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17. BOKU-Symposium TIERERNÄHRUNG

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Institut für Tierernährung, Tierische Lebensmittel und Ernährungsphysiologie

Interuniv. Department für Agrarbiotechnologie

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Using animal models to understand the mode of actions of carbohydrates and phytochemicals during digestion and absorption

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Abstract

Ileal-cannulated and multicatheterised pig models have been used to study digestion and absorption of the diverse group of compounds making up the carbohydrates and phytochemicals in plant materials. By using these and other models it has been found that the majority of sugars and starch are broken down during passage of the stomach and small intestine. Although no endogenous enzymes are present in stomach and small intestine to degrade dietary fibre (DF), modifications and degradations of DF occur during passage of the small intestine. Nevertheless, when provided in a concentrated form, soluble DF may influence the absorption of glucose. DF are the dietary residues flowing to the large intestine in quantitatively highest amounts. In the large intestine, DF are degraded and metabolised by the commensal microbiota through anaerobe fermentation and converted into short-chain fatty acids (SCFA) and gases. The majority of produced SCFA is absorbed and provides energy to the body. Plant lignans are a group of phytochemicals found in seeds, grains, berries and vegetables and with properties linked to promotions of human health. By the use of pig models, we have studied how the plant lignans are converted to enterolignans in the gastrointestinal tract and how both plant- and enterolignans are taken up to and excreted from the body. Among recent aspects studied are the impact of antibiotics on the formation of enterolignans and the absorption kinetics of plant and enterolignans in enzyme-modified bran. When comparing the acute plasma metabolome of pigs and humans after feeding different breads it was found that 21 out of 26 identified metabolites responded similar in the 2 species, suggesting that the pig in such studies is a suitable model for human nutrition.

Introduction

Carbohydrates constitute a major part of the diets for non-ruminant animals accounting typically for more than two thirds of the dry matter (Bach Knudsen and Lærke, 2018). Dietary carbohydrates make up a diverse group of compounds, which differ considerably in terms of their chemical structure and physicochemical properties. Since the transport processes in the intestinal enterocytes cannot accommodate anything larger than monosaccharides, the dietary carbohydrates have to be broken down to low molecular components to become available as energy for the animals. These processes all occur in the gastrointestinal tract, where endogenous enzymes in the stomach and small intestine of non-ruminant animals degrade sugars and starch to monosaccharides (glucose, fructose and galactose), or the carbohydrates are broken down by the microbiota through anaerobe fermentation and converted into organic acids (lactic acids, LA; short-chain fatty acids, SCFA), gases (CO_2, CH_4, H_2) to support the microbial growth (Bach Knudsen *et al.*, 2013).

While carbohydrates are present at high levels in almost all plant materials, the concentrations of phytochemicals is much lower (Bach Knudsen *et al.*, 2017). An example is plant lignans that are diphenolic compounds present in foods like seeds, grains, vegetables and berries. The main identified plant lignans are matairesinol, pinoresinol, medioresinol, lariciresinol, iso-lariciresinol, syringaresinol, secoisolariciresinol and the glycosylated form of secoisolariciresinol. The commonly held view is that

plant lignans are converted to enterolignans (enterodiol and enterolactone) by the gut microbiota and then absorbed from the large intestine.

We have used animal models for obtaining a more in-depth understanding of how the different types of carbohydrates and plant lignans are digested, fermented, absorbed, metabolised and excreted. The current paper will summarise part of the research on carbohydrates and phytochemicals.

Pig models

The animal models used have been intact pigs using slaughter technique, ileal-cannulated pigs and multicatheterised pigs; the latter with permanent catheters placed in a mesenteric artery and in the portal and hepatic veins (Figure 1) (Bach Knudsen *et al.*, 2006). With the slaughter-technique and ileal-cannulated pigs, it is possible to study the digestion, modifications and metabolism within the gastrointestinal tract whereas the multicatheterised pig model enable studies of the quantitative absorption of water-soluble metabolites (monosaccharides, amino acids, organic acids, lignans) to the portal vein (Bach Knudsen *et al.*, 2006).

(A) (B)

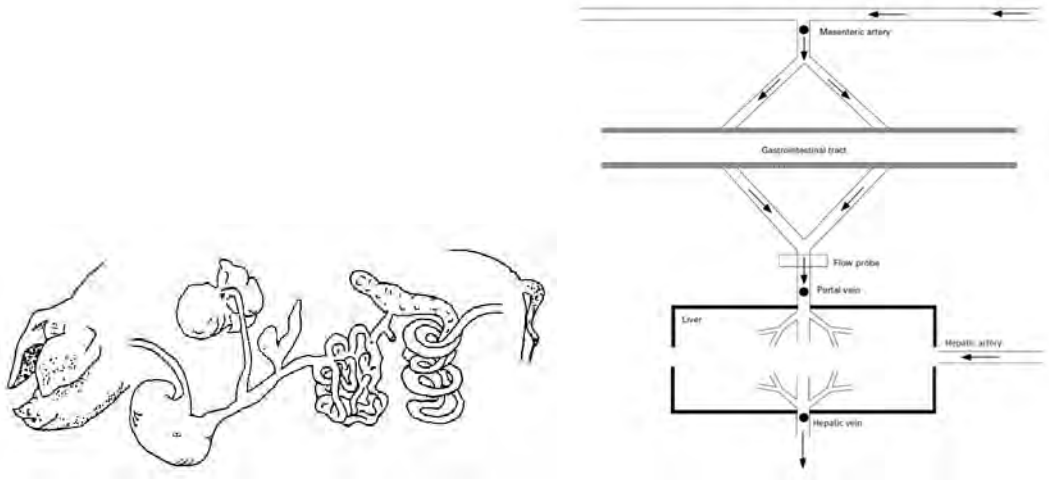

Figure 1. Pig model with a T-shaped cannula placed in the ileum (A) and multicatheterised pig model with permanent catheters placed in mesenteric artery and the portal and hepatic vein and with an ultrasonic flow-probe around the portal vein for monitoring portal flow rate (B).

Studies on carbohydrates

From the studies on carbohydrates it is clear that sugars and starch are almost completely digested during passage of the small intestine. The digestibility values for cereal starches are typically in the order of 96-97 % whereas it is lower for legume starches and more coarsely ground materials (Bach Knudsen *et al.*, 2013). Our studies have also shown that around 23 % of the polysaccharides in the dietary (DF) matrix are degraded during the passage of the small intestine. Nevertheless, DF is the most important dietary factor influencing the digestion and absorption processes at all sites of the gastrointestinal tract as well as the secretion of fluids from the pancreas and liver (Bach Knudsen *et al.*, 2013). Soluble DF may increase luminal viscosity and thereby prolong gastric emptying and potentially interfere with the digestion processes in the small intestine by hindering the contact between the substrate and digestive enzymes and slow down the movements of hydrolytic products of the digestion processes. These effects are observed particularly when the DF level in the diet is high by addition of

isolated DF sources, whereas the effects are less pronounced with diets naturally high in soluble DF (Christensen *et al.*, 2013; Ingerslev *et al.*, 2014).

Soluble as well as insoluble DF will provide substrate for fermentation by the microflora in the large intestine. Soluble DF is most readily fermentable, whereas insoluble DF is fermented more slowly and parts of it will not be fermented at all (Bach Knudsen *et al.*, 2013). The SCFA formed during fermentation reduce pH of the luminal content. However, the majority of the produced SCFA is absorbed and provides energy to the host (Bach Knudsen *et al.*, 2013). The type of fermentable substrate can influence the molar proportions of SCFA; e.g. arabinoxylan and resistant starch have been found to stimulate butyrate producing microorganisms, increase the large intestinal pool size of butyrate and result in a higher net portal flux of butyrate (Ingerslev *et al.*, 2014; Nielsen *et al.*, 2014b).

Studies on phytochemicals

Our studies on phytochemicals have so far primarily been concentrated on plant lignans; a group of non-nutritive phenolic compounds present in plant materials and of interest primarily because of its relation to chronic diseases including coronary heart disease, arteriosclerosis, type-2 diabetes and colonic and breast cancer. In our early work, we studied how the plant lignans were metabolised into enterolignans in the large intestine and how the enterolactone was absorbed and excreted in urine and faeces (Bach Knudsen *et al.*, 2003; Lærke *et al.*, 2009). While the majority of studies in the past have focused on enterolignans because of its use as biomarker in human epidemiological studies, recent studies with multicatheterised pigs have shown that around 7 % of ingested plant lignans were absorbed to the body (Bolvig *et al.*, 2016a). We also found that enzymatic modification of rye bran solubilises a significant proportion of the plant lignans from the plant matrix, which influences the kinetics of absorption of plant lignans but not of enterolignans (Bolvig *et al.*, 2017b). From human studies it is known that the bacteria strains capable of producing enterolactone are subdominant species probably constituting less than 1% of the total microbial community and sensitive to external disturbing factors among them antibiotic consumption. In our setting, intact pigs have been used to study how antibiotics provided under controlled conditions influence the formation and plasma levels of enterolignans. Intermuscular administration of antibiotics resulted in a changed microbial composition at the phyla level, changed metabolite composition of digesta, blood and urine and a blood enterolactone concentration that was 37 percent lower than in unmedicated pigs (Bolvig *et al.*, 2017a). These data support the findings of an epidemiological study where combined data from more than 2200 subjects from the Danish Diet, Cancer and Health cohort with data obtained from the Danish National Prescription Registry on the subjects' use of antibiotics were used. In that study, we found that antibiotics usage up to three months prior to blood sampling was related to a 41 percent lower enterolactone concentration when compared to women who did not use antibiotics (Bolvig *et al.*, 2016b).

Cross specie comparisons

To compare the metabolic response to a meal the acute plasma metabolome was studied in humans and pigs fed four breads varying in DF concentration. The breads were a low DF white wheat bread and three high DF breads provided as whole grain rye bread with whole rye-kernels and 2 white wheat breads supplemented with either wheat arabinoxylan or oat β-glucan (Nielsen *et al.*, 2014a). In this study, it was found that the postprandial response, as reflected in blood metabolomes, was similar in humans and pigs. Twenty-one of 26 identified metabolites that were found to be different between the species were qualitatively similar in response to the test breads, despite different basal metabolome concentrations in the plasma of pigs and humans. Humans had higher contents of metabolites reflecting intake of meats and fats and pigs had higher contents of metabolites reflecting higher doses of bread provided per kg body weight and/or because of their growing status. Acute metabolic differences in these metabolites induced by the breads were, however, comparable between the 2 species.

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Dietary fiber: more than a poor nutrient in swine nutrition

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Abstract

Dietary fiber is not hydrolysable by digestive enzymes released from the organism into the gut lumen and more or less fermentable by the guts microbiome. Dietary fiber is varying widely regarding different botanical origin as well as within plant and cell compartments and physical properties, such as the surface of the particles. Hence a lot of different mode of actions and are described in literature, resulting in different and sometimes counter findings. In summary dietary fibre is complicated and needs a lot of ongoing research work to understand the different mode of actions of the various fiber sources.

Introduction

Dietary fiber represents a group of nutrients originating from plants. Besides lignin they consist of non-starch polysaccharides (NSP) and so-called resistant oligosaccharides, analogous carbohydrates and substances associated with the NSP and lignin complex in plants. One of the most prominent properties of fiber is that it is not hydrolysable by digestive enzymes released from the organism into the gut lumen. Consequently, the so-called resistant starch has to be classified as a dietary fiber, too *(AACC 2001)*. Prebiotic carbohydrates, which are defined as food ingredients that stimulate selectively the growth and activity of bifidobacteria and lactobacilli in the gut and thereby benefit health *(Gibson & Roberfroid 1995, Cummings & Macfarlane 2007)*, belong from a physiological point of view to dietary fiber. Hence, the category of the so called „prebiotics" more or less overlaps with the definition of dietary fiber, with the exception of its selectivity for certain species. Prebiotic carbohydrates may also have additional, less specific benefits because they are fermented in the large intestine *(Cummings & Macfarlane 2007)*.

Dietary fiber is varying widely regarding different botanical origin as well as within plant and cell compartments and physical properties, such as the surface of the particles. Furthermore, their fermentation suitability may additionally affect living conditions of intestinal microbiota and hence the entire digestive processes to a significant extent *(Bach Knudsen 1997; Wenk 2001, Montagne et al. 2003)*. Consequently, it goes without saying that many studies addressing 'fiber' in general may produce different and sometimes counter results *(Montagne et al. 2003).* In this context, a clearer description as shown in table 1 and figure 1 inclusive the physiological properties of fiber is necessary. Table 1 and figure1 show a classification of the different dietary fiber constituents as well as a scheme of carbohydrate fractions.

Last but not least, it should be kept in mind, that most industrial by-products from the flour, the bio-fuel, or the vegetable oil production contain a high amount of dietary fiber. The knowledge about the physiological action of the different fiber sources in combination with the correct information of the available nutrient contents enables the inclusion of high amounts of such feed stuffs in diets for pigs *(Schedle 2016)*.

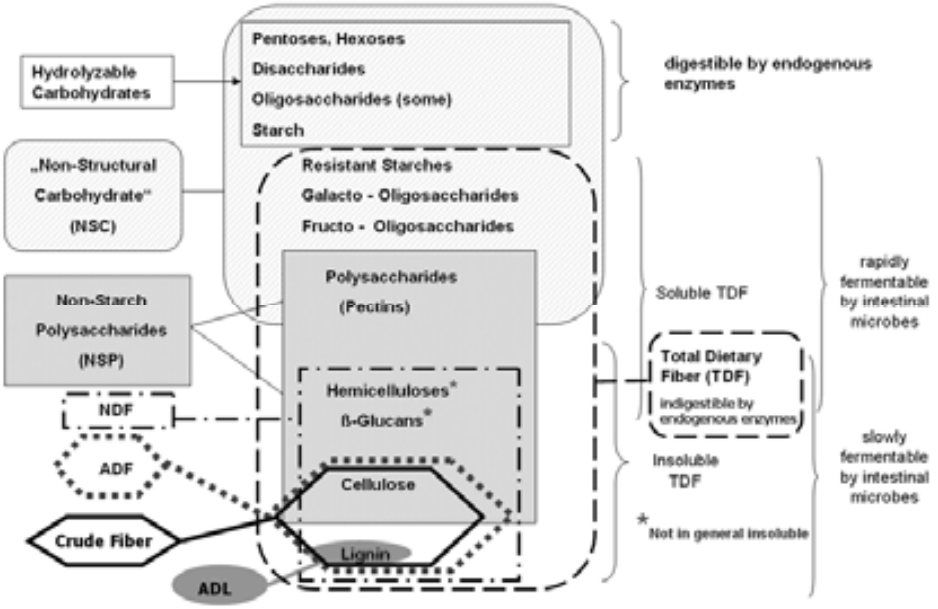

Figure 1: Scheme of carbohydrate fractions, modified according to AOAC (2000)

Mode of Action

Although dietary fiber is not considered as a nutrient *per se*, it shows secondary actions on digestion processes in different parts of the organism *(Bach Knudsen 2001, Wenk 2001, Montagne et al. 2003)*. Action starts with passive parameters like feed intake, and continues with affecting gut microbiology and their metabolites, tissue morphology, immunology, and finally zootechnical performance *(Wenk 2001, Montagne et al. 2003, Schedle et al. 2008)*. In general, dietary metabolisable energy content decreases with higher contents of dietary fiber *(Hador & Wenk 1996)*. Intensive research dealing with animal nutrition and dietary fiber started in the middle of the last century. Nevertheless, recent research has evaluated in depth the effects of dietary fiber on the development of the GIT, nutrient retention and growth performance *(Schedle et al. 2008;2008, Metzler-Zebeli et al. 2010, Schedle et al. 2012, Kraler et al. 2014, 2015, 2016)*. Furthermore, research evaluating the mode of action of dietary fiber necessary and still going on.

It is well known that additional insoluble dietary fiber (in nutritive amounts) tends to increase voluntary feed intake of pigs *(Jørgensen et al. 1996, Schedle et al. 2008)*. Fiber addition raises gut fill and in the case of viscosity increasing fiber gut volume, which in turn is probably associated with an overall increase of viscera organ weight *(Jørgensen et al. 1996, McDonald et al. 2001)*. Consequently, fiber intake may rise total dry matter (DM) consumption capacity and thus indirectly body weight gain. However, study designs showing a balanced metabolisable energy content and an increased fiber content between treatments, did not observe differences in feed intake or viscera organ weight *(Widmer et a.. 2008, Schedle et al. 2010; 2012, Kraler et al. 2015)*. Hence, observed difference in feed intake can be associated with differences in metabolisable energy contents, or variation in digesta viscosity, of diets.

In general, dietary fiber affects digestive processes by different mode of actions *(Wenk 2001, Montagne et al. 2003)*. In this context, soluble and insoluble fiber needs to be distinguished. Within soluble fiber, further classification separates fiber sources increasing viscosity of the intestinal contents (e.g. pectins, sugar beet pulp) or those without effect on physicochemical and rheological properties (e.g. inulin, oligofructose). The insoluble fraction can be divided into an insoluble and slowly fermented (wheat bran, soybean hulls) or in an insoluble and not fermentable one without effects on physicochemical properties with the exception of increasing the faecal bulk volume (maize cobs, linseed hulls) *(Van Nevel et al. 2006)*.

Viscous dietary fiber delays stomach emptying, saturation feeling, and increase transit time. In the small intestine, this type of fiber builds up a "cage effect" upon other dietary particles possibly containing valuable nutrients like proteins or fat, thus impairing access of digestive enzymes and causing an overall decrease of digestibility *(Montagne et al. 2003)*. This kind of dietary fiber is considered to reduce villi length in jejunum and ileum, which is positively correlated with germ counts of potential pathogens *(Drochner et al. 2004)*.

Table 1: Constituents and classification of dietary fiber (according to Bach Knudsen (1997), AACC (2001), Mussato & Mancilha (2007))

Category	Monomeres	Sources
Polysaccharides		
Resistant starches	Glucose	Partly milled grains and seeds, raw potato, banana, heat-treated starch products
Non- starch polysaccharides		
Cellulose	Glucose	Most cereals and legumes
Mixed linked β-glucans	Glucose	Barley, oats, rye
Arabinoxylans	Xylose, arabinose	Rye, wheat, barley
Arabinogalactans	Galactose, arabinose	Cereal coproducts
Xyloglucans	Glucose, xylose	Cereal flours
Rhamnogalacturans	Uronic acids, rhamnose	Hulls of pea
Galactans	Galactose	Soya bean meal, sugar beet pulp
(Pectins)	Uronic acids, rhamnose	Apple, sugar beet pulp
Resistant Oligosaccharides		
α-Galacto-oligosaccharides	Galactose, glucose, fructose	Soya bean meal, peas, rapeseed meal
Fructo-oligosaccharides	Fructose, glucose	Cereals, feed additives, Jerusalem artichokes
Transgalacto-oligosaccharides	Galactose, glucose	Feed additives, milk products
Lignin	Phenolic macromolecule	Cotton seed, cereal bran, pine pollen
Substances associated with the NSP and Lignin Complex in Plants		
Waxes, phytate, cutin, saponins, suberin, tannins	several components (e.g. furan rings, hydroxy fatty acids)	Leaves, cereals

Dietary composition may influence the carbohydrate structures of the mucosal and mucin glycoconjugates with marked consequences for the adherent microflora *(Kelly et al. 1994)*. Supplementation of insoluble dietary fiber may reduce transit time and vulnerability of the intestinal tissue to attack from potential pathogens *(Montagne et al. 2003)*. A possible reason for this action seems

to be a decrease of adherence possibilities for pathogen germs due to the alteration of the glycoproteins in the mucosa *(Drochner et al. 2004)*. In our studies a reduction of pathogen germs, affected by insoluble dietary fiber, was also confirmed by the decrease of pro inflammatory marker genes in the hind gut *(Schedle et al. 2008)*. In contrast to soluble dietary fiber, insoluble fiber may raise intestinal size and hence the release of brush border enzymes as well as adsorption surface *(Pluske et al. 1997)*. In the hindgut, water holding capacity (WHC) will be kept on by insoluble dietary fiber. Hence, mostly insoluble dietary fiber may increase WHC and produce more faucal volume *(Glitso et al. 1998)*.

Dietary fiber addition may decrease apparent digestibility of DM and crude protein *(Kreuzer et al. 1991, Hansen et al. 2006)*. Such an effect is typically characteristic of viscosity increasing soluble fiber in terms of bacterially fermentable substrates promoting bacterial growth in the hindgut. It reflects transformation of soluble N, mainly present as NH_3, into bacterial N and hence the raise in faecal N excretion *(Kreuzer et al. 1991, Hansen et al. 2006)*. Furthermore, such reduction in nutrient digestibility can also appear by insoluble dietary fiber *(Wenk 2001)*.

During the process of digestion in the small intestine, the content of highly digestible components (e.g. protein, fat, sugars, starch) decreases and the content of hardly digestible material such as fiber increases. This material – mixed with endogenous secretion - enters the hindgut and provides 'food' to a numerically great and diverse range of microbes *(Bach Knudsen & Hansen 1991, Kreuzer et al. 1991)*. Although these genera are present in the gut of most monogastric animals, quantity and composition shows somewhat inter-species specificity *(Montagne et al. 2003)*.

Fermentation of fiber is much higher for cell material from non-lignified than from lignified materials *(Bach Knudsen & Hansen 1991, Wenk 2001)*. Consequently, soluble dietary fiber represents the main source of fermentable material in the large intestine, especially at small particle size *(Bach Knudsen & Hansen 1991, Kreuzer et al. 1991)*. The most important products from microbial fermentation are SCFA, H_2O, various gases (CO_2, H_2, CH_4) and bacterial protein *(Montagne et al. 2003)*. Between 95% and 99% of total SCFA produced in the GIT are absorbed before reaching the rectum *(Von Engelhardt et al. 1989, Montagne et al. 2003)*. These SCFA still contain considerable amounts of energy providing up to 15-24% of maintenance requirement to pigs *(Montagne et al. 2003)*. SCFA are an important source of energy to the colonocytes, with butyric acid being the primary source *(Williams et al. 2001)*. Furthermore, butyric acid can play an important role in the immune response *(Furusawa et al. 2013)*. Acetate acts as an energy substrate to muscle tissue and propionate may be transformed to glucose in the liver *(Montagne et al. 2003)*. As carbohydrate sources become depleted due to microbial degradation and utilization, fermentation becomes more and more proteolytic. This may lead to the formation of potentially toxic metabolites like NH_3 or biogenic amines *(Williams et al. 2001)*. Biogenic amines can influence feed intake by provoking hypothalamic reactions along the serotonin pathway *(Eder et al. 2003)*. Special kinds of dietary fiber appear to increase protection against enteric infections with pathogenic germs, and/or infection and subsequent diseases in mongastric mammals *(Montagne et al. 2003).* In the case of insoluble dietary fiber a reduced proliferation of *E.coli* was reported post-weaning *(Bertschinger et al. 1979)*. Hence, dietary fiber seems to exert a protective effect against major problems of intensive pig production like diarrhea or ulcers *(Schedle et al. 2008; 2012)*.

In a nutrient balanced fiber-rich diet, without viscosity increasing effect, no deterioration in performance can be expected for pig *(Schedle et al. 2010, Schedle et al. 2012, Kraler et al. 2015)*. Furthermore, moderate amounts of insoluble dietary fiber can improve performance and health status of pigs *(Montagne et al. 2003, Schedle et al. 2008, González-Alvarado et al. 2010)*. Nevertheless, in future, a high consideration of the physiological properties of dietary fiber e.g. grinding size, water holding capacity, swelling capacity or ion exchange capacity, respectively, should be kept in mind.

From the perspective of sustainability, a high amount of usage of such fiber-rich feedstuffs would be desirable, to reduce the strong competition for food between the humans and the monogastric livestock.

Conclusion

Dietary fibre is complicated and needs a lot of ongoing research work to understand the different mode of actions of the different kinds of dietary fiber.

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Die Nutzung der Faser in der Ernährung der Sau

The importance of fiber in sow nutrition

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Abstract

Fibre can be defined as carbohydrates or lignin in plant materials that are indigestible by endogenous animal enzymes and have physiological effects on animal. Nevertheless, the amplitude of the effects of fibres depends on many factors related to the fibre per se (incorporation rate, fibre source, physicochemical properties), to environmental conditions (feeding and housing systems) and individual factors (parity). The requirement for fibre in the different performance phase (rearing gilts, gestation, farrowing, lactation) of sows are totally different. Gestation feeding systems expect a sufficient satiety, BCS-regulation, energy-dilution. On the other hand, at farrowing the sows need fibre fraction which improves the intestinal peristalsis and the transit/passage time as well as cause to no constipation and a good faecal consistency. In the lactation period the fibre of the sow feed should lead to less volume in the diets, high energy concentration and support intestinal health. Such different requirements for fibre could not be represented by just one analytical fibre parameter like crude fibre. Fibre may be analyzed using different methods and procedures. The crude fibre procedure is very robust and repeatable, but this analyse method have to be expanded to include values for soluble and in-soluble fibre, physicochemical properties like fermentability and viscosity, to meet the feeding requirements of hyperprolific sows.

Einleitung

Faser kann als Kohlenhydrat und Lignin definiert werden, das durch endogene Enzyme nur zum Teil verdaulich ist und spezifische physiologische Auswirkungen beim Schwein bewirken kann. Cellulose ist das in der Natur wohl am reichlichsten vorkommende Kohlenhydrat und es wird wichtig sein, Wege zu finden, Cellulose und andere Faserkomponenten oder Nicht-Stärke-Polysaccharide (NSP) für die monogastrische Tierernährung nutzbar zu machen (KERR und SHURSON, 2013). Gerade in den letzten Jahren steht auf Grund der gestiegenen Rohstoffnachfrage der Biokraftstoff- bzw. Bioethanolindustrie, eine Reihe von Nebenprodukten (z.B. DDGS, Rapskuchen) als Futtermittel aus dieser Branche für die Tierernährung zur Verfügung. Diese „neuen" Futtermittel haben oftmals einen sehr hohen Faseranteil. Schweine können Faser energetisch nutzen, jedoch nur nach mikrobieller Fermentation diese Faser und anschließender Absorption von freien Fettsäuren (FFS) im hinteren Intestinal-Trakt.

Des Weiteren ist auch auf Grund geänderter Gesetzgebungen wie z.B. der Tierschutz-Nutztierhaltungsverordnung, die für tragende Sauen ein Mindestgehalt bzw. eine Mindestaufnahme an Rohfaser von 200 g/Tier und Tag verlangt sowie durch die Veränderung von Haltungsverordnungen für Sauen (Gruppenhaltung), der Fokus wieder verstärkt auf eine geeignete Faserfütterung für Schweine gerichtet.

Einteilung der Faserfraktionen

Eine etablierte und häufig verwendete Bestimmungsmethode zur Analyse der Faser in Futtermitteln ist die Rohfaser (XF oder Rfa). Diese chemisch-gravimetrische Methode ist ein Teil der Weender

Rohnährstoffanalyse. Rohfaser ist als der in verdünnten Säuren und Laugen unlösliche fett- und aschefreie Rückstand definiert und beinhaltet die unlöslichen Anteile der pflanzlichen Zellwandbestandteile, die chemisch sehr heterogene Substanzen (Cellulose, Hemicellulose, Lignocellulose, Lignin und inkrustierende Substanzen wie Cutin und Suberin) umfasst. Alle Substanzen haben gemein, dass sie nicht durch körpereigene Enzyme der Säugetiere, sondern nur von Enzymen der intestinalen Mikroflora abgebaut werden können, weshalb sie auch oftmals als die Gruppe der „Ballaststoffe" bezeichnet werden. Die Rohfaser-Bestimmungsmethode gilt als sehr robuste und gut wiederholbare Prozedur, jedoch gibt es hier keinen direkten Zusammenhang zwischen Rohfaser und der Definition von verdaulicher Faser (dietary fibre) nach AACC (2001), da die unterschiedliche Abbaubarkeit von Cellulose (40-100%) Hemicellulose (15-20%) und Lignin (5-90%) bei der Rohfaserbestimmung nicht berücksichtig wird (MERTENS, 2003). Allerdings dienen die Rohfaserwerte in der praktischen Fütterung immer noch als Richtwerte in den Empfehlungen der Rationsplanung für Schweine. Da in der Fraktion der Rohfaser sehr heterogene Stoffe zusammengefasst werden, die sich chemisch, aber auch bezüglich des Abbaus im monogastrischen Gastro-Intestinal-Trakt unterscheiden, bietet es sich möglicherweise an, die Rohfaser nach ihrer Abbaubarkeit bzw. unterschiedlichen Fraktionen einzuteilen. Hierbei kann man zwischen schwer verdaulicher Rohfaser und leicht verdaulicher bzw. fermentierbarer Rohfaser unterscheiden: Schwer bzw. schlecht verdauliche Rohfaser wird oftmals durch ihren Lignifizierungsgrad (Verholzungsgrad) bestimmt, der wiederum von Anteil und Art der pflanzlichen Gerüstsubstanzen – z.B. lösliche bzw. unlösliche Polysaccharide – abhängig ist. Zur besseren Differenzierung der Kohlenhydrate wurde von VAN SOEST (1963) eine chemisch-gravimetrische Methode entwickelt. Die Summe der Gerüstsubstanzen wird dabei als Rückstand nach dem Kochen in neutraler Detergentienlösung erhalten (neutral detergent fibre - NDF). Der Rückstand nach dem Kochen mit schwefelsaurer Detergentienlösung (acid detergent fibre – ADF) enthält im Wesentlichen Cellulose und Lignin. In diesem Rückstand wird die Cellulose durch Schwefelsäure hydrolysiert und der verbleibende Rückstand als Lignin ausgewiesen (acid detergent lignin – ADL). Über die Differenz zwischen den analytisch erfassten Gehalten an NDF und ADF kann der Gehalt an Hemicellulose errechnet werden. Die Cellulose ergibt sich aus der Differenz von ADF und ADL. In der Abb. 1 (NRC, 2012) werden die verschiedenen in Futtermitteln enthaltenen Kohlenhydratfraktionen aufgrund der Abbaubarkeit durch körpereigene Enzyme bzw. der Geschwindigkeit der mikrobiellen Fermentation klassifiziert.

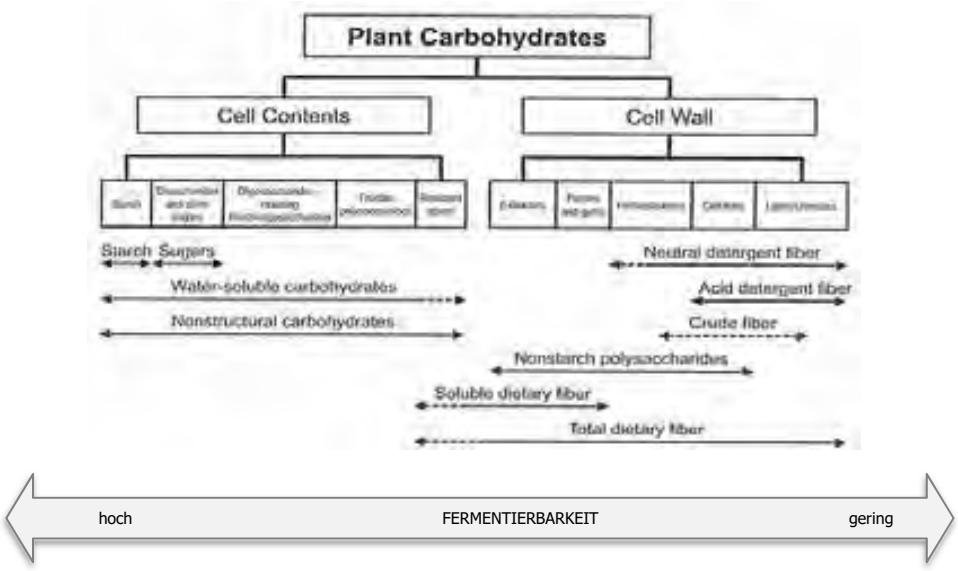

Abbildung 1: Einteilung der Kohlehydratfraktionen (NRC, 2012 – leicht abgeändert)

Effekte der Faser in der Sauenernährung

Bezüglich des Einsatzes von Rohfaser in der Schweinefütterung sollte eine klare Differenzierung nach Art der Faser erfolgen, da die Faserfraktionen je nach Verdaulichkeit, Abbaubarkeit, Fermentierbarkeit oder physikalischer Parameter wie Quellvermögen und Viskosität zu sehr unterschiedlichen Effekten im Verdauungstrakt bei monogastrischen Nutztieren und insbesondere bei den Sauen führen können. Während die so genannte inkrustierte Rohfaser (Haferspelzen, Sonnenblumenschalen, Stroh), die zu der langsam fermentierbaren bzw. unverdaulichen Rohfaser zu rechnen ist, einen mechanischen Reiz im Magen-Darmtrakt setzt und somit die Motorik fördert, steht bei anderen Rohfaserquellen insbesondere das hohe Wasserbindungs- bzw. das Quellungsvermögen sowie auch die Viskosität im Vordergrund (URRIOLA *et al.*, 2013).

Die Effekte der Faserfütterung bei monogastrischen Tieren werden neben der chemischen Faserzusammensetzung von der physikalischen Struktur der Faser beeinflusst. Nachfolgende physikalisch-chemische Eigenschaften der Faserfraktionen können für die Tierernährung/-fütterung relevant sein: Löslichkeit, Wasserhaltekapazität bzw. Wasserbindungskapazität sowie Viskosität und Fermentierbarkeit (HOODA *et al.*, 2011).

Verdauliche Faser lässt sich in lösliche und unlösliche Faser einteilen (WENK, 2001). Die Löslichkeit von verdaulicher Faser bezieht sich nicht nur auf die Fähigkeit sich in Wasser, sondern vielmehr auch in verdünnter Säure, Base, Puffer oder Enzyme-Solution aufzulösen. Der Grad der Löslichkeit von verdaulicher Faser ist größtenteils beeinflusst durch die Bindung zwischen Monosaccharideinheiten wie z.B. 1-3, 1-4 bzw. 1-6 Beta-Verbindungen von Glukoseeinheiten.

Verschiedene Rohfaserquellen wie z.B. Zuckerrübentrockenschnitzel können Wasser unter Bildung sogenannter Hydrokolloide binden. Faser-Fraktionen binden Wasser oftmals durch verschiedene Mechanismen wie ionische Wechselwirkung, Wasserstoffbrückenbindungen sowie Einschluss von Wasser durch die Kapillarwirkung. Dieses Maß für die Fähigkeit von Stoffen (Fasern) Wasser aufzunehmen, kann nach URRIOLA *et al.* (2013) und CHAPLIN (2003) auf verschiedenen Wegen dargestellt werden: Das Wasserhaltevermögen WHC (Water-holding-capacity) oder auch die Wasserbindungskapazität WBC (Water-binding-capacity). Dafür können verschiedene Methoden zur Bestimmung des Wasserhaltevermögens bzw. der -bindungskapazität von Fasern herangezogen werden. Wasserhaltevermögen wird durch die Filtrationsmethode ermittelt und für die Wasserbindungskapazität wird die Zentrifugation-Methode beschrieben (CHAPLIN, 2003). Eine europäische Gemeinschaftsstudie entwickelte eine standardisierte Methode zur Messung der Wasserbindungskapazität (WBC). Diese Methode basiert auf Zentrifugation modifiziert hinsichtlich Probengewicht und Zentrifugationsgeschwindigkeit (ROBERTSON *et al.*, 2000). In einer Studie von BRAACH *et al.* 2017, wurde eine praktikable Methode aufgezeigt mittels Wasserbindungsvermögen verschiedene Faserquellen, die in der praktischen Sauenfütterung von Bedeutung sind, zu charakterisieren.

Die WBC von Faser ist ein geeignetes Maß (hohe positive Korrelation) für die Bestimmung des Quellvermögens. Lösliche Faser hat gewöhnlich höhere WBC gegenüber unlöslicher Faser. Daher haben Cellulose und Lignin eine niedrige WHC und Hemicellulose (Arabinose/Xylose) eine vergleichsweise hohe WHC. So zeigen zum Beispiel eine Reihe von praktischen Untersuchungen mit Rübentrockenschnitzeln (RTS) oder auch Lignocellulose-Produkte, dass diese Futtermittel eine sehr große WHC aufweisen.

Des Weiteren hat das Quellvermögen von Faserfraktionen im Darmtrakt eine Zunahme des Darm- bzw. Ingestavolumens zur Folge und kann so über verstärkte Dehnungsreize auf Magen und Darmwand zu einer forcierten Darmperistaltik führen. Bei Schweinen, die ein Futter mit einem hohen Anteil an bakteriell fermentierbarer Substanz erhalten, zeigte sich in der Literatur ein deutlich langsamerer Anstieg des Glukose- und Insulinspiegels (DE LEEUW *et al.*, 2004). Da der Blutglukosespiegel eine wichtige Rolle bei der chemostatischen Sättigung spielt (hoher Blutglukosespiegel über längere Zeitphase), könnte ein gewisser Anteil an bakteriell fermentierbarer Rohfaser im Schweinefutter eine anhaltende Sättigung erzielen und somit die Sauenherde bzw. auch Ferkel- oder Mastschweinegruppen ruhiger und weniger

aktiv werden lassen, was sich positiv auf die Haltung und Leistung der Tiere auswirken könnte (DE LEEUW *et al.*, 2008).

Unbestritten ist der positive Effekt von Faserfraktionen auf die Passagerate durch den Gastro-Intestinal-Trakt (FREIRE *et al.*, 2000). Dadurch können die Darmträgheit, Verstopfungen und die Gefahr vor Mastitis-Metritis-Agalaktie (MMA) im geburtsnahen Zeitraum bei Sauen wirksam vermindert werden. Eine Studie von FREIRE *et al.* 2000 zeigt, dass sich insbesondere Luzernegrünmehl gegenüber anderen Rohfaserträgern (Weizenkleie, ZR-Trockenschnitzel und Sojabohnenschalen) sehr positiv auf die Passagerate bei Schweinen auswirkt.

Aber nicht nur das Quellungsvermögen der Rohfaser, sondern auch deren bakterielle Verdaulichkeit im hinteren Darmabschnitt ist von entscheidender Bedeutung für einen gesunden Darm und eine ausreichend schnelle Passagrate (FREIRE *et al.*, 2000). Die beim Abbau der leicht verdaulichen XF entstehenden Substanzen sind osmotisch wirksam und bedingen hierdurch – im Unterschied zu langsam fermentierbarer bzw. verholzter Rohfaser – einen weichen und verformbaren Dickdarminhalt und Kot (WARZECHA, 2006). Komponenten, die häufig in der Praxis eingesetzt werden, um diese Wirkung zu erzielen sind unter anderem Obst- bzw. Apfeltrester sowie Zuckerrübentrockenschnitzel. Über einen längeren Fütterungszeitraum betrachtet, kann Pektin und der noch vorhandene Restzucker aus diesen Rohwaren jedoch aufgrund der hohen Wasserbindungskapazität zu einem erhöhten Wassergehalt im Kot beitragen. Infolge dessen sind die Sauen sowie auch oftmals die Buchten und Spaltenböden ggf. mit schmierig-zähem Kot verschmutzter als bei einer pektinarmen Futterration.

Setzt man hingegen inkrustierte Rohfaser wie Stroh oder Sonnenblumenschalen in der Fütterung von Sauen ein, werden härtere Faeces aufgrund der geringeren Fermentierbarkeit der Faser produziert (NELSON *et al.*, 1992). Diese postileal schwer oder kaum fermentierbaren Rohfaserquellen, die eine vornehmlich mechanisch quellende Wirkung im Verdauungstrakt ausüben, können das mikrobielle Milieu nur schwer beeinflussen. Eine Unterstützung des eubiotischen Milieus im Sinne eines gezielten mikrobiellen Abbaus im Intestinaltrakt kann daher durch solche Faserquellen nur schwerlich erfolgen. Die Bedeutung der Rohfaser als Nährsubstrat für Vertreter der Hauptflora ist jedoch von nicht zu unterschätzender Bedeutung, weil die Tiergesundheit durch ein gesundes Milieu im Darm wesentlich beeinflusst werden kann.

Durch eine erhöhte postileale Fermentationsrate werden generell mehr flüchtige Fettsäuren entstehen (vornehmlich Essigsäure, Buttersäure und Propionsäure), die das intestinale Milieu positiv beeinflussen könnten. Eine pH-Wert Absenkung und damit eine kompetitive Verdrängung von Vertretern der Begleit- und Restflora, und eine Unterstützung von Vertretern der Hauptflora im Sinne einer Eubiose ist zu erwarten. Diese Eigenschaft ist besonders hervorzuheben, weil der Darm das größte Immunorgan im Organismus darstellt. Die mikrobiell gebildeten flüchtigen Fettsäuren können bspw. von der Mikroflora energetisch genutzt werden und unterstützen den Erhalt eines intakten Darmmilieus (BACH KNUDSEN, 2001; NOBLET und LE GOFF, 2001). Auch können sie absorbiert und im intermediären Stoffwechselbereich energetisch genutzt werden. Wie aus der wissenschaftlichen Literatur bekannt ist, können flüchtige Fettsäuren einen wesentlichen Beitrag zur Deckung des täglichen Energieerhaltungsbedarfs bei Schweinen leisten (NOBLET und LE GOFF, 2001).

Eine weitere wesentliche Eigenschaft von im Dickdarm bakteriell fermentierbaren Fasern besteht in der N-Fixierung und somit in einer Verringerung der Ammoniakemission (GERDEMANN *et al.*, 1999; KREUZER *et al.*, 1998). Dieses beruht auf einer erhöhten mikrobiellen N-Fixierung mit BFS, wodurch weniger leicht emittierbarer Harnstickstoff entsteht.

Effekte und Funktionen der Faserfraktionen in der Schweineernährung

- Verbesserte Darmperistaltik – unterstützt Darmgesundheit und Immunkompetenz – Wenk *et al.* 2001; Lindberg, 2014
- Steuerung der Passagerate und der Kotkonsistenz und somit verbesserte Kotabsetzung (z.B. Geburtsvorbereitung bei Sauen) – Wilfart *et al.* 2007; Warzecha 2006

- Bildung von bakteriellen Stoffwechselprodukten bei der Fermentation von Faser (z.B. FFS) – HAENEN *et al.*, 2013a/b, Jha *et al.* 2012, Bach Knudsen *et al.* 1993
- Erhöhung Enzymsekretion durch mechanische Stimulierung der Darmmucosa – Bach Knudsen et al. 1991
- N-Fixierung im Dickdarm durch BFS – durch weniger leicht emittierbaren Harnstickstoff – Zervas and Zijlstra, 2002; Bindelle *et al.* 2009)
- Quellvermögen steuert die Futteraufnahme (Sättigungsgefühl) – Sauenherde ist ruhiger (z.B. bei Gruppenhaltung von Sauen) – De LEEUW *et al.*, 2004, 2008; ROBERT *et al.*, 1993
- Gastro-Intestinal-Trakt (GIT) wird durch Quellvermögen der Faser voluminöser =>Futteraufnahmekapazität in der Laktation kann beeinflusst werden – QUENSEL *at al.*, 2009; DANIELSEN und VESTERGAARD, 2001

Tab. 1: Fütterungsrelevante Faserfraktionen in unterschiedlichen faserreichen Futtermitteln (Auswahl)

Rohwaren	Rohfaser g/kg (verd.%)	NDF g/kg	ADF g/kg	ADL g/kg	Hemi-Cellulose g/kg	Cellulose g/kg
Weizenkleie	120 (16)	396	119	34	277	85
ZR-Melasseschnitzel	144 (84)	405	206	19	199	187
Luzernegrünmehl	235 (39)	430	306	78	124	228
Sojabohnenschalen	351 (44)	564	404	21	160	383
Apfel-/Obst-Trester	205 (46)	580	470	17	110	453
Stroh (WW)	382 (-)	721	458	75	263	380
Ligno-Cellulose	600 (-)	820	700	200	120	500

	DF g/kg	BFS g/kg	WICW g/kg	WBC l/kg	WHC l/kg	VIS mPas
Weizenkleie	330	171	385	2,7	2,4	1,9
ZR-Melasseschnitzel	466	460	586	10,7	3,4	4,1
Luzernegrünmehl	160	276	438	4,9	2,7	1,4
Sojabohnenschalen	254	351	618	5,8	2,8	1,8
Apfel-/Obst-Trester	140	268	628	6,1	3,2	2,7
Stroh (WW)	260	--	--	--	--	--
Ligno-Cellulose	120	--	--	9,8	7,8	1,2

Abkürzungen: DF= verdauliche Faser (dietary fibre); BFS = bakteriell fermentierbare Substanzen; WICW = wasser-unlösliche Zellwandbestandteile (water insoluble cell walls); WBC = Wasserbindungskapazität (water binding capacity); WHC = Wasserhaltevermögen (water holding capacity); VIS = Viskosität

Quellen: DLG-Futterwerttabelle Schwein, 1991; NRC-Nutrient Requirement of Swine, 2012; INRA-Tables of composition and nutritional value of feed materials, 2004; Braach *et al.*, 2017; eigene Analysedaten

Schlussbetrachtung / Ausblick

Erhalt und Förderung des Tierwohls und Gesundheit des Gastro-Intestinal-Trakts sind derzeit wichtige Herausforderungen auch in der Tierernährung. In diesem Zusammenhang rückt die Faserfütterung bei Schweinen stark in den Fokus und wird oftmals auch kritisch diskutiert. Bei Betrachtung der Literatur wird sehr schnell deutlich, dass der oft genannte Slogan "Rohfaser ist nicht gleich Rohfaser" hier zutreffend ist. In der Futtermittelbewertung hat Rohfaser auf Grund der etablierten Weender-Analyse sowie der Energieschätzung (GfE-Mischfutterformel) sicherlich noch eine zentrale Bedeutung. Jedoch

ist es notwendig für die praktische Rationsgestaltung in naher Zukunft weitere Parameter wie die Zusammensetzung der Faserkomponenten aber auch physikalisch-chemische Parameter wie z.B. WBC, Quellvermögen, Viskosität oder Fermentierbarkeit heranzuziehen und zu optimieren.

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Dietary fiber in poultry nutrition: dispensable diluent or pivotal for digesta transit regulation?

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Abstract

Although the importance of dietary fibers to contribute to the nutrient supply in chickens is rather limited, fibers may interfere with the digestion of other nutrients through their effects on physicochemical properties of the digesta, thereby influencing nutrient accessibility, bulking properties, microbial activity, gut physiology and function, endogenous secretions, and flow of digesta through the GIT. Recent literature highlights the importance of dietary fibers for the regulation of digesta flow in the upper digestive tract, mainly through a prolonged retention of digesta in the gizzard. Furthermore, fiber may also be important for the regulation of digesta transit in other GIT segments. High fiber diets promote the separation of solid and liquid digesta and the amount of fiber directed to the ceca, seems to be influenced by fiber properties, as particle size and solubility.

Introduction

Nutrient digestion and absorption throughout the gastrointestinal tract (GIT) is a complex, dynamic process dependent on many factors, such as enzymatic hydrolysis, secretion of digestive juices, grinding, mixing and transit of digesta, microbial colonization, and fermentation including the formation of fermentation end-products. Hence, eventual fate of nutrients in the GIT of the animal is the result of many processes influenced by several animal-, environmental-, and diet-related factors.

The true nutritional value of diets results from complex, chemical and physical interactions between dietary components occurring inside the digestive tract, rather than being the simple sum of the assumed feeding values based on of the individual ingredients. Particularly fibers may considerably interfere with the digestion of other nutrients through their effects on physicochemical properties of the digesta, thereby influencing nutrient accessibility, bulking properties, microbial activity, gut physiology and function, endogenous secretions, and flow of digesta through the GIT[1-7]. It is generally believed that fiber degradation in poultry is low and that role of fiber in energy supply to the bird is minor[9], whereas the indirect effects on the nutritional value due to interactions with other nutrients may be considerable. For example, various soluble fiber sources have been found to reduce digestion of protein, starch, and fat in the upper GIT[10-12], whereas insoluble fiber sources as oat hulls, may improve nutrient digestibility[9, 14]. The adverse effects of soluble fibers are mainly explained by their viscous nature and fiber-degrading enzymes (as β- glucanases and xylanases) are generally added to poultry diets to reduce these effects. The beneficial effects of insoluble fibers likely result from improved development of the muscular gizzard and are generally ascribed to the physical structure of the fibrous ingredients. Hence, fibers or coarse particles are often added to the feed to improve gastrointestinal functioning and nutrient use[reviewed by 18, 19].

Digesta transit in chickens: From beak to cloaca AND reverse

The digestive system of chickens differs considerably from that of other monogastric animals. Soluble and insoluble fractions of the digesta are diverged into different sections of the digestive tract by

distinctive intestinal contractions and filtering mechanisms[22, 23]. Reverse peristalsis of the intestine, causes digesta to flow in reverse direction (reflux). In this way, digesta retention time can be adapted to the type of feed[25], presumably to optimize nutrient digestion or gut health.

Three major sites of reverse peristalsis in the digestive tract of birds are identified[23]. First, reflux of contents from the gizzard (the muscular stomach) to the proventriculus (glandular stomach) facilitates grinding and compensates for the bird's lack of teeth. Second, reflux from the small intestine into the gizzard and proventriculus enhances mixing of digesta and exposure to digestive juices[reviewed by 18, 19]. Third, reflux from the cloaca through the colon selectively delivers soluble contents, such as soluble fibers, to the ceca[21, 26-28]. This so-called hindgut reflux plays a crucial role in the degradation of nutrients that cannot be digested by the bird itself and have to be fermented by the microbes residing in the ceca[reviewed by 29, 30]. Also, reflux of urine from the cloaca to the ceca, represents a mechanism for recycling of urinary nitrogen (N) in a way analogous to urea recycling in mammals[31]. Microbes in the ceca can degrade this urinary-N and use the generated ammonia for protein synthesis[a.o.32, 33, 34]. The resulting microbial biomass can potentially become available to the host as N-source[reviewed by 30, 31]. In this way, birds are capable of using non-protein nitrogen as source for amino acid synthesis when dietary protein supply is limited[32, 35], although the contribution of urinary nitrogen to the total nitrogen supply in the bird has never been quantified.

Recent literature highlights the importance of dietary fibers for the regulation of digesta flow in the upper digestive tract[14, 18, 19], but fiber may also be important for the regulation of digesta transit in other GIT segments. The meshwork of ridges and villi at the opening of the ceca, prevents coarse particles from entering and hindgut reflux seems restricted to fluids and small particles (<0.2mm)[22]. Thus, physical properties of the feed, such as particle size, solubility, and viscosity likely influence also hindgut reflux[36, 37]. High fiber diets exacerbate the separation of solid and soluble GIT contents, possibly because of increased reverse flow of gut contents[26]. In addition, the amount of fiber directed to the ceca, seemed to be influenced by fiber content and fiber properties[21].

Analyses of dietary fiber and its degradation

The generic term fiber encompasses a very diverse group of polymers, with varying physicochemical properties. Traditional analytical methods to analyze fiber, as crude fiber (CF) and neutral detergent fiber (NDF), recover only a variable part of the fiber fraction which hampers prediction of the nutritive value of fiber fractions from raw materials in poultry diets[9]. In order to more accurately predict the nutritive effect of fiber from raw materials, a better characterization of fiber fractions, their degradation in the chicken, and their physiological effects are required. For scientific purposes, the enzymatic-chemical (Englyst or Uppsala) methods are more appropriate, whereas for routine analyses the AOAC (2009.01/2011.25) method for total, insoluble, and soluble dietary fiber can be used.

The separation of solid and soluble digesta fractions in the chicken GIT, may complicate digestion studies, particularly, but not exclusively, when fiber degradation is the matter of interest[26]. Unrealistic high ileal and cecal digestibility values for NSP, sometimes exceeding total tract digestibility[8, 16, 26, 38], clearly indicate that that the traditional marker method maybe inadequate to measure fiber degradation along the GIT in chicken. In future research, a dual phase marker system combined with mathematical modelling of digesta flow pathways, quantitative digesta collection, and stable isotope methods can be helpful to keep track of fiber fractions along the GIT[39]. For the measurement of total tract degradation of NSP, separation of digesta fractions does not have to be an issue provided that the collection time is sufficiently long and a representative sample of the excreta is collected. Short collection periods at single time points may promote selective recovery of excreta due to irregular evacuation of the ceca[40, 41], resulting in considerable over- or underrepresentation of cecal contents and erroneous digestibility estimates[reviewed by 9].

