Knaus W.F. (2017): Effect of variety and harvest date on nutritive value and ruminal degradability of ensiled maize ears. Arch. Anim. Nutr. 71:333-346

Udén P., van Soest P.J. (1984): Investigations of the in situ bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. J. Anim. Sci. 58:213-221

van der Koelen C.J., Goedhart P.W., van Vuuren A.M., Savoini G. (1992): Sources of variation of the in situ nylon bag technique. Anim. Feed Sci. Technol. 38:35-42

Vanzant E.S., Cochran R.C., Titgemeyer E.C. (1998): Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717-2729

Volden H. (1999): Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. J. Anim. Sci. 77:1905-1918

Volden H. (Ed) (2011): NorFor - The Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, Netherlands

Weisbjerg M.R., Bhargava P.K., Hvelplund T., Madsen J. (1990): Anvendelse af nedbrydningsprofiler i fodermiddelvurderingen, Report No. 679. National Institute of Animal Science, Foulum, Denmark

Wild K.J. (2019): Investigations on nutritional characteristics of microalgae with emphasis on ruminants. Dissertation, Stuttgart-Hohenheim, Germany

Zanton G.I., Heinrichs A.J. (2008): Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. J. Dairy Sci. 91:3579-3588

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Schätzung der Umsetzbaren Energie von Mischfuttermitteln für Schweine auf der Basis der Rohnährstoffe – Fortschreibung des bisherigen Vorgehens und Forschungsbedarf

Der Ausschuss für Bedarfsnormen (AfBN) hat 2008 die folgende Gleichung zur Schätzung der Umsetzbaren Energie (ME) von Mischfuttermitteln für Schweine empfohlen (GfE 2008):

$$\begin{aligned} &\mathrm{ME_S}\ \text{(MJ/kg Trockenmasse)} \\ &= 0{,}021503 \cdot \text{Rohprotein} \qquad [1] \\ &+ 0{,}032497 \cdot \text{Rohfett} \\ &- 0{,}021071 \cdot \text{Rohfaser} \\ &+ 0{,}016309 \cdot \text{Stärke} \\ &+ 0{,}014701 \cdot \text{Organischer Rest (alle Fraktionen in g/kg Trockenmasse)} \end{aligned}$$

Der Organische Rest entspricht hierin der Differenz aus Organischer Masse und der Summe von Rohprotein, Rohfett, Rohfaser und Stärke.

Die Ableitung und Validierung dieser Gleichung basierten auf einem Datensatz von 290 Rationen, mit denen in sechs Versuchsstationen Verdaulichkeitsversuche mit Ferkeln und Mastschweinen durchgeführt worden waren, die die Berechnung der ME aus den verdaulichen Rohnährstoffen gemäß der Gleichung 3 der GfE (2006) als Referenzwert ermöglichten (Bulang und Rodehutscord 2009). Seitdem sind weitere Verdaulichkeitsversuche durchgeführt worden, die den Umfang des Datensatzes auf 524 Rationen anwachsen ließen. Dieser erweiterte Datensatz wurde genutzt, um die Gleichung 1 mit aktuellen Futtermitteldaten zu validieren und zu prüfen, ob neu abgeleitete Gleichungen zu einer Verbesserung der Schätzgenauigkeit führen (Grümpel-Schlüter et al. 2021). Diese Erweiterung des Datensatzes veränderte die Spannweite der Nährstoffkonzentrationen im Futter mit Ausnahme von Stärke und Zucker nicht wesentlich. Die Schätzgüte unterschied sich zwischen einer neu abgeleiteten Gleichung und der Gleichung 1 nur sehr geringfügig. Der AfBN sieht daher keinen Bedarf für eine Änderung der Vorgehensweise und schließt sich der Schlussfolgerung von Grümpel-Schlüter et al. (2021) an, dass die Gleichung 1 zur ME-Schätzung von Mischfuttermitteln für Schweine einschließlich ihres Geltungsbereiches für die Nährstoffgehalte weiterhin Gültigkeit behält.

Ausgewachsene Schweine weisen eine höhere Verdaulichkeit der Faserfraktionen auf als wachsende (Noblet und Le Goff 2001). Es ist daher davon auszugehen, dass Futtermischungen für ausgewachsene Tiere mit der Gleichung 1, die auf Versuchen mit wachsenden Tieren beruht, energetisch unterschätzt werden, insbesondere wenn die Rohfasergehalte im oberen Geltungsbereich für die Formel liegen (80 g/kg Trockenmasse). Allerdings ist eine Berücksichtigung solcher Unterschiede bei der Schätzung der ME noch nicht möglich, da die stärker ausgebildete Fähigkeit ausgewachsener Tiere, Faserfraktionen zu verdauen, auch von der Quelle der Faserfraktionen abhängt.

Zur Charakterisierung der Faserfraktionen erfahren die Neutral-Detergenzien-Faser (NDF) und die Säure-Detergenzien-Faser (ADF) eine zunehmende Verbreitung bei der Analyse von Futtermitteln für Schweine. Bei der ME-Schätzung ist die Berücksichtigung dieser Fraktionen als Alternative zur Rohfaser daher erstrebenswert. Selbst in dem erweiterten Datensatz lagen jedoch nicht genügend Daten vor (Grümpel-Schlüter et al. 2021), so dass die Eignung von NDF und ADF bei der ME-Schätzung nicht geprüft werden konnte. Mit Nachdruck wird empfohlen, zukünftige Verdaulichkeitsversuche mit einer um NDF und ADF erweiterten Analytik zu begleiten, damit zu einem späteren Zeitpunkt auch diese Fraktionen als Schätzvariablen zur Verfügung stehen.

Im Bereich der Wiederkäuer sind *in vitro*-Verfahren zur Schätzung der Nährstoffverdaulichkeit und des energetischen Futterwertes lange etabliert und in der Anwendung (z. B. GfE 2020). Für die energetische Bewertung von Futtermitteln für Schweine ist das Potenzial von *in vitro*-Verfahren bislang unzureichend untersucht. Der AfBN empfiehlt daher, die Entwicklung von *in vitro*-Verfahren zur Schätzung der ME und der Verdaulichkeit von Nährstoffen beim Schwein weiterzuverfolgen. Die Ergebnisse zukünftiger Verdaulichkeitsversuche mit Schweinen sollten als eine Möglichkeit genutzt werden, um *in vitro*-Verfahren abzuleiten und zu validieren.

Literatur

Bulang M., Rodehutscord M. (2009): Development of equations for predicting metabolisable energy concentration in compound feeds for pigs. Arch. Anim. Nutr. 63:442-454

GfE [Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie] (2006): Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen. DLG-Verlag, Frankfurt a. M.

GfE [Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie] (2008): Prediction of metabolisable energy of compound feeds for pigs. Proc. Soc. Nutr. Physiol. 17:199-204

GfE [Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie] (2020): Gleichungen zur Schätzung der Umsetzbaren Energie und der Verdaulichkeit der Organischen Masse von Maisprodukten für Wiederkäuer. Proc. Soc. Nutr. Physiol. 29:171-175

Grümpel-Schlüter A., Berk A., Schäffler M., Spiekers H., Dänicke S. (2021): Evaluation of an equation for predicting metabolisable energy concentration in compound feeds for pigs. Arch. Anim. Nutr. 75:251-262

Noblet J., Le Goff G. (2001): Effect of dietary fibre on the energy value of feeds for pigs. Anim. Feed Sci. Technol. 90:35-52

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