Fiber degradation in chickens

Degradation of NSP in the animal depends to a large degree on the original cell wall matrix in which the polysaccharides are embedded[42]). Although it has been suggested that considerable NSP may be degraded in the upper GIT, presumably by microbial degradation in the crop[16], quantitative information on degradation of NSP in the crop is lacking. Considering the limited retention time in the crop under practical feeding regimes[43-47], substantial NSP degradation in the crop seems unlikely. Hence, it generally assumed that fiber is majorly fermented in the ceca[30], where digesta may be retained for up to 24h or longer, with a mean retention time of 7 to 15h (Figure)[24, 48]. As ceca access is restricted to fluids and small particles in chickens, it follows that NSP should be solubilized or finely ground in order to be fermented. However, a substantial fraction of canola meal NSP that were solubilized during transit through the GIT, remained undegraded in broilers[49] indicating that also the time available for fermentation or the lack of appropriate enzyme activities are possible limiting factors in NSP degradation.

Coefficients of apparent total tract digestibility (CATTD) of NSP in chicken, as reported in literature range between 0 and 0.4 and generally reflect differences in solubility of the NSP fractions of the various feed ingredients (Figure 1). Degradation of NSP is particularly low (0-0.1) in diets containing poorly solubilizable NSP from ingredients as maize or some sources of canola meal([15, 17, 20, 21]), whereas higher CATTD of NSP (0.2-0.4) are found for diets that contain more soluble NSP, originating mainly from cereal grains as barley, oats, wheat, and rye. Degradability of NSP from diets containing pea fiber in the study of Jørgensen *et al.* (1994)[3] are lower than expected based on the NSP solubility. Apparently, the soluble NSP of this product, which is produced by water extraction of peas and has a high water-binding capacity, were poorly degraded and likely pea fiber even impeded degradation of barley NSP.

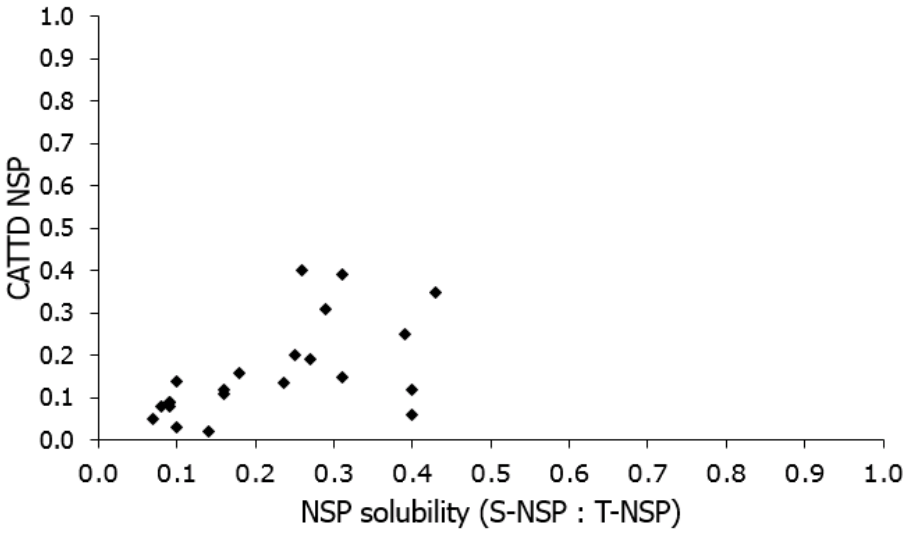

Figure 1: Coefficients of apparent total tract digestibility (CATTD) of non-starch polysaccharides (NSP) in chickens for diets with varying in NSP solubility (soluble to total NSP ratio). Data from: Jamroz *et al.*, 2002[8]; Jørgensen *et al.*, 1996[3]; Meng *et al.* 2006[13]; Meng and Slominski, 2005[15]; Petterson and Åman, 1989[16]; Slominski *et al.*, 1994[17]; and Slominski and Campbell, 1990[20]; de Vries *et al.*, 2014[21]; and de Vries *et al.*, unpublished[24].

The degradation of certain fibers, may also depend on the presence of other fibers in the diet, presumably due to effects on digesta flow and retention time or microbial colonization in the GIT[24, 50]. In common feed practices, NSP-degrading enzymes are usually added to broiler and laying hen diets. The use of such enzymes can facilitate degradation of specific NSP structures, when either appropriate enzyme activities are lacking or when time is limiting their full operation. Although such enzymes are

traditionally added to reduce the viscosity of the digesta matrix for cereal-based diets, also the fermentability of the fiber fraction can be affected. Depending on e.g. the NSP source and the type of enzymes used, CATTD of NSP can be improved over 0.2 units in broilers[13, 21, 51-54], whereas the effect in adult birds can be even greater[13, 20, 55]. Effects of common feed processing technologies on fiber degradation are typically smaller, and generally mainly affect easily solubilizable NSP such as cereal β-glucans[42].

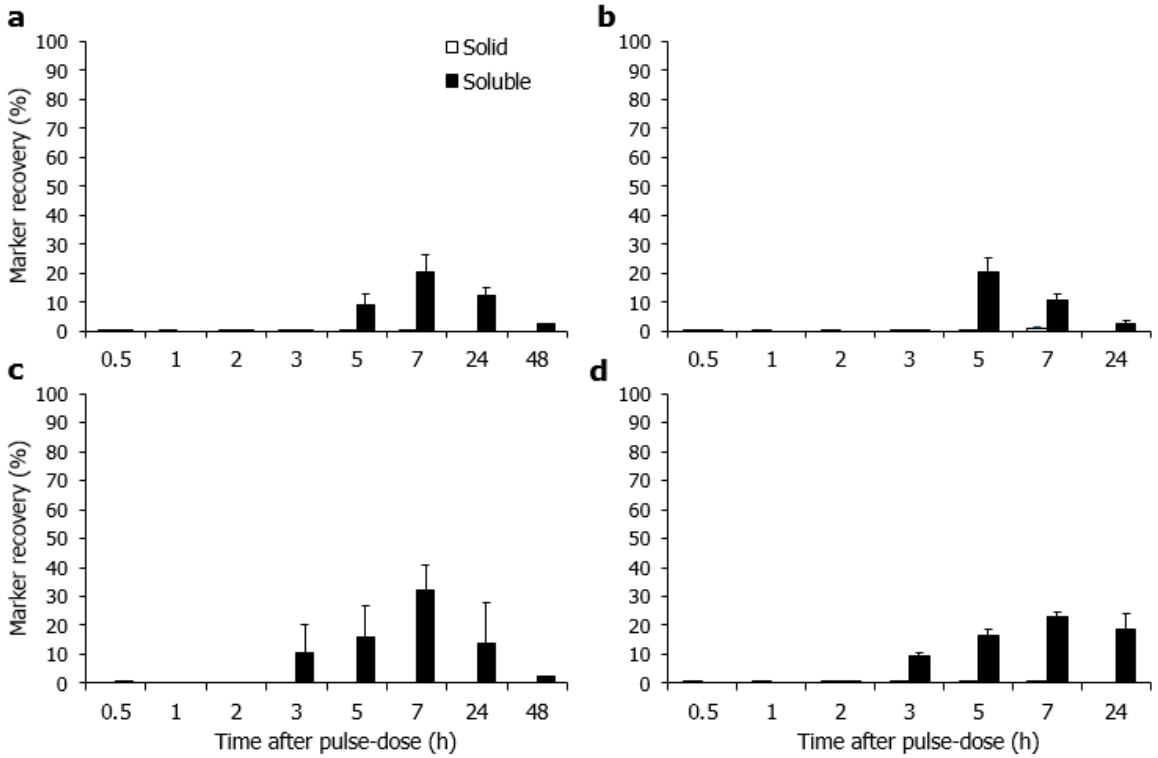

Figure 2: Recovery of chromium and cobalt in ceca of 35d broiler chickens after feeding a pulse dose of solid (Cr_2O_3) and soluble (Co-EDTA) markers. Birds were fed a wheat-soybean meal-maize based control diet (a), or the control diet diluted with 3% sugar beet pulp (b), oat hulls (c), or rice hulls (d). Data are presented as means. Error bars represent SEM.

Conclusion

The generic term fiber encompasses a very diverse group of polymers, with varying physicochemical properties. Not only the quantity of fiber, but also the type of fiber will determine the digestive utilization of the diet and, hence, affect its nutritional value, either positively or negatively. Coefficients of apparent total tract digestibility of NSP in chicken range between 0 and 0.4 and generally reflect differences in solubility of the fiber fraction. Besides, physical entanglement of polysaccharides in the cell wall matrix also time available for fermentation and the absence of appropriate enzyme activities as determined by the microbial colonization in the gastrointestinal tract are possible limiting factors for NSP degradation. Although the importance of dietary fibers to contribute to the nutrient supply in chickens is rather limited when compared with other species[56], recent literature highlights the importance of dietary fibers for the regulation of digesta flow in the upper digestive tract[14, 18, 19], mainly through a prolonged retention of digesta in the gizzard. Furthermore, high fiber diets promote the separation of solid and liquid digesta and the amount of fiber directed to the ceca, seems to be influenced by fiber properties, as particle size and solubility[21, 26].

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Starch digestion in poultry as affected by processing and diet structure interactions

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Abstract

The small intestine is under tremendous stress due to the large quantities of feed that needs to be handled by modern poultry breeds. The feed must be digested and the nutrients must be absorbed during the very short retention time in the small intestine. However, there is increasing evidence for impaired feed efficiency – at least for some birds in a broiler flock – due to suboptimal small intestinal function. This indicates that changes to improve digestive function and/or feeding management is increasingly required, both from a welfare/digestive health perspective and from a feed efficiency point of view. It is now clear that the gizzard plays a pivotal in nutrient digestion and absorption in the small intestine, both through the digestion process that takes place here, and in regulating flow of material into the small intestine. Stimulation of gizzard function through coarse grinding or addition of coarse fibres are therefore warranted. However, intermittent feeding and/or changes from pelleted diets to coarse mash feed may also be required to maximize intestinal function and gut health, at least in broiler chickens.

Introduction

A number of studies have shown that chicks are rapidly adapting to increased digestion when fed at hatch, as indicated by high activity levels of disaccharidase (Mahagna and Nir, 1996) and α-amylase (Sklan and Noy, 2000) two days after hatch. Even when measured on material collected from the ileum of broiler chickens, starch digestibility, using this quantitatively most important nutrient as example, has often been observed to be above 0.95 even for pelleted diets (Svihus, 2001; Hetland *et al.*, 2002; 2003; Svihus *et al.*, 2004; Hetland *et al.*, 2007). This high digestive capacity of poultry is truly impressive, not the least in broiler chickens, where pelleted diets and a high appetite results in material passing through the digestive tract in less than 5 hours (Svihus *et al.*, 2010; Svihus *et al.*, 2002). Extent of gelatinization during pelleting is normally below 20 %, so starch in poultry diets is to a large extent present in the form of complex semi-crystalline native starch granules, which in human- and pig-related experiments have been demonstrated to be slowly and incompletely digested even after several hours digestion time (Shrestha *et al.*, 2012; Willamil *et al.*, 2012; Stein and Bohlke, 2007).

The impressive capacity of broiler chickens to digest even unprocessed starchy ingredients is further illustrated by the fact that even when whole untreated cereals have been used in large quantities, starch digestibility has been observed to be very high. Svihus *et al.* (1997) found ileal starch digestibility to be 0.98 in a mash diet containing 70 % untreated whole barley as the only cereal source, and Svihus and Hetland (2001) found that 50 % of the birds exhibited an ileal starch digestibility above 0.94, even when given a pelleted diet with 38.5 % whole wheat. Even more astonishing were the results by Hetland *et al.* (2002), who observed an ileal starch digestibility of 0.98 in diets with 44 % whole wheat mixed with pelleted other ingredients. The starch digestion process in such cases cannot commence before grinding in the gizzard, and since digestibility was based on analyses of contents collected from the ileal segment between Meckel's diverticulum to the ileo-cecal junction, this means that the whole starch digestion and glucose absorption process has taken place during the short retention time in the duodenum and jejunum, estimated by Rougiere and Carre (2010) to be around one hour.

Despite the above indications of high starch digestion capacity, starch digestibility values below 0.9 (measured either on ileal and/or total tract level) have also been seen in a large number of experiments (Wiseman *et al.*, 2000; Maisonnier *et al.*, 2001; Marron *et al.*, 2001; Svihus, 2001; Svihus and Hetland, 2001; Weurding *et al.*, 2001; Carré *et al.*, 2002; Hetland *et al.*, 2002; Carré *et al.*, 2005; Zimonja and Svihus, 2009). In several of these experiments, the low digestibility for a group of birds has been characterized by a large individual variation and a very low digestibility of some individuals. Thus, some birds seems to fail to digest feed properly. This mini-review will explore this anomaly, under the hypothesis that suboptimal digestibility is a characteristic of at least a portion of birds in a flock of broilers. The focus will mainly be on starch. This review leans heavily on two previous reviews (Svihus 2014a,b).

Proventriculus/gizzard function

It is becoming increasingly clear that functionality of the gizzard is perhaps the most important cause for failure of digestion.

The proventriculus and gizzard are the true stomach compartments of birds, where hydrochloric acid and pepsinogen are secreted by the proventriculus and mixed with contents due to muscular movements in the gizzard. However, the gizzard has an important additional function in grinding feed material, since this is not done in the mouth. Thus, the gizzard contains strongly myolinated muscles and has a koilin layer which due to its sand-paper like surface will aid in the grinding process. Grinding activity and the regulation of this activity in the gizzard has been described in detail by Duke (1992).

It has been shown repeatedly that when structural components such as whole or coarsely ground cereals or fiber materials such as hulls or wood shavings are added, the pH of the gizzard content decreases by a magnitude of 0.2 to 1.2 units (Gabriel *et al.*, 2003; Engberg *et al.*, 2004; Bjerrum *et al.*, 2005; Huang *et al.*, 2006; Gonzales-Alvarado *et al.*, 2008; Jimenez-Moreno *et al.*, 2009; Senkoylu *et al.*, 2009; Sacranie *et al.*, 2012; Svihus *et al.*, 2013). The logical explanation for this is the increased gizzard volume and thus a longer retention time which allows for more hydrochloric acid secretion. Since feed usually has a pH close to neutral, high feed intake can be expected to result in an elevated gizzard pH unless gastric juice secretion is able to increase in accordance with intake. This is probably the main reason why gizzard pH is reported to be higher with pelleted diets when compared to mash diets (Engberg *et al.*, 2002; Huang *et al.*, 2006; Frikha *et al.*, 2009), although less structure due to the grinding effect of pelleting will also contribute to this effect (Engberg *et al.*, 2002; Svihus *et al.*, 2004). As reviewed extensively by Svihus (2011), the increase in size of the gizzard when the diet contains structural components in the form of coarse fibers or cereals improves digestive function both through an increased retention time, a lower pH and a better grinding. This, probably combined with a better synchronisation of feed flow, has been shown to improve nutrient utilization.

Yet unpublished experiments carried out at our lab have demonstrated that birds with a underdeveloped gizzard due to lack of structural components will allow for a very rapid flow of feed material into the small intestine when starved and then refed. As observed before in experiments with turkeys Jackson and Duke, 1995), refeeding of birds which have been starved to empty the digestive tract will result in that the entire length of the small intestine will be filled with contents within an hour. Although the flow is surprisingly fast independent of gizzard function, the amount was observed to be considerably larger for birds without a well-functioning gizzard. Even more conspicuously, the starch content in the ileal material was much higher for these birds. In fact, the starch content in ileal material one hour after refeeding was virtually nondigested feed material, with a starch content of between 30 and 40 % of the dry matter. Although this may indicate a dramatic loss of nutrients in such a situation, assessment of excreta has shown that although the nutrient content is higher than normal in this situation, most of the nutrients have disappeared. Although the microflora, for example through passage into the ceca, may have contributed, it is perhaps unlikely that such large quantities of nutrients can be removed by fermentation within a couple of hours. Thus, digestion in the small intestine remains a more plausible mechanism. Since Kadhim et al (2011) has shown that the ileum has a considerable amylase activity

and Ferrer *et al.* (1994) has demonstrated a considerable absorption capacity of the ileum, one possibility is that digestion and absorption continues through the whole small intestine. Another possibility is that reflux allows for digestion and absorption in more anterior parts of the small intestine.

Form of feed and digestive tract functionality

One important factor is the form of the feed, which to a large extent will determine feed intake. Pelleting of the diet will usually increase feed intake of broiler chickens by 10 to 20 % (Engberg *et al.*, 2002; Svihus *et al.*, 2004), and thus will increase the demands on an already high-performing digestive system. An increase in digestibility when diets were given as mash compared to as pellets was observed by Svihus and Hetland (2001), and indicates that pelleting may cause an overload of the digestive system. Engberg *et al.* (2002) found significantly higher levels of digestive enzymes when diets were given as mash compared to pellets, and also showed that pelleted diets resulted in a much more poorly developed gizzard than when mash diets were given. Thus, since the gizzard probably has an important role as a feed-flow regulator (Svihus, 2011), it is possible that the combined effect of a high feed intake and a low gizzard-stimulating effect increases the risk of a too rapid passage of material through the digestive tract. This fits with conclusions made by Rougiere and Carré (2010), who based on passage studies concluded that retention time in the proventriculus/gizzard was a major limiting factor for digestion in broiler chickens. A high feed intake due to pelleting may therefore have particularly detrimental effects when there are no structural components in the diet and therefore a small and under-developed gizzard. Environmental conditions may be important in this context, since birds will to some extent compensate for lack of structural components in the diet by eating litter materials such as wood shavings if available (Hetland *et al.*, 2005; Hetland and Svihus, 2007). Since pelleted diets are used commercially for broiler chickens, this means that the use of mash diets under experimental conditions may not reflect the commercial reality in terms of digestibility and digestive function. Further, it is possible that limitations to feed intake of broiler chickens would result in improvements in digestive function. One possible way of restricting feed intake would be to restrict access to feed at periods during the day. An advantage of such a feeding regime would be to increase retention time in the crop, which has been shown to have advantageous effects on feed efficiency (Svihus *et al.*, 2010; 2013). However, it is uncertain whether such intermittent feeding would restrict feed intake, since it has been shown that birds rapidly learn to fill up their crop and to use this as a reservoir for up to 5 hours after feeding. Thus, an alternative/additional way to restrict feed intake could be to switch from pelleted diets to mash diets. In addition to savings in processing costs, and additional advantage of such a feeding system would be that it would be easier to include coarse ingredients, since the structure of those would not be destroyed by the pelleting process.

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Chemische Zusammensetzung und Analyse der Gerüstsubstanzen sowie deren Bedeutung in der Ernährung der Wiederkäuer

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Abstract

Plant structural carbohydrates (SC) are very heterogeneous and have a very complex composition, influenced greatly by the plant species and the stage of vegetation. Cellulose, hemicellulose and lignin are the most important components. Cell wall protein, minerals and cuticular components are also present in smaller amounts. Fibre encompasses polymer substances which cannot be split by vertebrates' digestive enzymes.

Cellulose is a polysaccharide made up of thousands of glucose molecules bound together in a β-1–4-glycosidic linkage formed via a dehydration reaction. The β-position of the OH-group at the C_1-atom determines that the polymerized molecules are mainly ordered in linear chains. Parallel chains form fibrillae connected by hydrogen bridges between them. Hemicelluloses are a heterogeneous group of non-cellulosic polysaccharides (pentosans and hexosans). They make up the main portion of the cell wall matrix and are strongly associated with lignin. Pectin, a polymer made up of various acidic polysaccharides (galactouronic acid), represents the main component of the intercellular substance and is found mainly in the middle lamella. Lignins are a mix of polymers from phenylpropanes (cumaryl, coniferyl and sinapyl alcohols), which form a three dimensional linked structure to penetrate the cell wall. In the cell wall, lignin is formed by highly condensed phenylpropane fractions (so-called core lignin). Cross-linking occurs between core lignin and hemicelluloses mainly through the two phenolic monomers p-coumaric and ferulic acid via ester and ether links. Lignin is responsible for the reduced digestibility of cell wall substrate. The cell wall consists of several layers (the middle lamella consisting mostly of pectin, primary cell wall consisting of hemicellulose, secondary cell wall consisting of cellulose). In the primary cell wall lignin polymers are linked with arabinoxylan through ether and ester bondages of the ferulic acid.

The chemical analysis of SC is carried out by using a neutral-detergent solution (Na-lauryl-sulphate, EDTA). It has been shown that SC analysis by the detergent method represents a major improvement, because it allows the separation of fibre and non-fibre carbohydrates. This precise distinction is of great physiological relevance in ruminant nutrition. In contrast, in the crude fibre procedure certain SC constituents (hemicellulose and lignin fractions) are dissolved due to the use of improper solvents, and therefore erroneously added to the nitrogen-free extracts (i.e. non-fibre carbohydrates). Strictly speaking, it is not possible to calculate SC from crude fibre because the extent to which fibre fractions from the various botanical groups of species (grasses, legumes, composite, and umbel plants) are dissolved varies greatly. The Cornell Net Carbohydrate and Protein System (CNCPS) differentiates between fibre and non-fibre carbohydrates based on the detergent method, in which NDF and ADL play a major role. The division of protein into 5 fractions also requires the analysis of NDF and ADF, as well as of the nitrogen that is associated with these fibre constituents. This includes the protein fractions that are degraded in the rumen at medium and slow rates, as well as non-degradable protein. NDF includes the total fibre and can therefore be used as a means to judge the fibre supply and the fulfilment of fibre requirements of the ruminant animal. NDF supply also plays an important role in feed intake regulation, because it affects the rumen fill.

Einleitung

Die Kohlenhydrate sind die primären Syntheseprodukte der pflanzlichen Photosynthese und bilden den mengenmäßig größten Anteil der organischen Substanz auf der Erde. Bei der Photosynthese wird die Strahlungsenergie des Sonnenlichtes absorbiert und in die Form einer chemischen Bindung überführt (in erster Linie Kohlenhydrate). In chemischer Hinsicht wird Wasser unter Freisetzung von elementarem Sauerstoff oxidiert, der abgespaltene Wasserstoff auf Kohlendioxid übertragen und als metastabile C-Verbindung festgelegt. Die für die Trennung von Wasserstoff und Sauerstoff erforderliche und in der chemischen Bindung der Syntheseprodukte enthaltene Energie wird von den Organismen im Stoffwechsel im Wege der Dissimilation genutzt, wobei neben dem Energiegewinn wieder Wasser und Kohlendioxid entstehen (NULTSCH 2001). Durch Polymerisation der Hexosen entstehen die Polysaccharide, die je nach Bindungsform entweder Speicherfunktion (Stärke) haben oder als wichtiger Bestandteil der Gerüstsubstanzen (Zellulose) dienen.

Die Gerüstsubstanzen sind sehr heterogen und komplex zusammengesetzt, wobei die Pflanzenspecies und das Vegetationsstadium der Pflanzen von größtem Einfluss sind. Zellulose, Hemizellulose und Lignin sind die drei wichtigsten Komponenten der Gerüstsubstanzen. In geringerer Menge kommen auch Zellwandprotein, Mineralstoffe und Bestandteile der Cuticula (Kutin, Suberin, Wachse) vor (Van SOEST 1994, NULTSCH 2001). Unter dem Begriff „Faser" werden die polymeren Substanzen verstanden, die von den Verdauungsenzymen der Wirbeltiere nicht gespalten werden können (Van SOEST und ROBERTSON 1980). Neben den Hauptkomponenten Zellulose, Hemizellulose und Lignin sind dies auch Pektin, Gummi und Galaktane.

Auf Grund der heterogenen und komplexen Zusammensetzung der Gerüstsubstanzen gestaltet sich deren Analyse schwierig. Die ersten Faser-Analysen verschiedener Grob- und Kraftfuttermittel werden EINHOF (1806; zit. nach Van SOEST 1976) und GORHAM (1820; zit. nach Van SOEST 1976) zugeschrieben. Die auf HENNEBERG und STOHMANN (1864) zurückgehende Weender Futtermittelanalyse war bis in die jüngste Vergangenheit das Standardverfahren zur näheren Charakterisierung der Futtermittel auf der ganzen Welt. Der in der Weender Analyse gebräuchliche Ausdruck „Rohnährstoffe" (Rohprotein, Rohfett, Rohfaser, Rohasche) und auch die englische Bezeichnung „proximate analysis" weisen darauf hin, dass diese Analysenmethode nicht die Erfassung einzelner Stoffe ermöglicht, sondern nur die von Stoffgruppen. Als wesentlicher Nachteil der Weender Analyse hat sich allerdings die unzureichende und teils chemisch nicht zutreffende Beschreibung der Kohlenhydrate – definiert als Rohfaser und N-freie Extraktstoffe – erwiesen. Bedingt durch die teilweise ungeeigneten Reagenzien (Schwefelsäure und Natronlauge) bei der Bestimmung der Rohfaser werden die Gerüstsubstanzen nicht vollständig erfasst, da die Hemizellulose und auch Teile des Lignins in Lösung gehen und dadurch fälschlicherweise den Nichtfaser-Kohlenhydraten zugeordnet werden. Die AOAC (Association of Official Agricultural Chemists) in den USA hat schon 1940 empfohlen, den Gehalt an N-freien Extraktstoffen nicht anzuführen (siehe Van SOEST und ROBERTSON 1980). Einen entscheidenden Fortschritt brachten die Untersuchungen von Van SOEST (Beltsville, USA), der mit der sog. Detergenzien-Analyse eine im Wesentlichen zutreffende Aufteilung der Futtermittel in Gerüstsubstanzen und Zellinhaltsstoffe erreichte und eine weitere Fraktionierung der Gerüstsubstanzen in Neutral- und Säure-Detergenzien-Faser sowie Lignin (NDF, ADF, ADL) durchführte und somit die Bestimmung der Hauptkomponenten der Zellwandsubstanzen ermöglichte, nämlich Zellulose, Hemizellulose und Lignin (Van SOEST 1963a, 1963b, 1965, Van SOEST und WINE 1967, GOERING und Van SOEST 1970).

In den folgenden Jahren wurde die Detergenzien-Analyse mehrmals modifiziert (Van SOEST *et al.* 1991, MERTENS 2002); die NDF erlangte schließlich eine weltweite Akzeptanz als Analysenparameter zur Definition von Gerüstsubstanzen sowie als Parameter in der Fütterung der Wiederkäuer. Im Cornell Net Carbohydrate and Protein System (CNCPS) erfolgt die Trennung in Faser- und Nichtfaser-Kohlenhydrate auf der Grundlage der Detergenzien-Analyse (SNIFFEN *et al.* 1992). Auch bei der Aufteilung des Proteins in 5 Fraktionen spielen die Gerüstsubstanzen eine wesentliche Rolle, nämlich der N-Gehalt in der NDF und ADF zur Einschätzung des schwer bzw. nicht-verfügbaren Stickstoffs [NDIN, ADIN] (KRISHNAMOORTHY *et al.* 1982, SNIFFEN *et al.* 1992, LICITRA *et al.* 1996). Im Rumen Submodel des

CNCPS wird zur Bestimmung der mikrobiellen Umsetzungen von Pansenmikroben ausgegangen, die entweder Faser-Kohlenhydrate (FC) oder Nichtfaser-Kohlenhydrate (NFC) fermentieren (RUSSELL *et al.* 1992). MERTENS (1994) verwendete die NDF zur Beschreibung der Futteraufnahmekapazität von Milchkühen (12,5 g NDF pro kg Lebendmasse), und zwar für Situationen, in denen die Regulation der Futteraufnahme physikalisch erfolgt. Sehr häufig wird die NDF auch zur Beschreibung der physikalischen Struktur bzw. der Wiederkäuergerechtheit von Rationen herangezogen (MERTENS 1997, DE BRABANDER *et al.* 1999 und 2002, NRC 2001, ZEBELI *et al.* 2008).

Chemischer Aufbau der Gerüstsubstanzen

Komponenten der Gerüstsubstanzen

Grundlagen der Kohlenhydrate

In chemischer Hinsicht sind Kohlenhydrate primäre Oxidationsprodukte von mehrwertigen Alkoholen (KARLSON *et al.* 2005), wobei durch Dehydrierung die sog. Carbonyl-Gruppe (Oxo-Gruppe, C=O) entsteht. Sie ist die funktionelle Gruppe der Kohlenhydrate (NULTSCH 2001, KARLSON *et al.* 2005) und bestimmt im Wesentlichen deren chemische Eigenschaften.

Die mengenmäßig bedeutendsten Zucker bestehen aus 5 bzw. 6 C-Atomen (Pentosen, Hexosen). Von den C5-Zuckern haben die Xylose und die Arabinose als Bausteine des Xylans und Arabans in pflanzlichen Zellwänden praktische Bedeutung (sog. Pentosane). Universelle Bedeutung haben die Pentosen Ribose und Desoxyribose als Bestandteile von Nukleinsäuren (RNA, DNA) als Träger der genetischen Information. Die wichtigsten C6-Zucker sind Glukose, Galaktose, Fruktose und Mannose. Die Glukose ist Bestandteil der Speicherkohlenhydrate Stärke, Amylose und Amylopektin sowie des Gerüstkohlenhydrates Zellulose. Galaktose kommt in Laktose und Agar vor, Fruktose in Saccharose und Inulin (MENKE und HUSS 1987).

Die Monosaccharide Glukose, Galaktose und Mannose unterscheiden sich nicht in ihrer Summenformel ($C_6H_{12}O_6$), wohl aber durch die Position des oxidierten C-Atoms (C_1) und die sterische Anordnung der OH-Gruppen an den C-Atomen C_2, C_3 und C_4. Dadurch ergeben sich unterschiedliche chemische Eigenschaften dieser Zucker. Die Stellung der OH-Gruppe am C_1-Atom wird durch α oder β gekennzeichnet. Die α-Position bedeutet, dass sich die OH-Gruppe von C_1 auf gleicher Ebene wie in C_4 befindet, in der β-Position auf der gegenüberliegenden Seite (NULTSCH 2001, BERG *et al.* 2003). Dieser scheinbar geringe Unterschied ist biologisch von großer Bedeutung (daher Unterscheidung in α- und β-Glukose) und führt zu gänzlich unterschiedlichen Eigenschaften der bei der Polymerisation entstehenden Polysaccharide Stärke (α-glukosidische Bindung) und Zellulose (β-glukosidische Bindung). Neben der Art der Bindung (α bzw. β) beeinflussen innermolekulare Kräfte (durch die sterische Anordnung der Substituenten, nämlich äquatorial bzw. axial), die Bindungen zwischen den Ketten, das Molekulargewicht und das Lösungsmedium das Ausmaß und die Geschwindigkeit, mit der Polysaccharide durch Säuren oder Enzyme hydrolisiert werden können (Van SOEST 1994). In ernährungsphysiologischer Hinsicht ist die Chemie der Kohlenhydrate somit vorwiegend eine Beschreibung des Abbaues der Struktur- und Nichtstruktur-Kohlenhydrate und der Faktoren, die deren Verfügbarkeit für Tiere und Mikroben beeinflussen.

Die Atome sind in Form eines Sessels angeordnet (sog. „Sessel-Schreibweise", engl. chair). Dies bedingt die besondere Eigenschaft der C-Atome, sich gegenseitig bindend Ketten bzw. Ringe zu bilden (NULTSCH 2001). Als Folge können sich große Moleküle bilden, wobei eine überaus große Vielfalt verschiedener Stoffe möglich ist. Auf diesen Eigenschaften des Kohlenstoffs beruht letztlich die Evolution des Lebens auf der Erde. Der Kohlenstoff tritt in allen seinen Verbindungen vierwertig auf, er bindet also 4 einwertige Atome über sog. kovalente Bindungen. Die Bindungen des Kohlenstoffs sind allerdings nicht geradlinig, sondern zickzackförmig (NULTSCH 2001), da sich dessen Valenzen nicht auf einer Ebene befinden, sondern die Form eines Tetraeders mit dem Kohlenstoff als Mittelpunkt bilden. Die

Ursache dafür sind Abstoßungskräfte der (gleich geladenen) Elektronenwolken, um eine möglichst hohe Stabilität zu erreichen (Elektronenpaar-Abstoßungs-Modell nach GILLESPIE und NYHOLM; zit. nach BAARS und CHRISTEN 1995). Auch den Kohlenhydraten liegt diese Anordnung zu Grunde.

Zellulose

Die Zellulose ist ein Polysaccharid, das aus mehreren tausend Glukosemolekülen besteht. Ein Glukosemolekül beansprucht etwa 0,5 nm; dies ergibt eine Moleküllänge von etwa 7,5 µm (NULTSCH 2001). Im Prinzip ist der Grundbaustein von Zellulose das Disaccharid Zellobiose, bestehend aus zwei Glukose-Molekülen, die unter Wasserabspaltung in β-1–4-glukosidischer Bindung mit einander verbunden sind. Die β-Stellung der OH-Gruppe am C_1-Atom bestimmt die Anordnung des zweiten Glukose-Moleküls. Es wird dadurch um 180 Grad um die Längsachse gedreht und das resultierende Molekül ist weitgehend linear (Van SOEST 1994). Die β-Konfiguration ermöglicht der Zellulose die Bildung sehr langer Ketten. Parallel angeordnete Ketten bilden Fibrillen, die untereinander Wasserstoffbrücken ausbilden (BERG *et al.* 2003). Dagegen ergibt sich bei der α-glukosidischen Bindung der Maltose (Grundbaustein der Stärke) zwischen den beiden Glukose-Molekülen ein Winkel. Dies führt zu einer völlig unterschiedlichen Anordnung und damit biologischen Funktion der aus diesen Disacchariden gebildeten Polysaccharide Zellulose (aus Zellobiose) bzw. Stärke (aus Maltose). Durch die β-glukosidische Bindung sind die Glukose-Bausteine in langen, geradlinigen Ketten nach Art eines Faltblattes angeordnet (MENKE und HUSS 1987). Sie eignen sich daher hervorragend für die Bildung von Zellwänden und Gerüstsubstanzen mit hoher Zugfestigkeit. Durch die α-glukosidische Bindung ist das Stärke-Molekül nicht langgestreckt, sondern regelmäßig schraubig in Spiralform gewunden. Es bildet sich eine hohle Helix (BERG *et al.* 2003). Mit dieser Molekülform kann Stärke keine Funktion als Gerüstsubstanz übernehmen. Die Pflanze kann jedoch Glukose dadurch ohne größere Veränderungen in eine unlösliche und somit osmotisch unwirksame Form überführen. Daher ist Stärke der am weitesten verbreitete Reservestoff der Pflanzen (NULTSCH 2001). In der Natur kommt Zellulose nicht isoliert in reiner Form vor (mit Ausnahme der Baumwollhaare), sondern in Verbindung mit Pentosanen, Kutin und Silicium (Van SOEST 1994), wie sie in der Zellwand anzutreffen sind. Diese „Kontaminanten" können nicht von Zellulose getrennt werden, ohne sie zu zerstören.

Die Enzyme zur Verknüpfung bzw. Spaltung sind für die α- bzw. β-glukosidische Bindung spezifisch. Wirbeltiere haben kein eigenes Enzymsystem zur Spaltung der β-glukosidischen Bindung der Gerüstkohlenhydrate, sodass Wiederkäuer auf die Symbiose mit ihren Pansenmikroben angewiesen sind. Die Verdaulichkeit der Zellulose hängt stark von deren Lignifizierung ab. Der Abbau der Zellulose geht in mehreren Schritten vor sich, die durch unterschiedliche Enzyme bewerkstelligt werden. Zuerst greifen Oxidasen die Wasserstoffbrücken an und zerstören damit die Grundstruktur. Die Fasern werden gekürzt und Schichten aufgebrochen, erst danach können Zellobiasen (β-1–4-Glykosidasen) die Polymere spalten. Abschließend wirken Endoglukanasen (COUGHLAN 1991; zit. nach Van SOEST 1994).

Hemizellulose

Die Hemizellulosen sind eine heterogene Gruppe von nichtzellulosischen Polysacchariden (Zellulosane; NULTSCH 2001). Es kommen sowohl Pentosane als auch Hexosane vor, d. h. sie sind Polysaccharide, deren Makromoleküle aus Pentosen (z. B. Xylose, Arabinose) bzw. Hexosen (z. B. Glukose, Mannose, Galaktose) aufgebaut sind. Häufig treten sie als Heteroglykane auf (als Verbindungen verschiedener Zucker), wie z. B. Xyloglukane, Arabinogalaktane, Rhamnogalakturonane und Glukomannan (NULTSCH 2001). Kleinere Moleküleinheiten wiederholen sich und können auch verzweigt sein. Die Hemizellulosen bilden die Hauptmasse der Zellwandmatrix (Grundsubstanz) und erscheinen im elektronenmikroskopischen Bild strukturlos (NULTSCH 2001).

Die Zusammensetzung der Hemizellulosen hängt stark von der Pflanzenspecies ab und auch von den Teilen innerhalb einer Pflanze (Stängel, Blätter). Hemizellulosen sind im nativen Zustand unlöslich, jedoch löslich in Säure oder Lauge. Sie sind mit Lignin assoziiert und bilden gemeinsam das Inkrustierungsmaterial der Sekundärzellwand (Van SOEST 1994). In Grobfutterpflanzen kommt Hemizellulose vorwiegend in den lignifizierten Zellwänden vor. Wird Hemizellulose durch Lösung des

Lignins mit Lauge oder oxidierenden Reagenzien isoliert, wird auch die molekulare Struktur der Hemizellulose verändert bzw. abgebaut. Dadurch bleibt ihre Struktur im Dunkeln (Van SOEST 1994). Kein Polysaccharid ist enger mit Lignin assoziiert als die Hemizellulose (SULLIVAN 1966), und von dieser Lignifizierung hängt auch ihre Verdaulichkeit ab.

Pektine

Die Pektine bilden die Hauptmasse der Interzellularsubstanz, sie kommen besonders in der Mittellamelle vor (Van SOEST 1994, NULTSCH 2001). Sie sind Polymere aus verschiedenen sauren Polysacchariden. Hauptbestandteil ist die Galakturonsäure, deren Carboxyl-Gruppen zum Teil methyliert sind und die mit Rhamnose in α-1–2-Position verbunden ist. Zusätzlich sind Galaktose und Arabinose vorhanden (NULTSCH 2001). Die Unterscheidung zwischen Hemizellulose und Pektinen ist nicht ganz klar, ein wichtiges Kriterium ist die Löslichkeit. Pektine sind in heißen neutralen Lösungen von Ammoniumoxalat oder EDTA löslich, während Hemizellulose Säuren oder Laugen zur Lösung benötigt (Van SOEST 1994). Die Ketten sind untereinander vernetzt, indem jeweils zwei COOH-Gruppen durch zweiwertige Ionen (Ca^{2+}, Mg^{2+}) miteinander verbunden sind. Dadurch entsteht ein elastisches, leicht veränderliches Gerüstwerk, das die Eigenschaften der Pektine ausmacht. Es ist gelartig, sehr plastisch und hydrophil (NULTSCH 2001). Pektine sind im Pflanzenreich bei den Dikotyledonen wesentlich häufiger anzutreffen als bei den Einkeimblättrigen.

Lignine

Nach NULTSCH (2001) sind die Lignine Mischpolymere aus Phenylpropanen (Cumaryl-, Coniferyl- und Sinapyl-Alkohol), die sich zu einem dreidimensionalen Gitter vernetzen und so die Zellwand durchdringen. Diese Bausteine des Lignins gehören als Phenole zu den sekundären Pflanzeninhaltsstoffen. Phenole besitzen am aromatischen Ring mindestens eine OH-Gruppe oder deren funktionelle Derivate (OESTMANN *et al.* 1995).

Allerdings ist die genaue Struktur dieser Polymere bis heute nicht vollständig bekannt, da die oxidative Polymerisation der jeweiligen Phenylpropan-Monomere zu einem Verlust der Identität ihrer Vorläufer führt. Die Polymerisationsprodukte haben eine kondensierte, drei-dimensionale Struktur, hauptsächlich aus Ether- und C–C-Bindungen zwischen den Phenylpropanen. Dies macht Lignin sehr widerstandsfähig gegen eine Hydrolyse (Van SOEST 1994). Dagegen benötigt die chemische Analyse definierbare Rückstände, die extrahiert und abgetrennt werden können. Die meisten dieser Produkte sind allerdings unlöslich und können nicht extrahiert werden, ohne sie zu zerstören; daher ist ihre Identifizierung problematisch (Van SOEST 1994). Durch spektrometrische Verfahren werden die Veränderungen des Lignins vermieden, die bei gravimetrischen Analysenmethoden auftreten. Dabei nutzt man die Eigenschaft der Phenole, Licht bestimmter Wellenlänge zu absorbieren. Dadurch kann die komplexe Struktur der Zellwand eher aufgeklärt werden (OESTMANN *et al.* 1995).

Die heutigen Modellvorstellungen von diesem komplexen Makromolekül mit hohem Molekulargewicht gehen davon aus, dass in der Zellwand Lignin aus hoch-kondensierten Phenylpropan-Einheiten gebildet wird (sog. Kern-Lignin, engl. core lignin). Zwischen diesem Kernlignin und den Gerüstkohlenhydraten (Hemizellulosen, sehr wahrscheinlich jedoch nicht Zellulose) erfolgt eine Quervernetzung (cross linking) hauptsächlich über die beiden phenolischen Monomere p-Cumarsäure und Ferulasäure durch Ester- und Etherbindungen. Diese Monomere sind Zwischenstufen bei der Synthese der Phenylpropane aus Shikimisäure und werden als Nichtkern-Lignin (noncore lignin) bezeichnet (JUNG 1989, JUNG und DEETZ 1993, OESTMANN *et al.* 1995). Die p-Cumarsäure und Ferulasäure besitzen zwei funktionelle Gruppen, eine OH- und eine COOH-Gruppe, mit denen sie gleichzeitig eine Ether- und eine Ester-Bindung eingehen können. Bei der Quervernetzung besteht zu Lignin eine Ether-Bindung über die phenolische Gruppe und über die Carboxyl-Gruppe eine Ester-Bindung mit den Hemizellulosen (JUNG 1989. JUNG und DEETZ (1993) haben dieses Modell erweitert und gehen davon aus, dass zwischen den Lignin/Zimtsäure-Verbindungen auch eine solche mit phenolischen Dimeren bestehen (z. B. Di-Ferulasäure, Truxillsäure). Neben p-Cumarsäure und Ferulasäure gibt es noch eine Reihe weiterer phenolischer Monomere, wie Kaffeesäure, p-Hydroxy-Benzoesäure, Salizylsäure, Sinapinsäure etc.

(JUNG und FAHEY 1983b). Die Begriffe core und noncore lignin gehen auf GORDON (1975) und JUNG (1989) zurück. Einige Autoren folgen dieser Unterteilung des Lignins allerdings nicht (RALPH und HELM 1993, Van SOEST 1993), sondern sprechen von einem Lignin/Hydroxy-Zimtsäure–Komplex, der auf einer kovalenten Bindung zwischen Lignin und Hydroxy-Zimtsäure beruht.

Lignin ist der Hauptfaktor, der die Verfügbarkeit der pflanzlichen Zellwand für Pflanzenfresser und anaerobe Verdauungssysteme begrenzt (Van SOEST 1994). Die Zellinhaltsstoffe (Zucker, Stärke, Protein, Fett) sind von diesem negativen, verdauungshemmenden Einfluss des Lignins nicht betroffen, wie Van SOEST (1967) durch Anwendung des sog. Lucas-Tests gezeigt hat. Lignin selbst ist unverdaulich und die Lignifizierung vermindert die Verfügbarkeit der Zellulose und Hemizellulosen. Aus der Übersichtsarbeit von JUNG und FAHEY (1983a) über den Einfluss von phenolischen Monomeren und Lignin geht klar hervor, dass freie Phenole die Futteraufnahme senken und mehrere Wirbeltier-Enzyme *in vitro* behindert werden. Auch Lignin behindert mikrobielles Wachstum und die enzymatische Verdauung. Die Beziehung zwischen Lignin und Verdaulichkeit der Gerüstsubstanzen ist nicht linear, der negative Einfluss des Lignins ist bei niedrigem Lignin-Gehalt nämlich relativ größer (Van SOEST 1967, JUNG und VOGEL 1986). Diese nicht-lineare Beziehung zwischen Lignin und Verdaulichkeit lässt sich auch aus der Energie-Schätzformel von CONRAD *et al.* (1984) ablesen, in welche Lignin mit einem Exponenten von 0,66 (und nicht 1,0) eingeht. Obwohl Lignin nur mit Hemizellulosen chemische Bindungen eingeht, ist Zellulose im gleichen Ausmaß von der Reduktion der Verdaulichkeit betroffen (JUNG und VOGEL 1986).

Aufbau der Zellwand

Die Zellwand besteht aus mehreren Schichten, nämlich aus der Mittellamelle, der Primär-, Sekundär- und Tertiärwand (WILSON 1993, NULTSCH 2001). Die Mittellamelle bildet die Grenze zwischen benachbarten Zellen und ist der Ausgangspunkt für das Zellwachstum. Sie besteht vorwiegend aus Pektinen. Die Primärzellwand wird angelegt, wenn sich die Zellen teilen; sie besteht vorwiegend aus Hemizellulose und relativ wenig Zellulose. Die Primärzellwand ist elastisch und verformbar, was ein Wachstum der Zellen ermöglicht (NULTSCH 2001). Nach dem Aufbau der Primärwand bildet sich die Sekundärwand in das Innere der Zelle hinein. Der Abschluss zum Plasmalemma erfolgt durch die sehr dünne Tertiärwand. Die Sekundärwand ist überwiegend aus Zellulose aufgebaut, die in sog. Fibrillen angeordnet ist. Die kleinste Einheit stellen die Elementarfibrillen dar, die aus 50 – 100 Zellulosemolekülen aufgebaut sind und einen Durchmesser von etwa 3,5 – 5,0 nm haben. Diese Zelluloseeinheiten werden durch kovalente Bindungen und H_2-Brückenbindungen zusammengehalten (NULTSCH 2001). Mehrere Elementarfibrillen werden zu Mikrofibrillen (10 – 30 nm Durchmesser) zusammengefügt, welche die strukturelle Grundeinheit der Zellwände darstellen. Mehrere Mikrofibrillen werden zu Makrofibrillen gebündelt.

Obwohl die chemische Zusammensetzung von Zellwänden gut bekannt ist, bestehen über deren räumliche Anordnung nur Modellvorstellungen. NULTSCH (2001) führt ein Modell an, das auf ALBERSHEIM und Mitarbeiter zurückgeht. Demnach besteht die Primärwand einer Zelle gewebeartig aus zwei Polymeren, nämlich Zellulose-Mikrofibrillen, welche die Maschen eines Extensinnetzes (Zellwandprotein) durchdringen, eingebettet in ein hydrophiles Pektin-Hemizellulose-Gel, das als Matrix dient. Diese Extensinmoleküle tragen zwar Arabinose als Seitenketten, sie sind jedoch nicht mit Zellulose kovalent verbunden. Daraus kann abgeleitet werden, dass untereinander vernetzte Extensinmoleküle ein selbstständiges Gerüst bilden, das zusätzlich zum Gerüst der Zellulosefibrillen besteht und von diesem durchdrungen ist.

CHESSON (1993) bestätigt die Grundannahmen dieses Modells, führt aber an, dass genauere Analysen der Zellwandpolymere auf einige Unzulänglichkeiten hinweisen. So kann nicht von der im Modell ausgegangenen homogenen Zusammensetzung der Zellwandpolymere ausgegangen werden und dies verändert auch die Feinstruktur einzelner Polymertypen und die Verteilung der Polymere innerhalb der Zellwand. Das gilt besonders für Pektin-Polysaccharide.

JUNG und DEETZ (1993) haben daher ein Modell der Lignifizierung und der Abbaubarkeit von Zellwänden entwickelt, das der Zusammensetzung und den vielfältigen Bindungsarten zwischen den Molekülen eher Rechnung trägt. Die Grundzüge dieses Modells sind in Abbildung 1 dargestellt. Lignin-Polymere sind in der Primärzellwand über Ether-Bindungen der Ferulasäure mit Arabinoxylan verankert. Die Ferulasäure ist dabei mit dem Arabinose-Substitut des Arabinoxylans verestert. Die Primärzellwand enthält mehr verzweigte Lignin-Polymere, die einen hohen Guajakyl-Anteil aufweisen (aus Coniferyl-Alkohol, d. h. 1 Methoxy-Gruppe), während in der Sekundärzellwand eher unverzweigtes, lineares Lignin vorherrscht, das reich an Syringyl ist (aus Sinapyl-Alkohol, d. h. 2 Methoxy-Gruppen). Durch seine zweite Methoxy-Gruppe ist Syringyl nicht in der Lage, im gleichen Ausmaß Bindungen und Verzweigungen einzugehen wie Guajakyl. Infolgedessen ist das Lignin der Sekundärzellwand (mit hohem Syringyl-Anteil) nicht so nachteilig für die Abbaubarkeit der Zellwandkohlenhydrate. Dagegen führt Guajakyl zu mehr Verzweigungen und höherer Kondensation des Lignins mit dem Effekt, dass sich der Anteil und die Verzweigung des Lignins der Primärzellwand und der Mittellamelle erhöhen und durch die räumliche Behinderung des Enzymzutritts eine reduzierte Verdaulichkeit eintritt. Dies stimmt auch gut mit der Beobachtung überein, dass Primärzellwand und Mittellamelle von Pansenmikroben nicht angegriffen werden, wohingegen die Sekundärzellwand zum Teil abgebaut wird, obwohl auch diese lignifiziert ist (ENGELS 1989; zit. nach JUNG und DEETZ 1993). Während der Vegetation ändert sich sowohl die Zusammensetzung der phenolischen Monomere (p-Cumarsäure/Ferulasäure) als auch der Anteil der Phenylpropane (Cumaryl-, Coniferyl- und Sinapyl-Alkohol) im Lignin.

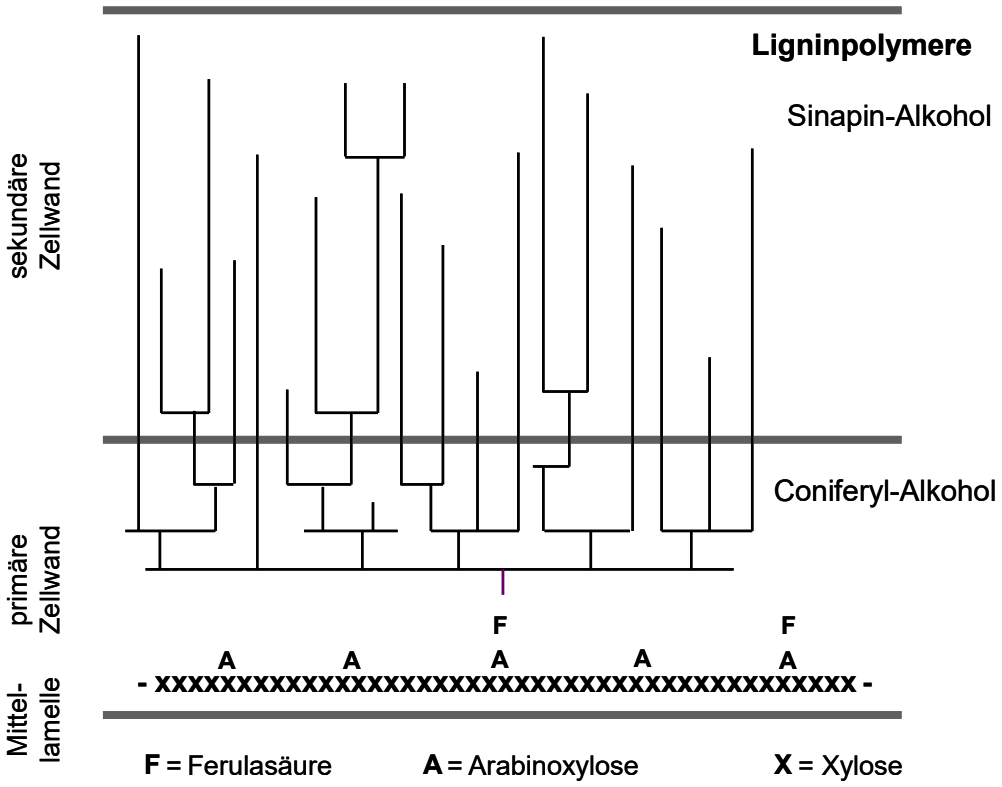

Abbildung 1: Modell der Zellwand-Lignifizierung (nach JUNG und DEETZ 1993)

Van SOEST (1994) bezeichnet die gröbere, räumliche Anordnung der Zellwandkomponenten als den übergeordneten Faktor für die Eigenschaften der Zellwand, wogegen die kovalenten Bindungen zwischen den Zellwandkohlenhydraten diese nicht vollständig erklären können. Er definiert die Zellwand als ein Riesenmolekül mit kovalenten Bindungen, die von β-Glukanen über Xylan und Araban zum Zellwandprotein (Extensin) laufen. Dabei spielen Querverbindungen mit Extensin und den phenolischen

Mono- und Dimeren von Ferula- und p-Cumarsäure sowie Lignin eine wichtige Rolle. Die physiko-chemischen Eigenschaften, welche die Nährstoffabbaubarkeit bestimmen, hängen daher vor allem von der Art der Bindung zwischen den chemischen Komponenten ab. Auch ÅMAN (1993) bezeichnet die komplexe dreidimensionale Struktur der Zellwand als entscheidender für deren Eigenschaften als die einzelnen Komponenten.

Analyse der Gerüstsubstanzen

Die Weender Analyse (HENNEBERG und STOHMANN 1864) dient seit vielen Jahrzehnten zur Charakterisierung der Futtermittel. Wie einleitend angeführt, entspricht die Rohfaser allerdings nicht der tatsächlichen Faser eines Futtermittels, wenn unter dem Begriff „Faser" die polymeren Substanzen verstanden werden, die von den Verdauungsenzymen der Wirbeltiere nicht gespalten werden können (Van SOEST und ROBERTSON 1980). Dies führt in der Folge auch dazu, dass der Gehalt an Nichtfaser-Kohlenhydraten falsch eingeschätzt wird, der üblicherweise durch Differenz errechnet wird. Die Ursache liegt in den bei beiden Verfahren sehr verschiedenen Reagenzien, die zu einer unterschiedlich starken Lösung der Futterinhaltsstoffe führen. Allen Methoden ist gemeinsam, dass in aufeinander folgenden Schritten der chemischen Behandlung die Nicht-Faserbestandteile gelöst werden und die Faser als Rückstand bestimmt wird. Die Vorgehensweise und die verwendeten Reagenzien werden nachfolgend kurz skizziert. Eine Übersicht über die wichtigsten Analysenschritte und die verwendeten Reagenzien gibt Übersicht 1 (nach VDLUFA 1976, Van SOEST *et al.* 1991, Foss Tecator 1997, MERTENS 2002). Um genaue und gut reproduzierbare Werte zu erhalten, sind in der Faseranalytik alle Maßnahmen entscheidend, eine repräsentative Probe zu erzielen (Probenziehung über mehrere Stufen, Probenzerkleinerung, Mahlfeinheit 1 mm-Sieb etc.). Auch die Entfettung (mit Azeton) und das Verhältnis Probe/Reagenzien spielen eine wichtige Rolle und beeinflussen das Ergebnis, wobei ein maximaler Kontakt der Reagenzien mit der Probe gewährleistet sein muss. Ein zu hoher Gehalt an Karbonaten ist ebenfalls störend, da die Wirkung der Säuren herabgesetzt wird (Rohfaser, ADF).

Rohfaser (XF)

Unter Rohfaser ist der aschefreie Anteil eines Futtermittels zu verstehen, der nach Behandlung mit verdünnter Säure und Lauge zurückbleibt. Die Probe wird in zwei Schritten jeweils 30 Minuten mit 1,25 % H_2SO_4 und 1,25 % NaOH (oder KOH) gekocht. Danach wird die Probe mit Aceton entfettet und getrocknet sowie verascht (VDLUFA 1976). Die Entfettung und Veraschung wird auch bei der Detergenzien-Analyse nach Van SOEST angewendet.

Der organische Anteil des Rückstandes gibt den Gehalt an Rohfaser an. Diese sollte ursprünglich die weniger verdaulichen Kohlenhydrate beschreiben, während unter den N-freien Extraktstoffen (NfE) – als Differenz errechnet – die besser verdaulichen Kohlenhydrate verstanden wurden. Zur Zeit von HENNEBERG und STOHMANN (1864) war die Bezeichnung „Holzfaser" üblich. In ihren „Beiträgen zur Begründung einer rationellen Fütterung der Wiederkäuer" empfahlen sie die Verwendung des Begriffes „Rohfaser".

Die Untersuchungen von Van SOEST (1977) haben allerdings gezeigt, dass die Behandlung der Futtermittel mit Säure und Lauge entsprechend der Rohfaser-Bestimmung nicht den vollständigen Gehalt an „Faser" zum Ergebnis hat. Vielmehr werden ein Großteil der Hemizellulosen und auch des Lignins gelöst, auch Teile der Zellulose gehen in Lösung. Das Ausmaß der Lösung von Lignin, Hemizellulosen und Zellulose bei der Rohfaser-Analyse ist allerdings stark von der Zugehörigkeit zu botanischen Gruppen bzw. von der Pflanzenspecies abhängig.

Übersicht 1: Wesentliche Analysenschritte und verwendete Reagenzien bei der Analyse von Rohfaser, NDF, ADF und ADL (nach VDLUFA 1976, Van SOEST *et al.* 1991, Foss Tecator 1997, MERTENS 2002)

	Rohfaser	NDF	ADF	ADL
Proben-menge (g)	1,00 *Gew. Einwaage (W_1)*	1,00 *Gew. Einwaage (W_1)*	1,00 *Gew. Einwaage (W_1)*	1,00 *Gew. Einwaage (W_1)*
Schritt 1	Heißextraktion 1 *100 ml 1,25 % H_2SO_4* *30 min Kochen*	Heißextraktion 1 *100 ml NDS kalt* *60 min Kochen*	Heißextraktion 1 *100 ml ADS kalt* *60 min Kochen*	Heißextraktion 1 *100 ml ADS kalt* *60 min Kochen*
	Filtrieren *Waschen mit heißem H_2O bis frei von Lösungsmitteln*			
Schritt 2	Heißextraktion 2 *100 ml 1,25 % KOH* *30 min Kochen* *Filtrieren* *Waschen mit heißem H_2O bis frei von Lösungsmitteln*			
Schritt 3	Kaltextraktion 1 *25 ml Azeton 10 min* *Filtrieren (3-mal)* *Waschen mit H_2O*	Kaltextraktion 1 *25 ml Azeton 10 min* *Filtrieren (1-mal)* *Waschen mit H_2O*	Kaltextraktion 1 *25 ml Azeton 10 min* *Filtrieren (1-mal)* *Waschen mit H_2O*	Kaltextraktion 1a *25 ml Azeton 10 min* *Filtrieren (1-mal)* *Waschen mit H_2O* *Trocknen*
Schritt 4				Kaltextraktion 1b *25 ml 72 % H_2SO_4* *3 h, Rühren* *Waschen mit H_2O*
Schritt 5	Trocknen *Verdampfen des Lösungsmittels 130 °C, 2 h; Gewicht nach Trocknung (W_2)*			
Schritt 6	Veraschen *525 °C, 3 h; Gewicht nach Veraschung (W_3)*			
Berechnung:	$y = (W_2 - W_3) / W_1 \times 100$			

XF = Rohfaser NDF = Neutral-Detergenzien-Faser ADF = Säure-Detergenzien-Faser
ADL = Säure-Detergenzien-Lignin NDS = neutral detergent solution ADS = acid detergent solution

Im Durchschnitt werden vom Lignin bei Leguminosen 30 %, bei Gräsern 82 % und bei anderen Species (vor allem Korbblütler und Doldenblütler) 52 % gelöst. Von den Hemizellulosen gehen 63, 76 bzw. 64 % in Lösung und von der Zellulose 28, 21 bzw. 22 %.

Die Rohfaser-Analytik ist also nicht in der Lage, die Gerüstsubstanzen (als Summe von Zellulose, Hemizellulosen und Lignin) eines Futtermittels exakt zu erfassen. Die sehr nachteilige Folge davon ist, dass in den N-freien Extraktstoffen nicht nur hochverdauliche Kohlenhydrate enthalten sind, sondern auch schwer bis unverdauliche Kohlenhydrate und das Lignin. Die Folge davon kann sein, dass die Verdaulichkeit der Rohfaser höher ist als die der N-freien Extraktstoffe (Van SOEST 1975). Das heißt, die klare und für die Wiederkäuerernährung sehr wichtige Trennung in Faser- und Nichtfaser-Kohlenhydrate war und ist mit der Rohfaser-Bestimmung nicht möglich.

Detergenzien-Faser

Um den tatsächlichen Gehalt der Pflanzen an Faserstoffen, d. h. an unlöslicher Zellwandmatrix, zu bestimmen, hat VAN SOEST die sog. Detergenzien-Analyse entwickelt (Van SOEST 1963a und b, 1964, 1965, Van SOEST und WINE 1967, GOERING und Van SOEST 1970). Damit ist auch die zutreffende Trennung der Kohlenhydrate in Faserstoffe (Zellwand) und Nichtfaserstoffe (Zellinhaltsstoffe) möglich (Van SOEST 1967). Die Zellinhaltsstoffe (lösliche Kohlenhydrate, Stärke, organische Säuren, Protein) sowie Pektin (Mittellamelle) sind mehr oder weniger vollständig verdaulich (90 - 100 %), während die Zellwände nur über die mikrobielle Fermentation in den Vormägen genutzt werden können. Das Ausmaß der Fermentierbarkeit hängt vom Grad der Lignifizierung ab. Die lignifizierte Fraktion selbst sowie Kutin, Silicium, Tannine etc. sind vollständig unverfügbar (Van SOEST 1994).

Die Detergenzien-Analyse erlaubt auch die Aufteilung der Faser in ihre Hauptkomponenten, nämlich Zellulose, Hemizellulosen und Lignin. Das Haupthindernis, die pflanzlichen Zellwandrückstände aufzubereiten, in denen die unverdaulichen Rückstände enthalten sind, ist die Entfernung des kontaminierenden Proteins. Aus diesem Grund wird bei der Präparation der Rohfaser Natronlauge verwendet. Leider wird dabei aber nicht nur Protein entfernt, sondern auch Hemizellulose und Teile des Lignins. In der von Van SOEST entwickelten Analyse werden Detergenzien angewendet, die in der Lage sind, lösliche Proteinkomplexe zu bilden (Van SOEST 1994), so dass diese auch entfernt werden.

Neutral-Detergenzien-Faser (NDF)

Die Gesamtheit der Gerüstsubstanzen, d. h. der Rückstand nach dem Kochen in neutraler Detergenzien-Lösung (NDS, neutral detergent solution) wird als Neutral-Detergenzien-Faser (NDF) bezeichnet (siehe Übersicht 1). Die Zellinhaltsstoffe werden dadurch gelöst. Die Detergenzien-Lösung besteht aus Na-Lauryl-Sulfat, Ethylen-Diamin-Tetraessigsäure (EDTA) und Triethylen-Glykol sowie den Puffern Na-Borat (Borax) und Na-Dihydrogen-Phosphat zur Einstellung des Milieus (pH = 7). Die genaue Einhaltung eines neutralen pH-Wertes ist entscheidend, da saure oder basische Bedingungen die Faser lösen können. Nach MERTENS (2002) ist hierbei ein pH-Bereich von 6,95 bis 7,05 einzuhalten.

Die Extraktion des Futters mit der neutralen Detergenzien-Lösung ergibt also einen Faserrückstand mit den wesentlichen Komponenten der Zellwand, nämlich Zellulose, Hemizellulose und Lignin. Außerdem sind als Nebenkomponenten noch geringe Mengen an Protein und gebundenem Stickstoff enthalten sowie Mineralien (Erdverunreinigungen) und die Cuticula. Die Extraktion mit der neutralen Detergenzien-Lösung wirkt nicht hydrolytisch und führt daher zur Gewinnung der unlöslichen Zellwandmatrix (Van SOEST 1994). Üblicherweise enthält der NDF-Rückstand noch Reste von Stärke, tierischem Keratin und Mineralstoffen.

Ursprünglich wurde die NDF-Methode zur Bestimmung der Gerüstsubstanzen in Grobfuttermitteln konzipiert. Die höheren Stärke-Gehalte in Kraftfuttermitteln stören die NDF-Analyse bzw. führen zu überhöhten NDF-Werten. Daher ist bei hohen Stärkegehalten die Entfernung der Stärke mit einer hitzestabilen Amylase während des Kochprozesses zusätzlich zu Triethylen-Glykol zwingend erforderlich (Van SOEST *et al.* 1991). Sowohl die Art der verwendeten Amylase als auch ihre Anwendung während der Analyse beeinflussen den NDF-Wert (MERTENS 2002). Die Behandlung der Probe vor der Extraktion birgt die Gefahr, dass durch gewisse Nebenaktivitäten (Protease, Hemizellulase, β-Glukanase) zu niedrige NDF-Werte erzielt werden, da Teile der Faser angegriffen und gelöst werden. Daher hat sich die Verwendung einer hitzestabilen Amylase während des Extraktionsprozesses als die beste Lösung herausgestellt (MERTENS 2002). Die Verwendung von Amylase bei der Analyse der Gerüstsubstanzen wird mit dem Buchstaben „a" angegeben, d. h aNDF (UDEN *et al.* 2005).

Pektine werden durch Kochen mit neutralen Detergenzien vollständig gelöst, obwohl sie Bestandteil der Zellwand sind. Dies wird häufig als Schwachpunkt dieses Analyseverfahrens angesehen. Die Lösung des Pektins ist auf die Wirkung des EDTA zurückzuführen. Laut Van SOEST *et al.* (1991) nehmen sie allerdings insofern eine Sonderstellung ein, als sie von Pansenmikroben rasch und vollständig abgebaut

werden. Dies zeigt, dass sie – im Gegensatz zu Hemizellulose – nicht kovalent mit der lignifizierten Zellwandmatrix verbunden sind. Sie werden daher als Neutral-Detergenz-lösliche Faser (NDSF, neutral detergent-soluble fiber) bezeichnet (HALL 2003).

Ein zu hoher Proteingehalt (> 30 % XP) kann die Analyse der Gerüstsubstanzen stören, indem die Kapazität des Detergenz überschritten wird, lösliche Komplexe zu bilden (Van SOEST und ROBERTSON 1980). In solchen Fällen soll der Proteingehalt durch Proteasen verringert werden. Ein gewisser Teil des Proteins kann nicht durch Proteasen angegriffen werden, nämlich tatsächlich unverdaulicher N, der sich in ADF und ADL befindet. Er stammt teils originär aus physiologisch reifen Pflanzen oder er ist auf Grund von Maillard-Reaktionen, Hitzeschädigungen sowie der Bildung von Tannin-Protein–Komplexen unverdaulich. Eine zu hohe Erhitzung (> 60 °C) bei der Probentrocknung führt daher zu Fehleinschätzungen bei der Beurteilung von ADIN (acid detergent insoluble nitrogen, siehe Abb. 3).

Die NDF-Methode hat seit ihrer Begründung mehrere Modifikationen erfahren. Diese waren erforderlich, da einige Reagenzien aus gesundheitlichen Gründen nicht mehr zulässig waren (Decalin eliminiert, Triethylen-Glykol anstelle von 2-Ethoxy-Ethanol) und da die ursprünglich für Grobfutter entwickelte Methode auch auf Kraftfutter ausgeweitet wurde. Zur Entfernung der im Kraftfutter enthaltenen, teilweise hohen Stärkegehalte ist die Behandlung mit Amylase zwingend erforderlich. Die wesentlichen Modifikationen sind bei Van SOEST *et al.* (1991) und MERTENS (2002) beschrieben. Die Terminologie zur Definition der verwendeten NDF-Methode wurde von UDEN *et al.* (2005) veröffentlicht.

Säure-Detergenzien-Faser (ADF)

Hemizellulosen sind in neutraler Detergenzien-Lösung (bei pH = 7) vollkommen unlöslich, gehen jedoch sowohl bei saurem als auch basischem pH-Wert leicht in Lösung (Van SOEST und ROBERTSON 1977, Van SOEST 1994). Durch Behandlung der Probe mit dem kationischen Detergenz Cetyl-Trimethyl-Ammonium-Bromid (CTAB) in 1 N Schwefelsäure (Säure-Detergenzien-Lösung, acid detergent solution = ADS, siehe Übersicht 1) wird Hemizellulose und ein Großteil des Proteins gelöst. CTAB ist eine quartäre Ammonium-Verbindung mit einer langkettigen Alkylgruppe (C12) und dient als Komplexbildner. Der Rückstand wird als Säure-Detergenzien-Faser (ADF) bezeichnet. Das im Rückstand noch befindliche Protein (ADIN) ist als für die Pansenmikroben nicht verfügbar anzusehen (KRISHNAMOORTHY *et al.* 1982). Der Gehalt an Hemizellulose kann durch die Differenz NDF minus ADF rechnerisch ermittelt werden.

Die ADF enthält also nicht mehr die gesamte Faser, sondern dient dazu, diese in Ligno-Zellulose und Hemizellulose aufzuteilen. Die säurelösliche Fraktion enthält vorwiegend Hemizellulose und Zellwandprotein, während der Rückstand Zellulose, Lignin und die am wenigsten verdaulichen Nicht-Kohlenhydrate enthält (Van SOEST 1994). Bei der ADF-Präparation werden viele Substanzen entfernt, welche die Analyse der Zellwandkomponenten stören. Daher ist der ADF-Rückstand sehr nützlich für die sequenzielle Bestimmung von Lignin, Kutin, Zellulose, unverdaulichem Stickstoff sowie Silicium.

Häufig wurde ADF anstelle von Rohfaser analysiert und auch in Regressionsgleichungen zur Vorhersage der Verdaulichkeit herangezogen. Da jedoch die ADF nur einen Teil der Faser darstellt, sind die Beziehungen zwischen ADF und Verdaulichkeit nach Van SOEST (1994) primär statistischer Natur und beruhen nicht auf den biologischen Zusammenhängen zwischen den Parametern. Die Verdaulichkeit und die Zusammensetzung der Faser wird vielmehr durch die Umweltbedingungen während des Pflanzenwachstums (Temperatur, Licht, Breitengrad, Wasser, Düngung, Boden) bestimmt (Van SOEST *et al.* 1978, Van SOEST 1994 und 1996).

Säure-Detergenzien-Lignin (ADL)

Die Lignifizierung der pflanzlichen Zellwand entscheidet über die Verdaulichkeit des Futters (JUNG und DEETZ 1993). Daher ist dessen exakte Analyse eine grundsätzliche Voraussetzung zur verlässlichen Bestimmung des Futterwertes. GIGER (1985) unterscheidet prinzipiell drei Möglichkeiten, nach denen Lignin analysiert werden kann:

1. Direkte gravimetrische Methoden durch Lösung der Zellulose (ausgehend von der ursprünglichen Klason-Methode)
2. Indirekte gravimetrische Methoden aus der Differenz nach Entfernung des Lignins
3. Messung der Absorption in den Lignin-Oxidationsprodukten

Im Folgenden wird auf die gravimetrischen Methoden eingegangen.

Die Klason-Methode besteht im Prinzip aus einer Behandlung mit Lösungsmitteln und einer daran anschließenden Lösung mit 72 % Schwefelsäure (THEANDER und WESTERLUND 1986, Van SOEST 1994). Lignin ist der unlösliche Rückstand. Die Klason-Methode wurde ursprünglich für die Holzanalyse entwickelt. Bei der Anwendung dieser Methode in der Analyse von Grobfuttermitteln besteht das Problem, dass Protein bei diesem Verfahren nicht eliminiert wird und daher im Lignin-Wert enthalten ist. (Der N-Gehalt von Holz ist sehr gering und zu vernachlässigen). Klason-Lignin enthält des Weiteren mehrere Nicht-Lignin-Komponenten wie Phenolaldehyd-Polymere, Tannin und Tannin-Protein-Komplexe, Maillard-Polymere bei Hitzeschädigung sowie Kutin (Van SOEST 1994). Klason-Lignin-Werte sind bei Gräsern üblicherweise 2 bis 4 mal höher als ADL-Werte und nur 30 % höher in Leguminosen (JUNG *et al.* 1997). Dies spricht für unterschiedliche Ligninstrukturen in verschiedenen botanischen Gruppen. HATFIELD *et al.* (1994) vertreten die Ansicht, dass das Klason-Lignin dem tatsächlichen Ligningehalt besser entspricht. Andere Autoren führen an, dass beim ADF-Schritt der ADL-Methode säurelösliche Komponenten des Lignins verloren gehen können (KONDO *et al.* 1987, LOWRY *et al.* 1994). JUNG *et al.* (1997) haben Klason-Lignin und ADL an einem bezüglich Species und Gehalt an Gerüstsubstanzen breit gestreuten Datenmaterial hinsichtlich deren Beziehung zur Verdaulichkeit der TM und NDF verglichen und festgestellt, dass beide Analysenmethoden annähernd gleichwertig in der Vorhersage der Verdaulichkeit sind, obwohl die Ligninwerte sich deutlich unterschieden. Die Differenz ADF minus ADL gibt den Gehalt an Zellulose an.

Beziehungen zwischen Rohfaser und Detergenzien-Faser

In Abbildung 2 sind die Beziehungen zwischen Rohfaser und NDF, ADF bzw. ADL für die drei wesentlichen Futtermittelgruppen Wiesenfutter, Silomais und Kraftfutter aus systematisch angelegten Versuchen dargestellt. Das Wiesenfutter entstammt einem Grünland-Nutzungsversuch mit drei Standorten, drei Nutzungshäufigkeiten und drei Düngungsniveaus im alpinen Grünlandgebiet der Steiermark in Österreich, deckt also einen sehr weiten Bereich an Gerüstsubstanzen ab (GRUBER *et al.* 2006a). Der Silomais kommt aus Versuchen zum Einfluss von Vegetationsstadium, Sorte, Standort und Konservierung auf den Futterwert von Silomais mit einer weiten Spannbreite an Qualität der Restpflanze und an Kolbenanteil (GRUBER *et al.* 2006b). Beim Kraftfutter handelt es sich um die in der praktischen Fütterung wichtigsten Energie- und Proteinträger (Getreide, Nebenprodukte der Müllerei, Stärke- und Zuckerherstellung sowie der Gärungsindustrie, Wurzeln und Knollen, Leguminosen-Samen und Ölsaaten, Extraktionsschrote und Ölkuchen bzw. Expeller). Dadurch ergibt sich ebenfalls ein weiter Bereich an Gerüstsubstanzen und deren Zusammensetzung (GRUBER *et al.* 2005). Dieses Datenmaterial deckt einen Bereich an Rohfaser von 2 bis 37 % ab, an NDF von 6 bis 74 %, an ADF von 3 bis 47 % und an ADL von 1 bis 15 % der TM. Die Beziehung Rohfaser vs. NDF zeigt, dass alle Futtermittelgruppen mehr oder weniger auf einer Linie liegen, mit ähnlichen Intercepts und Regressionskoeffizienten für die Kategorien Wiesenfutter, Silomais und Kraftfutter. Allerdings stammen die beiden Grobfutterkategorien Wiesenfutter und Silomais aus einer genau definierten Herkunft; dadurch ist die botanische Zusammensetzung des Wiesenfutters sehr ähnlich. Zahlreiche Untersuchungen von Van SOEST (1964, 1994) haben gezeigt, dass das Verhältnis Rohfaser zu Gerüstsubstanzen stark von der botanischen Species abhängt. Gräser beinhalten bei vergleichbarem Gehalt an Zellulose wesentlich mehr Hemizellulose und damit auch NDF. Daraus ergibt sich bei Gräsern ein deutlich anderes Verhältnis von Rohfaser zu NDF als bei Leguminosen. Daher ist gerade bei vielfältig zusammengesetzten Wiesen aus dem Dauergrünland eine „Umrechnung" von Rohfaser auf NDF nicht zulässig. Dazu kommt noch ein bei ähnlicher Verdaulichkeit höherer Grad an Lignifizierung in Leguminosen.

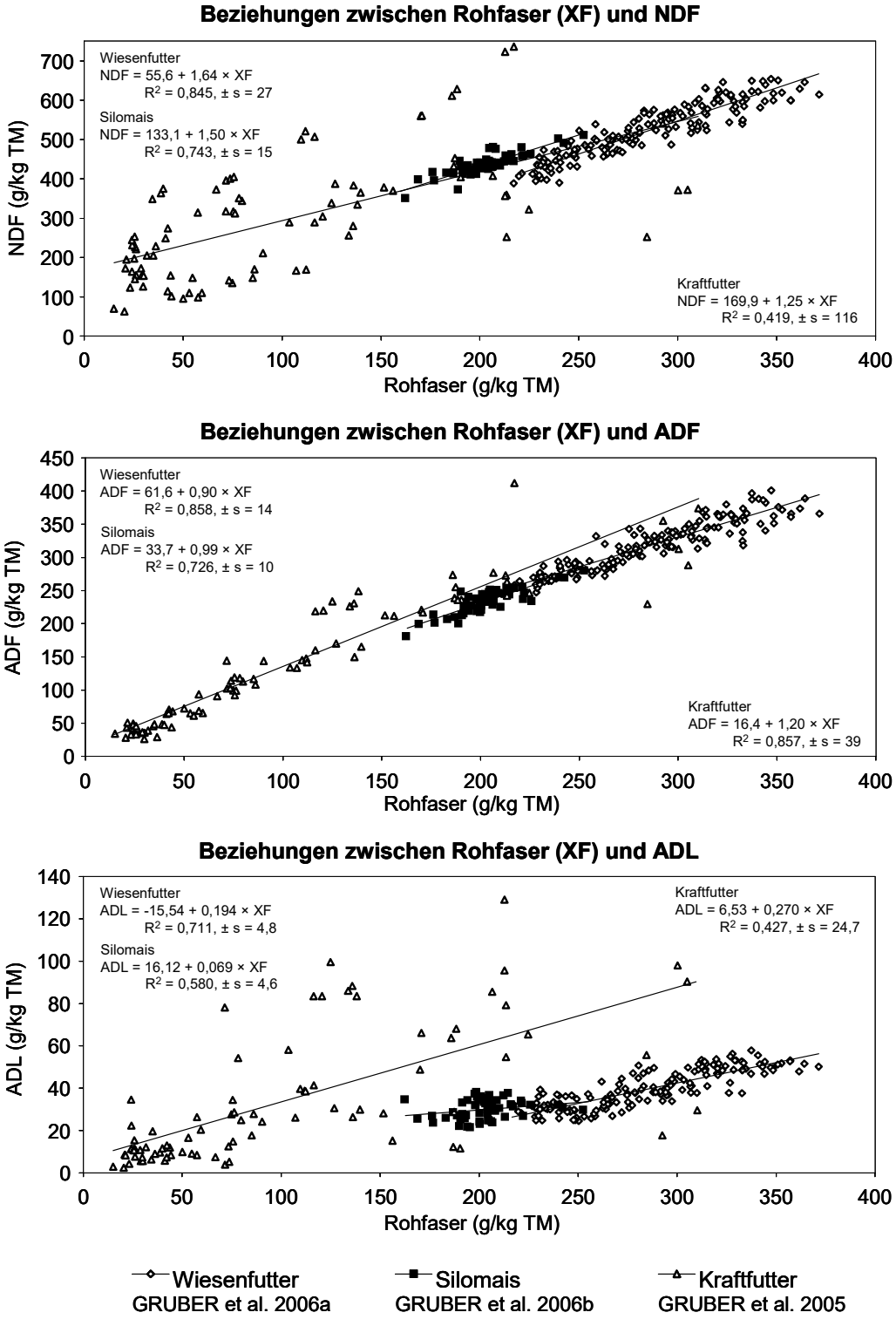

Abbildung 2: Beziehungen zwischen Rohfaser und NDF, ADF bzw. ADL
(nach GRUBER *et al.* 2005, 2006a, 2006b)

Es ist noch hervorzuheben, dass die Beziehung Rohfaser vs. NDF bei den Kraftfuttermitteln wesentlich schwächer ist. Dies ist darauf zurückzuführen, dass diese Gruppe botanisch sehr heterogen zusammengesetzt ist und die Schalen je nach botanischer Herkunft und technologischer Verarbeitung (z. B. Ausmahlungsgrad) sehr unterschiedlich aufgebaut sind. Ähnlich ist auch die Beziehung Rohfaser vs. ADF zu sehen. Besonders Wiesenfutter und Silomais liegen nahezu auf einer Linie. Und bei Kraftfutter ist R^2 höher als bei der Beziehung Rohfaser vs. NDF. Dies deutet darauf hin, dass sich die Zusammensetzung der Gerüstsubstanzen in Kraftfuttermitteln vor allem im Gehalt an Hemizellulose unterscheidet.

Auch in der Beziehung Rohfaser vs. ADL heben sich die Grobfuttermittel Wiesenfutter und Silomais vom Kraftfutter ab. Beim Grobfutter ist die Korrelation wesentlich höher, während bei Kraftfutter vor allem im Bereich hoher Rohfaser-Gehalte, d. h. bei Nebenprodukten, sich relativ zum Rohfaser-Gehalt hohe ADL-Werte zeigen. Daraus ist zu schließen, dass durch die Verarbeitung die Zusammensetzung der Gerüstsubstanzen selektiv verändert wird.

Die Gründe für die uneinheitlichen und unterschiedlichen Beziehungen zwischen Rohfaser und den Gerüstsubstanzen liegen im unterschiedlichen Ausmaß, mit dem Zellulose, Hemizellulose und Lignin in den botanischen Artengruppen Gräser, Leguminosen sowie Korbblütler und Doldenblütler bei der Bestimmung der Rohfaser (d. h. durch Säure und Lauge) gelöst werden. Daher führt Van SOEST (1976) an, dass „innerhalb einer botanischen Art gewisse Beziehungen zwischen Rohfaser und der tatsächlichen Faserkonzentration bestehen. Dies führe aber zu Chaos, wenn agronomische Grenzen überschritten werden".

Bedeutung der Gerüstsubstanzen in der Ernährung von Wiederkäuern

Nachfolgend werden einige wesentliche Aspekte der Wiederkäuer-Ernährung behandelt, die ohne Kenntnis des Gehaltes an tatsächlicher Faser kaum zu verstehen bzw. zu vermitteln sind. Es geht hierbei um die Charakterisierung der Nährstoffe in Form von Kohlenhydrat- und Protein-Fraktionen im Cornell Net Carbohydrate and Protein System (CNCPS), die Quantifizierung der mikrobiellen Proteinsynthese im Pansen (Rumen Submodel des CNCPS) und die Versorgung der Milchkühe mit Faser.

Anwendung der Detergenzien-Analyse im Cornell Net Carbohydrate and Protein System (CNCPS)

Das Cornell Net Carbohydrate and Protein System wurde in den 1990er Jahren von D.G. Fox und Mitarbeitern an der Cornell University (USA) entwickelt und berücksichtigt drei verschiedene Sachverhalte (Pansenphysiologie / Futtermittelkunde / Bedarfsformulierung), nämlich:

1. Ruminale Fermentation (RUSSELL *et al.* 1992)
2. Verfügbarkeit der Kohlenhydrate und des Proteins (SNIFFEN *et al.* 1992)
3. Bedarf der Tiere und Rationsbewertung (FOX *et al.* 1992)

Das CNCPS wurde laufend weiter entwickelt, bisherige Modifikationen sind von FOX *et al.* (2004), TYLUTKI *et al.* (2008) und Van AMBURGH *et al.* (2015) beschrieben. Das CNCPS ist ein mathematisches Modell, das einerseits den Bedarf der Tiere beschreibt und andererseits deren Nährstoffversorgung, und zwar unter Berücksichtigung des Leistungsniveaus und des physiologischen Status sowie der Zusammensetzung des Futters bzw. der Nährstoff-Fraktionen. Das CNCPS verwendet Abbau- und Passage-Raten für Kohlenhydrate und Protein, um die Fermentation im Pansen, die mikrobielle Synthese sowie die post-ruminale Absorption zu beschreiben. Daraus wird die Gesamtversorgung an umsetzbarer Energie und umsetzbarem Protein kalkuliert (FOX *et al.* 2004).

Fraktionen der Kohlenhydrate und des Proteins

Das CNCPS geht davon aus, dass die Futtermittel aus Kohlenhydraten, Protein, Fett, Asche und Wasser bestehen. Kohlenhydrate und Protein werden dabei in 4 bzw. 5 Fraktionen unterteilt. Diese Unterteilung erfolgt auf der Grundlage unterschiedlicher Abbauraten einzelner Fraktionen im Pansen. Mit "A" gekennzeichnete Fraktionen werden im Pansen vollständig und sofort abgebaut. Die Fraktionen "B" werden im Pansen von den Mikroben mit unterschiedlicher Geschwindigkeit abgebaut, während die Fraktion "C" im Pansen nicht abbaubar ist. Der tatsächliche, effektive Abbau der Fraktion "B" hängt entscheidend von der Passagerate dieser Fraktion ab (SNIFFEN *et al.* 1992). Folgende Fraktionen werden unterschieden:

Die Kohlenhydrate (CHO) werden in Faser- (FC) und Nichtfaser-Kohlenhydrate (NFC) unterschieden. Die Faser-Kohlenhydrate werden mit der Detergenzien-Methode nach Van SOEST analysiert und bestehen aus Zellulose, Hemizellulose und Lignin. Die Faser-Kohlenhydrate (FC) werden in nicht-abbaubare (C) und abbaubare Faser (B_2) unterteilt. Die Nichtfaser-Kohlenhydrate (NFC) werden als Differenz errechnet und bestehen aus Zucker (A) sowie der Fraktion B_1 (Stärke (XS) und Pektin).

Die Kohlenhydrate werden wie folgt ermittelt (SNIFFEN *et al.* 1992):

CHO =	TM – (XP + XL + XA)
	C = ADL × 2,4
	B_2 = NDF – (NDIP + C)
FC =	C + B_2
NFC =	CHO – FC
	B_1 = XS + Pektin
	A = NFC – B_1

Die Fraktionierung der Kohlenhydrate wurde inzwischen verfeinert. LANZAS *et al.* (2007) unterscheiden dabei 8 Fraktionen:

4 A-Fraktionen:	A_1 = flüchtige Fettsäuren, A_2 = Milchsäure, A_3 = weitere organische Säuren, A_4 = Zucker
3 B-Fraktionen:	B_1 = Stärke, B_2 = lösliche Faser, B_3 = abbaubare NDF
1 C-Fraktion:	nicht-abbaubare NDF

Die erweiterte CHO-Fraktionierung ermöglicht die Berücksichtigung der Silagequalität und der tatsächlichen Zusammensetzung der NFC-Fraktion. Die NFC selbst bestehen aus mehreren Fraktionen, die sich im Ausmaß und der Rate ihrer Fermentation unterscheiden, des Weiteren in ihren Fermentationsprodukten sowie in ihrem Beitrag zur mikrobiellen Proteinsynthese und damit letztlich zur Leistung der Tiere (NOCEK und TAMMINGA 1991, HALL und HEREJK 2001). Die Berücksichtigung dieser Fraktionen erlaubt eine genauere Einschätzung der flüchtigen Fettsäuren und des pH-Wertes im Panseninhalt, der Versorgung mit umsetzbarer Energie und umsetzbarem Protein sowie der Futteraufnahme.

Das Rohprotein wird in 5 Fraktionen unterteilt, die unterschiedlichen Proteinabbauraten entsprechen (SNIFFEN *et al.* 1992). Zuerst wird das lösliche Protein (SolXP) mit einem Phosphat-Borat-Puffer ermittelt. Das darin enthaltene echte („fällbare") Protein (B_1) wird durch Trichlor-Essigsäure präzipitiert. Der Rest ist NPN (Fraktion A). Das in der ADF-Matrix enthaltene Protein (ADIP = acid detergent insoluble protein) wird als nichtverfügbares Protein betrachtet (Fraktion C). NDIP (= neutral detergent insoluble protein) minus ADIP stellt das langsam abbaubare Protein dar. Die Fraktion B_2 (mittlere Proteinabbaurate) wird als Differenz zum Rohprotein ermittelt. Die Analyse dieser Protein-Fraktionen ist bei KRISHNAMOORTHY *et al.* (1982) sowie LICITRA *et al.* (1996) beschrieben (Abb. 3). Aus den NPN-Verbindungen (Ammoniak, Aminosäuren, Peptide) entsteht im Pansen entsprechend rasch Ammoniak. Besonders in Silagen liegt SolXP mehr oder weniger vollständig als NPN vor, d. h. in der Fraktion A.

Rohprotein (XP) =	Kjehldal-N × 6,25	
SolXP =	$A + B_1$	
	$A = SolXP - B_1$	[NPN]
	$B_1 = SolXP - A$	[Albumin, Globulin]
C =	ADIP	
	$B_3 = NDIP - ADIP$	[Extensin, Prolamin]
	$B_2 = XP - (A + B_1 + B_3 + C)$	[Glutelin]

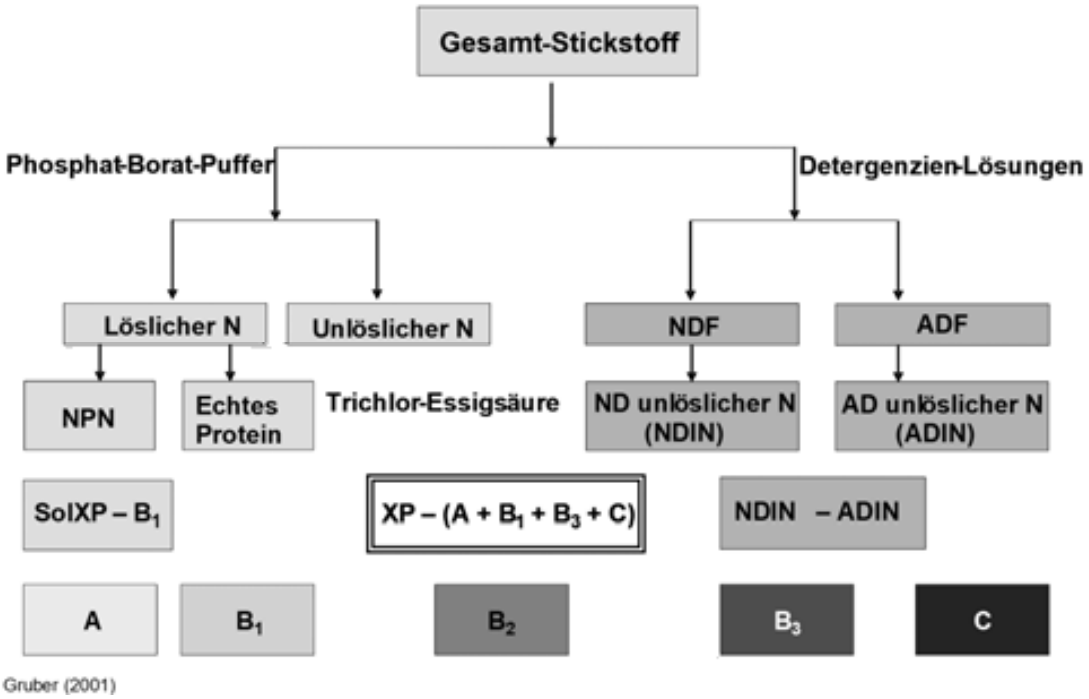

Abbildung 3 : Schema der Rohprotein-Fraktionierung nach dem Cornell Net Carbohydrate und Protein System (nach KRISHNAMOORTHY *et al.* 1982, LICITRA *et al.* 1996)

Die Fraktion B wird entsprechend ihrer Abbaurate dreifach unterteilt (B_1, B_2, B_3 ≙ hohe, mittlere, niedrige Abbaurate). Konserviertes Grobfutter (Silage, Heu) enthält eher geringere Anteile des Proteins in Form von B_1, frische Grünfutter und Kraftfutter dagegen mehr. Die Fraktion B_1 wird im Pansen vollständig abgebaut. Das nicht-abbaubare, gebundene Protein (Fraktion C) ist in Saurer Detergenz-Lösung (AD-Lösung) nicht löslich (ADIP ≙ acid detergent insoluble protein). Die Fraktion C enthält Lignin assoziertes Protein, Tannin-Protein–Komplexe und Maillard-Produkte. Diese ist weder durch mikrobielle noch durch Wirbeltier-Enzyme zu spalten, ist also im Pansen nicht-abbaubar und liefert auch im unteren Verdauungstrakt keine Aminosäuren. Die Fraktion B_3 ist löslich in AD-Lösung, jedoch nicht in Neutraler Detergenz-Lösung (ND-Lösung; NDIP ≙ neutral detergent insoluble protein). Sie ergibt sich aus der Differenz NDIP minus ADIP. Die Fraktion B_3 wird im Pansen langsam abgebaut, da dieses Protein an die Zellwand gebunden ist (vor allem Extensin). Daher entgeht ein großer Teil der Fraktion B_3 der Fermentation und gelangt in den nachgeschalteten Verdauungstrakt (Abbildung 3). Die Protein-Fraktion B_2 wird rechnerisch ermittelt, und zwar aus der Differenz des gesamten Proteins minus dem löslichen Protein sowie dem in der NDF enthaltenen Protein ($B_2 = XP - [A + B_1 + B_3 + C]$). Ein Teil von B_2 wird im Pansen fermentiert, ein Teil entgeht der Fermentation und gelangt in tiefere Abschnitte des Verdauungstraktes. Dies hängt vom Verhältnis der Abbaurate zur Passagerate ab (SNIFFEN *et al.* 1992).

Der effektive Abbau der einzelnen Kohlenhydrat- und Protein-Fraktionen im Pansen wird im CNCPS nach dem Modell von WALDO *et al.* (1972) kalkuliert. Dieses Modell besagt, dass die Nährstoffe den Pansen entweder durch Fermentation oder durch Passage verlassen (RUSSELL *et al.* 1992, SNIFFEN *et al.* 1992). Der effektive Abbau (deg, degradability) errechnet sich aus den relativen Raten der Fermentation (k_f) und der Passage (k_p) entsprechend der Formel:

$deg = k_f / (k_f + k_p)$

Die Passagerate hängt ab von der Partikelgröße, Dichte und Hydratation. Die Passagerate wird für Grobfutter, Kraftfutter und Flüssigkeit getrennt mit Formeln errechnet, wobei die Futteraufnahme und der Grobfutteranteil eine große Rolle spielen. Mit steigender Futteraufnahme erhöht sich die Passagerate und sinkt folglich die effektive Abbaubarkeit. Dadurch wird die mit steigendem Futterniveau einhergehende Verminderung der Energiekonzentration gezielt berücksichtigt (GfE 2001, NRC 2001).

Ruminale Fermentation

Das CNCPS verfügt über ein kinetisches Submodell, das die Fermentations-Endprodukte (ME aus den flüchtigen Fettsäuren, Mikrobenprotein und NH_3) und die unabgebauten Nährstoffe (Kohlenhydrate, Protein) quantitativ ermittelt (RUSSELL *et al.* 1992). Es teilt das mikrobielle Ökosystem das Pansens in zwei Gruppen: (1) Mikroben, die FC (Zellulose, Hemizellulose) fermentieren und (2) Mikroben, die NFC (Stärke, Pektin, Zucker) bevorzugt fermentieren. Diese beiden Gruppen unterscheiden sich also einerseits in den Nährstoffen, die sie abbauen, bzw. Substraten, die sie nutzen (FC, NFC), andererseits auch in der N-Verwertung und der Wachstums-Effizienz. Die FC-Bakterien verwenden ausschließlich NH_3 als N-Quelle und fermentieren keine Peptide oder Aminosäuren. Dagegen verwerten NFC-Bakterien neben NH_3 auch Peptide und Aminosäuren. Die FC-Bakterien wachsen langsam und haben einen geringeren Substratbedarf für ihre Erhaltung (0,05 g CHO pro g Bakterien pro Stunde). Die NFC-Bakterien wachsen rascher und haben einen höheren Erhaltungsbedarf (0,15 g CHO pro g Bakterien pro Stunde). Wenn ausreichend Stickstoff in geeigneter Form vorhanden ist, ist die Wachstumsrate beider Bakterien-Gruppen proportional der Abbaurate der Kohlenhydrate. Daraus erklären sich auch ihre unterschiedlichen Wachstumsraten. Die NFC werden wesentlich rascher fermentiert als die FC (Van SOEST *et al.* 1991, SNIFFEN *et al.* 1992). Die Wachstums-Effizienz der FC-Bakterien ist höher gegenüber den NFC-Bakterien, da ihr Erhaltungsbedarf geringer ist (Van SOEST *et al.* 1991). Bei sehr geringen Wachstumsraten ist der Erhaltungsanteil relativ hoch, sodass eine geringere Nährstoffeffizienz und damit geringere Mikrobenmasse zu erwarten ist. In solchen Fällen wird ein großer Teil der Energie für Nichtwachstums-Funktionen aufgewendet, wie auch von Tieren bekannt ist (Abbildung 4). Der mikrobielle Ertrag bzw. die Synthese mikrobieller Masse ist nach Untersuchungen von ISAACSON *et al.* (1975) maximal 50 %, d. h. 0,5 g Mikrobenmasse pro g fermentierter CHO. Da Protozoen im CNCPS Modell nicht berücksichtigt werden, wird der maximale Mikrobenertrag um 20 % auf 0,4 g pro g fermentierter CHO verringert (RUSSELL *et al.* 1992). Die mikrobielle Syntheseleistung ist geringer, wenn ungünstige pH-Verhältnisse vorliegen (pH-Wert < 6,3). Dies wird über den NDF-Gehalt aus Grobfutter geschätzt. Bei NDF-Gehalten aus Grobfutter < 20 % wird der Mikrobenertrag pro NDF-Prozent < 20 % um 2,5 % reduziert. Dagegen verbessert sich die mikrobielle Leistung der NFC-Bakterien um bis zu 18,7 %, wenn Stickstoff in Form von Peptiden oder Aminosäuren vorliegt (bis zu einem Aminosäurengehalt von 14 % der organischen Masse). Die mikrobielle Leistung wird im CNCPS nach dem doppelt reziproken Modell von PIRT (1965) errechnet (RUSSELL *et al.* 1992, FOX *et al.* 2004):

1 / Effizienz = (Erhaltung / Wachstumsrate) + (1 / theoretisch maximale Leistung)

Effizienz = 1 / [(Erhaltung / Wachstumsrate) + (1 / theoretisch maximale Leistung)]

Effizienz	= Wachstums-Effizienz (g Bakterienzellen pro g fermentierter CHO)
Erhaltung	= Erhaltungsbedarf der Bakterien
	FC = 0,05 g fermentierte CHO pro g Bakterien pro Stunde
	NFC = 0,15 g fermentierte CHO pro g Bakterien pro Stunde
theoretisch maximale	= 0,40

Leistung der Bakterien = 0,50 × 0,8 = 0,40 g Bakterienzellen pro g fermentierter CHO

(nach ISAACSON *et al.* 1975 bzw. RUSSELL *et al.* 1992)

Dieses Modell berücksichtigt die Tatsache, dass auch Bakterien einen Teil ihrer Energie für Nichtwachstums-Funktionen aufwenden müssen. Dieser Aufwand entspricht dem Erhaltungsbedarf von tierischen Zellen und dient dem Erhalt der Zellintegrität (RUSSELL und COOK 1995). Der Erhaltungsanteil ist umso höher und die Wachstums-Effizienz umso niedriger, je geringer die Wachstumsraten (d. h. Abbauraten der CHO) sind. Auf Grund des niedrigeren Erhaltungsbedarfs der FC-Bakterien weisen diese immer eine höhere Wachstums-Effizienz auf. Dies gilt ganz besonders im Bereich niedriger Abbauraten der CHO. Bei einer Fermentationsrate von 5 % erreichen FC-Bakterien 71 % und NFC-Bakterien nur 45 % ihrer theoretisch maximalen Leistung von 0,40 g pro g CHO, bei einer Fermentationsrate von 20 % dagegen 91 bzw. 77 %. Allerdings unterscheiden sich FC und NFC grundsätzlich in ihren Abbauraten (VAN SOEST *et al.* 1991; Abbildung 4).

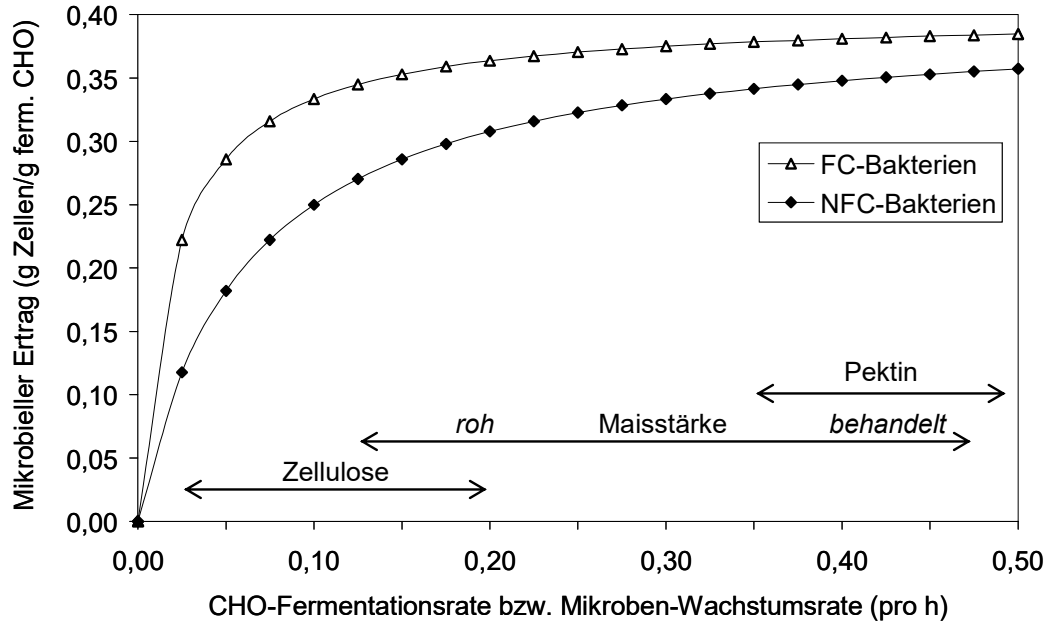

Abbildung 4: Einfluss der Kohlenhydrat-Fermentationsrate bzw. Wachstumsrate auf den Ertrag an Mikroorganismen-Masse im CNCPS (nach RUSSELL *et al.* 1992)

Gegenüber anderen Modellen zeichnet sich das CNCPS dadurch aus, dass die mikrobielle Leistung von der tatsächlich im Pansen fermentierten Masse an Kohlenhydraten abhängt und nicht von der Gesamtverdaulichkeit oder Energieaufnahme (ME), welche die Versorgung des Wirtstieres und nicht der Mikroben beschreiben. Des weiteren wird die mikrobielle Wachstums-Effizienz nicht als konstant angenommen, sondern wird mit der Wachstumsrate (d. h. Abbaurate der CHO) und mit dem Erhaltungsbedarf der Mikroben verknüpft. Schließlich ist die Unterscheidung in FC- und NFC-Bakterien sehr bedeutsam, da sich diese nicht nur in ihren Substraten unterscheiden, sondern auch in ihrem Erhaltungsbedarf, ihrer Wachstumsrate und ihren N- bzw. Protein-Ansprüchen. Über die mikrobielle Syntheseleistung sowie die Abbauraten der Kohlenhydrate und des Proteins wird die N-Bilanz des Pansens kalkuliert. Schließlich sind diese Abbauraten des Futters nicht fix, sondern verändern sich in Abhängigkeit von der Futteraufnahme und Ingesta-Passage. Damit ist das CNCPS ein dynamisches Modell, dass den tatsächlichen Verdauungsabläufen weitestgehend entspricht (RUSSELL *et al.* 1992).

Bewertung der Strukturwirkung von Total-Misch-Rationen für Milchkühe (nach GfE 2014)

Die Versorgung der Milchkühe mit ausreichend Struktur(futter) wird bei steigenden Leistungen immer schwieriger und limitierender. Da die Futteraufnahme mit der Milchleistung nicht linear ansteigt (sondern nur degressiv), muss die Energiekonzentration mit zunehmender Milchleistung erhöht werden – vor allem durch höhere Kraftfutteranteile in der Ration (und hochverdauliche Grundfutter), um eine ausreichende Energieversorgung zu erreichen. Dies vermindert die Speichelmenge und damit die Pufferwirkung der Ration, wodurch der pH-Wert im Pansen erniedrigt wird – mit der Konsequenz verminderter Verdaulichkeit und Futteraufnahme des Grundfutters. In weiterer Folge geht der Milchfettgehalt zurück und die Tiere erkranken an subakuter und letztlich an akuter Azidose. Typische Folgekrankheiten sind auch Labmagenverlagerungen, Leberschäden und Klauenerkrankungen.

Wiederkäuer benötigen also zur Aufrechterhaltung ihrer normalen Pansenfunktion neben der Zufuhr entsprechender Mengen an fermentierbaren Nährstoffen auch eine ausreichende Aufnahme an strukturiertem Futter. Dies sind die Faserbestandteile der Grundfutter, welche auch bestimmte physikalische Eigenschaften aufweisen müssen (Partikelgröße, Länge, Steifheit, Dichte), um die Speichelbildung auszulösen und zu fördern. Darüber hinaus wirken sich noch die Zusammensetzung des Kraftfutters (besonders der Stärkegehalt und dessen Abbauverhalten im Pansen) auf die Säurebildung aus. Die Gesellschaft für Ernährungsphysiologie (GfE) hat im Jahr 2014 ein System der Bewertung der Strukturwirkung der Futtermittel und des Strukturbedarfes der Milchkühe veröffentlicht, das nachstehend kurz beschrieben wird.

Definition der physikalisch effektiven Faser (peNDF)

Das Konzept der peNDF wurde von MERTENS (1997) in den USA entwickelt. Es verbindet den Gehalt an NDF (also die Summe eines Futtermittels an Gerüstsubstanzen) mit deren physikalischer Strukturwirkung. Die Strukturwirkung kann theoretisch Werte von 0 bis 1 annehmen und wird von der Art des Futtermittels und seinen physikalischen Eigenschaften bestimmt (im Wesentlichen von der Partikellänge). Die peNDF wird durch Multiplikation des NDF-Gehaltes mit dem Faktor "physikalische Effizienz" (pE) errechnet. Die physikalische Effizienz wird auf einfachem Weg mit der sogenannten Schüttelbox (PennState Particle Separator = PSPS) ermittelt. Sie errechnet sich aus dem Anteil der Futterpartikel, die eine Größe von mehr als 8 mm (Methode 1) bzw. von mehr als 1,18 mm (Methode 2) aufweisen. Die Auftrennung der Futterpartikel mit unterschiedlicher Größe erfolgt in der Schüttelbox mit Sieben verschiedener Lochdurchmesser, nämlich 19 mm, 8 mm (und 1,18 mm). Anstatt des 1,18 mm-Siebes wird neuerdings auch ein 4 mm-Sieb verwendet. Die nachstehend angeführten Auswertungen von Literaturdaten durch ZEBELI *et al.* (2010) erfolgten auf der Basis der 8 mm-Siebe.

Definition des physiologischen pH-Wertes im Pansen

Damit ein „Bedarf an Struktur" oder eine „Mindestversorgung an physikalisch effektiver Faser" angegeben werden kann, muss festgelegt werden, ab welcher Grenze der pH-Wert im Pansen nicht mehr als physiologisch (d.h. gesund) anzusehen ist. Um einen normalen pH-Wert im Pansen zu erreichen, ist eine bestimmte Kautätigkeit erforderlich. Außerdem muss der Panseninhalt klar in Pansenflüssigkeit, Pansenmatte und Gasblase geschichtet sein, die unwillkürlichen Pansenbewegungen müssen kontinuierlich ablaufen und die Pansenzotten müssen die von den Pansenmikroben gebildeten flüchtigen Fettsäuren rasch aufnehmen können, um einen starken Abfall des pH-Wertes zu vermeiden.

Auf der Basis einer Meta-Analyse vieler pansenphysiologischer Untersuchungen haben ZEBELI *et al.* (2008) ermittelt, dass eine „normale Pansenfunktion" dann gegeben ist, wenn folgende zwei Kriterien erfüllt sind. Erstens darf der mittlere pH-Wert während eines Tages nicht unter 6,2 absinken und zweitens darf ein pH-Wert von weniger als 5,8 nicht länger als 5–6 Stunden auftreten. Der pH-Wert zeigt nämlich – besonders in Abhängigkeit von den Fütterungszeiten (das heißt dem Nährstoffangebot an die Pansenmikroben) – einen typisch schwankenden Verlauf mit einem starken Abfall nach der Fütterung, bei dem die Fermentation und Bildung der Fettsäuren intensiv einsetzt. Sinkt der mittlere

pH-Wert unter 6,2 und dauert die Phase des pH-Wertes von weniger als 5,8 länger als 5–6 Stunden, ist von subklinischer Azidose auszugehen (Tabelle 1).

Tabelle 1: Abgrenzung von normaler Pansenfermentation und subklinischer Azidose (99 % Vertrauensgrenzen) (nach ZEBELI *et al.* 2008)

	Tagesmittelwert		**Dauer des pH-Wertes unter 5,8 (h)**	
	untere Grenze	obere Grenze	untere Grenze	obere Grenze
Normale Fermentation	**6,16**	6,49	1,62	**5,24**
Subklinische Azidose	5,82	**6,14**	**5,47**	15,54

Einfluss von peNDF auf den pH-Wert im Pansen, Futteraufnahme, Kautätigkeit und Verdaulichkeit

ZEBELI *et al.* (2010) haben aus diesen umfangreichen pansenphysiologischen Untersuchungen auch ermittelt, wie sich der Gehalt an physikalisch effektiver Faser ($peNDF_{>8}$) auf den pH-Wert im Pansen, Futteraufnahme und Wiederkautätigkeit sowie Verdaulichkeit der Faser auswirkt. Die Ergebnisse sind in Abbildung 6 dargestellt.

pH-Wert im Pansen

Mit steigendem Gehalt an $peNDF_{>8}$ steigt auch der pH-Wert im Pansen, allerdings nur bis 18,5 % $peNDF_{>8}$. Bei diesem Gehalt an $peNDF_{>8}$ beträgt der mittlere pH-Wert im Pansen 6,22 und liegt damit knapp über dem Grenzwert der subklinischen Pansenazidose. Mit steigendem Gehalt an $peNDF_{>8}$ verringert sich auch die Dauer, bei welcher ein pH-Wert von unter 5,8 festzustellen ist. Dieser Einfluss ist besonders stark im Bereich eines geringen Gehaltes an $peNDF_{>8}$ (bis 17 %) und weniger deutlich bei einem höheren Gehalt an $peNDF_{>8}$ (bis 26 %). Darüber hinaus wirkt sich der Gehalt an $peNDF_{>8}$ auf die Dauer des pH-Wertes von unter 5,8 nicht mehr aus. Es wird also in jedem Fall eine Dauer des pH-Wertes von unter 5,8 im Ausmaß von mindestens 2,5 Stunden gefunden, auch wenn ein höherer Gehalt an $peNDF_{>8}$ gefüttert wird. Dies ergibt sich aus der Tatsache, dass bei intensiver Fermentation (nach Beginn der Fütterung) mit einem deutlichen Abfall des pH-Wertes zu rechnen ist (Abbildung 6 oben).

Die Aussage, dass ein steigender Gehalt an peNDF ab einem gewissen (Schwellen-)Wert nicht mehr zu einer Erhöhung des pH-Wertes führt, kommt auch durch die Auswertungsmethode von ZEBELI *et al.* (2008, 2010) zustande (sog. broken line-Modell). MERTENS (1997) hat ein nicht-lineares Auswertungsmodell gewählt, nach dem bei niedrigen Gehalten an peNDF ein sehr starker Einfluss auf den Gehalt an Milchfett und den pH-Wert festgestellt wird, der sich im oberen Bereich der peNDF-Gehalte mehr und mehr abschwächt, allerdings nicht Null wird. Aus biologischen Gründen hat auch diese Auswertungsmethode ihre Berechtigung. Beide Modelle kommen zu ähnlichen Schlussfolgerungen (Abbildung 5).

Futteraufnahme und Kautätigkeit

Wie bereits diskutiert bedeutet die Fütterung, besonders der Hochleistungskuh, einen Kompromiss zwischen dem Bedarf der Kuh an Energie und den Ansprüchen der Pansenmikroben an nicht zu saure Milieu-Bedingungen im Pansen. Eine hohe Energieversorgung wird üblicherweise mit hohen Kraftfutteranteilen (und hochverdaulichem Grundfutter) erzielt, während genau diese Bedingungen zu tiefen pH-Werten im Pansen führen und besonders die Zellulose-Bakterien hemmen oder sogar schädigen.

In Abbildung 6 (Mitte) ist der Einfluss der $peNDF_{>8}$ auf Futteraufnahme und Wiederkautätigkeit dargestellt. Bis zu einem Gehalt von 14,9 % wirkt sich ein steigender Gehalt an $peNDF_{>8}$ nicht negativ auf die Futteraufnahme aus, höhere Gehalte an Gerüstsubstanzen senken dagegen die Futteraufnahme, weil diese im Pansen langsamer abgebaut werden und daher eine größere Füllwirkung erzeugen. Zusätzlich erhöht sich die Zeit für Kauen und Wiederkauen, wodurch weniger Zeit für Fressen bleibt. Doch selbstverständlich ist das Wiederkauen lebenswichtig für gesunde Pansenverhältnisse und einen

stabilen pH-Wert! Es ist gerade dieser positive Einfluss der Gerüstsubstanzen auf die Kau- und Wiederkautätigkeit, der für die Abpufferung der bei der Fermentation entstehenden flüchtigen Fettsäuren sorgt und günstige Lebensbedingungen für die Pansenmikroben schafft.

Verdaulichkeit der Faser

Wie bereits mehrfach angesprochen sind besonders die Zellulose abbauenden Pansenmikroben von sinkenden pH-Werten betroffen, da ihr pH-Optimum höher liegt als das der Stärke abbauenden Pansenmikroben. In Abbildung 6 (unten) ist daher der Einfluss des pH-Wertes im Pansen auf die Verdaulichkeit der ADF (Säure-Detergenzien-Faser) angeführt. Die Darstellungen zeigen, dass die Verdaulichkeit der Faser ab einem pH-Wert von 6,14 deutlich vermindert wird. Ebenso wirkt es sich negativ auf die Verdaulichkeit der Faser aus, wenn der pH-Wert länger als 3,5 Stunden unter 5,8 liegt.

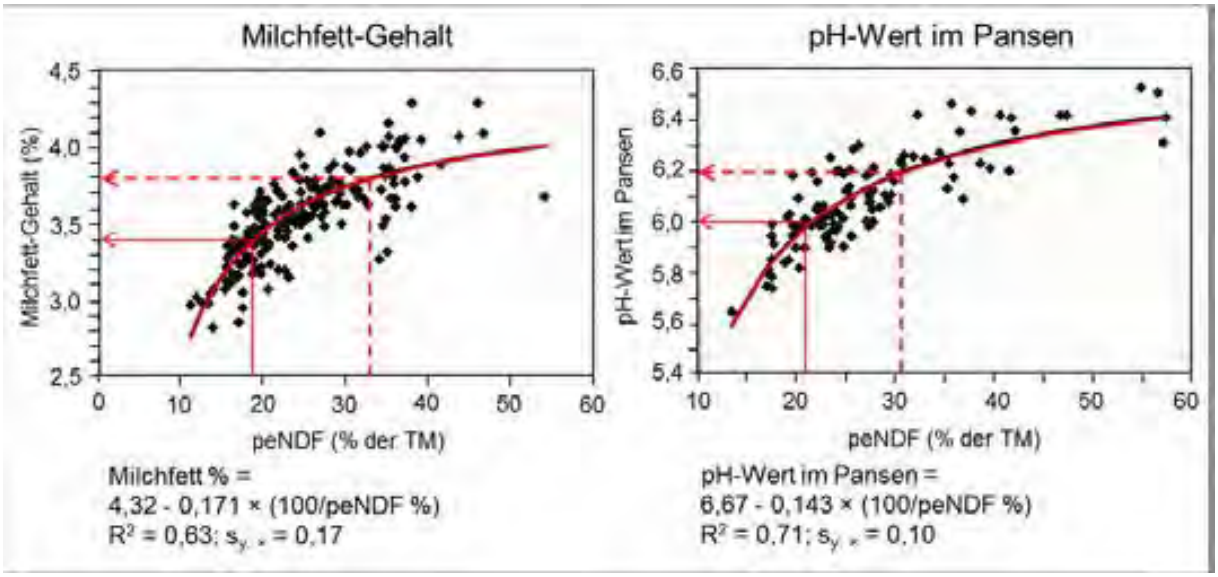

Abbildung 5: Einfluss des Gehaltes an physikalisch effektiver Faser ($peNDF_{>1.18}$) auf den Gehalt an Milchfett sowie den pH-Wert im Pansen (nach MERTENS 1997)

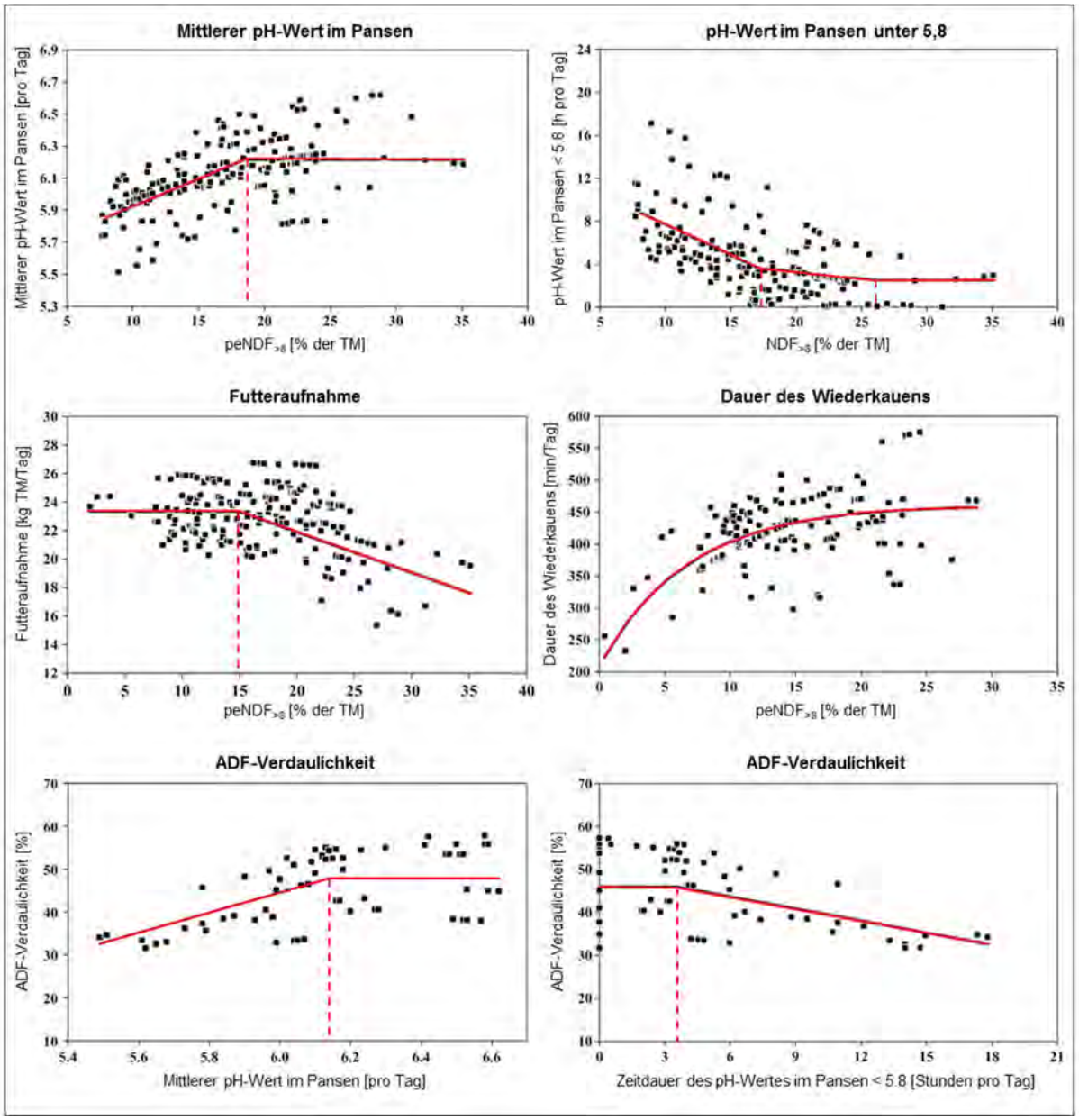

Abbildung 6: Einfluss des Gehaltes an physikalisch effektiver Faser (peNDF>8) auf den pH-Wert im Pansen, Futteraufnahme und Wiederkautätigkeit sowie Verdaulichkeit der Faser (nach ZEBELI *et al.* 2010)

Empfehlungen der GfE zur Versorgung der Milchkühe an peNDF

Sicherlich ist der Gehalt an peNDF hauptverantwortlich für die Regulation des pH-Wertes im Pansen. Doch zusätzlich üben auch der Gehalt an (abbaubarer) Stärke sowie die Höhe der Futteraufnahme einen Einfluss auf den pH-Wert im Pansen aus. Die im Pansen abgebaute Stärke wird zu Propionsäure fermentiert, welche stark sauer wirkt. Und es ist klar, dass auch eine höhere Futteraufnahme zu einem tieferen pH-Wert führt, da sie mehr organische Masse für die Fermentation der Pansenmikroben liefert. Abschließend werden daher die Empfehlungen der GfE zur Versorgung der Milchkühe an peNDF$_{>8}$ in Abhängigkeit von Stärkegehalt und Futteraufnahme angeführt (Tabelle 4).

Tabelle 4: Erforderliche Gehalte an peNDF$_{>8}$ (% der TM) für einen mittleren Tages-pH-Wert von 6,2 in Abhängigkeit von der TM-Aufnahme und dem Gehalt der Ration an Gesamtstärke(GfE 2014)

Gesamtstärke	**Futteraufnahme** (kg TM/Tag)				
(% der TM)	**18**	**20**	**22**	**24**	**26**
14	12	13	15	16	18
18	14	15	17	18	21
22	16	17	19	21	22
26	18	20	22	22	22

Je nach Stärkegehalt und Futteraufnahme ergibt sich, dass der Gehalt an peNDF$_{>8}$ 12 bis 22 % der TM betragen soll, um einen pH-Wert im Pansen von etwa 6,2 zu erreichen. Die umfangreichen Untersuchungen von ZEBELI *et al.* (2010) haben gezeigt, dass bei diesem pH-Wert sowohl die Lebensbedingungen für die Pansenmikroben (noch) ausreichend gut und auch die Futteraufnahme sowie Energieversorgung der Kühe entsprechend hoch sind. Insgesamt zeigen die dargestellten Zusammenhänge, dass es sehr schwierig ist, hochleistende Milchkühe ausreichend mit Energie zu versorgen und gleichzeitig optimale Lebensbedingungen für die Pansenmikroben zu schaffen (obwohl gute Pansenbedingungen die Lebensgrundlage für die Wirtstiere sind!). Die Züchtung auf höhere Milchleistungen muss daher auch die Futteraufnahme im Auge behalten, um die Pansengesundheit zu erhalten und Stoffwechselprobleme zu vermeiden.

Wiederkäuer benötigen also zur Aufrechterhaltung ihrer normalen Pansenfunktion neben der Zufuhr entsprechender Mengen an fermentierbaren Nährstoffen auch eine ausreichende Aufnahme an strukturiertem Futter. Das Konzept der peNDF vereinigt Informationen der chemischen Zusammensetzung mit der physikalischen Form des Futters. Beide Faktoren entscheiden über die Wiederkäuergerechtheit einer Ration. Das System peNDF geht vom pH-Wert im Pansen aus, der die aussagekräftigste Kennzahl für physiologische Pansenbedingungen darstellt. Darunter ist ein mittlerer pH-Wert von > 6,2 zu verstehen und eine Dauer des pH-Wertes < 5,8 von nicht länger als 5 Stunden. Der erforderliche Gehalt an peNDF wird zusätzlich vom Stärkegehalt der Ration und der Höhe der Futteraufnahme bestimmt, da beide Faktoren zu einer intensiveren Fermentation und Säurebildung im Pansen beitragen, welche über die durch strukturierte Faser hervorgerufene Speichelbildung abgepuffert werden muss.

Literatur

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Grain processing for high-producing dairy cattle

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Abstract

Cereal grains have long become an integral part of the diets of high-producing cattle. Starch is the major energy component of cereal grains but differs strongly regarding its absolute concentration and degradation characteristics among cereals. The feeding value of cereal grains can be markedly improved by processing. Main aim of this paper is to discuss the main trends of physical and chemical processing methods of grains and their effects on digestion, performance, and ruminal parameters in high-producing dairy cattle. Physical processing methods, such as grinding, rolling, pelleting, extrusion and steam flaking which are commonly applied in grain feed industry. These techniques aim at improving the digestion of grains by disrupting physicochemical barriers, to enable the rumen microbes and digestive enzymes access to the starchy parts of the kernel. However, research has also shown that although processing is essential to maximize the utilization of grains by ruminants, extensive grain processing increases ruminal starch degradation, which may negatively affect rumen health and production performance. The development of effective feeding strategies for ruminants requires the maintenance of an optimal rumen metabolism. Many attempts have been made to develop grain processing methods that enhance the nutritive value as well as ruminal tolerance of grains. In this regard, this paper will focus on several physical and chemical methods that have been investigated for their potential to modulate ruminal starch degradability. We conclude that due to the large variations among cereal grains in terms of their physical and chemical characteristics, the processing technique has to be adjusted to the type of the cereal and the performance level, as well as the forage fed in the diet.

Introduction

Cereal grains, such as barley, maize, wheat, and sorghum, have long been incorporated in the diets of high-producing dairy cattle to support high milk production. Starch is the main component of cereals and responsible for their high energy density; however, there is a strong variability in the total starch content (Fig. 1) as well as the extent of ruminal degradability of starch (Fig. 2) among cereals. Wheat, triticale, oats, rye, and barley have a very high level of rumen degradable starch compared to sorghum and maize (Offner *et al.*, 2003; Benninghoff *et al.*, 2015). Overall, the absolute amounts of ruminally degradable and undegradable starch have to be taken into account when formulating ruminant diets.

To optimize the feeding value in dairy cows, grains are typically processed. Mainly physical methods, such as grinding, rolling, and crushing, as well as thermal treatments, are applied. The application of these techniques breaks the outer layers of the grain, thereby allowing the rumen microbes and digestive enzymes access to the starchy parts of the kernel. However, although processing is essential to maximize the utilization of grains by ruminants, extensive grain processing increases ruminal starch degradation, which may negatively affect feed intake in ruminants as this process also enhances the risk of rumen fermentation disorders (Offner *et al.*, 2003; Drouillard, 2016). Thus, the development of effective feeding strategies for ruminants requires the maintenance of an optimal rumen metabolism.

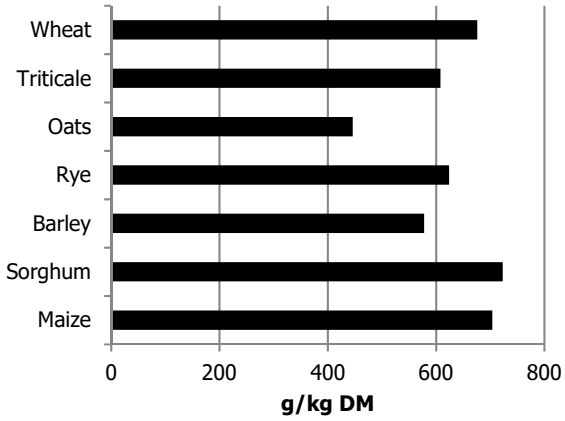

Figure 1: Starch content of common grains

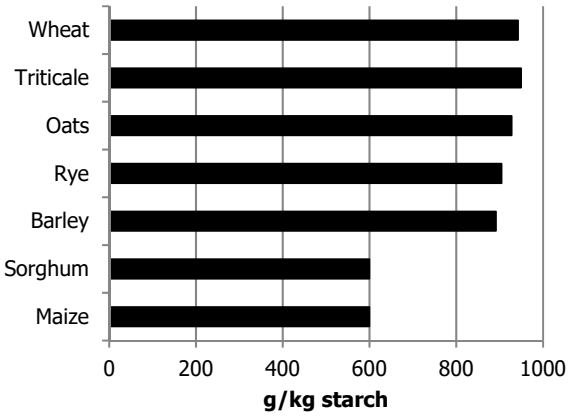

Figure 2: Ruminal starch degradability of grains

Slowing down the rate of ruminal degradation of starch-rich cereals would reduce the starch availability for microbial degradation in the rumen and shift some starch digestion to the small intestine (Svihus *et al.*, 2005). Thus, a higher amount of starch that bypasses the rumen is desirable in some feeding situations as this shift lowers the risk for rumen fermentation disorders and improves glucose supply to the host (Humer *et al.*, 2018). However, the limited capacity of cattle to digest starch postruminally, limits the extent that starch digestion can be shifted, as starch overload in the large intestine can induce hindgut acidosis (Gressely *et al.*, 2011).

Therefore, many attempts have been made to develop grain processing methods that promote milk yield, but without impairing rumen and animal health. In this regard, several physical and chemical methods have been investigated for their potential to modulate ruminal starch degradability (Svihus *et al.*, 2005; Humer and Zebeli, 2017). Overall, due to the large variations among cereal grains in terms of their starch degradability, the processing technique has to be adjusted to the type of the cereal as well as the performance level.

This paper discusses the effect of varying physical and chemical grain processing methods on digestion, performance, and rumen health in high-producing dairy cattle.

Physical methods

As cattle have a very limited ability to digest whole grain (Miron *et al.*, 1997), cereal grains have to be processed to improve their feeding value. However, the extent of processing required to enable digestion differs among grains (Beauchemin *et al.*, 1994). Physical grain processing methods generally aim at exposing starches embedded within the protein matrix of the endosperm and at increasing the surface area for bacterial and enzymatic digestion (Huntington, 1997). Commonly employed physical grain processing methods are rolling and grinding; however, also hydrothermal methods, such as pelleting, extrusion, expansion and steam flaking are in use. Further thermal processes, such as popping or micronizing are less common (Drouillard, 2016). Table 1 summarizes recent studies investigating the effect of different physical grain processing methods conducted in high-yielding dairy cows.

Rolling and grinding rely solely on the destruction of the outer layers to expose more of the endosperm to digestion and on increasing the surface area of the cereals so that they become exposed to the microbial enzymes (Svihus *et al.*, 2005). Previously it has been reported that grain processing by grinding lowers the dry matter intake (DMI) compared to rolling in barley- as well as maize-based diets (Mathison, 1996; Owens *et al.*, 1997). However, a more recent study conducted in high-yielding dairy cows found no differences in the DMI, milk yield, concentration of non-esterified fatty acids (NEFA), glucose and insulin in the blood as well as no effect on ruminal pH and concentration of volatile fatty

acids (VFA) between ground and rolled maize or barley (Mohammed *et al.*, 2010). However, dairy cows that received the ground cereals had a lower milk fat content and a lower concentration of milk urea nitrogen. These changes went along with an enhanced in situ rate of starch digestion and a decrease of the acetate to propionate ratio in the rumen fluid as well as with a lower concentration of ammonia and of branched-chain fatty acids. Thus, the increased degradability of carbohydrates by grain processing likely improved the nitrogen utilization in the rumen (Rezamand *et al.*, 2007). Finally, the increased microbial protein flow to the small intestine, increased the protein supply for the animal (Zinn, 1990), as reflected by the enhanced milk protein content.

In general, the mean particle size of the grain is inversely related to the starch digestion in the rumen. According to a meta-analysis conducted by Ferraretto *et al.* (2013) including 414 treatment means from 102 studies conducted in lactating dairy cows, decreasing the particle size from 3.5-4 mm to 0.5-1 mm enhanced the total-tract digestibility of starch from 78 to 93% in maize, while no significant effect on the neutral detergent fiber (NDF) digestibility was found. Finally, the DM digestibility was enhanced from 59 to 70%. Despite the changes in the digestibility, the particle size neither affected the DMI nor the milk yield and milk constituents.

Table 1: Effect of physical grain processing on performance, ruminal parameters and nutrient digestibility in high-yielding dairy cows

Grain treatment	Effect on performance	Effect on ruminal parameters	Effect on digestibility	Reference
Ground vs. rolled barley	- no effect on DMI - no effect on milk yield - ↓ milk fat (-13%) - ↑ milk protein (+1%)	- no effect on pH - ↓ acetate (-3%) - ↑ propionate (+3%) - ↓ iso-butyrate (-11%) - ↓ iso-valerate (-14%) - ↓ ammonia (-4%)	- ↑ in situ degradation rate of DM (+12%) - ↑ in situ degradation rate of starch (+42%)	Mohammed *et al.* (2010)
Pelleted vs. dry-rolled barley	- ↓DMI (-10%) - no effect on milk yield - ↓ milk fat (-30%) - ↑ milk protein (+6%)	- ↓ ruminal pH (-5%) - ↓ acetate (-16%) - ↑ propionate (+23%) - ↓ butyrate (-17%) - ↓ iso-butyrate (-25%) - ↓ iso-valerate (-56%) - ↓ ammonia (-21%)	- no effect on total-tract digestibility of DM, crude fat, NDF, ADF, OM - ↑ total-tract digestibility of starch (+10%)	Gozho *et al.* (2008)
Pelleted vs. dry-rolled barley	- ↓ DMI (-11%) - no effect on milk yield - ↓ milk fat (-17%) - no effect on milk protein	- ↓ ruminal pH (-5%) - ↑ duration of pH <5.8 (+183%)	- no effect on ruminal disappearance of DM, OM, ADF - ↑ ruminal disappearance of starch (+2%) - ↓ ruminal disappearance of NDF (-16%)	Mutsvangwa *et al.* (2012)
Steam-rolled vs. ground barley	- no effect on DMI - no effect on milk yield - no effect on milk fat - no effect on milk protein	- no effect on pH - no effect on acetate, propionate, butyrate - ↑ iso-butyrate (+200%) - ↑ valerate (+88%) - ↑ iso- valerate (+75%)		Sadri *et al.* (2007)

Steam-rolled vs. ground barley	- no effect on DMI - no effect on milk yield - no effect on milk fat - no effect on milk protein	- no effect on pH - no effect on acetate, propionate, butyrate	- no effect on total-tract digestibility of DM, OM	Soltani *et al.* (2009)
Steam-flaked vs. ground barley	- no effect on DMI - no effect on milk yield - no effect on milk fat - no effect on milk protein	- no effect on pH - no effect on acetate, propionate, butyrate	- no effect on total-tract digestibility of DM	Safaei *et al.* (2017)
Steam-flaked vs. ground barley	- ↓ DMI (-7%) - no effect on milk yield - no effect on milk fat - no effect on milk protein	- ↓ ruminal pH (-5%) - ↓ acetate (-6%) - ↑ propionate (+26%) - ↓ butyrate (-22%)	- ↑ ruminal degradation of OM (+22%), starch (+113%) - no effect on ruminal degradation of NDF and CP - ↑ total-tract digestibility of OM (+7%), starch (+4%) - no effect on total-tract digestibility of ADF and CP	López-Soto *et al.* (2014)
Ground vs. rolled maize	- no effect on DMI - no effect on milk yield - ↓ milk fat (-12%) - ↑ milk protein (+1%)	- no effect on pH - ↓ acetate (-2%) - ↑ propionate (+9%) - ↓ iso-butyrate (-13%) - ↓ iso-valerate (-12%) - ↓ ammonia (-15%)	- ↑ in situ degradation rate of DM (+39%) - ↑ in situ degradation rate of starch (+42%)	Mohammed *et al.* (2010)
Steam-flaked vs. ground or rolled maize	- no effect on DMI - no effect on milk yield - ↓ milk fat (-3%) - ↑ milk protein (+2%)		- ↑ ruminal starch degradation (+20%) - no effect on ruminal NDF degradation - no effect on total-tract digestibility of DM, OM - ↑ total-tract digestibility of starch (+2%) - ↓ total-tract digestibility of NDF (-3%)	Ferraretto *et al.* (2013)
Steam-flaked vs. finely ground maize	- no effect on DMI - ↑ milk yield (+3%) - no effect on milk fat - no effect on milk protein		- no effect on total-tract digestibility of CP, NDF, fatty acids - ↑ total-tract digestibility of DM (+1%), OM (+3%), starch (+6%)	Batistel *et al.* (2017)
Extruded maize vs. finely ground maize	- no effect on DMI - no effect on milk yield - ↓ milk fat (-4%) - ↑ milk protein (+5%)			Rezamand *et al.* (2007)

Pelleting combines the reduction of particle size and hydrothermal processing, which might additionally enhance ruminal starch degradation due to the effects of pressure and shear (Svihus *et al.*, 2005). This processing technique reduces feed sorting, thereby enhancing the palatability of finely ground grains. Together with their quick disintegration to small particles when masticated and mixed with the rumen fluid, this causes a rapid fermentation rate of pelleted concentrates (Drouillard, 2016). In this regard,

pelleting barley has been shown to enhance starch digestibility and cause a shift in the fermentation profile towards propionate at the expense of acetate and branched-chain fatty acids, compared to dry-rolled barley when fed to high-producing dairy cows (Gozho *et al.*, 2008). A lower ruminal pH was measured in cows that received the pelleted barley, which went along with a reduced DMI (Gozho *et al.*, 2008; Mutsvangwa *et al.*, 2012). Mutsvangwa *et al.* (2012) observed a decreased ruminal NDF degradability, which can be attributed to the higher acidosis risk in cows fed the pelleted barley compared to dry-rolled barley. However, Gozho *et al.* (2008) reported that the total-tract digestibilities of DM, fat, NDF, acid detergent fiber (ADF) and organic matter were not affected by the barley processing method. No effect was observed on the milk yield, whereas a decrease in the milk fat content was found. The milk fat depression observed in cows fed pelleted barley is likely attributed to the decline in ruminal pH (Humer *et al.*, 2017). Ruminal ammonia concentration was lower in cows fed pelleted barley (Gozho *et al.*, 2008), thereby reflecting a higher utilization of ammonia as a result of a higher proportion of readily available carbohydrates in the pelleted barley diets (Hristov *et al.*, 2001). However, while Mutsvangwa *et al.* (2012) found no effect on the milk protein content, Gozho *et al.* (2008) found an increase when pelleted barley was fed.

A processing method that is more prominent for high producing dairy cows is steam flaking. Overall, steam conditioning enhances the surface area without fracturing the grain into small pieces, as this process yields flattened kernels. Due to dissimilarities in the characteristics of the grain pericarp among different types of grain, the effect of flaking on the nutritive value varies among cereal grains, whereby maize and sorghum have been reported to respond more favorably (Drouillard, 2016). According to Ferraretto *et al.* (2013), starch digestibility was improved in dairy cows fed diets containing steam processed maize compared to dry maize. The improved starch digestibility is likely due to the disruption of the protein matrix surrounding the starch by heat and moisture during the steam treatment (Rooney and Pflugfelder, 1986) and gelatinization of the starch (McAllister *et al.*, 2006). However, the effect of steam flaking on starch gelatinization is dependent on the amount of steam added and treatment time (Svihus *et al.*, 2005). Most studies found no difference in the DMI and fiber digestibility between steam-flaked maize and dry-ground or rolled maize, whereas starch digestibility was enhanced (Ferraretto *et al.*, 2013; Batistel *et al.*, 2017). Although Batistel *et al.* (2017) found an improved milk yield in cows receiving steam-flaked maize, the meta-analysis conducted by Ferraretto *et al.* (2013) were not able to find an effect. However, effects on the milk constituents have been observed, with a trend towards enhanced milk protein content in lactating dairy cows fed steam-flaked cereals, whereas the milk fat content and milk urea nitrogen content were lowered (Ferraretto *et al.*, 2013).

Lack of difference in DMI, ruminal pH, milk production and milk components between steam-rolled or ground barley has been reported (Sadri *et al.*, 2007; Soltani *et al.*, 2009). In accordance, a recent study investigating the effect of steam flaking of barley compared to grinding, conducted in high-yielding dairy cows, revealed no difference in DMI, sorting behavior, milk yield, milk constituents, rumen pH and acetate and propionate molar proportions as well as total-tract dry matter (DM) digestibility (Safei *et al.*, 2017). However, López-Soto *et al.* (2014) found lowest pH values in cows fed steam-flaked barley, which was accompanied by a lowered acetate to propionate ratio. Also, Yang *et al.* (2001) found that increasing the extent of steam rolling in lactating cows caused a decline in ruminal pH.

A rigorous grain processing method is extrusion, as it combines mechanical and hydrothermal elements, which affect the starch and protein structure. The intense heat and pressure applied during the process of extrusion cause a more complete gelatinization and disintegration of starch granules, although the extrusion conditions are instrumental regarding the extent of starch gelatinization (Svihus *et al.*, 2005). Overall, extrusion causes higher energetic feed value compared to flaking. However, the extensive comminution of particles during extrusion processing enhances the ruminal degradation, therefore enhancing the risk of rumen fermentation disorders (Drouillard, 2016).

Overall, variable responses of extrusion on starch degradability have been reported. In general, the responses are greater for cereals with a relatively high fraction of starch resistant against rumen degradation, such as maize and sorghum, showing positive effects (Mills *et al.*, 1999; Offner *et al.*, 2003). Contrary, the results on cereals with a higher proportion of rumen degradable starch, such as

barley, wheat or oats, are more conflicting (Offner *et al.*, 2003; Svihus *et al.*, 2005). Reports regarding the effect of extrusion on the DMI of maize are controversial, showing either no effect (Dhiman *et al.*, 2002; Rezamand *et al.*, 2007) or a decrease (Shabi *et al.*, 1999; Burkholder *et al.*, 2004) compared to less-processed (ground) maize. Also, effects on the milk production are variable (Shabi *et al.*, 1999; Dhiman *et al.*, 2002; Rezamand *et al.*, 2007). A study conducted in high-yielding dairy cows fed either extruded maize or finely ground maize did not find a difference in the DMI and milk yield, as well as protein and milk fat yield, body weight and body condition score. However, a lowered milk fat content as well as a trend towards increased milk protein content was found in cows fed the extruded maize (Rezamand *et al.*, 2007). Therefore, it is likely that enhanced ruminal starch degradation caused a reduction in rumen pH and decreased production of acetate, which finally led to the observed changes in milk constituents.

In recent years also innovative methods, such as the usage of high-intensity laser beams and mechanical scarification, have been tested in several pilot studies (Drouillard, 2016). However, there is a need for intensive research, before these techniques can be applied as grain processing methods in the field.

Chemical methods

Chemical treatments with alkali have been reported to bring about an effect similar to that of rolling or crushing in increasing the digestibility of the seed coat and subsequently enabling access of rumen microbes and digestive enzymes (Kaiser, 1999). Furthermore, treatment of milled grains with chemical agents has been applied to retard the rate of digestion in the rumen of cereal starch and protein (McAllister et al 1992). Therefore, treating of whole grain with chemicals has been discussed as one strategy to reduce costs for physical processing as well as to decrease ruminal starch digestion and to improve fiber digestion with potential beneficial effects on DMI and performance (Humer and Zebeli, 2017).

Among the earliest chemical grain processing methods treatment with sodium hydroxide (NaOH) has been applied to disrupt the seed coat of cereals to improve digestibility (Berger *et al.*, 1981). In this regard, several cattle experiments indicate that digestibility and milk production on NaOH-treated whole grain can be similar to that on rolled grain, provided sufficient NaOH is applied, whereby the level of NaOH required depends on the type of the grain, with maize and sorghum requiring higher concentrations compared to barley (Humer and Zebeli, 2017). Treating grains with NaOH might result in a slower ruminal starch and protein degradation as well as decreased susceptibility to rumen acidosis. On the other hand, effects on the total-tract digestibility have been contradictory (Dehghan-Banadaky *et al.*, 2008; Miron *et al.*, 1997). Furthermore, several practical and commercial constraints have to be taken into account. For instance, negative side effects (e.g. nephrotoxicosis, soil salinification, risks for users as well as adverse effects on nutritional quality and grain flow characteristics) preclude its application as a routine technique in the practice (Dehghan-Banadaky *et al.*, 2008; Deckardt *et al.*, 2013).

Another chemical that has been used to decrease ruminal starch and protein degradation of grains is ammonia. Furthermore, the treatment enhances the crude protein (CP) content of grains and has shown promising effects for the preservation of high-moisture whole grains (Yaremico *et al.*, 1991; Mathison *et al.*, 1989). Despite these positive aspects, digestibility and animal production responses have been highly variable, and research regarding the effect on ruminal health is very limited (Humer and Zebeli, 2017). Moreover, limitations regarding the practicability, such as the need for long soaking periods and enhanced water intake by the animals have to be taken into account and additional research is required to identify effective ammoniation procedures (Ørskov, 1979; Humer and Zebeli, 2017).

Treating of (mainly milled) grains with formaldehyde (HCHO) has been used to retard the rate of ruminal starch and protein digestion, without impairing total-tract digestion, given that the concentration of HCHO is not excessive. However, while some studies found higher ruminal pH (McAllister *et al.*, 1990; Fluharty and Loerch, 1989), others found no effect (Erjaei *et al.*, 2012), or even detrimental effects

(McAllister *et al.*, 1992). Furthermore, results regarding the total-tract digestibility and animal performance are conflicting (Humer and Zebeli, 2017). Additionally, HCHO has been shown to coincidentally reduce the phytate solubility and thus the availability of Phosphorus (P) in the rumen that might impair the total-tract digestibility of P (Bravo *et al.*, 2003). Furthermore, also negative environmental and health aspects have to be considered, which preclude its application as a routine technique in the practice.

Due to the practical limitations of the use of NaOH, ammonia, and HCHO, increasing research has been conducted to identify new chemical grain processing techniques, which improve the nutrient degradation characteristics and are safe for the user and animal. In this regard, treatments with organic acids (especially propionic acid) have been used to enable storage of high-moisture grain to save drying costs or allow grain to be harvested at some earlier time, respectively (Campling, 1991). Furthermore, especially organic acids, that are found in biological tissues or produced in the gastrointestinal tract, such as fumaric and malic acid, are generally used to modify rumen fermentation. In this regard, malate has shown promising effects, such as an enhanced DM digestibility and decreased methanogenesis. Nevertheless, research regarding the ruminal fermentation characteristics of the above-mentioned acids is scarce. Solely Montano *et al.* (1999) observed improved ruminal pH without detrimental effects on ruminal starch, protein or fiber degradation in steers fed a high-grain finishing diet.

Also, in situ trials with tannic acid have shown its potential to decrease ruminal DM and protein degradation of barley grain in the rumen (Martinez *et al.*, 2005; Deckardt *et al.*, 2016). Therefore, treating cereals with tannic acid might be a promising method to shift starch digestion form the rumen to the intestine and improve ruminal pH dynamics; however, intensive in vivo research is required to evaluate its potential for practical application.

Recent studies investigated the effects of treating grains with other organic acids such as lactic acid (LA) or citric acid (Table 2), two acids that are widely used as a low-cost means of food preservation by different industries. In vitro studies have demonstrated modulation of the chemical composition of barley treated with these acids (Deckardt *et al.*, 2014; Harder *et al.*, 2015a,b).

Feeding dairy cows barley grain steeped in lactic acid has been shown to exert some beneficial effects, such as decreasing the starch degradation rate in the rumen (Khol-Parisini *et al.*, 2015), modifying ruminal fermentation towards enhanced concentration of propionate (Metzler-Zebeli *et al.*, 2015; Mickdam *et al.*, 2017) and enhancing the energy status of the animals (Iqbal *et al.*, 2009; Gruber *et al.*, 2016). However, effects on DMI and ruminal pH are controversial and no or only limited effects on milk yield and milk constituents have been reported (Iqbal *et al.*, 2009, 2012; Humer *et al.*, 2015; Gruber *et al.*, 2016; Khol-Parisini *et al.*, 2016). Moreover, it has been observed, that lactic and citric acid treatments of cereals trigger the hydrolysis of phytate and enhance ruminal P-disappearance (Khol-Parisini *et al.*, 2015). Furthermore, recent research revealed that treating a commercial compound feed with lactic acid or citric acid reduces the concentration of mycotoxins, such as deoxynivalenol and its derivatives (Humer *et al.*, 2016). However, further investigations are required to elucidate the mechanisms behind the phytate and mycotoxin degradation and further in vivo trials are required to tap the potential of this technique for practical applications.

Table 2: Effect of lactic acid treatment of barley on performance, ruminal parameters and nutrient digestibility in high-yielding dairy cows

Grain treatment	Effect on performance	Effect on ruminal parameters	Effect on digestibility	Reference
Rolled barley: steeped in tap water or in 50 g/kg LA, 48h in situ trial: 0, 5 or 10 g/kg LA, 48h	- no effect on DMI - no effect on milk yield - ↑ milk fat (+41%) - no effect on milk protein	- ↑ ruminal pH 10 and 12 h post-feeding, ↓ time pH <5.8 - ↓ acetate (-3%) - ↑ propionate (+3%)	- ↑ in situ DM degradation for 10 g/kg (+1.2%), ↓ for 5 g/kg (-0.9%)	Iqbal *et al.* (2009)
Rolled barley: steeped in tap water or in 10 g/kg LA and heat (LAH), 48h, 55°C	- no effect on DMI - ↑ milk fat (+15%) - no effect on milk protein	- ↑ pH - ↓ propionate (-8%) - ↑ butyrate (+17%)		Iqbal *et al.* (2012)
Ground barley: untreated vs. 10 g/kg LA vs. 10 g/kg LA and heat (LAH, 55°C for 12h), 24h soaking,	- no effect on DMI - no effect on milk yield - no effect on milk fat - no effect on milk protein	- ↓ time duration of pH <6.0 and 5.8 in primiparous cows (-121 min/d) -↑ time duration of pH <6.0 and 5.8 in multiparous cows (+56 min/d)		Humer *et al.* (2015), Gruber *et al.* (2016)
Ground barley: untreated vs. 10 g/kg LA vs. 10 g/kg LA and heat (LAH, 55°C for 12h), 24h soaking	-no effect on DMI	- no effect on ruminal pH - ↑ propionate - ↓ butyrate	- ↓ ruminal degradation of starch, DM and OM 2 and 4 h after incubation - ↑ ruminal degradation of ash and P - no effect on total-tract digestibility of ash, non-fibre carbohydrates, ether extract, CP, NDF - ↑ enhanced total-tract digestibility of DM (+2%)	Khol-Parisni *et al.* (2015), Metzler-Zebeli *et al.* (2015)
Whole concentrate: untreated, with P supplementation vs. 50 g/kg LA with inorganic P (LA+P) vs. 50 g/kg LA without inorganic P (LA-P), 24h soaking	- reduced DMI (-9%) - no effect on milk yield - no effect on milk protein - no effect on milk fat	- ↓ ruminal pH (-5%)		Khol-Parisni *et al.* (2016)
Whole concentrate: untreated, with P supplementation vs. 50 g/kg LA with inorganic P (LA+P) vs. 50 g/kg LA without inorganic P (LA-P), 24h soaking	- no effect on DMI	- no effect on ruminal pH - ↑ propionate (+14%) - ↓ ammonia in LA-P (-12%)		Mickdam *et al.* (2017)

Conclusion

In conclusion, processing techniques that enhance the nutritive value as well as ruminal tolerance of grains are becoming increasingly important, not only in terms of lowering the risk of metabolic disorders and promoting digestion but also in enhancing the nutrient supply for the host ruminants. Despite the progress made in using various processing methods, the impact of grain characteristics, both physical and chemical, on the response to various grain processing techniques require further research. Overall, it is important to optimize the conditions of physical and chemical treatments by taking several factors (such as the starch content and degradability, the proportion of concentrates in the diet, the content of physically effective fiber in the diet and the DMI level of the cow) into account.

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Influence of heat stress periods on three consecutive days on general health parameters, gut permeability and acute phase proteins in pigs

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Abstract

Heat stress plays an important role in livestock animals, due to increasing temperatures and extreme climatic events. Pigs seem to be especially sensitive to heat stress periods. Therefore, we conducted a trial with eight pigs, which were first kept at thermoneutral conditions (24 hours 28 °C) followed by three days with heat stress periods (6 hours 35 °C, 18 hours 32 °C). Health parameters (respiratory rate, body temperature) were measured every two hours during the heat stress periods. Gut permeability was assessed at day 0 (baseline) and day 2 using a non-invasive dual sugar assay (oral administration of lactulose and rhamnose). Furthermore, blood samples were collected at day 0 (baseline), 1 and 3 to measure lipopolysaccharide-binding protein (LBP) as well as pig-major acute phase protein (pig-MAP). Respiratory rate and body temperature were significantly increased during heat stress ($P < 0.05$). The dual sugar assay revealed a significantly increased lactulose/rhamnose ratio during heat stress conditions ($P < 0.05$). Furthermore, the LBP concentration as well as the pig-MAP concentration was significantly increased at the last day of heat stress ($P < 0.05$).
Taken together, heat stress did not only affect general health parameters but also impaired gut barrier and increased the concentration of acute phase proteins in blood.

Introduction

Heat stress plays an important role in livestock animals, due to increasing temperatures and extreme climatic events. Pigs seem to be especially sensitive to heat stress periods. Annual loss due to heat stress is estimated to be in total about $1.7 to $2.4 billion. Furthermore, a $299 - $316 million loss is estimated for the swine industry alone (St. Pierre *et al.*, 2003). Several studies have already shown that heat stress has detrimental effects on animal health (Baumgard *et al.*, 2003; Johnson and Lay, 2017). The gastrointestinal tract seems to be one of the first organs affected. A consequence of heat stress can be e.g. an impaired gut barrier (Liu *et al.*, 2016; Pearce *et al.*, 2014). When gut permeability is increased, pathogens (e.g. endotoxins) can enter the blood flow. Furthermore, this leads to an inflammation response with an increased production of cytokines and acute phase proteins.
The aim of the study was to assess the influence of heat stress in pigs on health parameters (respiratory rate, body temperature), gut permeability as well as the concentration of acute phase proteins in the blood.

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Material and methods

Trial design
Eight pigs (~ 20 kg body weight) were allocated to metabolic cages (two pigs per cage) 35 days after weaning. During an adaption phase of four days and on day 0 (baseline) of the trial period, pigs were kept at thermoneutral conditions (24 hours 28 °C). On day 1, 2 and 3, pigs were exposed to heat stress periods (6 hours 35 °C, 18 hours 32 °C) (Figure 1).

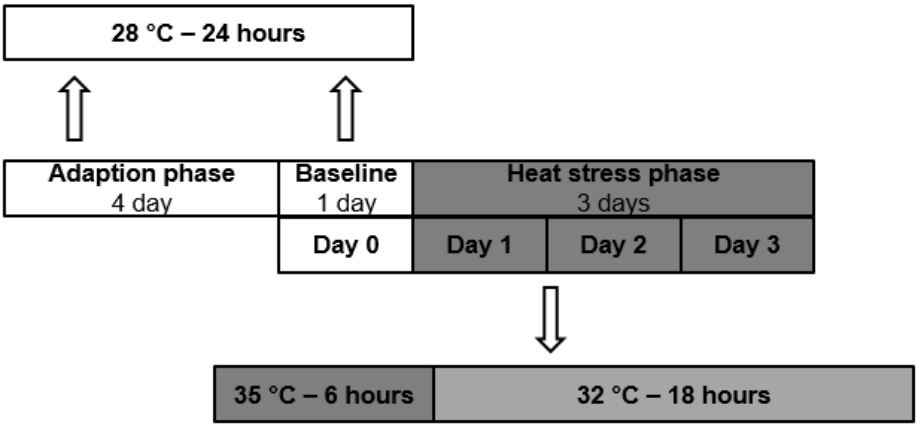

Figure 1: Schematic overview of the trial design. n = 8 pigs (2 pigs/metabolic cage).

Health parameters
Body temperature and respiratory rate (breaths per minute) were assessed every two hours during the heat stress period.

Gut permeability assay
A dual sugar assay was performed on day 0 and 2 to evaluate gut barrier function. Therefore, pigs were fasted overnight. Every animal received lactulose (500 mg/kg body weight) and rhamnose (100 mg/kg body weight) mixed with agar. Urine was sampled before, 2, 4 and 6 hours after sugar administration. Urine samples were frozen at -20 °C, and lactulose and rhamnose concentration was determined via HPLC-MS/MS.

LBP and pig-MAP concentration in the blood
Blood was sampled on day 0, 1 and 3. Acute phase proteins, LBP and pig-MAP, were measured in serum with commercially available ELISA kits.

Statistics
Statistical evaluation of the data was performed with the software Graphpad Prism (Version 7). When data were normally distributed, ANOVA with Dunnett's multiple comparisons test was performed. When data were not normally distributed, Kruskal-Wallis test was performed. Differences were considered significant when $P < 0.05$.

Results

Health parameters
There was a significant increase of the average respiratory rate (Figure 2) and average body temperature (Figure 3) on day 1, 2 and 3 during the six hours of heat stress compared to day 0 (baseline).

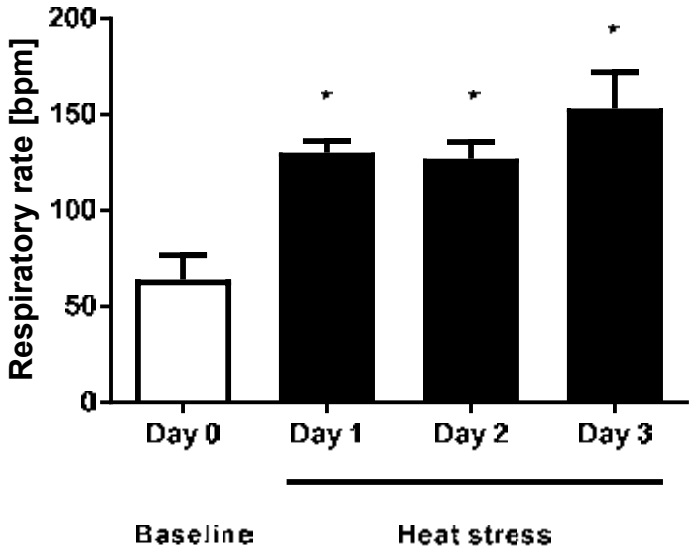

Figure 2: Average respiratory rate on day 0 (baseline, thermoneutral), day 1, 2 and 3 of the heat stress period. Error bars represent standard deviation. n = 8 pigs. * P < 0.05

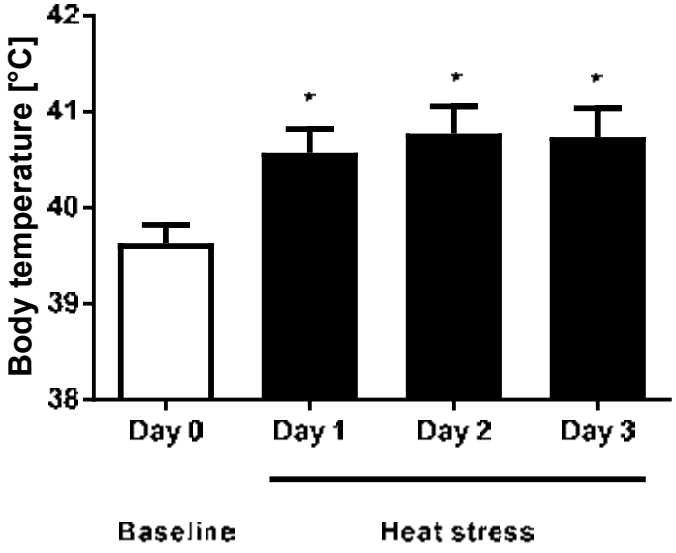

Figure 3: Average body temperature on day 0 (baseline, thermoneutral), day 1, 2 and 3 during the heat stress period. Error bars represent standard deviation. n = 8 pigs. * P < 0.05

Gut permeability (Sugar permeability) assay

There was no significant difference of the cumulative rhamnose and lactulose excretion between day 0 and day 2. However, the lactulose to rhamnose ratio was significantly increased by 3-fold in heat stressed pigs (P < 0.05).

LBP and pig-MAP concentration in the blood

There was a significant increase of the LBP concentration as well as of the pig-MAP concentration on day 3 by 2-fold (P < 0.05).

Discussion

Exposure to heat stress is known to negatively affect animal's health. In accordance with the literature, our study showed that pigs had a significantly increased body temperature as well as respiratory rate. Furthermore, our study confirmed that heat stress increases gut permeability. This is in line with other studies, using invasive technologies (e.g. Ussing chamber) to assess the impact of heat stress on gut barrier function (Pearce *et al.*, 2012). However, the presented study is the first study using the non-invasive dual sugar assay to measure impaired gut barrier during heat stress.
The increased gut permeability can induce inflammation processes, as pathogens (e.g. endotoxins) can enter the blood flow. This might lead to the production of cytokines as well as acute phase proteins in the liver. In our study, we could prove that two different acute phase proteins, LBP and pig-MAP, were significantly increased in heat stressed pigs.

Conclusion

Our study showed that heat stress did not only affect general health parameters in pigs but also led to an increased gut permeability. Furthermore, heat stress led to a higher concentration of the acute phase proteins LBP and pig-MAP in the blood.

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Lysophospholipids with monoglycerides and synthetic emulsifier enhance lipid digestion in broilers

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Abstract

Lysolecithin, with lysophospholipids as its key component, is used in feed to improve the digestion and absorption of nutrients, especially lipids. The present work investigates if the addition of selected quantities of synthetic emulsifier and monoglycerides to lysolecithin as a mixture (LYSOFORTE® EXTEND) could enhance the mode of action of lysolecithin in broilers.

Samples of rapeseed oil, oil with lysolecithin (0.5%) and oil with the mixture (0.5%) were assessed for emulsion stability. Emulsion stability was significantly improved by the mixture. Samples of animal fat, fat with lysolecithin (1.2%) and fat with the mixture (1.2%) were subjected to *in vitro* hydrolysis. The free fatty acid release rate ($k \times 10^{-3}$; min^{-1}) was the highest ($P<0.05$) in fat with the mixture ($k=15.06$) and the lowest in fat without ($k=10.00$). The digests were then applied to differentiated Caco-2 monolayers on permeable membranes to assess lipid absorption. The absorption of both monoglycerides and free fatty acids increased ($P<0.05$) by more than 70% with the addition of lysolecithin or the mixture. Whereas the synthetic emulsifier and monoglycerides improved emulsification and hydrolysis respectively. Lysophospholipids were found to be essential for improving lipid absorption.

To confirm these findings, Ross 308 broilers (8 birds/pen, 9 pens/diet) were fed a positive control diet (PC) fulfilling all requirements, a negative control diet (NC) with lower AME (- 60 to 80 kcal/kg) or a NC with 500ppm of LYSOFORTE EXTEND dry (LEX). At 14 days of age, birds fed the LEX diet had a higher ($P<0.05$) average body weight (+18 g/bird), higher average daily gain (+1.3 g/bird/day) and lower FCR (-7 points) than birds fed the NC diet. Birds fed either PC or LEX also had a higher ($P<0.05$) carcass (+1%) and breast yield (+1.3%) than those fed NC. Birds fed the LEX diet had significantly lower abdominal fat pad content (LEX: 0.91% vs. PC: 1.19% and NC: 1.07%). The mixture thus confirmed to result in overall improved performance in broilers.

Introduction and objectives

Lysolecithin is added to feed in order to promote the intestinal absorption of nutrients, more in particular of dietary fats. It is postulated that lysolecithins act, together with bile salts, as an emulsifier within the first stages of lipid digestion (ZHANG *et al.*, 2011). Additionally, it has been proposed that lysolecithins are able to participate in the formation of mixed micelles and they are known to improve the absorption of lipids and other nutrients (JANSEN, 2015).

The present work investigates if the addition of selected quantities of synthetic emulsifier and monoglycerides to lysolecithin as a mixture (LYSOFORTE EXTEND) could improve respectively lipid emulsification and hydrolysis to fully exploit the benefits of lysolecithin usage in feed.

Material and methods

Lipid emulsification. First, 0.25 g of lysolecithin and the mixture were each dispersed in 49.75 g of rapeseed oil (n=2). Next, aliquots of 50 ml of a salt solution (2.6 mol/l NaCl) were then added to the

different rapeseed oil dispersions and a rapeseed oil control (50 g oil). The contents were then successively mixed for 5 seconds with a high shear mixer (Bamix, ESGE Ltd., Mettlen, Switzerland), poured in 100 ml cylinders and the emulsion stability was recorded over time.

Lipid hydrolysis. The lipid hydrolysis model previously described by JANSEN *et al.* (2015) was used. In brief, lysolecithin (1.2%) and the mixture (dosed to 1.2% lysolecithin) were each dispersed in animal fat (for use in animal feed; ILVO, Merelbeke, Belgium). Aliquots of each fat treatment were added to simulated intestinal fluid (n=3). The content of each tube was mixed with a high shear mixer, pancreatin was added and tubes were incubated (2h, 40°C). The final contents in the digests were 106 mM NaCl, 1.6 g/L pancreatin, 1.6 g/L bile salts and 16.7 g/L animal fat. During incubation, samples of each digest were taken to analyze the degree of lipid hydrolysis and to determine the apparent rate constant for free fatty acid release ($k \times 10^{-3}$ min^{-1}). At the end of the incubation, hydrolysis samples were submerged in liquid nitrogen and stored at -80 °C.

Lipid absorption. The lipid absorption model previously described by JANSEN (2015) was used. In brief, Caco-2 cells were seeded on collagen-coated Transwell-COL inserts (Corning Costar Corporation, Cambridge, MA) and incubated to allow the cells to differentiate. Next, the different hydrolysis samples obtained with the hydrolysis model were diluted in simulated intestinal fluid and applied at the apical side of the monolayer (n=3). At the start and after 60 minutes of incubation, a sample of the apical fluid was taken and subjected to lipid analysis. The absorption of monoglycerides and free fatty acids (mg/g) in each well was calculated.

Broiler performance trial. A broiler performance trial was carried out at the Banat's University of Agricultural Science and Veterinary Medicine (Timişoara, Romania). Birds were fed either a basal diet fulfilling all dietary requirements (PC; positive control, Table 2), a basal diet with lower metabolizable energy (NC; negative control) or a basal diet with lower metabolizable energy with 500 ppm of a mixture of lysolecithin, monoglycerides and synthetic emulsifier (LEX; LYSOFORTE® EXTEND dry, Kemin Europa NV, Herentals, Belgium). A total of 216 day-old male Ross 308 broilers were housed with eight birds per pen. Replicates (pens, n=9) were allocated to the treatments for a homogeneous distribution of treatments within the room. A dynamic ventilation and heating system provided optimal poultry house temperature and ventilation. During the whole trial period a lighting scheme of 23 hours light and 1 hour dark was used. Feed (mash) and drinking water was provided *ad libitum*.

The average body weight (ABW, g) and the feed intake (g) were recorded and the average daily gain (ADG, g/bird/day) and feed conversion ratio (FCR) were calculated over the starter (0 – 14 d) and whole rearing (0 – 42 d) period. At the end of the rearing period (42 days of age), two chickens per pen were sacrificed. The birds were then processed according to standard slaughter procedures and the live weight, weight of the whole carcass (eviscerated), the breast (with bone) and the abdominal fat pad was recorded to calculate carcass yield, breast yield and abdominal fat pad content.

Statistics. The apparent rate constants for free fatty acid release, the monoglyceride and free fatty acid absorption values, the performance data (ABW, ADG, FCR), carcass yield, breast yield and abdominal fat pad contents were subjected to analysis of variance (ANOVA, STATGRAPHICS Centurion XVI software, Statpoint Technologies Inc., Warrenton, VA) and means were separated by the least significant differences (LSD) procedure. All statements of significance were based on a *P*-value equal to or less than 0.05.

Table 1: Ingredients and nutrient composition of the experimental diets

Item (g/kg, unless noted)	Basal diet			Basal diet with reduced AMEn		
	starter	grower	finisher	starter	grower	finisher
Nutrient						
Corn	360.4	382.1	405.4	360.4	390.5	416.2
Soybean meal (44% CP)	385.0	331.0	295.5	384.0	334.7	295.5
Wheat	150.0	150.0	150.0	150.0	150.0	150.0
Lard	45.0	57.0	65.0	37.0	43.0	51.4
Sunflower meal (34% CP)	20.0	44.0	50.0	20.0	33.0	41.0
Limestone	13.5	12.1	12.0	13.5	13.0	12.0
Wheat bran	0.0	0.0	0.0	9.0	12.0	11.8
Monocalcium phosphate	8.0	7.5	7.0	8.0	7.5	7.0
Vitamin and mineral premix	5.0	5.0	5.0	5.0	5.0	5.0
Lysine HCl	3.8	3.2	2.8	3.8	3.2	2.8
Methionine	3.4	2.8	2.0	3.4	2.8	2.0
NaHCO3	2.0	2.0	2.0	2.0	2.0	2.0
NaCl	1.9	1.9	2.0	1.9	1.9	2.0
Threonine	1.5	0.9	0.8	1.5	0.9	0.8
KEMZYME® Plus P Dry	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient content						
Crude protein	221.2	205.5	192.8	225.2	106.3	192.8
AMEn (kcal/kg)	3,063	3,109	3,143	3,005	3,027	3,064

CP: Crude protein; AMEn: Apparent Metabolizable Energy

Results and discussion

Lipid emulsification. Emulsion stability (Fig. 1) was significantly improved by the mixture. For example, after 30 min. the emulsion stability of oil (8%) was improved to 39% with lysolecithin and to 84% with the mixture.

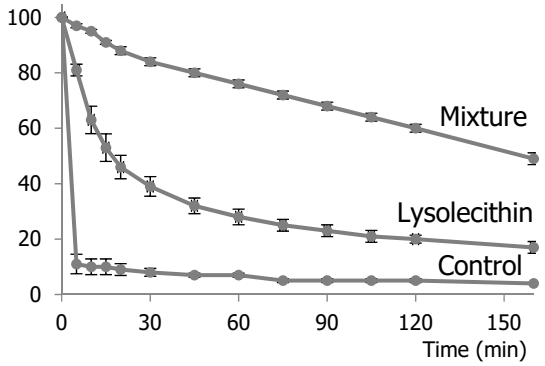

Figure 1: Emulsion stability (%; n=2).

Lipid hydrolysis. The apparent first-order rate constants for free fatty acid release (k, $\times 10^{-3}$ min^{-1}) were 10.00, 14.12 and 15.06 for the *in vitro* hydrolysis of animal fat (Control), animal fat with lysolecithin, animal fat with the mixture, respectively. The addition of lysolecithin or the mixture had a significant (P<0.05) impact on the free fatty acid release rate (Fig. 2). Addition of lysolecithin increased the free fatty acid release rate by 41%. Even though the same amounts of lysolecithin were added, the mixture was more successful and increased the free fatty acid release rate by 50%.

Lipid absorption. The absorption of monoglycerides generated during *in vitro* hydrolysis (Fig. 2) was significantly higher in lysolecithin and in the mixture (+121% and +142%, respectively) than in the Control. Similarly, the absorption of free fatty acids generated during *in vitro* hydrolysis was significantly higher in lysolecithin and in the mixture (+72% and +77%, respectively) than in the Control. The observed improvements confirm that indeed the lysolecithin is the active component for improving lipid absorption. Moreover, as the emulsifi-cation and hydrolysis process was significantly higher with the mixture than with lysolecithin alone, more fatty acids and other hydrolysis products would reach the enterocytes and the mixture thus would result in higher absorption of the hydrolysis products over all.

Broiler performance trial. At the starter period, ABW and ADG were significantly higher (18 g/bird and 1.3 g/bird/day, respectively) in birds fed LEX than in those fed NC (Table 2). Over the whole rearing period, the highest ABW and ADG were observed for birds fed LEX. Additionally, during the starter period, the FCR was significantly lower (-7 points) in birds fed LEX than in birds fed NC. Similarly, the

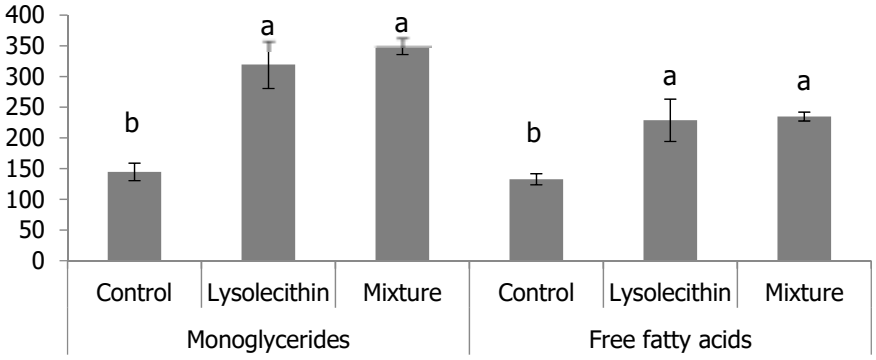

Figure 2: Absorption of monoglycerides and free fatty acids generated during *in vitro* hydrolysis of animal fat (Control), animal fat with lysolecithin and animal fat with a mixture of lysolecithin, monoglycerides and synthetic emulsifier (LYSOFORTE® EXTEND) by Caco-2 cells and expressed as mg per g of applied monoglycerides and free fatty acids (n=3). [a-b] Values within one parameter with different superscripts are significantly different (P<0.05).

FCR over the whole rearing period was reduced (P>0.1) by 6 points with the addition of the mixture. Hence, due to a better nutrient digestion and absorption, birds fed a diet supplemented with the mixture were able to recover an energy gap of 60 kcal/kg in the starter diet and of 80 kcal /kg in the grower and finisher diets. The carcass yield and breast yield were significantly higher in birds fed PC diet or LEX than in birds fed NC (Table 2). Moreover, the abdominal fad pad content of birds fed the LEX was significantly lower than in birds fed PC or NC. The latter suggests that addition of the mixture resulted in a better utilization of the absorbed nutrients for meat production.

Table 2. Growth and slaughter performance of broilers fed a basal diet fulfilling all dietary requirements (PC), a basal diet with lower metabolizable energy (NC) or a basal diet with lower metabolizable energy with 500 ppm of a mixture of lysolecithin, monoglycerides and synthetic emulsifier (LEX; LYSOFORTE® EXTEND dry, Kemin Europa NV, Herentals, Belgium)

Treatment	Average body weight (g/bird)		Average daily gain (g/bird/day)		Feed conversion ratio		Carcass yield (%)	Breast yield (%)	Abdominal fat pad content (%)
	14 days	42 days	0-14 days	0-42 days	0-14 days	0-42 days			
PC	402^{bc}	2754	26.1^{bc}	64.7	1.22^{bc}	1.61	72.8^{a}	28.9^{b}	1.07^{a}
NC	397^{c}	2686	25.7^{c}	63.1	1.26^{c}	1.67	71.6^{b}	27.6^{c}	1.19^{b}
LEX	415^{ab}	2775	27.0^{ab}	65.2	1.19^{ab}	1.61	72.6^{a}	28.9^{a}	0.91^{a}

[a-c] Values within columns with different superscripts are significantly different (P<0.05)

Conclusion

The addition of selected quantities of synthetic emulsifier and monoglycerides to lysolecithin and applying it as a mixture (LYSOFORTE EXTEND) resulted in improved *in vitro* lipid emulsification and hydrolysis. The mixture more than doubled the absorption of monoglycerides and increased the absorption of free fatty acids by more than 75%. With the use of this mixture, diets could be reduced with 60 to 80 kcal /kg, while maintaining the same performance and slaughter yield of the broilers. Moreover, as a result of better utilization of the absorbed nutrients for meat production, birds fed a diet with the mixture had a lower abdominal fat pad than those fed the positive control diet.

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Festphasenfermentation von Weizenkleie zur Verbesserung der Verdaulichkeit für Legehennen

Solid-state fermentation as a valorization strategy of wheat bran - a preliminary study

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Abstract

Cereals play an important role in human and animal nutrition. In parallel with growing population, the demand for cereals and cereal-based food has constantly increased. In this context, cereal technology, especially the milling process, can be regarded as one of the key technologies in the food supply chain. However, the need for sustainable use of bio-based products also has to take into account the side-streams of the Agrifood chain. Among this, wheat bran accumulates at high quantities attaining around 150 million tons per year world-wide. Due to this fact, there is some increasing need for innovative strategies to sustainably utilize and valorize wheat bran. Despite its nutritionally valuable compounds (13-18% protein, 10-14% starch, 4% lipids and 7% minerals), so far wheat bran has been mainly established as a low cost feed compound. The restricted implementation of wheat bran in animal diets results from its high amount of non-starch polysaccharides. In this respect this study was carried out to investigate the valorization of wheat bran by solid-state fermentation (SSF) using *Pleurotus* strains, having the capability to degrade lignocellulose through their lignocellolytic system. Pre-digestion of wheat bran via SSF may offer novel application fields in animal feeding, while mushrooms cultivation significantly contributes to modern nutrition.

Einleitung

Durch die weltweite Verknappung an Lebensmitteln steigt die Nahrungskonkurrenz von Mensch und Tier zusehends. Hinsichtlich einer zukunftsorientierten tierischen Produktionskette gewinnt die Nutzung von Nebenprodukten in der Tierernährung zunehmend an Bedeutung. Weizenkleie ist ein Nebenprodukt der Müllereiindustrie und fällt pro Jahr weltweit in Größenordnungen von bis zu 150 Millionen Tonnen an. Derzeit wird Weizenkleie vor allem als günstiges Futtermittel mit hohem Fasergehalt eingesetzt.
Um den Fasergehalt zu reduzieren oder zumindest dessen Verdaulichkeit zu verbessern gibt es verschiedene Vorbehandlungsmethoden. Ein sehr zukunftsorientiertes Verfahren scheint die Festphasenfermentation zu sein: Dabei wird mit bestimmten Mikroorganismen wie Pilzen oder Bakterien das Substrat durch den enzymatischen Abbau von Cellulose und Lignin vorverdaut.
Der Einsatz von Pilzen verfolgt zwei Ziele: Einerseits können wertvolle Speisepilze auf Weizenkleie erzeugt werden. Andererseits wird das Substrat besser verdaulich und kann für die Ernährung monogastrischer Nutztiere in höheren Mengen eingesetzt werden. Besonders Legehennen reagieren aus Sicht der Nährstoffverdaulichkeit sensibel auf eine zu hohe Zufuhr an Nahrungsfaser, weshalb es bei der Eiproduktion wünschenswert wäre die Weizenkleie „vorzuverdauen", um keine Leistungseinbußen zu provozieren. Für den menschlichen Konsum kann durch die Fermentation von Weizenkleie eine gute

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Versorgung mit hochwertigem Protein in Form von Eiern aber auch dem Nebenpunkt Pilz gewährleistet werden. Gleichzeitig wird durch die Supplementierung von hoch verdaulichem Getreide durch Weizenkleie im Legehennenfutter die Nahrungskonkurrenz (Mensch – Nutztier) vermindert.

Material und Methoden

Dieser Pilotversuch wurde an der Universität für Bodenkultur am Christian Doppler Labor für innovative Kleie Bioraffenerie durchgeführt. Die Fermentation mit Pilzen wurde im Doppelansatz in Substratsäcken mit 98% Kleie und 2% Calciumcarbonat durchgeführt. Um optimales Wachstum zu gewährleisten wiesen die Säcke einen Wassergehalt von 64% auf. Nachdem diese autoklaviert wurden um Kontaminaten abzutöten, wurden sie mit 5% Körnerbrut von *Pleurotus eryngii* oder *Pleurotus ostreatus* beimpft und bei 23°C und 85% Luftfeuchtigkeit für 21 Tage inkubiert. Danach wurde die Temperatur auf 18°C gesenkt und die Luftfeuchtigkeit auf 95% erhöht um die Fruchtung einzuleiten. Schließlich wurden Rohasche, Rohfett, Rohprotein, Trockenmasse, Acid detergent fiber (ADF), Neutral detergent fiber (NDF) und Phytatgehalt vom nativen sowie fermentierten Substrat bestimmt. Die Viskosität wurde mit einem Viskosimeter RVA 4500 (Perten Instruments, Hamburg) gemessen. Zusätzlich wurden die Pilzausbeute und die Trockenmasse sowie Proteingehalt der Pilze bestimmt und die Biologische Effizienz nach Wang *et al.* (2013) berechnet: BE = (Gewicht der frischen Pilze (g)/ Substratgewicht (g). Um die Produktsicherheit zu gewährleisten wurde das fermentierte Substrat einem Mykotoxinscreening unterzogen. Alle Experimente und Analysen wurden im Doppelansatz durchgeführt. Die statistische Auswertung basierte auf Median und Range und wurde mit BiAS für Windows, Version 11 (Epsilon-Verlag, Frankfurt) durchgeführt. Um die Unterschiede zwischen nativem und fermentiertem Substrat zu berechnen wurde ein Wilcoxon-matched-pairs-test ($\alpha = 0{,}05$) durchgeführt.

Ergebnisse

Die Fermentation von Weizenkleie führte zu einem Rohproteinanstieg und einem Anstieg der Rohasche (*P. eryngii*: +24%; *P. ostreatus*: +38%). Im Gegensatz dazu sank der Rohfettgehalt um 63% in der Kleie, die mit *P. ostreatus* fermentiert wurde. Im Zuge der Fermentation mit *P. eryngii* wurde der Phytatgehalt um 69,2% verringert, während durch *P. ostreatus* der Phytatgehalt nicht merklich gesenkt wurde. Zusätzlich wurde eine Reduktion an NDF (*P. eryngii*: -19%; *P. ostreatus*: -23%) und ADF (*P. eryngii*: +6%; *P. ostreatus*: +9%) beobachtet. Allerding sank die Viskosität durch die Fermentation mit beiden *Pleurotus* Pilzen merklich (Abb. 1). Es wurde keine Belastung mit Mykotoxinen nachgewiesen. Verglichen mit herkömmlichen Substraten, wurde durch die Verwendung von Weizenkleie als Pilzsubstrat für *P. eryngii* der Proteingehalt, die biologische Effizienz sowie der Trockenmasseanteil der Pilze gesteigert. *P. ostreatus* bildete keine Fruchtkörper aus.

Diskussion

Beide Pilze, *P. eryngii* und *P. ostreatus* waren in der Lage auf allen Substraten ein Myzel auszubilden. Allerding konnte nur *P. eryngii* erfolgreich Fruchtkörper ausbilden. Laut Literatur benötigt *P. ostreatus* einen Kälteschock um die Fruchtkörperbildung zu triggern (Philippoussis 2009; Yildiz *et al.*, 2002). Aufgrund des Versuchsaufbaus und technischer Voraussetzungen wurde auf diesen Kälteschock in unserem Experiment verzichtet. Verglichen mit herkömmlichem Substrat wurde in unserem Experiment eine wesentlich höhere Biologische Effizienz des Weizenkleiesubstrates beobachtet. Diese Behauptung wurde auch von andern Autoren festgestellt (Wang *et al.*, 2001) und scheint vermutlich auf den erhöhten Anteil leicht verdaulicher Kohlehydrate in Form von Stärke zurückzuführen zu sein.

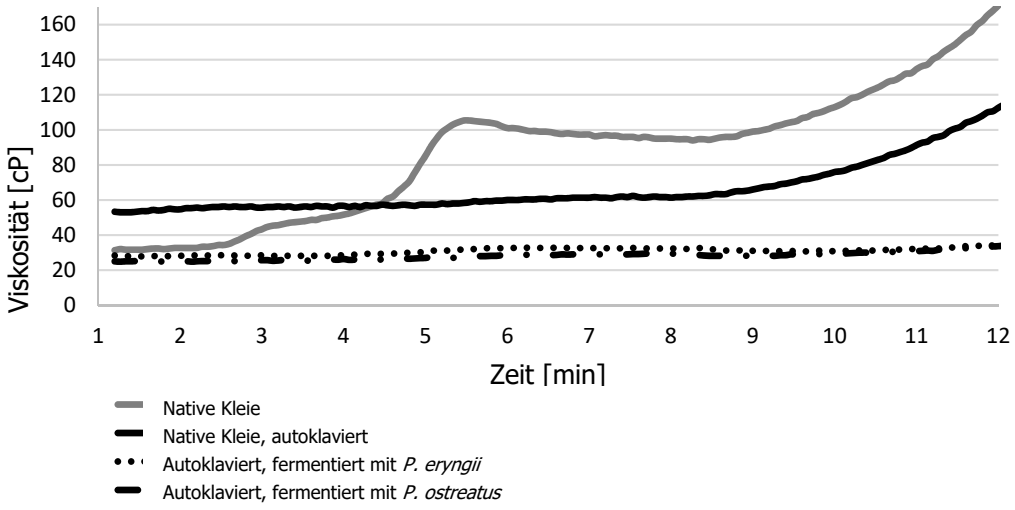

Abbildung 1. Viskosität von nativer, autoklavierter und fermentierter Kleie

Um den Abbau von nicht Stärke Polysacchariden (NSP) zu untersuchen wurde die Viskosität des nativen und fermentierten Substrates gemessen. Um den Einfluss von nativer Stärke auf die Viskosität auszuschalten, wurde analog zur Substrat Behandlung auch die native Weizenkleie vor der Messung autoklaviert. Wie erwartet verschwand der Gelatiniesierungspeak bei Minute fünf (Abbildung 1). Unabhängig vom Autoklavierungsprozess sank die Viskosität durch die Fermentation merklich, was auf einen Abbau von NSP durch die Pilze hindeutet. Bis dato werden hauptsächlich Enzyme wie Xylanase und beta-Glucanase eingesetzt um die NSP Verbindungen im Tierfutter abzubauen – gebundene Nährstoffe werden dadurch freigesetzt und Viskosität wird verringert (Koutrotsios *et al.*, 2014). Der Einsatz von *Pleurotus sp.* könnte deswegen eine natürliche Alternative zu den zugesetzten Enzymen darstellen. Phytat behindert die Aufnahme an Mineralstoffen im Darm und hindert Proteasen beim Proteinabbau. Die meisten Phytasen die in der Tierfütterung eingesetzt werden, werden aus Bakterien oder Pilzen gewonnen und es ist bekannt dass auch essbare Pilze diese Enzyme Produzieren (Collopy *et al.*, 2004; Selle *et al.*, 2007). In unserem Experiment konnte der Phytatgehalt der Weizenkleie durch die Fermentation mit *P. eryngii* drastisch gesenkt werden. Auf Grund dieser Tatsachen ist eine Fermentation als Vorbehandlungsstrategie ein adäquates Mittel um den NSP Gehalt zu reduzieren. Zusätzlich scheint der Abbau von Phytat durchaus mit der Effizienz von zugesetzten Enzymen vergleichbar.

Schlussfolgerung

Durch die Fermentation von Weizenkleie konnte eine Biologische Effizienz von 21,5% erreicht werden. Zusätzlich wiesen die Pilze einen höheren Protein und Trockenmasse Gehalt als herkömmliche Pilze auf. Die verminderte Viskosität und verringerten NDF Gehalte im fermentierten Substrat weisen auf einen Abbau von Lignozellulose hin und außerdem verringerte *P. eryngii* den Phytatgehalt der Weizenkleie deutlich.
Einerseits ist Weizenkleie ein geeignetes Substrat für die Kultivierung von Speisepilzen und andererseits wird durch die Fermentation die Eignung von Weizenkleie als Tierfutter verbessert.

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Changes of metabolizable energy content of functional group in permanent grassland after N fertilization levels

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Abstract

Intensive management of permanent grassland implements increasing N-fertilization which influences the proportion of functional groups in plant community. An experiment was established at AREC Raumberg-Gumpenstein (Austria) almost 50 years ago, and for the present study the period of 2014-2016 was evaluated. The field experiment is based on a randomized block design, with four replications. There were five different N-fertilization levels: treatment 1 – no fertilizer at all (control), treatment 2 - PK dynamic, treatment 3 - PK dynamic + 80 N, treatment 4 – PK dynamic + 120 N and treatment 5 - PK dynamic + 180 N. The plots were cut and harvested three times per year and beside bulk samples, additional separated samples of grass, legumes and herbs were taken. Fresh forage was dried at 58°C and milled to pass a 1 mm sieve. Metabolizable energy (ME) was calculated according the equation generated by GfE (2008). The results showed highly significant differences ($P<0.01$) among the treatments concerning the proportion of grass and legumes, ME concentration of grass, and total yearly ME production of grass and legume. The results of this experiment suggest that keeping a 3-cut harvest regime for highly fertilized treatments is not suitable to obtain higher ME content as MJ ME/kg DM, although higher DM yield is achievable. Increasing cutting frequency at higher N-fertilization level is therefore recommended for this long-term experiment.

Introduction

Grassland-based production systems play an important role in ruminant nutrition with low production costs in mountainous areas (Ferris, 2007; O'Donovan *et al.*, 2011). In those regions, permanent grassland is supposed to supply sufficient feedstuff for year-round feeding, particularly during summer season, and conserved forage (hay or silage) in the winter time. In order to maintain and increase productivity, intensively management is needed. Management (cutting regime and N-fertilizer application) clearly influence grassland productivity and quality as well (Nevens & Rehuel 2003). Applying N fertilizer to grass swards increases the proportion of grass and dry matter (DM) yield but it may inhibit legume growth. However, changing the botanical composition contributes to transform nutritive value of grassland. There are several indicators regarding nutritive value such as crude protein (CP), ME, ADF and neutral detergent fibre (NDF) (Van Soest, 1994; Loyra-Tzab *et al.*, 2011; Ball *et al.*, 2001). Obviously legumes provide higher nutritive value than grass, due to differences in fibre composition and protein content. Kolver & Muller (1998) reported that milk production correlates with ME intake and with protein which reaches the small intestine. The ME content of forages may be the limiting factor for higher production efficiency of grassland-based dairy cow diets. Therefore it is necessary to investigate the changes in ME content, which is provided by grassland to assist appropriate management strategies.

Material and methods

The experiment was established at AREC Raumberg-Gumpenstein, Austria in 1967 and the samples for the present study originate from the period of 2014 to 2016. The field experiment is following a block design with four replications. This long term experiment includes five different fertilization levels which are: T1 - no fertilizer at all; T2 - P (phosphorous) and K (potassium) dynamic (dynamic means that PK was applied according to the removal of the previous year), no N; T3 - PK dynamic + 80 kg N fertilizer/year; T4 - PK dynamic + 120 kg N fertilizer/year; and T5 - PK dynamic + 180 kg N fertilizer/year.

A 3-cut system was fixed for all treatments independent on the intensity of fertilization since 1993 (Table 1). Forage was cut 5-6 cm above ground and dry matter content (DM) of the bulk sample was determined after drying at 58°C for yield determination. In addition forage was separated manually into aliquot samples of functional groups (legume, grass and herbs). Approx. 200 g fresh samples were taken, dried, and milled in a Cyclotech mill to pass 1 mm sieve for further analysis. All samples were scanned using Perkin Elmer FT-IR/NIR spectrometer. ADF was determined using ANKOM 200 Fiber Analyzer apparatus. The method used to analyse ELOS adopting the technique by De Boever *et al.* (1988). The ME content was estimated according to the equation of GfE (2008).

$$ME = 5.51 + 0.00828\ ELOS - 0.00511\ CA + 0.02507\ CL - 0.00392\ ADF_{org}$$

where,
ELOS = soluble organic matter
CA = crude ash
CL = crude fat
ADF_{org} = organic acid detergent fibre

Table 1. Harvest dates for the experimental period

Year	Harvest dates		
	1	2	3
2014	26 May	22 July	30 September
2015	1 June	23 July	5 October
2016	25 May	21 July	28 September

All data were subjected to analysis of variance using the general linear model (GLM) procedure of SAS (2008). Differences were tested using the Tukey-Kramer test and were declared significant at $P<0.05$.

Result and discussion

The botanical composition clearly responded to the N-fertilization level (Figure 1-3). Grass is highly responsive to N-fertilizer, higher level of N-fertilizer consequently increases substantially the proportion of grass in permanent grassland ($P<0.01$). It can be seen from Figure 1 that in treatment 4 and 5 the proportion of grass was more than 80%. In contrast, legume proportion was reduced significantly due to the higher proportion of grass. This result is in line with McDonagh *et al.* (2017), who observed lower white clover proportion with increasing amounts of N-fertilizer. Despite this, the proportion of legumes was highest at T2, with P and K application only (Figure 2). With regard to Andrade *et al.* (2010) P and K fertilization tend to increase the percentage of legume.

The highest level of N-fertilizer resulted in the highest total DM yield (Figure 4). Table 2 provides data on DM yield, ME content and total ME yield of grass and legume functional groups. Grass contributes to DM yield performance more than legume. However, ME content of grass significantly declines by increasing N-fertilizer level ($P<0.01$), as the cutting frequency was kept constant among treatments.

The lowest ME content of grass was observed in treatment 5. There was no statistical difference on the ME content of legumes.

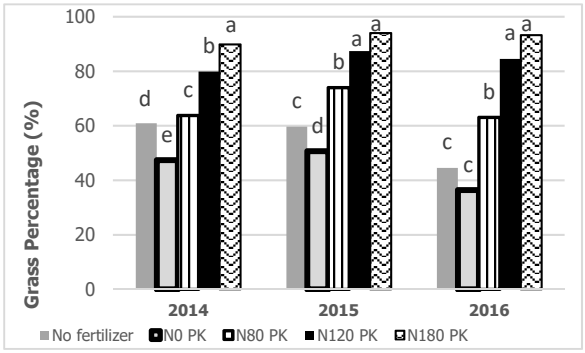

Figure 1. Average grass proportion per year

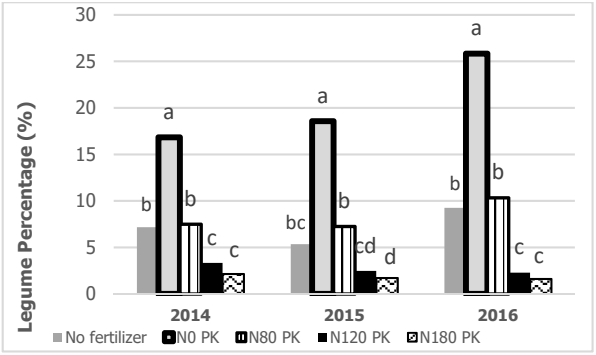

Figure 2. Average legume proportion per year

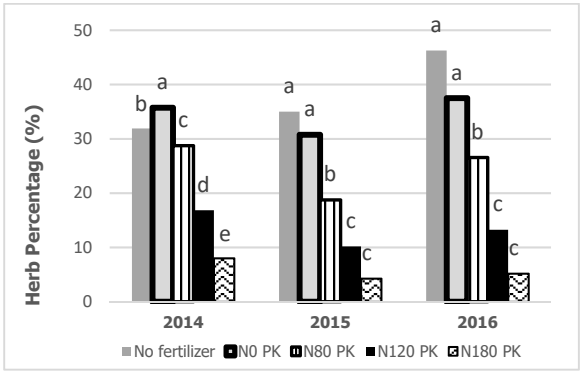

Figure 3. Average herb proportion per year

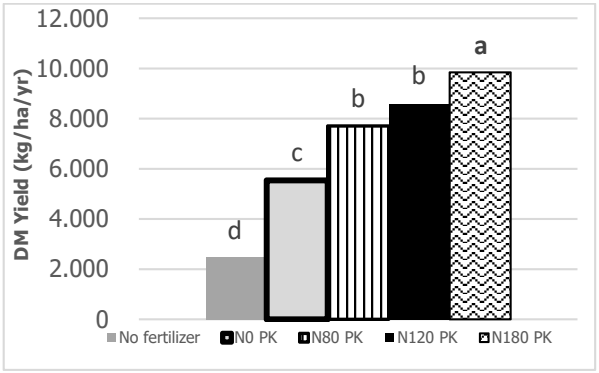

Figure 4. Average DM yield/ha (2014-2016)

Grass responded to N-fertilization and showed higher and faster growth rates than legumes and herbs, contributing to higher DM yields (Figure 4). However, at the same cutting date grasses were at different maturity stages in the observed treatments. We assume that lignin increases as part of the process of cell maturation, causing negative impact on organic matter digestibility, which again is affecting the ME content negatively (Table 2).

Table 2. Average yearly DM yield, ME content and ME yield of grass and legume (2014-2016)

	Functional Group	Fertilization level					SEM	P-value
		1	2	3	4	5		
DM yield (kg/ha)	Grasses	1,266^{e}	2,487^{d}	5,247^{c}	7,238^{b}	9,098^{a}	403	<0.0001
ME (MJ/kg DM)		9.56^{a}	9.31^{b}	9.07^{bc}	9.02^{c}	8.88^{c}	0.04	<0.0001
ME (MJ/ha)		12,070^{e}	22,926^{d}	47,480^{c}	65,202^{b}	80,772^{a}	3544	<0.0001
DM yield (kg/ha)	Legumes	221^{c}	1,236^{a}	684^{b}	250^{c}	188^{c}	63.8	<0.0001
ME (MJ/kg DM)		10.78	10.67	10.79	10.79	10.62	0.65	NS
ME (MJ/ha)		2,363^{c}	13,181^{a}	7,433^{b}	2,665^{c}	2,115^{c}	718	<0.0001

SEM: standard error of the mean
NS: not significant
Different superscripts (a-e) indicate significant differences between treatments

Grasses are the dominant functional group, and they substantially influence yield and the nutritive value of permanent grassland as well.

Conclusion

The increasing level of N-fertilization decreased the ME content of grass, whereas the ME content of legume remained at a constant level. The application of 180 kg N/ha and year in combination with PK dynamic fertilization resulted in highest total ME yield. The results of this experiment suggest that keeping the 3-cut harvest regime for highly fertilized grasslands is not suitable for higher ME content as ME MJ/kg DM, although high DM yield is achievable. Therefore higher N-fertilization level should absolutely be combined with increasing cutting frequency.

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Impact of the combination of synergistically acting probiotic cultures, organic acids and adsorbents to productivity and production quality of dairy cows

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Abstract

In the interest of animal health as well as future consumers, natural additives are increasingly being given to animals, such as probiotics, prebiotics and synbiotics. Defined as "natural growth promoters" stimulate growth and proper functioning of the body, which primarily affects the health of individuals as well as the productivity and consequently the quantity and quality of the products they receive.
A total of 20 Lithuanian black and white cows (10 animals in the control group and the test group 2 of 10 animals each) were selected for feeding test and fed with the experimental diets for 90 days. The experimental group of cows are fed with a diet added by the combination of synergistically active probiotic cultures, organic acids and adsorbents *Fibramax plus* (20 g/day). The results shown that Fibramax plus increased these parameters: cows productivity 11.7 % ($P>0.05$), fat content in the milk 0.37 % ($P>0.05$), protein content – decreased by 0.13 % ($P>0.05$) and lactose content decreased by 24 % ($P<0.05$) compared to the control group. The results of this study clearly demonstrate that combination of synergistically acting probiotic cultures, organic acids and adsorbents FIBRAMAX PLUS had positively effect on productivity and production quality of dairy cows.

Introduction

In intensive production systems, the nutritional requirements of livestock can be met through supplementation of the limiting nutrients in concentrated form, so that they can produce large quantities of products rapidly. However, the use of feedstuffs that are rapidly fermented in the rumen can create conditions in the rumen that are suboptimal for the fibrolytic microorganisms, thereby impairing fiber digestion (Beuchemin *et al.*, 2006). Moreover, nutritional quality of a feed is not only influenced by nutrient content but also by many other aspects such as,hygiene, content of anti-nutritional factors, digestibility, palatability and effect on intestinal health. Hence, the use of feed additives has been an important part of achieving this success (Fanelli, 2012). One of the most important feed ingredients is additives. These are substances, microorganisms or preparations that are intentionally added to improve the properties of feed or animal products, to meet the animal nutritional needs, to have a positive effect on the gastrointestinal microflora, feed digestibility (Eirich, 2017). Factors affecting the composition of milk are genetics, lactation stage, level of productivity, age of cows, environmental conditions, health and nutrition. Inheritance affects milk composition by 55%, the rest - 45%. Remain to environmental factors such as feeding (www.fao.org). The activeness of fermentation processes in the rumen content

is not constant, it may depend on feeding time, the composition of the diet, the quality of feed, the time elapsed after feeding, the movement speed of the large rumen content to the other parts of the gastrointestinal tract, and so on (Laugalis *et al.*, 2007a). Dairy farm managers have long noticed that it is worth considering not only milk research but also manure changes when changing cows' diet. This gave an incentive to analyse the excrement structure more closely. Adsorbents are substances that can absorb. This is a phenomenon of accumulation of some substance from gas or solution on the surface of a liquid and solid form. Adsorption is based on physical properties of some solids to selectively absorb and concentrate gas, vapour components or dissolved materials by the surface or volume of its pores. The absorbent component (substance) is called the adsorbent, while the adsorbed substance is addorbate (Migliarati *et al.*, 2007). In scientist literature about *Saccharomyces cerevisiae, Aspergillus oryzae, Kluyveromyces marxianus* and malic acid, there is absence about supplements effect for dairy cow's production quality, health and feed digestibility. So the aim of the trial was to investigate the impact of the combination of synergistically acting probiotic cultures, organic acids and adsorbents *Fibramax plus* on cow productivity, product quality parameters with feeding tests of dairy cows.

Material and methods

The experimental was conducted of 20 Lithuanian black and white cows (10 animals in the control group and the test group 2 of 10 animals each) were selected for feeding test (by age, performance and calving time). Healthy dairy cows housed and fed in equal conditions are selected for the tests. The farm cows are tied, watered from the automatic drinkers and being milked in lines and fed twice a day. The experimental group of cows are fed with a diet (Table 1) supplemented by the combination of synergistically active probiotic cultures, organic acids and adsorbents *Fibramax plus (*20 g/day)*.* The test was consist of two periods - the preparatory period of 14 days and the investigation period of 90 days. During control milking the amount of milk was determined the content of milk fat, protein, lactose and urine was determined by means of Lactoscope 550 and LactoScope FTIR device (FTI.O.2001; Delta Instruments, The Netherlands) using the infrared rays absorption method. The test of somatic cells number is performed using the meter Somascope working based on the principle of flow cytometry.
The acquired data of productivity, quality and composition of the milk, rumen were processed using Microsoft Office Excel 2010. Arithmetic averages of characteristics and the deviations of the averages will be calculated. The reliability of arithmetic averages difference (P) is determined according to the Student. The data are considered to be statistically significant when $P<0.05$.

Table 1.

	Content
Dry matter (DM) total consumption/kg	23.99
DM consumption from grass p., per cent., DM from g.p.	62.16
Neto energy lactation, MJ NEL/kg DM	6.79
Sugar, g/kg DM	33
Starch, g/kg DM	277
Resistent starch, g/kg DM	69
Sugar + non- resistent scratch, g/kg DM	241
Raw fat, g/kg DM	36
Raw proteins, g/kg DM	162
Available raw proteins	
g/kg DM	150
g.	3593
Indigestible proteins, percent. RP	20
Ruminal nitrogen balance, g	45
Raw fibre, g/kg DM	156
g/kg DM	131
g/100 kg DM k.s.	543
Acid detergent fibre ADF	187
Neutral detergent fibre NDF	306
Calcium	
g/kg DM	6.4
g.	153.5
Phosphorus	
g/kg DM	4.31
g.	103.5
Magnesium g/kg DM	2.66
Sodium g/kg DM	3.24
Potassium g/kg DM	16.42
Manganese g/kg DM	42
Copper g/kg DM	13
Cobalt g/kg DM	0.52

Specification of the *Fibramax plus*

Hydrated sodium calcium aluminosilicates (HSCAS)	72,460 %
Tannines	2,200 %
Fermentation extracts of Saccharomyces cerevisiae, Aspergillus oryzae, Kluyveromyces marxianus	7,890 %
Carrier & anticaking	7,650 %
BHT (butylated hydroxytoluene)	0,100 %
Calcium propionate	0,500 %
Malic acid (E296)	8,350 %
Essential oils, plant extracts and derivatives	0,850 %

Results and discussions

The main form of probiotic commonly used in dairy cows is various strains of yeast (mostly Saccharomyces cerevisiae). The most consistent effects following the addition of yeast cultures to the diet include improved productivity in both lactating and growing animals (Lettat *et al.*, 2012). Modification of rumen fermentation by using feed additives, such as antibiotics, has proved to be a useful strategy to improve production efficiency in ruminants (Anantasook *et al.*, 2015).

Data of performance parameters are given in Table 2. At the beginning of the experimental period, on average, there was more milk by 1.66 kg or by 5.16 % (P> 0.05). During the whole experimental period,

the average milk yield from cows of group I was more by 11.7 kg or by 14.63% (P> 0.05), compared to the control group. Quantity of basic milk during the experimental period (kg), in experimental group increased by 388.8 kg or 11.51%, compared to the control group (P<0.05). Average quantity of basic milk during the preparation and after experimental period (90 days), kg/d, in I period this parameter decreased by 2 % (P>0.05), but at the end of trial it increased by 11.7 % (P>0.05) compared to the control group.

Table 2: Impact of the combination of synergistically acting probiotic cultures, organic acids and adsorbents FIBRAMAX PLUS to dairy cows productivity

Parameter	Groups	
	I (control) group	II (experimental) group
At the beginning of the trial		
The change in productivity of cows, kg	32.20±6.09	33.86±10.04
After 90 days of the trial		
The change in productivity of cows, kg	26.24±3.90	29.12±4.95
Quantity of basic milk during the experimental period, kg	3377.4	3766.2
At the beginning of the trial		
Average quantity of basic milk during the preparation period, kg/d.	39.04	38.40
After 90 days of the trial		
Average quantity of basic milk during the after experimental period, kg/d.	37.45	41.85

Impact of the combination of synergistically acting probiotic cultures, organic acids and adsorbents FIBRAMAX PLUS to milk composition is presented in Table 3. At the beginning of the experimental period, the fat content in milk in the I experimental group was less by 0.2% at the end of the trial it increased 0.37 % compared to the control group (P>0.05). When analysed milk protein content, in whole trial period it decreased by 0.16 % (P>0.05) and 0.13 % (P>0.05) compared to the control group. Quantity of lactose various very low (P>0.05). Somatic cell content in the milk at the begging of the trial was 22 % lower, at the end of the trial – 24 % lower compared to the control group. Urea concentration during the preparation period decreased by 10 % (P>0.05), at the end of the trial – decreased by 24 % (P<0.05) compared to the control group.

Table 3. Impact of the combination of synergistically acting probiotic cultures, organic acids and adsorbents FIBRAMAX PLUS to milk composition

Parameter	Groups	
	I (control) group	II (experimental) group
Milk fat during the preparation period, %.	4.1±0.85	3.9±0.76
Milk fat after the experimental period period, %.	4.76±0.97	5.13±1.10
Milk protein during the preparation and period, %	3.33±0.32	3.17±0.36
Milk protein after the experimental period, %	3.62±0.30	3.49±0.29
Lactose quantity in the milk during the preparation period, %	4.76±0.14	4.78±0.13
Lactose quantity in the milk after the experimental period, %	4.54±0.11	4.51±0.13
Somatic cells content in the milk during the preparation period, thousand/ml.	59.60	46.22
Somatic cells content in the milk after the experimental period, thousand/ml.	141	107.2
Urea concentration during the preparation period, mg%	18.00±5.60	20.50±4.62
Urea concentration after the experimental period, mg%	16.20±4.66	17.80±8.39

Conclusions

The results of this study clearly demonstrate that combination of synergistically acting probiotic cultures, organic acids and adsorbents FIBRAMAX PLUS had positively effect on productivity and production quality of dairy cows.

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A rumen-protected capsicum product improves performance of dairy cows in early lactation: review of three controlled trials

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Abstract

The objective of this work was to assess the effect of a rumen-protected capsicum oleoresin (NEX, NexUlin, Pancosma, Switzerland) on performance of dairy cow in early lactation through a review of controlled trials. The productivity results of three published studies comparing a standard diet (Control) to the same diet supplemented with NEX were pooled using a mixed procedure. Selected outcomes were milk yield, milk fat and protein %, as well as energy corrected milk (ECM, standardized to 4.0% fat and 3.3% protein). Results showed that NEX increased milk and ECM by respectively 6.8% and 7.8% ($P < 0.10$), without affecting milk fat and protein levels ($P > 0.20$). The 90% confidence interval of the difference between NEX and Control was also determined for milk yield and ECM. The lower limit of this 90% confidence interval was of 664 and 703 g respectively, confirming that NEX has the potential to consistently improve performance and profitability of dairy cows during the first 100 days of lactation.

Introduction

Profitability of dairy farms relies on animal productivity and feed efficiency more than on milk price. Over the past 40 years, the level of production of dairy cows has reached production levels that were never thought to be achievable. Today, European economic indicators forecast an increase of total milk production by more than 7% between 2016 and 2026, combined to a decrease of the total number of heads (OECD/FAO 2017). This necessarily implies an additional improvement of cow's productivity by more than 10% for the same period. Until now, the rumen has been considered as the main driver of ruminants' efficiency. Many nutritional tools (feed management, raw materials, feed additives) has been developed and are now widely known to optimize rumen function, at a point that this organ may not be the limiting factor to further improvement in ruminants' performance and efficiency. Recently, the lower gut of the cow has become the new potential target to achieve this. Effectively, the partitioning of energy between maintenance, storage and production is mediated by post-absorptive pathways that can be modulated by additives having a post-ruminal effect. Among them, a rumen-protected capsicum oleoresin was shown to reduce glucose absorption by peripheral tissues of dairy cows in early lactation via a reduction of insulin secretion, increasing therefore the amount of glucose available for milk production (Oh *et al.* 2017). This should result in an improvement of cow's productivity in early lactation.

The objective of the present work was to evaluate the global effect of this additive on performance of dairy cows in early lactation via a summary of three controlled trials.

Experimental procedure

Since January 2016, a total of 3 trials assessing the effect of a rumen protected capsicum product (NEX, NexUlin, Pancosma, Switzerland) on Holstein cows' performance were published in peer-reviewed journals or during scientific conferences. Dose of supplementation was set at 100 mg/hd/d, and stage of lactation was restricted to less than 100 days in milk (DIM) at the start of the trials. This represented 3 side by side comparisons of NEX to an unsupplemented control (Control). Primary outcomes selected

for analysis were milk yield, milk fat and protein %, and Energy Corrected Milk production standardized at 4.0% fat and 3.3% protein and calculated according to the following equation:

$$ECM = \frac{(0.383 * fat\% + 0.242 * true\ protein\% + 0.7832) * kg\ milk}{3.1138}$$

Secondary outcomes consisted in the prevalence of subclinical ketosis, determined as the proportion of cows having a milk fat to protein ratio above 1.5, and the change in body weight between the end and the start of the trial.

The effect of the treatments on the primary outcomes was assessed using a mixed model with the study variable as a random effect and the treatment variable as fixed effect. Mean values were calculated using the LSMEANS procedure of XLstat, weighting the data for the variance among trials. For ECM and milk yield, the mean difference NEX relative to the control group and its respective 90% confidence intervals (CI) was calculated. This interval represents the range of improvement enabled by NEX in 90% of the situations.

Results and Discussion

Details of the studies involved in the analysis are given in the table.

	Oh *et al.* 2017		Wall et Bravo. 2016		Stelwagen *et al.* 2016	
Trial characteristics						
Number of animals	9 cows		67 cows		50 cows	
Design	Latin square design		Side by side comparison		Side by side comparison	
Duration	28 days		42 days		100 days	
Diet	Total Mixed ration		Total Mixed ration with ionophores and probiotic		Pasture + supplement with ionophores	
Outcomes						
Milk Yield (kg/d)	42.8	44.7	40.7	44.4	26.1	27.9
Milk fat %	3.89	3.92	4.40	4.50	5.07	5.08
Milk protein %	3.12	3.11	3.09	3.1	3.92	4.03
ECM (kg/d)	41.6	43.6	42.0	46.5	30.8	33.2
Subclinical ketosis (%)			22.1%	23.9%	10.2%	1.7%
Body weight change(kg)			29	32	-13	-17

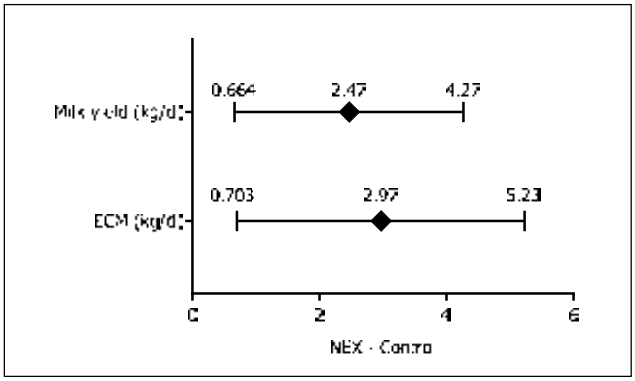
Results showed that in average, NEX increased milk yield by 6.8% (P = 0.06) but did not affect the concentration of fat and protein in milk (P > 0.20), as shown in the table. As a result, NEX supplementation improved ECM by 7.8% (P = 0.06).

Outcome	LSMEANS		Standard deviation	P-value
	Control	NEX		
Milk yield (kg/d)	36.5	39.0	8.5	0.06
Milk fat (%)	4.45	4.50	0.52	0.24
Milk protein (%)	3.38	3.41	0.45	0.43
ECM (kg/d)	38.1	41.1	6.2	0.06

These results confirm the beneficial glucose sparing effect of NEX on milking performance of dairy cows in early lactation (before 100 days in milk). This suggests that more dietary glucose is directed to the mammary gland, without any negative effect on milk composition. In addition, dry matter intake was reported to be un-affected in the two trials where cows were fed a total mixed ration, and capsicum oleoresin is known to have no effect on rumen function (Oh *et al.* 2015). Moreover, the effect of NEX on cows' performance seems to be independent from the presence or absence of a rumen modifier. Therefore the increased performance is mediated by a greater feed efficiency, itself driven by a post-ruminal effect of this product. This is in line its described mode of action on insulin secretion (Oh *et al.* 2017). In parallel, metabolic status seems to be un-altered by NEX supplementation, as revealed by no negative effect of the supplementation on the prevalence of subclinical ketosis and body weight change.

In other words, NEX re-routes dietary glucose to the mammary gland, which limits the pressure applied by the udder to mobilize reserves. This results into more milk, improved feed efficiency, without altering metabolic status of dairy cows in early lactation. More applied trials are currently on-going to validate this assumption.

Then, the 90% confidence interval of the difference between NEX and Control for milk yield and ECM are presented in the figure.



This suggests that in 95% of the situations, NEX improves milk yield and ECM respectively by at least 700 and 664 g/cow/d during the first 100 days of lactation. From an economic standpoint, if one considers a low milk price of 30 €/100 kg ECM recorded for Western Europe in 2017, this represents an increase in income for the farmer of 31 €/cow.

Conclusion

The supplementation of NEX to cows during their first 100 days of lactation has the potential to improve their performance without altering milk quality, and resulting in a better animal's profitability.

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Effekte unterschiedlicher Palmitinsäuregehalte in pansenstabilen Futterfetten auf das Fettsäuremuster im Milchfett von Holsteinkühen

Effect of different levels of palmitic acid in rumen stable fats on fatty acid composition of milk fat from Holstein cows

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Abstract

The recommended energy level in rations for high-yielding cows is often difficult to reach without disturbing the rumen. Rumen stable fats have become common feed ingredients in lactating cow rations because of their high energy content and versatility on farm and in concentrates. Typical fat products differ in type, origin and fatty acid profile. The fatty acid profile in milk fat is influenced (among others) by nutrition. The aim of this study was to evaluate the effect of different levels of palmitic acid in rumen stable fats on fatty acid composition of milk fat. After a control period without rumen fats four different fat supplements (A-D) were supplemented periodically (A: C16-rich (82%) fractionated palm fatty acid, B: palm fatty acid, hydrogenated (44% C16:0 / 55% C18:0), C: palm fat, hydrogenated (44% C16:0 / 55% C18:0) D: rapeseed oil, hydrogenated (92% C18:0)). The test was run on two farms, fat levels were 280 g/d (farm 1) and 430g/d (farm 2). Feeding C16-rich fractionated palm fatty acid increased C16:0-levels in milk fat. Feeding C18:0-rich (>50%) fat products increased C18:1 content in milk due to desaturation of C18:0 in the mammary gland. C18:0 content in milk fat only increased moderately. These results are confirmed by Loften *et al.*, 2014. Moderate proportions of C16:0 in rumen stable fats had no negative effect on milk fatty acid composition. Based on this study and reviewed literature, feeding of C16:0 should be limited to avoid negative effects on milk fat.

Einleitung

Das für hohe Milchleistungen benötigte Energieniveau in Rationen für Milchkühe kann häufig auch bei einer hohen Grundfutterqualität und Zulage von Kraftfuttermitteln nicht erreicht werden, da die Einsatzmenge von Kraftfuttermitteln zur Vermeidung einer Pansenübersäuerung und dadurch bedingter Folgeschäden begrenzt ist. Pansenstabile Fette ermöglichen aufgrund ihrer hohen Energiedichte eine energetische Aufwertung der Ration ohne die Pansenfunktion negativ zu beeinträchtigen und werden daher vielfach in der Praxis eingesetzt.

Aus der Literatur ist bekannt, dass die Fütterung einen Einfluss auf das Fettsäuremuster in der Milch hat. Die verschiedenen marktüblichen Futterfette unterscheiden sich teilweise erheblich in der Fettsäurezusammensetzung und haben dadurch einen unterschiedlichen Effekt auf die Fettsäurezusammensetzung in der Milch. Im Herbst 2017 wurde in den Niederlanden das Milchfettsäuremuster als zusätzliches Qualitätskriterium der Tankmilch eingeführt.

Der genaue Einfluss der Einsatzmenge und Art der eingesetzten Futterfette auf die Milchfettzusammensetzung ist jedoch noch nicht ausreichend bekannt. Ziel dieser Studie war es daher, einen Beitrag zur Klärung der Effekte unterschiedlicher Anteile an Palmitinsäure in pansenstabilen Futterfetten auf das Fettsäuremuster im Milchfett von Holsteinkühen zu liefern.

Material und Methoden

Auf zwei Milchviehbetrieben in den Niederlanden wurden in aufeinanderfolgenden Perioden nach einer Kontrollperiode ohne Fettzulage verschiedene pansenstabile Futterfette supplementiert, die sich u.a. im Palmitinsäuregehalt unterschieden. Je Betrieb gab es eine spezifische Kontrollration. Anschließend wurden die Mischrationen periodisch jeweils über mindestens 14 Tage mit den unterschiedlichen Fetten in gleicher Zulagenhöhe ergänzt. Als Fette standen pflanzliche Fettsäuren aus Palmöl (fraktioniert vs. hydrogeniert) und pflanzliche Fette als Triglycerid aus Palmöl bzw. Rapsöl (beide hydrogeniert) zur Verfügung. Alle Fette wurden im Sprühkühlverfahren in Pulverform überführt. Das Fettsäuremuster der eingesetzten Fette (Tabelle 1) unterschied sich besonders im Anteil an C16:0 und C18:0 und der Bindungsform (freie Fettsäure bzw. Triglycerid).

Tabelle 1: Fettsäureanteile in den eingesetzten Futterfetten (Anteile in %)

Anteil der Fettsäuren (%)	PFLANZENFETTSÄURE, FRAKTIONIERT (PALM)	PFLANZENFETTSÄURE, HYDROGENIERT[1] (PALM)	PFLANZENFETT, HYDROGENIERT[2] (PALM)	PFLANZENFETT, HYDROGENIERT[3] (RAPS)
freie Fettsäuren (FFA)	>95	>85	<2	<2
≤ C14:0	1	1	1	1
C16:0 (Palmitinsäure)	82	44	44	4
C18:0 (Stearinsäure)	3	54	54	92
C18:1 (Ölsäure)	11	-	-	-
C18:2 (Linolsäure)	3	-	-	-
≥ C20:0	-	1	1	3

[1] BEWI-SPRAY® 99 FA [2] BEWI-SPRAY® 99 M [3] BEWI-SPRAY® RS 70

Im Betrieb 1 wurden über die Perioden hinweg im Schnitt 80 laktierende Holsteinkühe mit Frischgras und einer zusätzlichen Mischration aus Maissilage, Grassilage, Pressschnitzeln und Konzentrat gefüttert. Die mittlere tägliche Milchleistung lag bei 31 kg. Die Fettzulage betrug 280 g pro Kuh und Tag.

Auf dem zweiten Betrieb wurden im Schnitt 140 laktierende Holsteinkühe gemolken (mittlere Tagesmilchmenge 33 kg) und die bestehende Ration aus Grassilage, Maissilage, CCM und Konzentratfutter wurde mit 430 g Fett pro Kuh und Tag ergänzt. Auf beiden Betrieben wurde am Ende jeder Periode eine Probe der Tagesmilch in Form von Käse (Betrieb 1) bzw. Tankmilch (Betrieb 2) erfasst. Nach standardisiertem Verfahren wurden die Proben aufbereitet, das Milchfett extrahiert und anschließend die Fettsäurezusammensetzung mittels Gaschromatografie bestimmt.

Ergebnisse

Im Laufe des Versuches konnten bei beiden Betrieben beim Einsatz des palmitinsäurereichen Futterfettes subjektiv rheologische Veränderungen bei der Käsegewinnung im Betrieb bzw. bei der Verarbeitung der Tankmilchprobe im Labor festgestellt werden.

Die Ergebnisse der Fettsäurenanalysen des Milchfettes der beiden Betriebe sind in Tabelle 2 (Betrieb 1) und Tabelle 3 (Betrieb 2) dargestellt. Im Sinne der Übersichtlichkeit werden nur die mengenmäßig relevanten Fettsäuren C6 bis C18:2 dargestellt.

Bei beiden Betrieben hatte die Fütterung der palmitinsäurereichen, fraktionierten Palmfettsäure eine Reduzierung der Anteile an C6-C12 und C14:0 zur Folge. Dies zeigte sich gegenüber der Kontrolle bei beiden Betrieben und bei Betrieb 2 auch gegenüber den anderen Fettvarianten. Demgegenüber war bei beiden Betrieben durch den Einsatz des C16-reichen Fettes der Anteil an C16:0 im Milchfett deutlich erhöht. Dies zeigte sich bei beiden Betrieben gegenüber der Kontrollperiode und allen anderen Fettvarianten. Mehr als 50% C18:0 im pansenstabilen Fett führte zu keiner Erhöhung bzw. einer Reduzierung der C16:0-Anteile und überwiegend zu einer leichten Erhöhung der C18:0-Anteile im

Milchfett. Der Einsatz der stearinsäurereichen (>50%) pansenstabilen Fette führte zu einer deutlichen Erhöhung der C18:1-Anteile im Milchfett.

Tabelle 2: Anteile ausgewählter Fettsäuren im Milchfett aus Käse von Betrieb 1 (Anteile in %)

	Kontrolle (ohne Futterfett)	Pflanzenfettsäure fraktioniert (Palm)	Pflanzenfettsäure hydrogeniert (Palm)	Pflanzenfett, hydrogeniert (Palm)	Pflanzenfett, hydrogeniert (Raps)
C6:0 - C12:0	9,7	7,5	8,7	7,5	7,2
C14:0	11,7	10,3	9,6	9,9	11,1
C16:0	32,0	36,2	29,3	30,1	29,8
C16:1	1,8	2,0	1,8	2,0	1,1
C18:0	8,7	8,4	10,1	9,5	10,4
C18:1	19,7	20,0	23,9	24,5	23,9
C18:2	1,9	1,7	1,6	1,8	2,1

Tabelle 3: Anteile ausgewählter Fettsäuren im Milchfett aus Tankmilch von Betrieb 2 (Anteile in %)

	Kontrolle (ohne Futterfett)	Pflanzenfettsäure fraktioniert (Palm)	Pflanzenfettsäure hydrogeniert (Palm)	Pflanzenfett, hydrogeniert (Palm)	Pflanzenfett, hydrogeniert (Raps)
C6:0 - C12:0	11,6	9,6	10,4	11,6	11,0
C14:0	11,4	10,3	10,5	11,0	11,7
C16:0	31,9	35,2	31,9	31,1	31,2
C16:1	1,7	1,8	1,7	1,7	0,5
C18:0	8,1	7,8	9,0	8,0	8,8
C18:1	18,3	18,7	19,8	19,7	19,8
C18:2	2,4	2,2	2,1	2,3	3,0

Diskussion

Im Milchfett findet sich ein breites Spektrum an rund 400 verschiedenen Fettsäuren. Es enthält praktisch alle gesättigten und ungesättigten, kurz-, mittel- und langkettigen Fettsäuren. Die allermeisten sind nur in Anteilen unter 1% vertreten. Ungefähr 50% der Fettsäuren des Milchfettes werden de-novo im Eutergewebe synthetisiert, die übrigen stammen ursprünglich aus dem verdauten Futter bzw. aus mobilisiertem Körperfett (bis zu 10%) (Palmquist und Jenkins, 1980). Fettsäuren von C4 bis C14 werden de-novo synthetisiert, C16:0 wird sowohl de-novo synthetisiert als auch direkt aus dem Blut absorbiert. Längerkettige Fettsäuren werden aus dem Blut absorbiert (in erster Linie C18:0). Ein Großteil der C18:1-Fettsäure stammt aus der Desaturierung von C18:0. Enjalbert *et al.* (1998, 2000) fanden bei direkter Infusion von C16:0 bzw. C18:0 ins Duodenum in Übereinstimmung mit anderen Fütterungsstudien beim Einsatz von C16:0-reichen Fetten deutlich erhöhte C16:0-Gehalte im Milchfett.

Im Gegensatz dazu wurde C18:0 aus Futter bzw. Duodenum-Infusion scheinbar nur zu 12% ins Milchfett überführt. Ein großer Teil von C18:0 wurde über Desaturierung in C18:1 umgewandelt. Betrachtet man C18:0 und C18:1 zusammen, wurden 50% der zugeführten Stearinsäure in Milchfett überführt.

In der aktuellen Untersuchung führte der Einsatz eines palmitinsäurereichen Futterfettes ebenfalls zu einer Erhöhung des Anteils an Palmitinsäure (C16:0) im Milchfett. Gleichzeitig reduzierte sich der Anteil der de-novo-synthetisierten C6-C12 und C14. Der Einsatz höherer Anteile (>50%) an Stearinsäure im Futterfett erhöhte hingegen nicht nur den C18:0-Anteil, sondern in erster Linie den Anteil an C18:1. Beim Einsatz moderater Anteile an C16:0 im Futterfett, wie in der vorliegenden Untersuchung mit hydrogeniertem Palmfett bzw. -fettsäure, zeigten sich keine negativen Effekte auf das Milchfett. Dies steht auch in Übereinstimmung mit der zusammenfassenden Auswertung bei Loften *et al.* (2014). Diese

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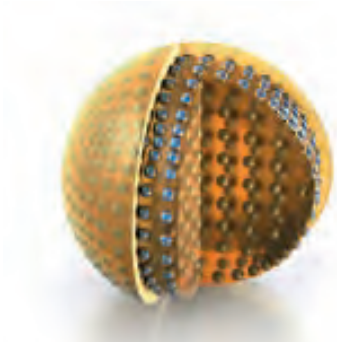
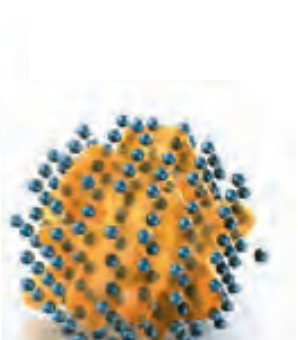


Matrixschutz, kein Abbau im Pansen



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Autoren schließen weiterhin aus der bisher vorliegenden Literatur, dass C18:0 sich (im Gegensatz zu C16:0) zu Zeiten negativer Energiebilanz nicht in der Leber ablagert, sondern in der Leber zur Energiegewinnung genutzt werden kann. Mangels alternativer Nutzung wird ein hoher Anteil der zugeführten C16:0 wieder über das Milchfett ausgeschieden oder in der Leber abgelagert. Der Einsatz von C16:0 im Futter sollte besonders in der frühen Laktation begrenzt werden.

Durch eine veränderte Fettsäurezusammensetzung im Milchfett ändert sich auch die Festigkeit des Milchfettes. Ein erhöhter Anteil an Palmitinsäure, die einen Schmelzpunkt von über 60°C hat, führt zu einem höheren Festfettgehalt (SFC). Milchfett mit höheren Palmitinsäureanteilen hat in der späteren Verarbeitung daher teilweise erhebliche Nachteile. Dies konnte bei beiden Betrieben im Versuch bestätigt werden.

Schlussfolgerung

Der Einsatz palmitinsäurereicher Futterfette führt zu einer Erhöhung des Anteils an Palmitinsäure im Milchfett, was in der Praxis negative Effekte auf die Verarbeitbarkeit der Milch haben kann. Dies konnte durch die vorliegende Untersuchung bestätigt werden. In den Niederlanden wurden daher bereits im Herbst 2017 Maßnahmen zur Begrenzung des C16:0-Anteiles eingeleitet. Der Einsatz palmitinsäurereicher Futterfette wird dabei auf freiwilliger Basis begrenzt (NZO & Nevedi, 2017) Beim Einsatz stearinsäurereicherer (>50%) Futterfette auf Palmöl bzw. Rapsöl-Basis konnte kein negativer Einfluss auf das Milchfett festgestellt werden. Der als positiv zu bewertende Ölsäure-Anteil im Milchfett wurde sogar erhöht. Die Auswahl des Futterfettes sollte daher zukünftig auch unter dem Aspekt der Beeinflussung des Milchfettes erfolgen.

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Effects of an encapsulated grape extract supplementation on the antioxidant and immune status of bovine colostrum

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Abstract

Colostrum is an essential element for the good health of calves during their first days of life because it will allow the transfer of immunity from the cow to the calf. The antioxidant supplementation is described as being able to support the immunity, in particular by increasing the immunoglobulins concentration. Grape polyphenols have a recognized antioxidant activity and a trial was carried out in order to study the effects of an encapsulated grape extract on the antioxidant and immune status of bovine colostrum.

Colostrum from control and supplemented cows were sampled and analyzed using DPPH method for the determination of antioxidant activity and radial immunodiffusion method for the determination of total immunoglobulin G. Results evidenced that the supplementation with an encapsulated grape extract significantly improved the colostrum's quality by increasing its antioxidant activity and immunoglobulin G concentration.

Introduction

The immunity of calves is an extremely important lever to ensure their good health. At birth, calves are born agammaglobulinemic, which means that they are totally devoid of immune defenses due to a placentation which doesn't allow the transfer of immunoglobulins from the mother to the fetus during pregnancy (Quigley, 2004). Indeed, in cattle, the placenta is epitheliochorial, meaning that six placental tissue structures separate the mother and the fetus bloods. Molecules weighing more than 150 kDa such as immunoglobulin G (IgG) cannot pass through it (Jacques, 2012). Colostrum uptake thus represents the main route of IgG transfer from the cow to the calf. Colostrum indeed contains, among the total nitrogenous matter contained in colostrum (160 g/kg), 87.5% of proteins, of which, both casein (34.3%), and soluble proteins which are albumin (6.4%) and immunoglobulins (42.9%) (Foley and Otterby, 1978).
The good health of calves will then depend in part on the quality of the colostrum ingested. One of the main markers of the quality of colostrum is therefore its IgG concentration. It depends on many factors such as the amount of milk produced (dilution effect), the breed and the genetic potential of the animal, the rank of lactation, the duration of the maternal drying period, the health status, and the diet (Eichinger, 2014).
In addition to be an IgG transfer vector, colostrum also allows the transfer of compounds with antioxidant activity. Indeed, studies have shown that when cows were supplemented with antioxidants such as α-tocopherol (vitamin E), the concentration of α-tocopherol in colostrum was then higher (Weiss *et al.*, 1994, Meglia *et al.*, 2006).
Authors have also investigated the potential existence of a link between the antioxidant status of colostrum and immune status. Regarding the effects of selenium supplementation which has antioxidant properties, it has been shown to have a significant effect on the IgG concentration of colostrum (Sweker *et al.*, 1995), particularly depending on its intake level (Awadeh *et al.*, 1998). Finally, a study conducted by Panousis *et al.* (2001) showed that selenium and/or vitamin E positively influence the immune response, including the production of immunoglobulins.

Considering these elements, and the fact that grape extracts are rich in polyphenols (molecules with recognized antioxidant capacity), the objective of this study was to determine the effects of an encapsulated grape extract on the antioxidant and immune status of colostrum.

Materials and methods

In order to test the effects of an encapsulated grape extract supplementation on the antioxidant and immune status of colostrum, a study was conducted on 21 Holstein dairy cows which were on late-stage of gestation. The animals were separated into two groups, one of them was used as a negative control (CTL, 9 cows) and the other group (NG, 12 cows) received a commercial encapsulated grape extract (Nor-Grape by Pass®) supplement at a rate of 2g per cow per day. The supplement was introduced in the ration composed of roughage (hay, corn silage), nitrogen corrector (soy/rapeseed 70:30) and concentrate. The average supplement duration of the group was 16.5 days before the scheduled date of calving. A colostrum sample was recovered for each cow and frozen (-20°C) for later analysis.

Antioxidant capacity of the colostrum was measured using the DPPH assay. Briefly, 1 mL of colostrum was treated with 0.5 mL of glacial acetic acid diluted to 1/5. The mixture is vortexed during 1 min and then centrifuged at 7800g, 15 min. DPPH assay consists in dissolving 100 µL of the colostrum extract obtained with 3.3 mL of DPPH solution (0,2mM). The reaction was carried out in the dark for 30 minutes at room temperature. Once the reaction was complete, 1 mL of chloroform was added. The solution was then centrifuged 5 min at 3000g and the absorbance was measured at 515 nm. The antioxidant capacity was then expressed in mg Trolox equivalents/L.

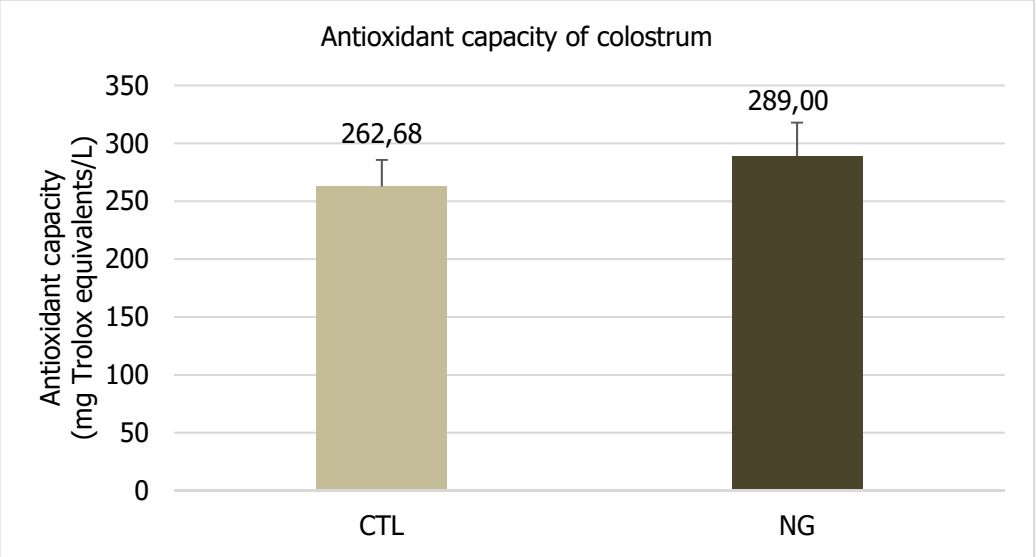
Immunoglobulin G determination was assessed via radial immunodiffusion method, using an analysis kit of bovine colostral IgG (IdBiotech®, France). The recommendations provided with the kit were followed. After depositing the samples, the plates were incubated in a humid box for 23 hours in a thermostatically controlled oven at 40°C.

Statistical analyses (Shapiro-Wilk normality test and Student t-test) were performed using R software (v. 3.4.3).

Results

The study of the colostrum's antioxidant capacity using the DPPH assay evidenced a significant increase of this parameter in samples obtained from supplemented cows (NG group, + 10%, $p<0.05$).

The determination of the colostrum IgG concentration showed that colostrum from control cows (CTL) had an average IgG concentration significantly lower than that of the NG group (49.3 g/L, and 77.5 g/L respectively, $p<0.05$).



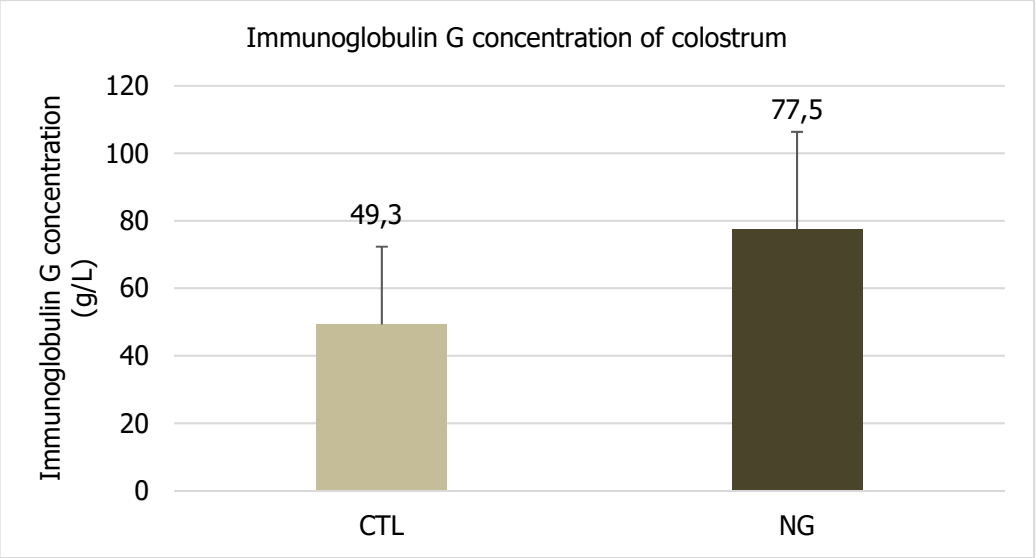


Figure 1.

Discussion

Results evidenced that the supplementation was beneficial in order to improve the colostrum's quality. The supplementation of 2g of encapsulated grape extract per day during the late-stage of gestation did provide stronger antioxidant and immune status of colostrum.
To our knowledge, very few studies have been carried out on the antioxidative status of bovine colostrum. Despite this, Przybylska *et al.* (2007) underlined the fact that antioxidant properties of colostrum seem to be as much important as nutritive or immunological properties.

Regarding the immunological status, the increase of the IgG concentration observed in samples from supplemented cows is consistent with a study conducted by Singh *et al.* (2013) which showed that a supplementation with antioxidant compounds (vitamin E) significantly increased the colostral IgG level of buffaloes.

Conclusion

The supplementation of dairy cows in a late-stage of gestation with an encapsulated grape extract has evidenced beneficial effects both on antioxidant and immunity status of colostrum produced. Such an increase in colostrum quality could therefore have interesting repercussions in suckling cows.

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Einfluss von Huminsäuren auf die Aufzuchtleistung und Gesundheit von Ferkeln

Influence of humic acids on the rearing performance and health of piglets

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Abstract

Based on a number of positive effects, humic acids have the potential to influence intestinal health positively. At the beginning of 2017 started a trial on a practical farm in the north of Germany. The aim of the trial was to record the effect of humic acids on the growth and health performance of weaned piglets under practical conditions. Altogether 335 piglets from a litter group were included in the feeding trial. The use of humic acids led to significantly higher daily weight gains. The piglets in the test group with humic acids added to the feed achieved 4.7 % higher daily weight gains than the control group. Accordingly, they weighed on average 1 kg more at the time of sale on rearing day 48. The piglets in the test group display a statistically significantly higher average feed intake. The difference between the control group and the test group was 4.9 %. No significant differences between the control group and the test group were ascertained on examining the health parameters in this trial. The loss rates in both groups during the trial period were 0.00 %.

Einleitung

Die Zeit nach dem Absetzen stellt für Ferkel aufgrund von zahlreichen Stressfaktoren (Trennung vom Muttertier, Transporte, neue Umgebung, Rangkämpfe, Wechsel von Milch auf Festfutter, u.a.) eine sehr kritische Phase dar (ALTMEYER 2016, BENARBIA *et al.* 2016). Die größte Herausforderung der Ferkelaufzucht bildet der immer wiederkehrende Absetzdurchfall, der häufig eine antibiotische Behandlung nach sich zieht (GERHARDY 2017). Erfolgsversprechende Alternativen zum Einsatz von Antibiotika sind von hohem wissenschaftlichen Interesse und gewinnen somit an Bedeutung (BASSITTA 2016, BENARBIA *et al.* 2016). Die durch den Humifizierungsprozess entstehenden Huminsäuren bilden einen natürlichen Bestandteil von Boden und Braunkohle (ZIECHMANN 1961, WILDENHAIN 1969, KNAUF *et al.* 1983). Im Tier entfalten Huminsäuren viele nachgewiesene Wirkungseigenschaften. Dazu zählt die Schleimhautabdeckung im Gastrointestinaltrakt. Zudem entfalten Huminsäuren adsorptive, antiphlogistische und paraimmunologische Wirkungen (KÜHNERT u. HAUFE 2011).

Material und Methoden

Zu Beginn des Jahres 2017 wurde ein Fütterungsversuch auf einem Praxisbetrieb in Norddeutschland mit insgesamt 335 abgesetzten Ferkeln durchgeführt. Die Tiere wurden nach dem Absetzen in zwei

Gruppen auf zwölf Buchten zu je 28 Tieren pro Bucht aufgestallt. Um Geschlechtseffekte zu minimieren, bestanden die einzelnen Buchtengrößen aus je 14 männlichen und 14 weiblichen Tieren. Es sollte geprüft werden, ob die Zugabe von Huminsäuren zum Ferkelaufzuchtfutter einen Einfluss auf die Leistung und Gesundheit von Aufzuchtferkeln hat. Alle Tiere erhielten eine identische Standardration deren Futterzusammensetzung sich zwischen den Gruppen allein in der Zugabe der Huminsäuren, welche dem Futter der Versuchsgruppe zugesetzt wurden, unterschied. Die Dosierung betrug hierbei 0,3 % (Ferkelaufzuchtfutter 1) bzw. 0,2 % (Ferkelaufzuchtfutter 2 u. 3). Zu den erfassten Daten, die am 1., 14., 26. und 48. Tag erhoben wurden, zählten zum einen die Leistungsparameter tägliche Zunahmen, Futteraufnahme und Futteraufwand und zum anderen die Gesundheitsparameter Verluste, Medikamenteneinsatz, Verletzungen und Kotbeschaffenheit. Es erfolgte eine tierindividuelle Wiegung und Bonitierung. Da bei der Untersuchung zwei Faktoren (Futter- und Geschlechtseffekt) eine Bedeutung hatten, wurde bei der statistischen Auswertung der täglichen Zunahmen eine zweifaktorielle Varianzanalyse durchgeführt. Eine einfaktorielle Varianzanalyse diente zur Auswertung der Futteraufnahme und des Futteraufwandes. Ab einem p-Wert von $p \leq 0,05$ wurde die Nullhypothese, dass keine signifikanten Unterschiede zwischen Versuchs- und Kontrollgruppe bestehen, verworfen.

Ergebnisse

Leistungsparameter

Die Leistungsergebnisse in Tabelle 1 zeigen, dass der Einsatz von Huminsäuren zu signifikant höheren Tageszunahmen geführt hat. Die Ferkel der Versuchsgruppe wiesen zudem eine statistisch absicherbare höhere durchschnittliche Futteraufnahme, im Vergleich zur Kontrollgruppe, auf. Gleiches gilt somit auch für die Energieaufnahme.

Tabelle 1: Zusammenfassung der Ergebnisse (Gesamtzeitraum, Tag 0 – 48 nach dem Absetzen)

Parameter	Einheit	Kontrolle (n = 168)	Versuch (n = 167)	Differenz	p
Tägliche Zunahmen	g/Tag	457,16[a]	478,51[b]	21,35	0,007
Futteraufnahme	g/Tag	706,12[a]	740,87[b]	34,75	0,045
Energieaufnahme	MJ/Tag	10,87[a]	11,39[b]	0,52	0,047
Futteraufwand	kg/kg	1,55	1,55	0,00	0,736
Energieaufwand	MJ/kg	23,77	23,81	0,04	0,839

(Signifikante Unterschiede mit hochgestellten Zeichen (a, b) gekennzeichnet ($p \leq 0,05$))

Die Ferkel der Versuchsgruppe mit den zugesetzten Huminsäuren erzielten somit 4,7 % höhere tägliche Zunahmen und eine um 4,9 % höhere Futteraufnahme als die der Kontrollgruppe. Infolgedessen konnte ein um durchschnittlich 1 kg höheres Gewicht zum Verkaufstermin am 48. Aufzuchttag erreicht werden. Aufgrund der höheren täglichen Zunahmen und der höheren Futteraufnahme der Tiere, auf Seiten der Versuchsgruppe, lag der Futteraufwand bei Kontroll- und Versuchsgruppe auf gleichem Niveau (1:1,55). Bei Betrachtung des Energieaufwandes ergibt sich ebenfalls kein signifikanter Unterschied zwischen den beiden Gruppen.

Gesundheitsparameter

Die Bonitierungsergebnisse der Verletzungen haben gezeigt, dass nur ein sehr geringer Anteil der Tieren (2,39 %) Auffälligkeiten zeigte. Somit kann keine Aussage darüber getroffen werden, inwieweit Huminsäuren die Entstehung von Verletzungen und Nekrosen beeinflussen. Die Kotkonsistenz und der Medikamenteneinsatz wurde durch die Zugabe von Huminsäuren nicht signifikant beeinflusst. Mit Ausnahme eines direkt bei der Einstallung ausselektierten Ferkels, lag die Verlustrate bei beiden Gruppen während des Versuchszeitraums bei 0,00 %.

Diskussion

Am 48. Versuchstag konnte ein mittleres Endgewicht von 28,87 kg (Kontrolle) bzw. 29,87 kg (Versuch) erreicht werden. Somit konnte das von der DLG (2008) angegebene Ziel, einer Lebendmasse zwischen 28 bis 30 kg zum Ende der zehnten Lebenswoche, nahezu erreicht werden. Mit einer durchschnittlichen Gesamtzunahme von 21,49 kg und Tageszunahmen von 457,16 g erreichte die Kontrollgruppe das von der LFL (2014) und von STALLJOHANN *et al.* (2016) angegebene Ziel von über 450 g täglichen Zunahmen. Das hohe Leistungsniveau der Kontrollgruppe konnte die Huminsäurengruppe (478,51 g) übertreffen. Die signifikant höhere Futteraufnahme der Versuchsgruppe, in Phase 2 und auf dem gesamten Versuchszeitraum bezogen, kann mit der besseren Gewichtsentwicklung in der ersten Phase (Tag 1-14) und dem daraus resultierenden höheren Futteraufnahmevermögen erklärt werden. Als Folge der höheren Zunahmen und einer höheren Futteraufnahme der Tiere ergibt sich ein Futteraufwand, der zwischen Versuchs- und Kontrollgruppe vergleichbar ist und keine Unterschiede aufweist (1:1,55). Die LFL (2014) gibt zu dem Futteraufwand den Zielbereich < 1,8 (kg/kg) an. Der von STALLJOHANN *et al.* (2016) angegebene Zielbereich des Energieaufwandes von < 23 MJ ME je kg Zuwachs wurde mit 23,77 und 23,81 MJ ME nur knapp verfehlt. Die Bonitierungsergebnisse zeigen, dass im Laufe des Versuchszeitraumes nur ein sehr geringer Anteil an Tieren (2,39 %) Verletzungen an Ohren oder Schwanz aufwies. Somit kann in diesem Punkt keine Aussage darüber getroffen werden, ob Huminsäuren die Entstehung von Verletzungen und Nekrosen beeinflussen. Um eine Aussage darüber treffen zu können inwieweit Huminsäuren das Potential haben Verletzungen und damit das Risiko von Schwanzbeißen zu reduzieren, sind weitere, zukünftige Untersuchungen notwendig. Bei Untersuchungen mit Geflügel von ISLAM *et al.* (2005) konnte eine mortalitätssenkende Wirkung der Huminsäuren festgestellt werden. Da ab dem Versuchstag 1 bis zum Ende des Versuchszeitraumes keine Verluste zu beklagen waren, kann keine bestätigende oder wiederlegende Aussage dazu getroffen werden. So konnten die Zielwerte von der LFL (2014) (< 2 % Verluste) und von STALLJOHANN *et al.* (2016) (< 3 % Verluste) in dem Parameter Verluste sowohl bei den Kontroll- als auch bei den Versuchstieren mit Abstand erreicht werden (0,00 %). Die höheren Leistungen der Versuchsgruppe können sich möglicherweise damit erklären lassen, dass die Tiere dieser Gruppe eine bessere Darmgesundheit und daher eine höhere Futteraufnahmekapazität aufweisen konnten.

Schlussfolgerungen

Im Rahmen eines Fütterungsversuches führte der Einsatz von Huminsäuren zu folgenden Ergebnissen:

1. Die Ferkel der Versuchsgruppe (Huminsäuren) erzielten signifikant höhere tägliche Zunahmen als die der Kontrollgruppe (+21,35 g/Tier und Tag; $p = 0,007$).
2. In der Versuchsgruppe konnte eine signifikant höhere Futteraufnahme festgestellt werden (+34,75 g/Tier und Tag; $p = 0,045$).
3. Für die Parameter Futteraufwand, Verletzungen, Kotbeschaffenheit, Medikamenteneinsatz und Verluste ließ sich kein statistisch signifikanter Unterschied zwischen den Gruppen beobachten.

Die vorliegenden Ergebnisse lassen auf eine grundsätzlich hervorragende Gesundheitslage der im Versuch verwendeten Tiere schließen. Somit deutet sich an, dass Huminsäuren in Betrieben mit einer tendenziell guten Gesundheitslage und guten Leistungen zusätzliche positive Leistungseffekte erzielen können.

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Einfluss unterschiedlich hoher Gehalte an Natrolith-Phonolith im Ferkelfutter auf zootechnische Parameter und Blutbild

Effect of different levels of natrolite-phonolite in diets of weaned piglets on feed intake, growth performance and blood count

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Abstract

The aim of this study was to evaluate the effect of different levels of natrolite-phonolite in diets of weaned piglets on daily feed intake, daily weight gain, feed conversion ratio and blood count. The diets were supplemented with 0 (treatment A, control group), 8,000 (treatment B), 25,000 (treatment C) and 50,000 (treatment D) ppm of natrolite-phonolite. The average daily feed intake was 724 g in the control group and 735, 672 and 602 g in the groups with 8,000, 25,000 and 50,000 ppm natrolite-phonolite respectively. The average daily feed intake was significantly different between treatment A and D and treatment B and D. Piglets of the control achieve 480 g daily weight gain. The average daily gains in the groups fed natrolite-phonolite were 507 g (treatment B), 453 g (treatment C) and 338 g (treatment D). The differences between treatment A and D, treatment B and C and treatment B and D were significantly different. The feed conversion ratio was 1.51 kg/kg (control group), 1.47 kg/kg (treatment B), 1.48 kg/kg (treatment C) and 1.82 kg/kg (treatment D). The feed conversion ratio was significantly different between treatment B and all other groups.

Einleitung

Natrolith-Phonolith ist ein seit 1991 unter der Nummer E 566 ein zugelassener Futterzusatzstoff. Er ist der Gruppe der Bindemittel, Fließhilfsstoffe und Gerinnungshilfsstoffe zugeordnet. Aus der landwirtschaftlichen Praxis wird von weniger Problemen mit Durchfall und Schwanzbeißen berichtet, wenn Natrolith-Phonolith in das Ferkelfutter eingemischt wird. Hohe Anteile an diesem Zusatzstoff im Futter vermindern jedoch die Energie- und Nährstoffdichte im Ferkelfutter und können zu Leistungseinbußen führen. In vorliegender Untersuchung wurden deshalb unterschiedlich hohe Gehalte an Natrolith-Phonolith im Futter auf zootechnische Parameter getestet.

Material und Methoden

Der Fütterungsversuch wurde am Lehr-, Versuchs- und Fachzentrum Schwarzenau durchgeführt. Dazu wurden 80 frisch abgesetzte, schwanzkupierte Ferkel der Rasse Pi x (DL x DE) nach Lebendmasse (LM), Abstammung und Geschlecht ausgewählt und gleichmäßig auf Versuchsgruppen mit 0,0 %, 0,8 %, 2,5 % und 5,0 % Natrolith-Phonolith in der Ration aufgeteilt. Der Versuch gliederte sich in zwei Fütterungsphasen. Die Ferkel wurden in 8 Buchten zu je 10 Tieren auf Kunststoffspalten ohne Einstreu gehalten. Sie waren bei der Aufstallung im Durchschnitt 27 Tage alt und wogen ca. 8,8 kg. Pro

Behandlungsgruppe wurden 2 Buchten gemischtgeschlechtlich aufgestallt. Die Futterzuteilung erfolgte über Abrufstationen mit integrierter Futterverwiegung für das Einzeltier (Compident Station CID2006 MLP-Ferkel, Schauer Agrotronic GmbH). Die LM wurden wöchentlich am Einzeltier erfasst. In den Buchten wurde einmal pro Woche der Kot der Tiere von hart=1 bis 4=wässerig bewertet. Nach Abschluss der Versuchsphase wurden Blutproben gezogen. Es wurden die Gehalte an Spurenelementen und Mineralstoffen sowie die Aktivitäten von Leberenzymen bestimmt. Zur Beurteilung des Gesundheitsstatus wurden ein klinisches Blutbild sowie ein Differenzialblutbild angefertigt.

Die Ferkelaufzuchtfutter (FAF) wurden in der Versuchsmahl- und Mischanlage Schwarzenau hergestellt, in der Schraubmühle Volkach pelletiert und im Labor der Abteilung Qualitätssicherung und Untersuchungswesen der Bayerischen Landesanstalt für Landwirtschaft in Grub nach VDLUFA-Methoden analysiert (VDLUFA, 2012). Die Versuchsmischungen basierten auf Getreide, Sojaextraktionsschrot, Mineralfutter, Pflanzenöl und Fumarsäure (Tabelle 1).

Tabelle 1: Zusammensetzung, Gehalte an umsetzbarer Energie und analysierte Inhaltsstoffe der Versuchsrationen (Gehaltsangaben bei 88 % TM)

		Behandlung A		Behandlung B		Behandlung C		Behandlung D	
		FAF I	FAF II	FAF I	FAF II	FAF I	FAF II	FAF I	FAF II
Natrolith-Phonolith	%	0,0	0,0	0,8	0,8	2,5	2,5	5,0	5,0
Weizen	%	45,0	45,0	43,7	45,7	44,0	44,0	50,0	50,0
Gerste	%	30,0	32,0	30,0	30,0	27,0	29,0	17,5	19,5
Sojaextr.-Schrot, LP	%	19,0	17,0	19,0	17,0	19,5	17,5	20,0	18,0
Sojaöl	%	1,0	1,0	1,5	1,5	2,0	2,0	2,5	2,5
Mineralfutter[1)]	%	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0
Fumarsäure	%	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
Rohasche	g	58	49	55	53	69	69	91	89
Rohprotein	g	175	163	173	167	173	172	170	154
Rohfaser	g	35	36	30	35	31	30	27	31
Rohfett	g	32	31	37	32	39	36	30	40
Stärke	g	434	453	441	446	424	433	407	426
Zucker	g	26	23	26	24	26	25	27	22
aNDFom	g	114	117	111	116	117	103	120	109
ADFom	g	55	48	38	47	54	38	34	42
Umsetzb. Energie[2)]	MJ	13,31	13,31	13,61	13,32	13,37	13,37	12,97	12,96
Lysin	g	11,8	11,7	11,6	11,8	11,9	12,3	11,9	11,1
Methionin	g	3,4	3,0	3,3	3,4	3,4	3,4	3,2	3,1
Cystin	g	2,6	2,5	2,5	2,5	2,6	2,5	2,5	2,3
Threonin	g	7,1	6,8	7,2	7,3	7,4	7,5	7,3	6,8
Tryptophan	g	2,3	1,8	2,3	2,1	2,0	2,0	2,0	1,8
Kalzium	g	11,0	8,0	8,3	7,5	8,2	8,0	9,6	9,3
Phosphor	g	4,9	5,0	5,0	4,9	4,4	4,7	4,2	4,3
Natrium	g	2,1	2,3	2,5	2,4	3,0	3,1	4,4	4,3
Magnesium	g	2,2	2,1	2,0	2,0	2,0	1,9	2,1	1,9
Kalium	g	7,2	6,7	7,2	6,8	6,9	6,7	6,9	6,1
Kupfer	mg	136	140	145	157	146	158	151	153
Zink	mg	108	94	99	91	103	85	108	83
pH		5,4	5,3	5,3	5,2	5,2	5,2	5,3	5,3
SBV[3)]	meq	827	673	714	669	781	763	885	865

[1)]11 % Lysin; 3,0 % Methionin; 4,5 % Threonin, 0,4 % Tryptophan [2)]nach Mischfutterformel
[3)] Säurebindungsvermögen

Die analysierten Inhaltsstoffe sowie die nach Mischfutterformel ermittelten Gehalte an umsetzbarer Energie (ME) sind ebenfalls in Tabelle 1 zusammengestellt. Für die FAF von Phase 2 wurden die Gehalte an ME zusätzlich in Verdauungsversuchen ermittelt. Es ergaben sich Verdaulichkeiten der organischen Substanz von 90 % (Gruppen A, B), 89 % (Gruppe C) und 88 % (Gruppe D). In Abhängigkeit der Zulage von Natrolith-Phonolith stieg der Gehalt an Natrium von im Mittel 2,2 bzw. 2,5 g in Gruppe A bzw. B auf

3,1 g in Gruppe C und 4,4 g in Gruppe D an. Auch der Gehalt an Rohasche stieg von ca. 55 g in den Gruppen A und B auf 69 bzw. 90 g in Gruppe C bzw. D an.
Die statistische Auswertung wurde mit Hilfe des Statistikprogramm SAS 9.4 (SAS Institute Inc., Cary, NC, USA) mit der Prozedur GLM durchgeführt. Im Modell wurden als fixe Effekte die Behandlung, das Geschlecht und die Abstammung sowie die Interaktion von Behandlung und Geschlecht berücksichtigt.

Ergebnisse und Diskussion

In Tabelle 2 sind die täglichen Zunahmen, der Futterverbrauch sowie die daraus errechneten Futtereffizienzzahlen für die einzelnen Abschnitte sowie für die gesamte Aufzucht zusammengestellt. Die Versuchsfuttermischungen wurden ab Einstallung in das Versuchsabteil vorgelegt. Bis zum Versuchsstart konnten sich die Ferkel 5 Tage lang an die Abrufstationen gewöhnen. Während dieser Zeit erzielten die Ferkel einen LM-Zuwachs zwischen 300 g (Gruppen B, C, D) und 600 g (Gruppe A). Die Unterschiede waren in dieser Versuchsperiode statistisch nicht signifikant. Im Mittel des Versuchs wurden mit 507 g in Gruppe B die höchsten täglichen Zunahmen ermittelt. In der Kontrollgruppe A wurden 480 g und in Gruppe C 453 g erzielt. Die Unterschiede zwischen den Gruppen B und C waren statistisch signifikant. In der Versuchsgruppe mit der doppelten maximal empfohlenen Dosierung von 5 % Natrolith-Phonolith (Gruppe D) lagen die täglichen Zunahmen bei 338 g. Dieser Wert ließ sich gegenüber allen anderen Behandlungsgruppen statistisch absichern. Mit einem Futterabruf pro Tier und Tag zwischen 672 g in Gruppe C und 735 g in Gruppe B waren keine statistisch signifikanten Unterschiede zwischen den Gruppen A, B und C zu erkennen. In Gruppe D wurden 602 g pro Tier und Tag abgerufen. Gegenüber den Gruppen A und B war der Unterschied signifikant. In den Gruppen A, B und C ergaben sich beim Futteraufwand je kg Zuwachs Werte zwischen 1,47 und 1,51 kg. Diese unterschieden statistisch von Futteraufwand in Gruppe D mit 1,82 kg pro kg Zuwachs.

Tabelle 2: Aufzuchtleistungen, Futterverbrauch und Futteraufwand (LSQ-Werte)

		a	B	C	D	sign. p[1)]
Tiere/Ausfälle	n	19/1	20/0	18/2	17/3	
Lebendmasse						
Aufstallung	kg	8,8	8,8	8,8	8,8	0,988
Versuchsbeginn	kg	9,4	9,1	9,1	9,1	0,471
Beginn Phase II	kg	16,1^{ab}	17,1^{a}	15,8^{bc}	14,8^{c}	0,006
Versuchsende	kg	29,0^{ab}	29,9^{a}	27,7^{b}	23,0^{c}	<0,001
Tägliche Zunahmen						
Phase 1	g	322^{b}	378^{a}	316^{b}	272^{b}	0,002
Phase 2	g	645^{a}	642^{a}	597^{a}	407^{b}	<0,001
gesamt	g	480^{ab}	507^{a}	453^{b}	338^{c}	<0,001
Futterabruf pro Tag						
Phase 1	g	526^{a}	537^{a}	458^{b}	461^{b}	0,020
Phase 2	g	931^{a}	944^{a}	896^{a}	750^{b}	<0,001
gesamt	g	724^{a}	735^{a}	672^{ab}	602^{b}	<0,001
Futteraufwand pro kg Zuwachs						
Phase 1	kg	1,64^{bc}	1,44^{a}	1,49^{ab}	1,77^{c}	<0,001
Phase 2	kg	1,45^{a}	1,50^{a}	1,51^{a}	1,88^{b}	<0,001
gesamt	kg	1,51^{a}	1,47^{a}	1,48^{a}	1,82^{b}	<0,001

[1)] Irrtumswahrscheinlichkeit

Insgesamt wurden sechs Ferkel nicht in die Auswertung einbezogen. Vier Tiere mussten wegen Problemen mit den Gliedmaßen nach der 1. bzw. 2. Versuchswoche aus dem Versuch genommen werden. Betroffen waren die Gruppen A und D mit jeweils einem Tier und Gruppe C mit zwei Tieren. Zwei weitere Tiere aus Gruppe D wurden aufgrund von Minderwachstum gegen Versuchsende eliminiert. Eines dieser Tiere wurde bereits vorher aufgrund von Gliedmaßenproblemen tierärztlich behandelt. Die

Anzahl von Tierausfällen und die Häufigkeit der Anwendungen von Tierarzneimittel waren nicht durch die Behandlungsmaßnahmen bedingt. Auf die Konsistenz des Kots zeigten die steigenden Zulagen von Natrolith-Phonolith nur einen geringen Effekt. Im Mittel wurde der Kot mit der Note 2,0 (Behandlungen A und D) bzw. 2,1 (Behandlungen B und D) als optimal bewertet. Nekrosen an Schwänzen und Ohren sowie Infektionen an den Klauen wurden bei keinem Tier diagnostiziert. In keiner Bucht trat Caudophagie auf. Die untersuchten Blutparameter lagen im bzw. nahe am angegebenen Referenzbereich. Die Glutamatdehydrogenase (GLDH), die Glutamat-Pyruvat-Transaminase (GTP), das Totalprotein (TP), das Bilirubin (Bili) sowie die Konzentrationen an Kupfer (Cu) und Zink (Zn) lagen bei allen Behandlungsgruppen innerhalb des Referenzbereichs. Bei den Konzentrationen an Kalzium (Ca), Magnesium (Mg), Phosphor (P) und Eisen (Fe) gab es in einzelnen Gruppen geringfügige Abweichungen vom Referenzbereich. Gegenüber dem Referenzbereich höhere Werte wurden bei der Glutamat-Oxalacetat-Transaminase (GOT) und bei der Gamma-Glutamyl-Transferase (GGT) in allen Behandlungen gemessen. Bei der GOT, der GGT, beim TP sowie beim Ca wurden mit zunehmender Dosierung von Natrolith-Phonolith im Mittel höhere Werte im Blut analysiert, die sich z.T. statistisch unterschieden. Beim P verhielt es sich umgekehrt. Bei den übrigen Mineralstoffen und Spurenelementen, der GPT, der GLDH sowie beim Bili war kein bzw. kein gerichteter Effekt durch die steigende Zulage von Natrolith-Phonolith zu erkennen. Beim Blutbild bzw. Differenzialblutbild lagen die Erythrozyten (RBC), der Hämatokrit (HCT), das Erythrozytenmerkmal MCV (=mittleres corpuskuläres Volumen), die Lymphozyten (LYM), die eosinophilen Granulozyten (EOS), die basophilen Granulozyten (BASO) sowie die Thrombozyten (PLT) im Mittel aller Gruppen im angegebenen Referenzbereich. Geringfügige Abweichungen vom Referenzbereich zeigten sich bei den Leukozyten (WBC) in Behandlung B, bei den neutrophilen segmentkernige Granulozyten (NEU) in Behandlung B und C sowie beim Hämoglobin (HGB) in Behandlung A, B und C. Geringfügig unterhalb des Referenzbereiches lagen in allen Gruppen die Werte der Erythrozytenmerkmale MCH (= mittleres corpuskuläres Hämoglobin) und MCHC (=mittlere corpuskuläre Hämoglobin Konzentration). Oberhalb der Referenzwerte lagen in allen Gruppen die Retikulozyten (RETIC) und Monozyten (MONO). Statistische Unterschiede zwischen den Gruppen wurden bei 9 Parametern des Blutbildes ermittelt, jedoch war nur bei 2 Parametern ein gerichteter Effekt durch die steigende Zulage von Natrolith-Phonolith zu erkennen. Insgesamt zeigten sich durch die steigenden Zulagen von Natrolith-Phonolith keine bzw. nur geringe Auffälligkeiten.

Zusammenfassung und Schlussfolgerung

Natrolith-Phonolith lässt sich bis zu einem Anteil von 2,5 % ohne negative Effekte auf die zootechnischen Parameter im Ferkelfutter einsetzen. Die absolut besten Leistungen zeigten sich mit 507 g täglichen Zunahmen, 735 g Futterabruf pro Tier und Tag und einem Futteraufwand von 1,47 kg pro kg Zuwachs bei 0,8 % Natrolith-Phonolith im Futter. Gegenüber der Kontrollgruppe waren bei dieser Einsatzmenge die Leistungen in der Tendenz höher.

Literatur

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Autorenanschrift

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Einfluss eines Futterzusatzstoffes aus natürlichen Pflanzen-extrakten, Kräutern, Gewürzen und Gummi Arabicum auf zootechnische Parameter und Caudophagie bei Ferkeln

Effect of a feed additive based on plant extracts, herbs, spices and gum arabic in diets of weaned piglets on feed intake, growth performance and tail biting

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Abstract

The aim of this study was to evaluate the effect of a feed additive based on plant extracts from hops and licorice and gum arabic in diets of weaned piglets on feed efficiency, feed intake, growth performance and tail biting. The diets were supplemented with 0 and 500 ppm of the feed additive. Both diets were fed to piglets with long and shortened tails, therefore following groups were generated: Group CS (control, shortened tails), group CL (control, long tails), group FS (feed additive, shortened tails) and group FL (feed additive, long tails). Average daily gains were 516 g in group CS, 487 g in group CL, 486 g in group FS and 499 g in group FL. The differences between the groups were not significantly. Average daily feed intake was lower in the groups fed the feed additive (722 g in group FS and 744 g in group FL versus 813 g in group CS and 763 g in group CL). Therefore the feed conversion ratio (FCR) was better in the groups fed the feed additive (1.49 kg/kg in group FS and 1.51 kg/kg in group FL versus 1.59 kg/kg in group CS and 1.58 kg/kg in group CL). The feed additive showed no effect on tail biting.

Einleitung

Eine gesunde Darmflora und eine damit einhergehende effiziente Futterverwertung sind wesentliche Ziele in der Aufzucht von Ferkeln. Um diesen näher zu kommen wurde ein Futterzusatzstoff entwickelt, der die Futteraufnahme fördern und eine gesunde Darmflora sowie die Entwicklung der Darmzotten unterstützen soll. Dieser Zusatzstoff setzt sich aus natürlichen Pflanzenextrakten (aus Hopfen, Süßholzwurzel) sowie Gummi Arabicum zusammen. Für die Inhaltsstoffe sind antimikrobielle, präbiotische, schleimhautprotektive sowie beruhigende Wirkungen beschrieben. In der Mast von Puten zeigte dieser Zusatzstoff positive Effekte bezüglich der Einstreuqualität und der Fußballengesundheit (Korzekwa *et al.*, 2015). In vorliegender Untersuchung sollte geklärt werden, ob und wie sich der Zusatzstoff auf Futtereffizienz, Futteraufnahme und Leistung sowie auf das Schwanzbeißgeschehen bei Ferkeln auswirkt.

Material und Methoden

Der Fütterungsversuch wurde am Lehr-, Versuchs- und Fachzentrum Schwarzenau durchgeführt. Dazu wurden 40 schwanzkupierte und 40 nicht schwanzkupierte, abgesetzte Ferkel der Rasse Pi x (DL x DE) nach Lebendmasse (LM), Abstammung und Geschlecht ausgewählt und gleichmäßig auf folgende vier Versuchsgruppen aufgeteilt: Gruppe CS (Kontrolle, gekürzter Schwanz), Gruppe CL (Kontrolle, ungekürzter Schwanz), Gruppe FS (Futterzusatzstoff, gekürzter Schwanz) und Gruppe FL (Futterzusatzstoff, ungekürzter Schwanz). Die Ferkel wurden in 8 Buchten zu je 10 Tieren auf Kunststoffspalten ohne Einstreu gehalten. Die Futterzuteilung erfolgte über Abrufstationen mit integrierter Futterverwiegung für das Einzeltier (Compident Station CID2006 MLP-Ferkel, Schauer Agrotronic GmbH). Die LM wurden wöchentlich am Einzeltier erfasst. Der Versuch gliederte sich in zwei Fütterungsphasen, Phase 1 bis ca. 17 kg LM, Phase 2 bis ca. 30 kg LM. Während des Versuchs wurde der Kot der Tiere einmal pro Woche bonitiert (Note 1-4 von hart bis wässrig). Bei den Ferkeln mit ungekürzten Schwänzen wurde zweimal pro Woche der Verletzungsgrad der Schwänze dokumentiert.

Die Ferkelaufzuchtfutter (FAF) wurden in der Versuchsmahl- und Mischanlage Schwarzenau hergestellt, in der Schraubmühle Volkach pelletiert und im Labor der Abteilung Qualitätssicherung und Untersuchungswesen (AQU) der Bayerischen Landesanstalt für Landwirtschaft (LFL) in Grub nach VDLUFA-Methoden analysiert (VDLUFA, 2012). Die eingesetzten FAF hatten eine vergleichbare Zusammensetzung (Tabelle 1). Der Zusatzstoff wurde mit 500 g pro Tonne Futter eingesetzt.

Bei allen FAF wurde an jeweils vier Ferkeln die scheinbare Verdaulichkeit der Nährstoffe bestimmt. Die ermittelten Verdauungsquotienten (vgl. Tabelle 2) gingen in die Berechnung der Gehalte an umsetzbarer Energie (ME) ein.

Die analysierten Inhaltsstoffe sowie die ermittelten Gehalte an ME sind in Tabelle 1 dargestellt. Mit 11,9 bzw. 12,1 g (FAF I) und 10,8 bzw. 11,2 g (FAF II) ergaben sich beim Lysin nur geringe Unterschiede, die sich im Rahmen der Analysenspielräume bewegten. Auch die Gehalte an ME je kg Futter waren mit 13,4 bzw. 13,3 MJ im FAF I und jeweils 13,6 MJ im FAF II vergleichbar. Bei den weiteren Inhaltsstoffen zeigten sich ebenfalls nur geringe Unterschiede zwischen den Versuchsrationen der einzelnen Fütterungsabschnitte.

Tabelle 1: Zusammensetzung der Versuchsrationen sowie Gehalte an umsetzbarer Energie und analysierten Inhaltsstoffen (Gehaltsangaben bei 880 g TM)

		Ferkelaufzuchtfutter I		Ferkelaufzuchtfutter II	
		Kontrolle	Futterzusatzstoff	Kontrolle	Futterzusatzstoff
Weizen	%	35,5	35,45	37	36,95
Gerste	%	40,0	40,0	40,0	40,0
Sojaextr.-Schrot, HP	%	18,5	18,5	17,5	17,5
Sojaöl	%	1,0	1,0	1,0	1,0
Mineralfutter[1)]	%	4,0	4,0	3,5	3,5
Fumarsäure	%	1,0	1,0	1,0	1,0
Zusatzstoff	%	-	0,05	-	0,05
Rohasche	g	50	50	45	45
Rohprotein	g	170	175	166	170
Rohfaser	g	34	37	31	32
Rohfett	g	29	30	28	29
Stärke	g	443	431	455	444
Zucker	g	22	24	25	24
aNDFom	g	121	125	111	113
ADFom	g	49	52	46	46
Umsetzb. Energie[2)]	MJ	13,4	13,3	13,6	13,6
Lysin	g	11,9	12,1	10,8	11,2
Methionin	g	3,3	3,4	3,4	3,3
Cystin	g	2,5	2,6	2,4	2,5
Threonin	g	7,5	7,6	7,1	7,4
Tryptophan	g	2,1	2,0	1,7	2,2
Kalzium	g	7,4	8,0	6,4	6,3
Phosphor	g	4,2	4,6	4,5	4,2
Natrium	g	2,2	2,1	1,8	1,8
Magnesium	g	2,1	2,2	2,0	2,0
Kalium	g	7,5	7,9	7,5	7,7
Kupfer	mg	161	151	135	143
Zink	mg	106	101	112	121
pH		5,1	5,1	5,2	5,1
SBV[3)]	meq	632	639	592	591

[1)]10 % Lysin; 2,5 % Methionin; 3,5 % Threonin, 0,5 % Tryptophan [2)]aus Verdauungsversuch
[3)] Säurebindungsvermögen

Tabelle 2: Scheinbare Verdaulichkeit der Rohnährstoffe

		Ferkelaufzuchtfutter I		Ferkelaufzuchtfutter II	
		Kontrolle	Futterzusatzstoff	Kontrolle	Futterzusatzstoff
Trockenmasse	%	88	86	88	88
Organische Substanz	%	89	87	89	89
Rohprotein	%	85	86	87	87
Rohfett	%	82	79	80	81
Rohfaser	%	37	38	34	39
N freie Extraktionsstoffe	%	93	91	93	93

Ergebnisse und Diskussion

In Tabelle 3 sind die täglichen Zunahmen, der Futterverbrauch sowie der daraus errechnete Futteraufwand für die einzelnen Abschnitte sowie für die gesamte Aufzucht dargestellt.

Tabelle 3: Aufzuchtleistungen, Futterverbrauch und Futteraufwand (LSQ-Werte)

		Kontrolle (C)		Futterzusatzstoff (F)		
		kupiert (CS)	nicht kupiert (CL)	kupiert (FS)	nicht kupiert (FL)	sign. p[1)]
Tiere/Ausfälle	n	20/0	20/0	19/1	18/2	
Lebendmasse						
Aufstallung	kg	8,7	8,6	8,8	8,5	0,612
Versuchsbeginn	kg	9,8[a]	9,0[b]	10,0[a]	9,0[b]	<0,001
Beginn Phase 2	kg	17,9[a]	16,2[b]	17,4[a]	16,3[b]	0,002
Versuchsende	kg	31,0	28,9	29,9	29,4	0,192
Tägliche Zunahmen						
Phase 1	g	388	343	354	347	0,153
Phase 2	g	652	638	623	657	0,742
gesamt	g	516	487	486	499	0,483
Futterabruf pro Tag						
Phase 1	g	567	526	522	511	0,139
Phase 2	g	1071[a]	1011[ab]	931[c]	989[bc]	0,003
gesamt	g	813[a]	763[ab]	722[b]	744[b]	0,009
Futteraufwand pro kg Zuwachs						
Phase 1	kg	1,48	1,56	1,50	1,50	0,609
Phase 2	kg	1,67[a]	1,60[a]	1,51[b]	1,52[b]	0,006
gesamt	kg	1,59[a]	1,58[ab]	1,49[c]	1,51[bc]	0,026

[1)] Irrtumswahrscheinlichkeit

Ab Einstallung in das Versuchsabteil wurden die Versuchsfuttermischungen vorgelegt. Bis zum Versuchsstart konnten sich die Ferkel fünf Tage lang an die Abrufstationen gewöhnen. Während dieser Zeit erzielten die Ferkel mit ungekürzten Schwänzen einen geringeren LM-Zuwachs. Aufgrund der dadurch signifikant unterschiedlichen LM bei Versuchsbeginn wurde das Startgewicht als Kovariable in das statistische Auswertungsmodel mit einbezogen. Das Zunahmeniveau war mit knapp 500 g im Mittel aller Behandlungen als gut einzustufen. Signifikante Unterschiede zwischen den Behandlungsgruppen traten bei den täglichen Zunahmen nicht auf. Das Nichtkupieren der Schwänze hatte den stärksten Einfluss auf die Tageszunahme. In der Kontrollgruppe (C) reduzierte das Nicht-Kupieren die Tageszunahmen durchschnittlich um -29 g. In der Futterzusatzstoffgruppe (F) konnte der Effekt des Nicht-Kupierens kompensiert werden (+13 g).

Der Futterabruf aus den Stationen war in den Futterzusatzstoffgruppen mit 722 g und 744 g pro Tier und Tag niedriger als in den Kontrollgruppen mit 813 g und 763 g. Gegenüber der Kontrollgruppe mit schwanzkupierten Tieren war der Unterschied statistisch signifikant. Eine Erklärung für die reduzierte Futteraufnahme könnte die gewählte hohe Dosierung sein. Von den Bitterstoffen des Hopfens ist bekannt, dass sie - abhängig von der sonstigen Rationsgestaltung -in hohen Dosen verzehrsdepressiv wirken können. Beim Futteraufwand schnitten die Futterzusatzstoffgruppen mit 1,49 und 1,51 kg Futter pro kg Zuwachs deutlich günstiger ab als die Kontrollgruppen mit 1,59 und 1,58 kg Futter je kg Zuwachs. Die Unterschiede waren z.T. hoch signifikant. Der Zusatzstoff hatte also einen positiven Effekt auf den Wirkungsgrad des Magen-Darm-Trakts. Kaum Unterschiede wurden in der Bewertung der Kotbeschaffenheit festgestellt. In allen Gruppen wurde der Kot mit der Werten zwischen 2,0 und 2,1 als normal bewertet. Auf das Beißgeschehen zeigte der Futterzusatzstoff keine Wirkung. Aufgrund von Schwanzverbiss waren in der Kontroll- und Futterzusatzstoffgruppe jeweils 8 Ferkel nicht vermarktungsfähig. Als bedingt vermarktungsfähig wurden in der Kontrollgruppe 3 und in der Futterzusatzstoffgruppe 4 Ferkel eingestuft. Die Hopfenkomponente konnte in diesem Versuch ihre beruhigende Wirkung nicht zeigen. Aus im selben Versuchsabteil durchgeführten Fütterungsversuchen mit einer weiterentwickelten Zusatzstoffvariante des gleichen Herstellers ist bekannt, dass mit einer anderen Zusammensetzung der Hopfenkomponenten eine signifikante Verbesserung des Schwanzbeißgeschehens erreichbar ist (Wilke *et al.* 2017).

Zusammenfassung und Schlussfolgerung

Der Futterzusatzstoff verbesserte den Futteraufwand signifikant von 1,6:1 auf 1,5:1. Durch den Futterzusatzstoff wurden die Tageszunahmen in der Ferkelaufzucht nicht beeinflusst. Der Futterabruf aus den Futterstationen war durch den Zusatzstoff z.T. signifikant niedriger. Auf das Beißgeschehen bei nicht schwanzkupierten Tieren zeigte der Zusatzstoff keine Wirkung. Eine positive Wirkung auf die Futteraufnahme konnte nicht bestätigt werden.

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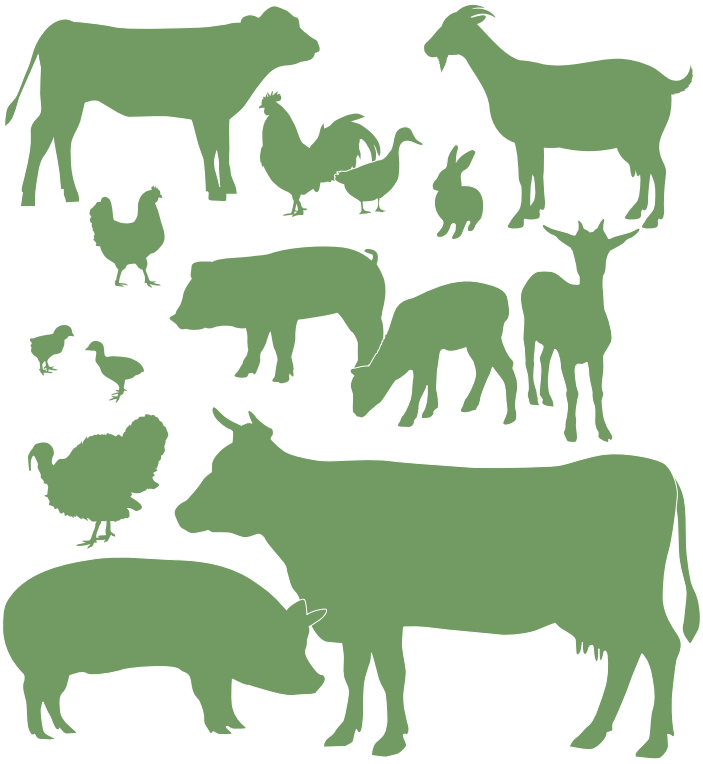

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Combination of a bacillus-based probiotic and yeast-based prebiotic improves fecal consistency and reduces individual antibiotic treatment in early weaned piglets

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Abstract

A feeding trial was conducted to investigate the effect of a combination of a defined probiotic (*Bacillus subtilis* + *B. licheniformis*) and a yeast-based prebiotic on intestinal health (fecal score) and zootechnical performance in early weaned piglets. Male and female early weaned piglets (DanZucht x Piètrain, 21 days of age, 5.66 ± 0.12 kg BW) were randomly allocated into two treatment groups (n = 4, 45 piglets per replicate). Piglets had *ad-libitum* access to feed and drinking water. Two different basal diets were fed (diet I: d 1-20, diet II: d 21-42). For control group the basal diets were without any pro- or prebiotic feed supplements. The treatment group received a combination of bacillus based probiotic and yeast-based prebiotic (Triple P®) with a dosage of 2 g per kg in diet I and 1 g per kg in diet II. In first week after weaning reduced fecal scores compared with normal values were observed in both groups. However, dietary supplementation of Triple P® improved fecal scores in first week after weaning and reduced the time period of reduced fecal scores compared with control. At day 7 after weaning Triple P®-fed piglets showed normal fecal scores of 4, whereby fecal scores from control piglets were reduced until day 15. Less individual antibiotic treatments were necessary in treatment groups compared with control. Average daily weight gain and average daily feed intake were numerically but not significantly higher in treatment group.

Introduction

Weaning is considered as one of the most significant events in the life of pigs accompanied by a high incidence of intestinal disturbances and diarrhea and finally by suppressed growth performance (Pluske *et al.*, 1997). Gastrointestinal disturbances immediately postweaning cause large economic losses in pig industry. Within the pig population, total losses of all those born in the EU amount to approximately 17% and a substantial proportion of these losses during suckling and weaning period can be associated with infections via intestinal mucosal surfaces (Lallès *et al.*, 2007).

For more than 50 years, sub-therapeutic dosed antibiotic growth promoter (AGP) have been an effective way to overcome these weaning problems and to maintain high production efficiency in pig production (Cromwell, 2002). Since the withdrawal of AGP as feed additives in the EU in January 2006, the attempts to identify alternatives have steadily been increasing, and new products and feeding concepts have been evaluated (Heo *et al.*, 2013; Gresse *et al.*, 2017). Furthermore, a reduction of therapeutic dosages of antibiotics is desired due to public concerns. At present, high dosages of zinc oxide (ZnO) are widely used in pig industry as they have been proposed to be one of the most effective feed additives to replace antibiotics. However, some disadvantages have been found when high dosages of ZnO were extensively used such as environmental pollution and increased risk of antibiotic resistance in gram-negative bacteria (Vahjen *et al.*, 2015; Gresse *et al.*, 2017). In December 2016, the European Medicines Agency's Committee for Medicinal Products for Veterinary Use proposed a ban of high levels of zinc oxide in swine rations (CVMP, 2016). As a result, a ban of highly dosed ZnO (2000 – 3000 ppm) may be expected in near future. Consequently, there is still an ongoing demand of finding non-antibiotic

alternative strategies to maintain intestinal microbial balance for reduced incidence of intestinal disturbances and improved weaning transition.

In contrast to the antimicrobial active feed additives such as AGP and ZnO, pre- and probiotics positively modulate the composition and activity of the intestinal microbiota with the result of a higher diversity (Gresse *et al.*, 2017). Consequently, pre- and probiotics are showing a high potential to be part of a non-antibiotic, alternative feeding strategy by improving and stabilizing intestinal microbiota and immune function. Therefore, the aim of the current study was to investigate the effect of a combination of a defined probiotic and a yeast-based prebiotic on intestinal health (fecal score) and zootechnical performance in early weaned piglets.

Materials and methods

The feeding trial was conducted at a commercial farm in Northern Germany. Early weaned piglets (DanBred x Piètrain, 21 days of age, 5.66 ± 0.12 kg BW) were randomly allocated into two treatment groups. The trial lasted six weeks in total (d 21 to 63 days of age). All piglets were housed in the same compartment with 8 pens and 45 piglets per pen (♂ + ♀). One pen was considered as a replicate (n = 4). Piglets had *ad-libitum* access to feed (mash form) and drinking water. Two different diets were fed (diet I: d 1-20, diet II: d 21-42). Diets were mainly based on wheat and soybean meal meeting the nutritional requirements of postweaning piglets as recommended by GfE (2006). During suckling period, piglets were offered a piglet milk (d 5-13) and later a commercial creep feed (d 14-21). The creep feed was also fed in first 5 days after weaning together with diet I. The control group did not receive any pro- or prebiotic feed supplements. The treatment group received a combination of highly dosed bacillus based probiotic (*Bacillus subtilis* + *B. licheniformis,* 1.3 x 10^9 CFU per g) and yeast-based prebiotic with a dosage of 2 g per kg in diet I and 1 g per kg in diet II (Triple P® 1000, Biochem Zusatzstoffe GmbH, Lohne).
Fecal scoring was performed every day on a pen basis. For fecal scoring the following subjective scoring system was used: 1 - liquid diarrhea; 2 - pasty feces falling out of shape upon contact with surfaces; 3 - formed feces, soft to cut; 4 - well-formed feces, firm to cut; 5 - hard and dry feces. Scores 1 and 2 were considered as signs of diarrhea, whereby score 4 represent optimal feces consistency. All piglets were monitored daily throughout the experiment for any abnormalities, abnormal behavior, and clinical signs of disease. Medical treatments such as antibiotic treatments and mortality were documented. Piglets with signs of diarrhea were colour-coded and treated individually with therapeutic doses of antibiotic (Enro-Sleecol® 50mg (Enrofloxacin), Albrecht, Germany) following the dosage and administration advices for piglets. The antibiotic was injected intramuscular. Piglets body weight and feed intake was documented on a pen basis at day 1, 21 and 42 of the experiment. The body weight and feed intake per piglet was estimated by dividing feed intake or body weight per pen by the number of piglets per pen. The feed conversion ratio (FCR) was calculated from feed intake per pen and the recorded body weight gain per pen for each period. Average daily weight gain (ADWG) and average daily feed intake (ADFI) was calculated by dividing weight gain or feed intake per piglet by the number of days for each period.
Results are presented as means ± standard deviation (SD) unless otherwise stated. Normally distributed data were analyzed by one-way ANOVA. The statistical analyses were performed with the software package SPSS (IBM SPSS Version 21). Differences at $P < 0.05$ were considered as significant, also P-values ranging from 0.06 to 0.10 were accepted as trends.

Results

In first week after weaning reduced fecal scores compared with optimum values (Score = 4) were observed in both treatment groups. However, dietary supplementation of Triple P® improved fecal consistency in first week after weaning (Figure 1.A.). Piglets from Triple P® group recovered much earlier

from the reduced fecal scores after weaning. At day 7 after weaning Triple P®-fed piglets showed normal fecal scores of 4, whereby fecal scores from control piglets were reduced until day 15. As a result of intestinal disturbances, antibiotic treatment was only necessary in the first two weeks after weaning. Less antibiotic treatments were needed in Triple P® group compared with control (-27%). Mortality was numerically reduced from 2.2% to 1.6% (Figure 1.B.).

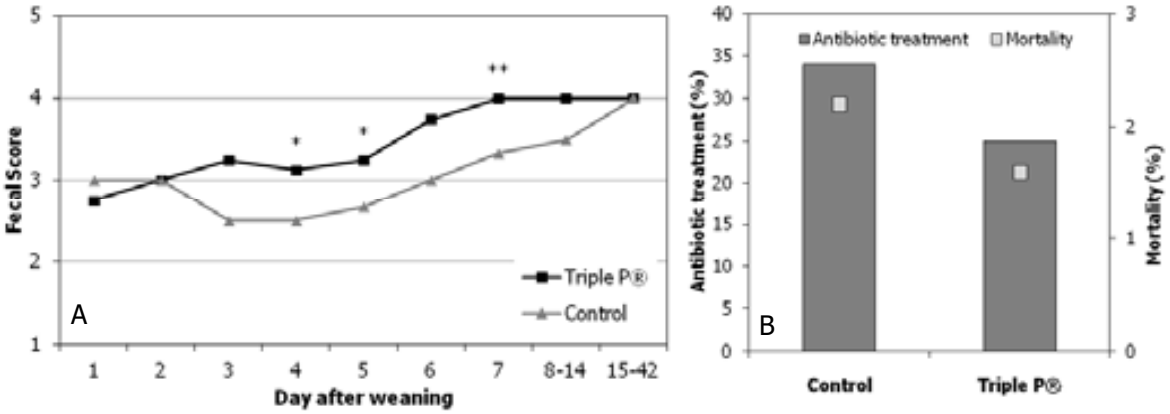

Figure 1. Effect of dietary supplementation of Triple P® on fecal consistency (A), individual antibiotic treatment from day 1 to 14 after weaning and mortality rate (B) in early weaned piglets, *P < 0.1; **P < 0.05

No statistical difference was observed in zootechnical performance. Piglets from control group weighed 8.37 ± 0.67 kg at day 20 and 23.43 ± 1.72 kg at day 42 and piglets from Triple P® group weighed 8.68 ± 0.62 kg at day 20 and 24.33 ± 1.60 kg at day 42. The results on ADWG and ADFI are shown in Figure 2. Feed conversion ratio was not influenced by dietary treatments (Control, 1.36 ± 0.08; Triple P®, 1.35 ± 0.04 from d 1 to 42 of experiment).

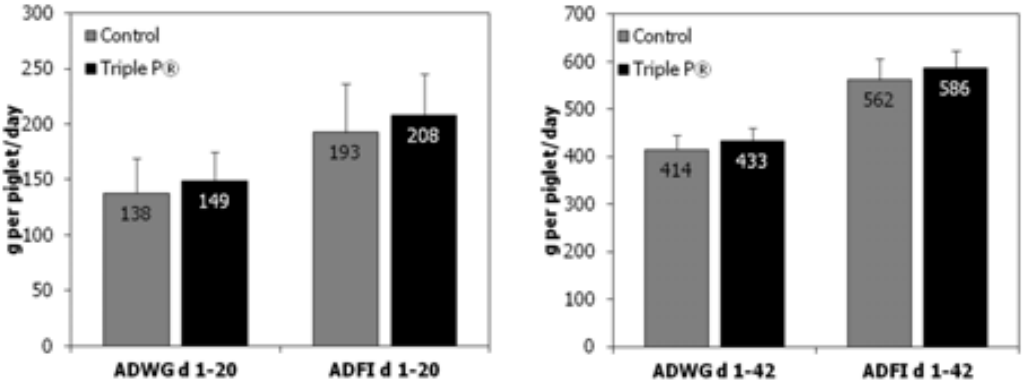

Figure 2. Effects of dietary supplementation of Triple P® on average daily weight gain (ADWG) and average daily feed intake (ADFI) in first feeding period (d 1-20) and total period (d 1-42) of the experiment

Discussion

In practice, postweaning diarrhea typically occurs in the first two weeks after weaning, often from day 4 to day 10. This time period can be considered as a critical period after weaning for intestinal disturbances and reduced growth performance. Triple P®, the combination of bacillus based probiotic (*B. subtilis* + *B. licheniformis*) and yeast-based prebiotic improved fecal scores in the critical period after weaning, which may indicates a lower incidence of intestinal disturbance. The period of reduced fecal

scores after weaning was markedly reduced in Triple P® group compared with control. At day 7 after weaning, Triple P®-fed piglets had significantly better fecal scores compared with control and fecal score was close to optimum value. Piglets from control group showed reduced fecal score until day 15 after weaning. Based on this results it can be assumed, that Triple P® reduces the incidence and the time period of intestinal disturbances in early weaned piglets. As a result, less individual antibiotic treatments and lower mortality can be observed. It should be considered, that fecal scores were already reduced on day 1 of the experiment. It is not clear, how far an earlier supplementation of Triple P® before weaning would have higher potential to improve fecal scores and growth performance during weaning transition. In the current study, zootechnical performance was only numerically, but not significantly, improved with Triple P®. However, the values for zootechnical performance as well as for fecal scores scattered considerably and more replicates would have allowed for a higher statistical power to investigate the effects on zootechnical performance. The low number of replicates was accepted due to practical reasons as this study was conducted on a commercial farm.

Conclusion

The symbiotic combination product Triple P® has been shown to improve fecal scores and to reduce the need for individual antibiotic treatments in early weaned piglets. Therefore, Triple P® has a high potential to be part of alternative concepts to antimicrobial feed additives such as AGP and ZnO. It should be considered, that pro- and prebiotics are more potent to prevent and not to treat intestinal disturbances such as postweaning diarrhea. A supplementation of Triple P® before and after weaning may allow even better effects and warrants further investigations.

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Positive Effekte der Xylanase 4a8 auf Leistung und Fußballengesundheit bei Broilern

Positive effects of Xylanase 4a8 on performance and foot pad health of broilers

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Abstract

The aim of this study was to investigate the effects of Xylanase 4a8 (Econase® XT; 9600 BXU/kg; 60 g/t feed) on broilers fed wheat based diets. In comparison with two multienzymes, the application of xylanase 4a8 led to considerable improvement of two major animal welfare criteria: litter quality and foot pad health.

Einleitung

Arabinoxylane sind der natürliche Hauptbestandteil von Nicht-Stärke-Polysacchariden (NSP) im Getreide. Insbesondere im Weizen liegen sie in sehr hoher Konzentration vor. Diese Moleküle sind für Geflügel kaum verdaulich und bewirken einen anti-nutritiven Effekt (Choct, 1990). Sie reduzieren die Bioverfügbarkeit von Nährstoffen und erhöhen die Viskosität der Ingesta. Das hat eine geringere Verdaulichkeit und schlechtere Einstreuqualität zur Folge (Choct, 1999). Die Tierleistungen sinken und das Risiko für Fußballenerkrankungen steigt (Fairchild, 2010). Daher werden Enzyme eingesetzt, die in der Lage sind, NSP zu spalten. Diese sind entweder Monoenzyme wie Xylanasen oder Multienzyme. In der Folge werden zusätzliche Nährstoffe verfügbar und die Zähigkeit des Verdauungsbreis sinkt.

Die vorliegende Studie hatte das Ziel, den Effekt von Econase® XT (Xylanase 4a8) auf Broiler bei einer weizenbasierten Fütterung im Vergleich zu zwei Multienzymen zu ermitteln. Dabei wurden die Auswirkungen auf die Einstreuqualität und Fußballengesundheit detailliert untersucht.

Material und Methoden

675 Eintagsküken der Linie Cobb 500 aus einer kommerziellen Brüterei wurden zufällig in fünf verschiedene Gruppen eingeteilt. Diese erhielten unterschiedliche Diäten mit neun Wiederholungen pro Diät, so dass sich in jeder Gruppe 15 Broiler befanden. Die Fütterung und Wasserversorgung der Broiler erfolgte *ad libitum*. Das Licht- und Temperaturprogramm entsprach den Empfehlungen der Zuchtorganisation. Die Versuchsgruppen wurden mit einer Weizen-Soja-basierten Diät gefüttert. Der ernährungsphysiologische Wert aller Gruppen entsprach dem der Kontrollgruppe. Die Versuchsdiäten enthielten entweder keine Enzymzulage (Negativkontrolle), Multienzymprodukte Multi A (100 g/t) oder Multi B (50 g/t) oder die Monoenzym-Xylanase 4a8 (Econase® XT) mit einer Dosierung von 60 g/t (9600 BXU/kg) (Tabelle 3). Alle Rationen wurden bei 80°C konditioniert und anschließend pelletiert. Die Fütterung erfolgte in drei Phasen: Starter von Tag 1 – 7 gekrümelt, Aufzuchtfutter von Tag 8 – 21 gekrümelt, Endmastfutter von Tag 22 – 42 pelletiert. Alle Diäten enthielten die Phytase Quantum® Blue (750 FTU/kg) die mit der von AB Vista empfohlenen Matrix eingesetzt wurde.

Am Ende jeder Fütterungsphase (Tag 21 und 41) wurden das restliche Futter und die Tiere gruppenweise gewogen. Daraus wurden die Lebendmassezunahmen, die Futteraufnahme und die um die Mortalität korrigierte Futterverwertungsrate (mFVW; g Futter/g Körpergewicht) errechnet. An Tag 21 erfolgte eine Bonitur der Einstreuqualität (Note 1 – 5, 1 = sehr gut, 5 = sehr schlecht). Der Zustand der Fußballen wurde an Tag 21 und am Versuchsende (Tag 42) mithilfe eines Bonitursystems (Note 1 – 3, 1= gesunder Fußballen, 3 = schwere Läsion) bewertet. Für die parametrischen Einflussgrößen (Tierleistung und Zustand des Tierkörpers) wurde eine Varianzanalyse (ANOVA) durchgeführt und der Mittelwert wurde mit dem Student's t-test ($p < 0,05$) untersucht. Nicht-parametrische Einflussgrößen (Einstreuqualität und Fußballendermatitis) wurden mit dem Kruskal-Wallis-Test berechnet und das Signifikanzniveau auf $p < 0,05$ festgelegt. Die statistische Auswertung wurde mit dem Softwareprogramm JMP Pro 13 durchgeführt.

Ergebnisse

Bezüglich der Mortalität gab es bei allen Gruppen keine Unterschiede ($p > 0,05$). An Tag 21 gab es außerdem keine Unterschiede bei den Tierleistungen ($p > 0,05$, Tabelle 1). Bei den Broilern, die mit einer Diät aus Weizen und Xylanase 4a8 gefüttert wurden, war am Tag 21 der Zustand der Einstreu am besten und der Grad der Fußballendermatitis am geringsten ($p < 0,05$, Tabelle 2). Bezüglich der Prävalenz der Fußballenläsionen gab es bei den Tieren mit enzymsupplementierter Diät unter den Enzymen keine Unterschiede. Am Tag 42 waren bei den unterschiedlichen Fütterungsregimen keine Unterschiede bei den Zunahmen zu sehen ($p > 0,05$). Die Broiler, die mit einer Weizendiät und Xylanase 4a8 oder Multi A-Gemischen gefüttert wurden, hatten die besten durchschnittlichen Futterverwertungsraten (mFVW). Die Tiere, die Weizen mit dem Enzym Multi B erhielten, wiesen durchschnittlich mittlere Futterverwertungen auf. Die schlechteste Futterverwertungsrate hatten Broiler bei Diäten ohne Enzyme ($p < 0,05$). Am Tag 42 war die Anzahl der Fußballenläsionen bei den Broilern geringer, die Futter mit Weizen und Xylanase 4a8 erhalten hatten. 98 % dieser Tiere hatten keine oder nur geringgradige Verletzungen im Gegensatz zu den Broilern, die eine Diät ohne Enzyme oder Multienzyme erhalten hatten.

Tabelle 1: Mortalitäts-korrigierte Futterverwertungsrate (mFVW) von Broilern, die mit einer weizenbasierten Diät mit unterschiedlichen Enzymen gefüttert wurden

Diät	Tag 1 – 21	Tag 1 – 41
	mFVW, g:g	mFVW, g:g
ohne Enzym	1,40	1,688[a]
Xylanase 4a8	1,40	1,635[b]
Multi A	1,40	1,622[b]
Multi B	1,39	1,652[ab]
P-Wert	0,45	< 0,01

Unterschiedliche Buchstaben in den Reihen zeigen statistische Unterschiede an ($p < 0,05$)

Tabelle 2: Mittelwerte der Einstreuqualitäten und Häufigkeiten der Fußballenläsionen bei Broilern, die mit einer weizenbasierten Diät mit unterschiedlichen Enzymen gefüttert wurden

Diät	21 Tage				41 Tage		
	Einstreu-qualität	Häufigkeit Fußballenläsionen, %*			Häufigkeit Fußballenläsionen, %*		
		A	B	C	A	B	C
Ohne Enzym	1,7[b]	92,6[a]	6,7[a]	0,7	14,5[b]	84,2[a]	7,8[b]
Xylanase 4a8	1,27[a]	96,6[a]	4,0[a]	0,7	25,5[a]	75,0[a]	1,2[a]
Multi A	1,54[b]	98,6[a]	2,7[a]	0,7	16,5[ab]	73,2[a]	10,4[ab]
Multi B	1,55[b]	96,6[a]	2,7[a]	0,7	16,5[ab]	75,2[a]	5,9[ab]
P-Wert	< 0,01	0,02	0,04	0,366	< 0,01	< 0,01	< 0,01

* A: keine Läsionen; B: geringgradige Läsionen; C: hochgradige Läsionen, unterschiedliche Buchstaben in den Reihen zeigen statistische Unterschiede an ($p < 0,05$)

Tabelle 3: Formulierung und kalkulierte Inhaltsstoffe in der Grunddiät

Inhaltsstoffe, g/kg	Starter (Tag 1 – 7)	Aufzucht (Tag 8 – 21)	Mast (Tag 22 – 41)
	Weizen	Weizen	Weizen
Weizen	573	622	622
Mais	0	0	0
Vollfettsoja	74	80	154
Sojaöl	20,0	26,0	29,0
Sojabohnenmehl	292	237	168
Austernschalen	8,5	8,5	8,3
Knochenmehl	20,9	16,9	10,8
Kokzidiostatika	0,5	0,5	0,5
Vit-Min Vormischung	2,0	2,0	2,0
Salz	3,7	3,0	3,2
Lysin HCL	1,3	1,1	0,2
DL-Methionin	2,6	2,2	1,7
L-Threonin	0,4	0,3	0
Cholin Chlorid	0,5	0,5	0,3
Phytase 4a19	0,15	0,15	0,15
		Inhaltsstoffe	
Protein, %	23,0	21,0	20,1
Fett, %	5,0	5,6	6,8
Rohfaser, %	3,6	3,4	3,4
Calcium, %	0,9	0,84	0,76
Verfügbarer P, %	0,45	0,42	0,38
Na, %	0,22	0,19	0,19
ME, kcal/kg	3,035	3,108	3,222
dLys, %	1,18	1,05	0,95
dM+C, %	0,88	0,8	0,74
DThr, %	0,77	0,69	0,65

Diskussion

Arabinoxylane sind der natürliche Hauptbestandteil der NSP im Getreide. Insbesondere im Weizen liegen sie in sehr hohen Konzentrationen vor. NSP schließen durch den sogenannten Käfigeffekt Nährstoffe ein und reduzieren die Bioverfügbarkeit (Bedford, 2017). Außerdem erhöhen Arabinoxylane die Viskosität des Verdauungsbreis, da lösliche NSP das 100-fache ihres eigenen Gewichts an Wasser aufnehmen und dabei aufquellen (Choct, 1997). Das führt zu einer weiteren Minderung der Verdaulichkeit ebenso wie zur Verschlechterung der Einstreuqualität (Choct, 1999).

In dieser Studie wurde der positive Effekt von NSP-spaltenden Enzymen (Xylanase 4a8/Econase® XT, Multienzym A und B) auf die Leistung und Fußballengesundheit von Broilern beim Einsatz in einer praxisüblichen weizenbasierten Diät gezeigt.

Im Vergleich zur Negativkontrolle wurde bei den Gruppen mit NSP-Enzymen eine signifikante Verbesserung der Futterverwertung während der gesamten Versuchsdauer (Tabelle 1) erzielt. Dies ist auf die zusätzlich verfügbaren Nährstoffe und die geringere Viskosität des Verdauungsbreis durch die enzymatische Spaltung der NSP zurückzuführen (Bedford, 2017). Möglicherweise sind dafür auch kurzkettige Xylooligosaccharide verantwortlich, die bei der Spaltung von NSP entstehen und einen präbiotischen Effekt auf die Darmintegrität haben (Bedford, 2012).

Abhängig von den getesteten NSP-Enzymen variierte die erzielte Leistungssteigerung der Broiler. Mit Multienzym A und Xylanase 4a8 wurde die Futterverwertungsrate (um Mortalität korrigiert) im Vergleich zur Kontrolle um 3,2 – 4,0 % verbessert.

Am Ende des ersten Versuchsabschnitts wurde durch den Einsatz von Xylanase 4a8 im Vergleich zur Kontrolle und den NSP-Multienzymen eine signifikant höhere Qualität der Einstreu erzielt (Tabelle 2, Note 1,27).

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Gegenüber den Vergleichsenzymen spaltet dieses Enzym die Arabinoxylane offenbar in noch kurzkettigere Bruchstücke und reduziert die Viskosität der Ingesta wirkungsvoller.

Die höhere Einstreuqualität wirkte sich auf die Fußballengesundheit der Tiere am Ende des Mastdurchgangs aus (Tabelle 2). Mit Econase® XT war der Anteil der Broiler mit gesunden Fußballen im Gegensatz zu den Tieren in der Kontrollgruppe und den Gruppen, die Multienzyme erhalten hatten, um 55 – 76 % erhöht. Zusätzlich waren schwerwiegende Läsionen im Vergleich zu den anderen Gruppen um 80 – 89 % reduziert (Tabelle 2).

Diese Ergebnisse zeigen den Zusammenhang zwischen einer verbesserten Einstreuqualität und einem höheren Gesundheitsstatus der Fußballen. Gerade in der heutigen Zeit, wo am Schlachthof die Fußballen auf Veränderungen bonitiert werden, nimmt deren Zustand und damit Gesundheit und Gesamtwohl der Tiere eine immer größere Bedeutung ein.

Schlussfolgerung

Es konnte gezeigt werden, dass die Xylanase 4a8 (Econase® XT, 9600 BXU/kg; 60 g/t Futter) bei weizenbasierten Diäten für Broiler positive Effekte im Vergleich zu zwei Multienzymprodukten bewirkt. Die Futterverwertung, Einstreuqualität und Fußballengesundheit wurden verbessert. Damit konnte sowohl die Wirtschaftlichkeit als auch das Tierwohl in der Broilermast gesteigert werden.

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Influence of NSP-degrading enzymes on growth performance and carcass traits of broiler chikens fed wheat-based diets

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Abstract

Background: Improving digestibility of NSP in poultry diets is one way in which producers can positively impact both feed efficiency and animal nutrition. NSP-degrading enzymes can save money by uplifting the average feedstuff quality, increase the flexibility to use a wider range of raw materials and reduce the risk of animal gut disturbances. Objective: objective of this trial was to evaluate the influence of NSP-degrading enzymes on growth performance and carcass traits of broiler chickens fed wheat-based diets. Methods: 500 Ross 308 chicken broilers were fed with two basal wheat-based diets. Dietary treatments were divided into two groups: control diet without enzyme supplementation and experimental diet supplemented with NSP-degrading enzymes. Results: NSP-degrading enzymes increased broiler chicken‘s preslaughter weight by 2.5% ($p \leq 0,05$) and decreased feed consumption by 1.3%. Furthermore, boneless chicken breast fillet without skin was 4% bigger ($p \leq 0,05$) comparing to control group‘s chicken breast. Chicken breast external fillet and inner fillet in the experimental group were respectively 5.6% and 3.8% bigger. Conclusion: the obtained results demonstrate that adding NSP-degrading enzymes to chicken broilers diets had a positive effect on their carcass traits and feed consumption.

Introduction

Cereal grains are the major source of energy for commercial poultry and represent from 60-70% of the diet (1). Wheat is the leading grain in Western Europe, Australia, New Zealand and certain provinces in Canada. However, the utilization efficiency of wheat is lower comparing to other types of grains because it contains more anti-nutritive factors, especially non-starch polysaccharides (NSP) (2).

These complex non-starch polysaccharides (NSP) are a heterogeneous group of compounds that include cellulose, pectins, β-glucans, pentosans, heteroxylans, and xyloglucan, which cannot be hydrolyzed by the endogenous digestive enzymes of humans and monogastric animals (3).

Soluble NSP increase digest viscosity due to their high capacity to absorb water and gelatinize the intestinal tract contents of birds (4). This phenomenon disturbs digestion with consequences for feed energy, feed efficiency and broiler performance (5). Hence, cereal grains high in non-starch polysaccharides (NSP) such as wheat (cellulose, and therefore largely insoluble) exhibit poor nutritional value (low apparent metabolizable energy, AME). Along with this, high dietary levels of NSP lead to high nitrogen and phosphorus excretion, which is a major environmental concern in densely populated regions (6).

Therefore nowadays, there is no doubt about the usefulness of exogenous NSP-degrading enzymes in wheat-based poultry diets (7).

The NSP degrading enzymes are known to increase the digestibility of raw materials for monogastrics and help digesting feed ingredients, thereby increasing the use of nutrients and the energy available for growth and production (8).

Materials and methods

Housing and management
A total of 500 Ross 308 chicken broilers were randomly allocated to 2 dietary treatments with 5 replicate pens/diet and 50 broiler chicks/pen. Birds were housed in an environmentally controlled system and growing conditions were similar in all treatments.
Dietary treatments
The two basal wheat-based diets were used. These diets were formulated to meet Ross 308 catalogue recommendations. Dietary treatments were divided into two groups: control diet (i.e. wheat-based diet without enzyme supplementation) and experimental diet (i.e. wheat-based diet supplemented with NSP-degrading enzymes). Broiler chickens were fed *ad libitum*.
Enzyme mixture
The enzyme mixture used was a commercial multi-enzyme preparation. The mixture includes endo-1,3(4)-ß-glucanase (30,000 U/g) and endo-1,4-ß-xylanase (22,000 U/g) produced by a nongenetically modified strain of *Penicillium funiculosum* Pf 8/403. + 17 other activities with cellulase and arabinofuranosidase. Enzyme dosage - 0,1 L/t of feed.
Performance traits
Broiler chickens were grown for 42 days. The amount of feed appointed for one group/diet was 1.280 t. Following indexes were recorded during the experiment: Feed consumption (FC, g/bird/period) and body weight gain (BWG, g/bird/period) were recorded for the period of 1st, 7th, 21st, 35th and 42nd day of age.
Statistical analysis
Data were analyzed by MS Office Excel 2010 and "SPSS 20" ("IBM, USA)" statistical package. Data was considered significant at $p \leq 0.05$.

Results

From the obtained results, we can see that experimental diet's chicken broilers had 3.3% lesser weight at 7 days of age. Though at 21st day chicken broiler's that had experimental dietary treatment applied already had a 0.4% bigger comparing to control group. Comparing the results of all the growth period, i.e. 42 days, chicken broiler's from experimental group weight gain was 2.5% ($p \leq 0,05$) bigger.

Table 1. NSP-degrading enzyme's influence on chicken broiler's weight.

Chicken broiler's age, d	Dietary Treatment	
	Control diet	Experimental diet
	22.90±0.32	23.03±0.74
7	202.92±2.05	196.27±1.15
21	1120.18±3.17	1124.65±6.15
35	2634.39±9.15	2629.01±8.45
42	2677.00±7.64	2746.01*±6.15

*;– $p \leq 0,05$.

The results of the experiment demonstrated that during the first experimental period (1-7 days) experimental group's chicken broilers had a 9% lesser feed consumption comparing to the control group. During the next period (8-21 days) the analysed index was bigger in the experimental group by 0.04%. In the 3rd chicken broiler's growth period (22-35 days) feed consumption was already lesser by 4.6% in the experimental group. During the whole chicken broiler's growth period, i.e. 42 days, experimental group's chicken broilers had a 1.3% lesser feed consumption comparing to control group.

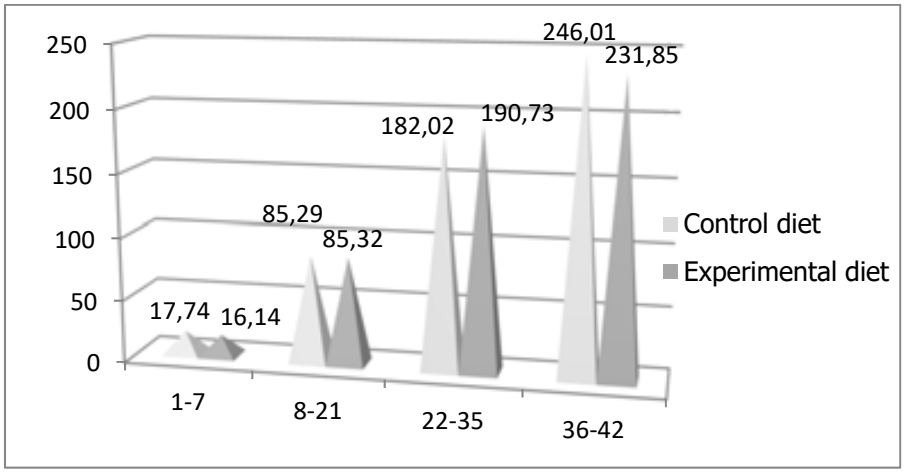

Figure 1. NSP-degrading enzyme's influence on chicken broilers feed consumption, g

The results of the experiment demonstrated that chicken broiler's preslaughter weight was 69.01g or 2.5% ($p \leq 0,05$) bigger in experimental group. Chicken broiler's fully dissected carcass (without inner organs) mass varied only slightly – it was 0.7% bigger in the experimental group. Experimental group's chicken broiler's breastfillet mass was 4% ($p \leq 0,05$) bigger comparing to control group. Moreover, it was determined that experimental group's external breast fillet mass distinguished for being 5.6% bigger, while inner fillet mass was 3.8% bigger comparing to the control group.

Table 2. NSP-degrading enzyme influence on chicken broiler's carcass development.

Indexes	Dietary Treatment	
	Control diet	Experimental diet
Preslaughter weight, g	2677.00±4.19	2746.01*±5.16
Carcass mass, g	1811.33±14.15	1823.82±5.16
Wings mass, g	153.35±2.14	162.78±2.16
Thighs mass (with bone and skin), g	260.44±3.12	274.58±3.45
Drumstick mass (with bone and skin), g	241.18±5.16	256.14±6.26
Thighs mass (without bone and skin), g	241.18±4.17	254.4±5.89
Drumstick mass (without bone and skin), g	173.87±2.05	175.03±1.79
Breastfillet mass (without skin), g	593.33±4.67	618.31*±6.02
External breastfillet mass, g	379.15±3.18	402.06±4.67
Internal breastfillet mass, g	89.49±0.54	96.14±1.64

*;– $p \leq 0,05$.

Discussion

The obtained results had shown that NSP-degrading enzymes increased chicken broilers growth intensity and the prelaughter weight, which was 2.5% ($p \leq 0,05$) bigger comparing to control group, moreover, it decreased feed consumption by 1.3%. Z. F. Fang. *et al.* (9) obtained similar results when after adding cellulase and xylanase into chicken broiler feeds, chicken broiler's weight increased by 5.9% during their 4-21 growth days, and the feed consumption decreased by 7.1%.

After chicken broiler's slaughter it was determined that the chicken broiler's which had NSP-degrading enzymes added to their diet had better carcass traits, including increased weight of chicken breast external fillet and inner fillet respectively by 4% ($p \leq 0,05$) and 3.8%. These results match with FC.

Tavernali. *et al.* (10) where in the trial with NSP-degrading enzymes, the enzymes increased such chicken broiler‘s carcass traits as chciken breast fillet, inner fillet, thighs‘ and drumstics mass.

Conclusions

NSP-degrading enzymes had a positive effect on chicken broilers weight and carcass traits. It increased broiler chicken‘s preslaughter weight and decreased feed consumption. Furthermore, boneless chicken breast fillet without skin was bigger comparing to control group‘s chicken breast. Chicken breast external fillet and inner fillet in the experimental group were as well bigger.

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Effects of different supplementation levels of choline and total sulfur amino acid on growth performance and tibio-metatarsal characteristics of broiler chickens

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Abstract

Choline is an essential nutrient for poultry and has roles in the methyl donation, membrane function, transportation of lipids, and neurotransmission. An experiment was conducted to evaluate growth performance and tibio-metatarsal joint characteristics of broilers chickens fed corn-soy based diets supplemented with increasing levels of choline from choline chloride (60%) and 3 levels of ratio of digestible total sulfur amino acid (TSAA) to digestible lysine (Lys) from 1 to 21d. A total of 525 one-day-old Cobb 500 chicks were distributed in a completely randomized design in 75 battery cages, 7 birds per cage. A 73% corn semi-purified basal diet (342 ppm of choline) was supplemented, using a 3 x 5 factorial arrangement (3 levels of digestible total sulfur amino acid (TSAA) ratio to digestible Lys - 70, 75 and 80%; and 5 levels of choline supplementation: 0, 700, 1,400, 2,100, and 2,800 ppm). A 2-phases feeding program was used and growth performance was evaluated weekly and in the cumulative period. At 21 d all birds were evaluated for valgus, varus and tibial rotation deformities. Considering a corn-soybean meal common diet (1,500 ppm of choline), 1,047 and 939 ppm of choline inclusion are appropriate to improve BWG and FCR response in starter phases, which are above breeder's recommendation (400 ppm of choline for starter phase).

Introduction

Choline (also known as Vitamin B4) is considered an essential nutrient for animals with high participation in development and cells structure maintenance. Differing to other vitamins, it could be synthetized in the liver from the amino acid Serine in addition to vitamin B6. Methionine is also involved in the choline training for given methil to the conversion of choline precursors, as well as the opposite process might also happen with the choline participation in the cystine training. Thus, there is a high correlation between choline levels and total sulfur amino acids (TSAA).

Choline chloride is the main commercial source used in the industrial feed formulations for poultry and its concentration is around 60 and 70%. Rostagno *et al.* (2017) suggests choline levels of supplementation for broiler chickens diets from 1 to 7 d and from 8 to 21 d at 550 and 496 ppm respectively. Pompeu *et al.* (2011) suggested that supplementation of 400 ppm in male broilers until 21 d improves the feed conversion ratio. Thus, this study was conducted to evaluate the effects of choline supplementation at different levels and TSAA concentration on growth performance and legs abnormalities in male chickens from 1 to 21 d.

Material and methods

Experimental design:

A total of 525 one-day-old male chicks (Cobb x Cobb 500) were distributed in 15 treatments with 5 replications in a completely randomized design, using 75 battery cages with 7 birds each.

Experimental diets and treatments:

A 73% corn semi-purified basal diet (342 ppm of choline) was supplemented using a 3 x 5 factorial arrangement with 3 levels of TSSA ratio to digestible Lys - 70, 75 and 80%; and 5 levels of choline chloride supplementation: 0, 700, 1,400, 2,100, and 2,800 ppm). A 2-phases (pre-starter and starter) feeding program was used. The choline source was Choline Chloride with minimum of 52.2% of choline. Levels of total choline (supplemented + basal diet) were 347, 1,047, 1,747, 2,447 and 3,147 respectively.

Growth performance:

Body weight gain (BWG), feed conversion ratio (FCR) and feed intake (FI), were evaluated weekly from 7 to 21 d and in the cumulative period.

Legs abnormality:

At 21 d all birds were visually evaluated for the occurrence of valgus, varus and tibial rotation deformities presence with all the animals in anatomic position. Birds were submitted to a panel with 3 different evaluators. The terms varus and valgus always refer to the direction that the distal segment of the joint points. Valgus deformity is a term for outward angulation of the distal segment of a bone or joint. The opposite deformation, medial deviation of the distal bone, is called varus. Tibial rotation above 90° was considered abnormal. At 22 days 7 birds per replication were euthanized by electrical stunning using 45 V for 3 s in order to evaluate legs thickness abnormalities: width (distance between the medial and lateral borders of tibio-metatarsal joint) and length (distance between malleollus tibiotarsal and trochlea tarsometatarsal).

Statistical Analysis:

Data were analyzed using the GLM procedures of SAS and when significant the means were compared by Tukey test at 5% significance. A factorial arrangement was used (3 levels of ratio of digestible TSAA to digestible Lys x 5 levels of choline chloride supplementation). Performance data were also fitted to linear and quadratic polynomial regressions and the maximum response of choline supplementation was estimated.

Results

No interactions between TSAA and choline levels were observed from growth performance of broilers ($P>0.05$). Broilers fed diets with 80% TSSA ratio to digestible Lys presented higher BWG than those fed diets with 70% from 1 to 21 d. The BWG of broilers fed diets with increasing levels of choline increased quadratically ($P<0.05$) and FCR decreased quadratically ($P<0.05$) from 1 to 14 d and 1 to 21 d. From 1 to 14 d, quadratic regression estimates were 2,462 ppm for BWG and 2,608 ppm for FCR. From 1 to 21 d, requirements were estimated as 2,547 ppm for BWG and 2,439 ppm for FCR.

The occurrence of valgus deformity was not different among broilers fed diets supplemented with increasing levels of choline as TSAA ($P>0.05$). Broilers fed diets without choline supplementation presented higher occurrence of varus and tibial rotation deformities ($P<0.05$) compared broilers fed increasing levels of choline. Additionally, at 22 d no differences on legs joint width and length of slaughtered chickens were observed among choline supplementation levels. Thus, choline levels that provide better BWG and FCR responses were determined as 2,547 and 2,439 ppm for starter phases until 21 d, respectively.

Discussion

It is known that the most common first results of choline deficiency are decreasing in growth performance and increased legs abnormalities, as chondrodistrophy (Tacconi *et al.*, 1988). It was supported in the present study, in which birds fed diets without choline supplementation (basal diet with 342 ppm of choline) until 21 d presented the worst results on BWG, FCR and also presented increased occurrence of varus and tibial rotation deformities.

Although using amino acids to spare other nutrients is not economical under practical conditions, it was reported that when methionine is provided at levels exceeding use for protein synthesis, the additional methyl groups may decrease the dietary choline requirement (Pesti *et al.*, 1980). Broilers fed diets with 80% TSSA ratio to digestible Lys presented higher BWG than those fed diets with 70%, however, it was not sufficient to improve BWG of broilers fed diets with total choline under 2,447 ppm. This might be supported by the absence of interaction between TSAA and choline levels from 1 to 21 d. Finally, more studies are required in order to evaluate the efficiency and behavior of the relation between sulfur amino acids and choline supplementation on broiler chickens responses.

Conclusion

Considering a corn-soybean meal common diet (1,500 ppm of choline), 1,047 and 939 ppm of choline supplementation are appropriate to improve BWG and FCR responses in starter phases, which are above breeder's recommendation (400 ppm of choline for starter phase).

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Effects of mineral sources and phytase on calcium and phosphorus retention in broiler chickens

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Abstract

An experiment was conducted to evaluate the effects of phytase and mineral sources of zinc (Zn), copper (Cu) and manganese (Mn) supplementation on growth performance and nutrient digestibility of male broilers from 1 to 25 d. A total of 528 1-d-old Cobb 500 chicks were distributed in a completely randomized design in 48 battery cages with 11 birds each. Corn-soy based diets were supplemented using a 2 x 2 factorial arrangement (with and without phytase) and 2 mineral sources (inorganic and organic). Enzyme supplementation was 500 FTU/kg whereas Zn-Cu-Mn were supplemented in a concentration of 32-30-32 ppm or 100-120-100 ppm in organic and inorganic forms, respectively. A two-phase feeding program was used, from 1 to 12 and 13 to 25 days. Tibiae were collected at 12 and 25 d to measure ash, Calcium (Ca) and Phosphorus (P) content. Ileal contents were collected at 25 d to determine apparent ileal digestibility. The use of organic minerals and phytase was found to affect positively performance parameters, bone mineralization and digestibility parameters. It is concluded that using organic minerals and phytase, combined or individually, is a well-spent investment that translates into productive improvement in poultry production.

Introduction

It is well known that supplementing exogenous phytase to animal diets enhances the utilization of P from vegetable ingredients, by liberating the phosphate from phytic acid. This improves growth performance as well as digestibility of nutrients. Also, an improved bone mineralization converts into stronger bones and legs conformity. The use of phytase allows feed-formulators to avoid P supplementation, since the enzyme will liberate the phosphate that, in other case, would be supplemented of inorganic P. Thus, enables to reduce feed costs, since P is an expensive mineral.

Trace minerals (TM) are of great importance in several metabolic processes, since enzyme reactions to cellular walls integrity. TM in adequate concentrations in the diet and also translates into performance gain in animal production, and these molecules are commonly supplemented in animal diets in the form of inorganic salts to meet bird's requirements. Uncertainties related to the absorption and utilization of inorganic trace minerals (ITM) and low-cost of inorganic salts lead to the excessive use of TM in animal diets (Saripinar-Aksu *et al.*, 2012). However, the use of high amounts of ITM may reduce nutrient absorption and bioavailability (Underwood & Suttle, 1999), besides dietary antagonism, which causes significant reduction in bioavailability. The capacity of trace minerals to interact with other molecules present in diets, such minerals as P that could be liberate in higher concentrations by phytase, as well as possible improved bioavailability of organic forms (Saripinar-Aksu *et al.*, 2012), hypothesized that

performance, nutrient digestibility and bone mineralization could be improved by the use of exogenous phytase and organic mineral source.
Thus, this study was conducted to evaluate the effectiveness of exogenous phytase in combination to mineral sources of zinc (Zn), copper (Cu) and manganese (Mn) supplementation in corn-soy based diets on growth performance and nutrient digestibility of broiler chickens from 1 to 25 days.

Material and methods

Experimental design:

A total of 528 one-day-old male chicks (Cobb x Cobb500) were distributed in 4 treatments with 12 replications in a completely randomized design, using 48 battery cages with 11 birds each.

Experimental diets and treatments:

A corn-soy meal bean basal diet was supplemented using a 2 x 2 factorial arrangement, with 2 enzyme supplementation – with or without, and 2 mineral sources – organic and inorganic. A 2-phase feeding program was used; pre starter from 1 to 12 and starter from 13 to 25 d. Celite was used as indigestible marker in the starter diet.

Growth performance:

Body weight gain (BWG), feed conversion ratio (FCR), feed intake (FI) and mortality, were evaluated in both periods, from 1 to 12 and from 13 to 25 d, as well as cumulative period.

Bone collection:

At 12 d 4 birds per cage were humanely euthanized to allow tibia collection to evaluate ash, Ca and P content. At 25 d, the remaining birds were submitted to the same procedure. Also at 25 d, ileal digesta contents of all dead animals were collected to digestibility parameters analysis.

Calculations:

Apparent ileal digestibility was calculated using the following equation (Kong and Adeola, 2014). Digestibility (%) = [1 − (Mi/Mo) × (Eo/Ei)] × 100, where Mi represents the concentration of acid insoluble ash in the diet in grams per kilogram of DM; Mo represents the concentration of acid insoluble ash in the ileal digesta in grams per kilogram of DM output; Ei represents the concentration of DM, Ca, or P, in the diet in milligrams per kilogram of DM; and Eo represents the concentration of DM, Ca, or P in the ileal digesta in milligrams per kilogram of DM.

Statistical Analysis:

Statistical analyses were conducted using a 2 × 2 factorial having 2 enzyme supplementation (with and without phytase) and two mineral sources (organic and inorganic) using a completely randomized design. Data were analyzed using the GLM procedure of SAS Institute (SAS, 2009). Significance were accepted at 5% and mean differences were separated using Tukey's test.

Results

No interaction between phytase and mineral sources supplementation was observed to performance parameters and bone mineralization ($P > 0.05$). BWG, FCR and FI of broilers from 1 to 12, 12 to 25 days and in the cumulative period were not affected by supplementing the two mineral sources ($P > 0.05$). These performance parameters were all improved ($P < 0.05$) by phytase supplementation, in all periods evaluated. Apparent ileal digestibily of dry matter (DM) was improved by organic mineral source and phytase ($P < 0.05$). Supplementing exogenous phytase improved ($P < 0.05$) ileal digestibility of

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DM, Ca and P. Interaction between mineral sources and phytase was observed to ileal digestibility of P ($P < 0.05$), with treatment supplemented with inorganic mineral + phytase elected as the best improvement of this parameter. To bone mineralization, in both periods variables performed similarly: mineral sources did not affected ($P > 0.05$) any of the parameters evaluated, whereas phytase supplementation was found to improve ($P < 0.05$) ash content in percentual as well as in grams of DM.

Discussion

Supplemental phytase was found to improve digestibility parameters, in agreement with several studies upon the effects of the enzyme on digestibility and retention of P in chickens (Żyła *et al.*, 2000 and Juanpere *et al.*, 2005). Phytate limits the efficiency of digestion, by decreasing the access of digestive enzymes to substrates, after forming indigestible complexes especially with Ca and Zn. Therefore, the use of exogenous phytase in broiler diets is expected to improve digestibility not only of P itself, but of nutrients in general, as found in the present study with phytase supplementation, which was associated with an improvement on DM, Ca and P ileal digestibility. Additionally, although there was interaction between mineral sources and phytase in ileal digestibility, it should be further investigated with future studies in order to understand better the mechanisms of this interaction, especially because the utilization of organic sources by the poultry industry has been considerably investigated.

Conclusion

Considering a common corn-soy bean meal diet, the use of phytase in concentration of 500 FTU/kg was found to improve performance, digestibility and bone mineralization parameters. Although several authors have found that organic trace minerals have positive effects on performance, this study did not observed such improvements. Further studies upon the bioavailability of organic minerals in a phytase-supplemented diet should be made in order to better understand the interaction of organic molecules with diet-origin molecules.

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Feed utilization and some plasma constituents as affected by probiotic and/or synthetic enzymes in broiler diets

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Abstract

Microbial ecosystem balance and digestive enzymes are an important aim to improve nutritional utilization by increasing the digestibility and absorption in broilers supplemented with dietary bio additives (probiotic and/or synthetic enzymes).Three hundred and sixty one day-old Ross (308) broiler chicks were randomly divided into four experimental groups, fed a basal diet (control) or diets supplemented with a probiotic and/or enzymes. Results showed that live body weight at 42 days of age was significantly increased by bio additives supplementation, and recorded the best feed conversion ratio compared with non-supplemented group. Furthermore; Chicken fed diet supplemented with bio additives had a lower concentration of plasma triglycerides, HDL and cholesterol, while an increase in glucose and LDL levels was recorded. Histomorphology examination revealed that villi height and crypt depth were increased significantly with probiotic or probiotic supplementation with enzymes compared with control or enzymes groups. In conclusion, additions of bio additives to broiler diets enhanced productive performance, improved some plasma constituents and ileal histomorphology.

Introduction

Dietary carbohydrates are the main sources for energy in poultry diets. Most of the carbohydrates in poultry diets is provided by cereal grains. Carbohydrates in feed are composed of sugars, starches and cellulose. Cellulose is the insoluble portion of carbohydrates and cannot be utilized efficiently by birds. Some enzymes that can be able to destroy the cellulose are add to diets to improve the utilization of carbohydrates such as supplementation of rye or barley-containing diets with appropriate supplemental enzyme preparations improves nutrient utilization of poultry (Friesen *et al.*, 1992). Chickens are not able to digest carbohydrates completely due to their lack of cellulase and xylanase that involved in carbohydrate digestion. Cecal portion in chickens plays an important role in cellulose and hemicellulose by fermentation and hydrolysis, yielding short chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. The SCFAs can be utilized by chickens as energy and carbon source (Tellez *et al.*, 2006). SCFAs are absorbed across the epithelium intestinal and it can serve as an important energy source for intestinal epithelial cells (Pryde *et al.*,2002).Some studio reported that SCFAs play an important role to stimulate enterocyte growth and proliferation, and catalyze intestinal immune responses of the birds (Sanderson, 2004). Probiotic or synthetic enzymes involving in improvement of both microbial ecosystem and carbohydrate balance or carbohydrate digestion is one of the main objectives of the present study.

Materials and methods

A total of 360 broiler chicks of a commercial strain (Ross 308) were used in a randomized completely design with 4 groups; 3 supplemented groups and a control group; 3 replicates in each group and 30 birds in each replicate. Birds were raised on cages for 42 days of age. The chicks were fed a basal diets formulated to cover all nutrient requirement of broiler chicks according to nutritional requirements of

Ross 308 (table 1). Treatments were randomly assigned to the following treatments: T1= control (non-supplemented or basal diet); T2= Basal diet supplemented with 100g/ton Lactobacillus and 500g/ton mixture enzymes (Amylase 5.5 million / unit, Beta-glucoses 30000 unit, Lipase 150000 unit, ∞-Ylanase 500000 unit and Cellulose 15000 unit); T3= Basal diet supplemented with 500g/ton mixture enzymes; T4= Basal diet supplemented with 100g/ton Lactobacillus. Live body weight (LBW), Feed Consumption (FC) and Feed Conversion ratio (FCR) were determined at 42 days of age. Nine birds of each treatment were slaughtered randomly on day 42 days of age and blood samples were obtained, to determine biochemical examination. The plasma concentration of glucose, triglycerides, cholesterol, HDL and LDL in plasma samples were analyzed by an Automatic Biochemical Analyzer. For each segment from ileum, a 4-µm cross-section was sectioned using a microtome, placed on a glass slide, and stained with periodic acid Schiff reagent for 2 to 3 min. Villus height and crypt depth were measured using a light Microscope (Olympus CX31, Tokyo, Japan).The villus height was measured from the villus tip to the valley between individual villus. The crypt depth was measured from the valley between individual villus to the basolateral membrane. Mean villus height and crypt depth, were calculated and used for statistical analysis. All data were analyzed statistically by using the General linear Model Procedure of the Statistical Analysis System (SAS). Significance of differences between groups was determined using the Duncan Multiple Range Test (Duncan,1955).

Results and discussion

Effects of dietary probiotic and enzymes supplementation on LBW and FCR at 42 day of age compared with that of control group are shown in table (2). No significant effects due to dietary treatments were observed on FC at 42 days of age. Groups that fed a diet supplemented with a mixture of probiotic with enzymes and enzymes had a higher LBW of 2058g and 2004g at 42days of age respectively compared with 1897g in the control group at the same age. A different trend was observed for the values of FCR which showed a significant improvement in all supplemented groups when compared with the control group. These results are in agreement with (Chen *et al.*, 2013).

The results revealed also that FCR as a good indicator for economic performance of broiler chickens was improved in all supplemented groups. However, Sherif, (2009) found that dietary supplementation with Avian plus and Natuzyme improved feed conversion and economic efficiency of growth for broiler chicks, but feed intake was not affected. Positive effects of bio additive on the performance of chicks return possibly to improvement of digestion and adsorbent of nutrient such as carbohydrates. Data of productive traits presented in this study are in agreement with the findings of Nematallah *et al.*,(2015) who reported that, the dietary addition of probiotic to broiler chickens may make better nutrients utilization, metabolism and absorption and these substances would maintain a better environment in digestive tract. This reflects on LBW and FCR.

The overall means of some biochemical blood constituents were presented in table (3). Plasma glucose and HDL levels was significantly higher in supplementary groups than the control. Highly significant decrease in triglycerides, cholesterol and LDL was recorded in treated chicks compared with those chicks fed a basal diet. In this concern, Friesen *et al.*, (1992) found that enzyme complex increased the digestion rate of starch, while the product of starch digestion was absorbed into blood in the form of glucose and so increased blood sugar content.

The effects of dietary bio additives on ileum histomorphology are shown on table (4). Birds fed on the probiotic with mixture enzymes (T2) and probiotic (T4) had significantly higher ileum villi height and crypt depth than those fed on control and mixture enzymes (T3). However, Jin *et al.*, (1998) revealed that probiotic administration also increased villi height, probably associated with increased concentrations of short-chain fatty acids in the lower intestine. Villi height is known to correlate positively with nutrient absorption (Tarachai and Yamauchi, 2000).

Table 1. Composition of the basal diet

Ingredient (%)	Starter	Grower	Finisher
Yellow corn	55.00	59.32	64.60
Soybean meal 44%	30.40	26.90	22.20
Corn gluten meal 62%	8.00	6.90	6.20
Di-Calcium phosphate	2.10	1.85	1.70
Calcium Carbonate	1.30	1.25	1.10
Corn Oil	2.00	2.70	3.20
Salt	0.30	0.30	0.30
Premix*	0.30	0.30	0.30
HCl -Lysine	0.40	0.30	0.25
DL-Methionine	0.20	0.18	0.15
Total	100	100	100
Calculated Analysis			
ME, Kcal/Kg	3015	3110	3205
Crude Protein (%)	23.01	21.16	19.10
Calcium (%)	1.071	0.986	0.881
A. Phosphorus (%)	0.513	0.459	0.424
Lysine (%)	1.439	1.246	1.076
Methionine (%)	0.604	0.554	0.495
Meth+Cys	0.992	0.915	0.826

*Vitamins and minerals premix provided per kilogram of the diet: Vit. A, 1000 IU; D3 2000 ICU; Vit. E, 10 mg; Vit. K, 1mg; B1, 10 mg; B2, 5 mg; B6, 1500 mg; B12, 10mg Pantothenic acid, 10 mg; Nicotinic acid, 30 mg; Folic acid, 1mg; Biotin, 50 mcg; Chloride, 500 mg; copper, 10 mg; iron, 50 mg; Manganese, 60 mg; Zinc, 50mg, and selenium, 0.1 mg.

Table 2. Effect of dietary bio additive on LBW, FC and FCR of broiler chickens at 42d of age

Items	T1	T2	T3	T4	SEM	Sig.
LBW	1897^{c}	2058^{a}	2004^{b}	1975^{b}	11.3	**
FC	3316	3357	3346	3329	13.61	NS
FCR	1.749^{a}	1.631^{c}	1.669^{b}	1.686^{b}	0.56	**

Table 3. Effect of dietary bio additive on plasma biochemical constituents of broiler chickens

Items	T1	T2	T3	T4	SEM	Sig.
Cholesterol	176^{a}	116.0^{b}	101.7^{c}	122.8^{b}	4.18	**
Glucose	189.4^{c}	231.1^{a}	217.2^{b}	209.1^{b}	7.25	**
Triglycerides	98.6^{a}	68.4^{b}	70.3^{b}	73.2^{b}	1.36	*
HDL	75.9^{b}	84.3^{a}	83.6^{a}	81.8^{ab}	1.58	*
LDL	69.4^{a}	61.9^{b}	60.2^{b}	63.4^{b}	0.96	*

a ,b, c, d Means within the same row with different superscripts are significantly different sig.=significance **($p<0.01$) , *($p<0.05$) , NS = Non-Significant. SEM =Standard Error Mean. T1=Control; T2= Basal diet supplemented with 100g/ton probiotic and 500g/ton mixture enzymes ;T3= Basal diet supplemented with 500g/ton mixture enzymes;T4= Basal diet supplemented with 100g/ton probiotic.

Table 4. Effect of dietary bio additive on ileum histomorphology of broiler chickens

Items	T1	T2	T3	T4	SEM	Sig.
Villus height (um)	532^{b}	687^{a}	561^{ab}	673^{a}	19.20	*
Crypt depth (um)	123^{b}	139^{a}	126^{b}	137^{a}	6.32	*

a ,b Means within the same row with different superscripts are significantly different sig.=significance **($p<0.01$) , *($p<0.05$) , NS = Non-Significant. SEM =Standard Error Mean. T1=Control; T2= Basal diet supplemented with 100g/ton probiotic and 500g/ton mixture enzymes ;T3= Basal diet supplemented with 500g/ton mixture enzymes;T4= Basal diet supplemented with 100g/ton probiotic.

Conclusion

The current study showed that bio additives, such as Probiotic and Enzymes, can improve growth performance and nutrient utilization of broiler chickens through positive effects of histomorphology development of the gut.

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Effects of dietary sodium diformate in broilers in Eastern Europe – a performance analysis

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Abstract

Dietary sodium diformate (NDF) has been tested in poultry production since 2006 and numerous publications and conference contributions on its use in broilers have been published. A word-wide data analysis on its impact on broiler performance is available; however those data have not been regionalized. This study analyzed the average impact from all studies carried out in Eastern Europe on the effect of the additive on the performance parameters weight gain, feed efficiency, mortality and productivity (EBI). The final data-set contained the results of 11 trials with NDF-inclusion, which ranged from 0.1% to 0.5%. Results are expressed as percentage difference from the negative control. The average level of dietary NDF from the data-set in all treated broilers was 0.21%. The performance of broilers based on daily gain was significantly increased by 5.4% (P=0.001). Furthermore, the FCR was also significantly improved (5.0%; P=0.004). Survival was increased on average by 2.5% (P=0.004). Finally, the EBI improved significantly due to the inclusion of NDF by 13.7% (P=0.002). It is therefore concluded that dietary sodium diformate can improve broiler production under Eastern European conditions.

Introduction

Overuse of antibiotics, the development of resistant bacteria and its ill effects on the human population eventually leading to the ban on prophylactic use of antibiotics in animal farming, is currently the hottest topic of discussion everywhere. The ban on the prophylactic use of antibiotics in animal farming is well deserved, however, looking at the bacterial challenges experienced here, it is imperative to have some kind of a tool to control the bacterial infections and improve the performance of the farmed animal. Organic acids are looked upon as the most promising alternative to the antibiotics (Papatsiros and Billinis, 2012), as, in addition to its antimicrobial property, organic acids provide many extra benefits such as improving the intestinal health, optimising the intestinal pH and thereby improving the nutrient digestibility. They have long been used in animal nutrition, usually in order to stabilize compound feed, but also to enhance animal performance. Most of the early studies on this group of additives however, were carried out within the pig production chain (Cole *et al.*, 1968); covering sows, piglets and fatteners. Available data on organic acid use in poultry production indicate a later focus on such additives. One of the first reports of improved broiler performance when diets were supplemented with single acids was for formic acid (Vogt *et al.*, 1981). Later, similar effects were noticed for fumaric acid (Patten and Waldroup., 1988; Kirchgessner *et al.*, 1991; Skinner *et al.*, 1991). Izat *et al.* (1990a) found significantly reduced levels of *Salmonella spp.* in carcass and caecal samples after including calcium formate in broiler diets. In another trial from Izat *et al.* (1990b), buffered propionic acid was used to counteract pathogenic microflora in the intestine and carcass of broiler chickens, and resulted in a significant reduction in *E. coli* and *Salmonella spp*. The use of pure formic acid in breeder feed reduced the contamination of tray liners and hatchery waste with *S. enteritidis* drastically (Humphrey and Lanning, 1988). Kirchgessner *et al.* (1992) found significantly better feed utilization in laying hens after adding fumaric acid, but only when the feed was low in protein and methionine and cysteine. Performance enhancement was influenced by both quantity and quality of the protein.

Although growth performance benefits of organic acids and their salts have been shown in numerous

studies over the past half-century, though mainly in pigs, the ban on antimicrobial growth promoters (AGP) in the European Union in 2006, and subsequently in an increasing number of other countries (e.g. Japan, South Korea, Thailand, Vietnam, Indonesia) has resulted in an increased scientific and commercial focus on organic acids.
Improving broiler performance or hygienic conditions with the aid of organic acids has been reported by many sources, as reviewed by Desai *et al.* (2007). An important limitation, however, is that organic acids are rapidly metabolised in the fore-gut (crop to gizzard) of birds, which will reduce their potential impact on growth performance. A new molecule (sodium diformate, traded as Formi NDF, ADDCON, Germany) has been proven to be effective against pathogenic bacteria, including salmonella, along the whole gastro-intestinal tract (Lückstädt and Theobald, 2009). The reduced impact of pathogenic bacteria on the broiler, as well as the improved gut microflora, leading to a state of eubiosis in treated chickens, due to the inclusion sodium diformate (NDF) in broiler diets resulted in improved bird performance. Several trials have been carried out over the last decade world-wide which have documented the positive effects on broiler performance – and were additionally confirmed using meta-analysis (Lückstädt, 2013; Table 1).

Table 1: Performance analysis of 17 trials with broilers, fed diets with NDF, expressed as an average percentage difference from negative control (after Lückstädt, 2013)

Dosage	Feed intake	Weight gain	FCR	Survival	EBI
0.28	+1.1	+5.2	-4.1	+2.3	+12.4
P-value	0.22	0.0001	0.002	0.034	0.0005

However, since poultry production conditions, including, environment, housing and dietary ingredients, differ considerably from region to region, it was therefore interesting to estimate the potential impact of NDF on broiler performance in a certain region – in this case Eastern Europe, through an analysis of the results of trials carried out.

Material and methods

This study analyzed the average impact from all studies available on the effect of the additive on the performance parameters weight gain, feed efficiency, mortality and productivity, as measured using the European Broiler Index, (EBI). EBI is calculated using the following equation:

EBI = ADG [g] × survival [%] / (10 × FCR)

The final data-set contained the results of 11 negatively controlled studies which tested NDF-inclusion, whose dosages ranged from 0.1% to 0.5%. Those studies were carried out between 2009 and 2017 in Russia, Serbia and the Ukraine under commercial, semi-commercial and institutional conditions and included more than 1.63 million broilers raised to between 38 and 60 days.
The above mentioned performance parameters are expressed as percentage difference from the negative control. The results are given as means and were statistically analysed using the t-test. A confidence level of 95% was defined for these analyses.

Results and conclusions

The average level of dietary NDF from the data-set in all treated broilers was 0.21% (Table 2). The typical dosage for NDF in broilers ranges from 1-3 kg/tonne feed, depending on age (dietary protein level) and hygienic status of the farm.

Table 2: Performance analysis of 11 Eastern European trials with broilers, fed diets with or without sodium diformate (NDF), expressed as an average percentage difference from negative control

Dosage	Weight gain	FCR	Survival	EBI
0.21	+5.4	-5.0	+2.5	+13.7
P-value	0.001	0.004	0.004	0.002

As seen in Table 2, the performance of broilers based on daily gain was significantly increased by 5.4% (P=0.001). Furthermore, the FCR was also significantly improved (5.0%; P<0.01). Survival was increased on average by 2.5% (P<0.01). Finally, the EBI improved significantly due to the inclusion of NDF by 13.7% (P<0.01). Similar trends had been reported by Lückstädt (2013) for the world-wide performance analysis of NDF in broilers. In broilers, improved zootechnical performance is thought to stem from both improvements in the intestinal microflora, as a result of suppressing pathogenic bacterial species; and improved protein digestion. As often seen with other additives, hygiene challenge also plays some role in the performance observed. In the present performance analysis, a range of hygiene conditions were included, representing both university and farm trials. The average impact of NDF inclusion on performance remained above that normally expected. It can therefore be concluded that dietary sodium diformate can play an important role in improving broiler nutrition in Eastern Europe, leading to a sustainable poultry production also without the use of prophylactic in-feed antibiotics.

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Effect of *B. amyloliquefaciens* CECT 5940 on health status and live performance in broilers

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Abstract

The objective of this trial was to determine the efficacy of feeding *Bacillus amyloliquefaciens* CECT 5940 (Ecobiol®) on broiler performance. Three hundred male Ross 308 broiler chicken were randomly allocated in two treatments; basal diet (Control) and basal diet supplemented with *B. amyloliquefaciens* CECT 5940 at 1.0×10^6 CFU/g of feed (Trial) . Body weight was numerically higher in birds fed Ecobiol® compared to the unsupplemented group (2,114 vs 2,086 g). Feed conversion ratio was significantly lower in the probiotic group at day 20 (1.316 vs 1.344; p=0.003) and at day 35 (1.508 vs 1.533; p=0.037). Additionally, a numerical reduction of both cecal coliform and *E. coli* count was observed. The results demonstrate that Ecobiol® is able to improve growth performance even in birds already performing on a high level.

Introduction

In the last years the veterinary authorities have been putting a lot of pressure on antibiotic usage in animal production and on stopping the transfer of the products into human food chain. In reaction to these demands, farmers are searching for appropriate alternatives to antibiotics. The range of the potential replacers is really wide, and one of the most promising groups are the probiotics. Probiotics, in general, can improve feed conversion, decrease mortality, modulate the immune response, and protect against enteric pathogens (Siragusa, 2012; Ducatelle et al., 2015). Some probiotics are marketed in spore form, being heat-stable and offering additional advantages over other non-spore formers such as *Lactobacillus* spp. Thus, spore form-products can be stored at room temperature in a desiccated form without any deleterious effect on viability (Cutting 2010), and, contrary to some species of *Lactobacillus* (Tuohy et al., 2007), they are capable of surviving the low pH of the gastric barrier (Barbosa et al., 2005; Spinosa et al., 2000). In principle, a specified dose of spores can be stored indefinitely without refrigeration, and the entire dose of ingested bacteria will reach the small intestine intact. Research in human nutrition and physiology also supports understanding the benefits of probiotics in animal nutrition, showing that the interrelation between of gut microflora on and health status does not stop at the intestinal level (Corthesy, 2007).

Ecobiol® is a probiotic designed for gut microbiota management that can be used to stabilize performance during intestinal challenges. It contains a fast growing, naturally occurring strain of *Bacillus amyloliquefaciens* CECT 5940 with an inherent capacity to produce lactic acid, and the ability to influence interactions between different bacterial populations. This strain is closely associated with intestinal mucosa and tolerates the gastric, and bile secretions. Ecobiol® is a spore form-product and stable under different pelleting conditions.

Material and methods

The experiment was conducted in the trial facility of University of Agricultural Studies and Veterinary Medicine of Banatului Timisoara, Romania. Three hundred male Ross 308 broiler chicken were randomly allocated in two groups, each with 25 replicas, 6 birds per pen. The average initial body weight (BW) was 42 g/chicken (+/- 3g). The diets were based on corn, wheat and soybean meal with no enzymes used in any diet (Table 1). The test feed contained 500 g/t Ecobiol® 500. The diets were formulated according to Evonik recommendations (Evonik 2012). Feed analyses confirmed calculated figures.

Table 1. Composition of the diets

Treatment	T1	T1	T1	T2	T2	T2
Phase, days	Starter 0 – 10	Grower 11 – 20	Finisher 21 – 35	Starter 0 – 10	Grower 11 – 20	Finisher 21 – 35
Ingredients (%)						
Soybean meal 46%	43.8	34.0	29.6	43.8	34.0	29.6
Wheat 11,57%	25.0	30.0	30.0	25.0	30.0	30.0
Corn 6,71%	21.1	26.6	31.0	21.1	26.6	31.0
Sunflower oil	5.99	5.72	5.83	5.99	5.72	5.83
Monocalcium-Phosphate	1.52	1.31	1.19	1.52	1.31	1.19
Limestone	1.48	1.25	1.21	1.48	1.25	1.21
Premix 0,5%	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.26	0.24	0.24	0.26	0.24	0.24
DL-Methionine	0.24	0.19	0.17	0.24	0.19	0.17
L-Lys HCl	0.12	0.15	0.15	0.12	0.15	0.15
L-Threonine	0.04	0.04	0.05	0.04	0.04	0.05
Sodium bicarbonate	0.01	0.04	0.04	0.01	0.04	0.04
Ecobiol® 500				0.05	0.05	0.05
Nutrients (%)						
Dry matter	88.1	87.8	87.8	88.1	87.8	87.8
AME_N (MJ)	12.6	13.0	13.2	12.6	13.0	13.2
Crude protein	24.7	21.2	19.4	24.7	21.2	19.40
Crude fiber	3.50	3.11	2.94	3.50	3.11	2.94
Crude fat	7.99	7.81	7.99	7.99	7.81	7.99
SID Lys poultry	1.29	1.10	1.00	1.29	1.10	1.00
SID Met poultry	0.61	0.52	0.49	0.61	0.52	0.49
SID M+C poultry	0.93	0.81	0.76	0.93	0.81	0.76
SID Thr poultry	0.82	0.70	0.65	0.82	0.70	0.65
SID Arg poultry	1.55	1.28	1.16	1.55	1.28	1.16
SID Ile poultry	0.94	0.79	0.72	0.94	0.79	0.72
SID Val poultry	1.02	0.87	0.80	1.02	0.87	0.80

The diets were fed ad libitum in mash form within a three-phase feeding program: starter: 0 – 10 days, grower: 11 – 20 days, and finisher: 21 – 35 days. The feeds did not contain any coccidiostats and no vaccination was done. Feed intake and body weight were recorded at the end of each feeding phase. Mortality was monitored on a daily base. Additionally, 25 chickens from each treatment were slaughtered at day 35. The cecum of these birds was collected, packed in a freezing box and sent to a microbiology lab for bacterial analyses (*E. coli* and coliforms). The ileum from 15 birds was used to determine the *Lactobacilli* count (6 dilution with 2 plates each).

Statistic assessment of data was done using the software package R, t-test routine.

Results and discussion

Weight gain during all the phases was similar for both treatments, with values of 2,086 g for control and 2,114 g for Ecobiol group (Table 2). This growth performance was slightly below the recommended Ross value of 2,283 g (Aviagen 2014). Mortality was at zero level due to very good housing and management conditions.

Feed intake was also similar in both treatments, but still below the respective Ross recommendation of 3,510 g. The lack of additional enzymes might explain this finding.

Feed conversion ratio (FCR) was statistically different in the period 0 to 20 days, and over the entire trial. In particular, in the first 20 days when the broilers are facing more gut challenges, the positive effect of Ecobiol® on the intestinal tract and on gut health became obvious. Results for FCR are in line with the respective Ross recommendation of 1.537 g/g.

Table 2. Broilers performances at 10, 20 and 35 days of age

	Body weight (g)			Feed intake (g)			Feed conversion (g/g)		
	10 days	20 days	35 days	10 days	20 days	35 days	10 days	20 days	35 days
Control	238	820	2086	216	1051	3134	1.094	1.344[b]	1.533[b]
Ecobiol® 500	239	831	2114	215	1043	3120	1.082	1.316[a]	1.508[a]
p value	0.628	0.303	0.312	0.622	0.340	0.672	0.425	**0.003**	**0.037**

The number of lactic bacteria in the intestine (ileum) of broilers was measured in CFU's (colony forming units). This technique indirectly determines the number of viable cells by counting colonies on the surface or depth of a culture medium. Table 3 shows the average count (log) of lactic bacteria, *E. coli*, and coliforms. Within the trial no differences in the bacterial population could be detected.

Table 3. Total count of lactic bacteria, *E. coli* and coliforms at day 35 (log_{10} CFU/g)

Treatment	Lactobacilli	Coliforms	*E. coli*
Control	8.60	7.97	7.76
Ecobiol® 500	8.65	7.86	7.53
p value	0.725	0.603	0.285

The ceca from probiotic-supplemented group had a 68% lower coliform count (4.25×10^8 CFU *vs*. 1.37×10^8 CFU) and a 79% lower *E. coli* count (3.6×10^8 vs 7.61×10^7) than in control group. These numeric modification in the total bacterial count (in CFU/g) is shown in Figure 1.

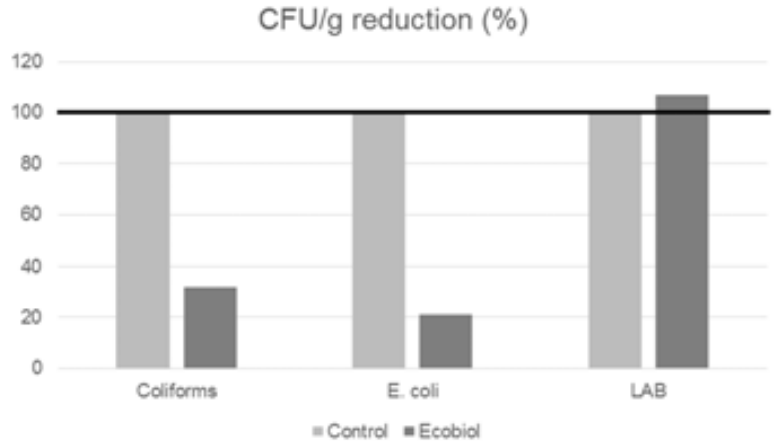

Figure 1. Relative bacterial count (CFU/g) comparison at day 35. Coliforms, *E. coli* and Lactic bacteria (LAB)

Conclusion

Dietary inclusion of Ecobiol® (*B. amyloliquefaciens* CECT 5940) was able to significantly improve the feed conversion ratio during the entire period (0 – 35 days), with the main effect on period 10 – 20 days, when animals are facing the most severe gut health challenges. The product stabilized the gut microflora by numerically reducing the number of *E. coli* and coliforms in the cecum of birds fed Ecobiol®. This indicates that a well-balanced intestinal microflora can contribute to a higher performance of the birds. The results demonstrate that Ecobiol® is able to improve growth performance even in birds already performing on a high level.

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KRAFT FÜR NEUES

Effect of medium chain fatty acids, emulsifier and Sanquinarine on laying hens productivity and gut health

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Abstract

Supplying qualitative, safe, healthy feed livestock industry will be able to improve animal health, welfare and production levels. The aim of this study was to investigate the influence of medium chain fatty acids, emulsifier and sanguinarine on the physiological laying hens productivity and health. The study was performed on 30 Lohman Brown laying hens which were 30 week old assigned to three treatment groups (10 hens per each treatment group) and feeding experiment lasted 56 days. Laying hens were fed for 8 wk MCFA group with a basal diet+medium chain fatty acids (MCFA) 1 kg/t, of feed), MCFA+LIP group received basal diet supplemented with medium chain fatty acids1 kg/t + 0.5 emulsifier LIPIDOL® kg/t, of feed), MCFA+LIP+S group received basal diet supplemented with medium chain fatty acids1 kg/t + 0.5 emulsifier LIPIDOL® kg/t + phytobiotics preparat Sangrovit® Extra 60 g/t, of feed). During the whole trial period, the mean weight of eggs in the experiment groups increased by 1.9-2.22% ($P<0.05$). Feed conversion ratio in both experimental groups increased by 4.26% ($P<0.05$).
The results shows that *duodenal* villi increased by by 13,8 and 21,6% ($P<0.05$), then experimental groups of lying hens fed was supplemented with medium chain fatty acids, LIPIDOL®, Sangrovit®. In both experimental groups in the *ceacum* increased acetic acid by acetic acid respectively by 15.2-12.3% ($P<0.05$) and butyric acid 18.2-26.4% ($P<0.05$).
The results clearly demonstrate that MCFA, emulsifers and Sangrovit had statistically significant effect on villi lengt and depth acetic and butyric acid in intestinal tract of laying hens.

Introduction

Medium chain fatty acids have specific nutritional, metabolic and antibacterial effects (Wang *et al.*, 2015; Devi *et al.*, 2014; Skrivanova *et al.*, 2010; Batovska *et al.*, 2009). The use of emulsifiers in bird's diets can increase lipids absorption growth performance and feeding efficiency and modified the blood lipids (Aguilar *et al.*, 2013). Emulsifiers can be interspersed into feeds as they can improve egg weight, egg yolk color, nutrient digestibility and have beneficial effects on egg production, besides that, they can also influence egg`s flavor and odor alterations (Mandalawi *et al.*, 2015, Sureh *et al.*, 2014). Moreover, fitobiotics constitute an alternative to antibiotic growth promoters in poultry feeding. Herbs and essential oils improve digestion and the conversion of feed ingredients, consequently improving livestock rearing results. At the same time they have antibacterial and antioxidative properties, and they contribute to improving the dietetic and palatability value of compound feed (Alp *et al.* 2012, Lipinski *et al.* 2011).

The aim of this study was to determine the effect of the supplemented of medium-chain fatty acids (MCFA), emulsifier LIPIDOL® and phytobiotics preparat Sangrovit® Extra on the performance, crypt depth, villi lenght and concentration of short-chain fatty acids (SCFA) of laying hens.

Material and methods

Feeding trial was conducted laying hens of Lohman Brownn strain at the age of 30 weeks. The laying hens were divided into 3 groups, 10 laying hens in each group. The MCFA group received basal diet supplemented with medium chain fatty acids 1 kg/t, of feed), MCFA+LIP group received basal diet supplemented with medium chain fatty acids1 kg/t + 0.5 emulsifier LIPIDOL® kg/t, of feed), MCFA+LIP+S group received basal diet supplemented with medium chain fatty acids1 kg/t + 0.5 emulsifier LIPIDOL® kg/t + phytobiotics preparat Sangrovit® Extra 60 g/t, of feed). Feeding trial was conducted with laying hens of Lohman Brown strain of 38 weeks age. The laying hens were fed with compound feed 125 g per day (NRC, 1994). During the feeding trial, the laying hens were held in the individual cages with stationary drinking–bowl and feed box under the same feeding and holding conditions. The content of short chain fatty acids (SCFA): acetic, propionic, iso-butyric, butyric, iso-valeric, pantothenic, iso-caproic, hexanoic, n-heptanoic in caecum was determined by Gas Chromotography Ultra Shimadzu GCMS-QP 2010 with mass spectrometric detector (Shimadzu corp., Kyoto, Japan) using Guahua et al, 2006 method. Post-mortem and histopathological (*Duodenum*) mucosal histomorphometry. Samples were fixed with 10 % neutral formalin solution. Using standard procedures for histologic evaluation the tissues were embedded into paraffin, cut with rotary microtome by 4 µm thick tissue sections, which were painted with hematoxylin and eosin. Prepared histological samples were examined using an "Olympus BX63" microscope, "Olympus DP72" video camera and the computer "Image Pro Plus" program (Olympus) system, control and test groups of broiler duodenum, ileum villi height and crypt depth, caecum crypt depth were morphometrically measured and microscopically. Villi height measurement were carried out from top of the villi to villi-crypt connector and total mucosal thickness was measured from the top of the papillae to the muscularis mucosa (caecum samples was carried out only one – the latter measurement). Each sample was subjected to 10 intestinal villi measurements (best expressed) to measure the height and in the same places for 10 measurements to measure the thickness of the lining. The thickness of the lining of the villi height was obtained by subtracting the crypt depth ratio (Pockevičius *et al.*, 2014).

Results and discussion

There is little literature regarding the effect of MCFA on broiler production. Solis de los Santos *et al.* (2008) found that supplementation with 1.4% caprylic acid (an 8-carbon MCFA) in a regular chick starter diet reduced feed consumption and weight gain, but did not affect feed conversion.

Table 1. Effect of MCFA, emulsifier, sanquinarine on lying hens productivity

Parameter	Groups		
	MCFA	MCFA+LIP	MCFA+LIP+S
Laying intensity, (%)	91.07±8.32[a]	90.67±9.66[a]	92.26±7.91[a]
Dynamic of egg mass, g	61.74±3.73[a]	62.93±5.00[b]	63.12±4.95[b]
Feed conversion, kg/kg	1.96±0.04[a]	1.88±0.06[b]	1.88±0.05[b]

a, b – averages in the rows of the table are indicated by different letters, there is a statistically significant difference between them ($p<0.05$)

Our results are regarding with Wang *et al.* (2015). The feed additives influence to the productivity of laying hens is presented in Table 1. Feed conversion in both tested groups increased by 4.26% ($P<0.05$).

During the whole trial period, the mean weight of eggs in the test group increased by 1.9-2.2% (P<0.05). Dynamic of eggs mass in both groups increased by 9.5-4.5% (P<0.05).

In addition to organic acids, MCFAs are also used as alternatives to antibiotics due to their similar effects as those of organic acids on antimicrobial activities outside the intestinal tract and on reducing pH (Van Imerseel *et al.*, 2006). High concentrations of MCFAs had mainly antibacterial effects and lower concentrations of MCFAs exerted more selective effects in the upper digestive tract and led to a change in the spectrum of the intestinalmicrobiota (Dierick *et al.*, 2003; Zentek *et al.*, 2012).

Table 2. Effect of MCFA, emulsifier and sanquinarine on duodenum intestine crypt depth and intestinal villi length, µm

Parameter	Groups		
	MCFA	MCFA+LIP	MCFA+LIP+S
Crypt depth	288.85±54.88[a]	284.23±65.93[a]	283.59±66.48[a]
Villi length	1579.87±340.20[a]	1797.89±176.89[b]	1922.18±227.85[b]

a, b – averages in the rows of the table are indicated by different letters, there is a statistically significant difference between them (p<0.05)

The Duodenum intestine crypt depth and intestinal villi length is presented in Table 2. The experimental group II of laying hens fed was supplemented with MCFA, LIP and S result was that *duoden*um villi increased by 13.8 and 21.6 (P<0.05).

The Medium-chain fatty acids, emulsion and phytobiotic effect SCFA concentration in the laying hens intestinal tract is presented in Table 3. In both test groups in the *ceacum* increased acetic acid by 12.3-15.2% (P<0.05) and butyric acid 13%-34%.

Table 3. Effect of MCFA, emulsifier and sanquinarine on medium-chain fatty acids, emulsion and phytobiotic effect SCFA concentration in the chickens intestinal tract, mmol/l

Parameter	Groups		
	MCFA	MCFA+LIP	MCFA+LIP+S
Acetic acid	59.91±6.37[a]	73.21±8.78[b]	69.02±2.96[ab]
Propionic acid	11.69±1.47[a]	10.83±2.10[a]	12.64±0.71[a]
Iso-butyric acid	2.80±0.48[a]	3.31±0.99[a]	3.54±0.88[a]
Butyric acid	12.43±1.27[a]	14.06±2.71[ab]	16,64±1.35[b]
Isovaleric acid	3.13±0.48[a]	3.52±0.88[a]	3.91±0.64[a]
Pantothenic acid	3.04±0.39[a]	2.92±1.10[a]	3.81±0.64[a]
Iso-caproic acid	1.87±0.03[a]	1.90±0.10[a]	1.83±0.01[a]
Hexanoic acid	1.35±0.01[a]	1.34±0.02[a]	1.33±0.03[a]
n-heptanoic acid	1.17±0.02[a]	1.17±0.01[a]	1.17±0.01[a]

a, b – averages in the rows of the table are indicated by different letters, there is a statistically significant difference between them (p<0.05);

Conclusion

The results clearly demonstrate that MCFA, emulsifers and Sangrovit had statistically significant effect on villi lengt and depth acetic and butyric acid in intestinal tract of laying hens.

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Effects of prebiotics (mannan-oligosaccharides) and probiotics (*Pediococcus pentosaceus* and *Lactobacillus sakei*) on growth performance and health status of carp (*Cyprinus carpio*) farming in a closed recirculating system

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Abstract

The aim of this study was to evaluate the influence of prebiotics and probiotics, growth intensity of carp grown in a closed recirculation system, intestinal absorption surface and morphological parameters of blood, carp Fish was living in three different basins for nine months.The first (I) study group of carp with commercial diet feed get 0.2% prebiotic mannanoligosaccharides, the second (II) study group received an additional 0.2% mannanoligosaccharides and 3% probiotics (*Pediococcus pentosaceus* and *Lactobacillus sakei*), and the control group received only commercial feed. After the end of the experiment, the fish were weighed, the lengths of the anatomical parts of the body were measured, weights were calculated, body, intestinal samples were taken and histology performed, which made it easy to measure and record the length of the intestinal tract with micrometres (µm) with a computer program. Blood samples were also taken three times (April, May, June). The results showed that the study group I had higher weight gain ($P<0.05$), the anatomical parts of the body also showed better development ($P<0.05$), while the second group had the smallest weights ($P<0.05$) and the anatomical parts of the body developed worse ($P<0.05$) compared to the control group. The length of the intestinal mucus was highest in study group I and in the second group it was the lowest ($P<0.05$). The morphological parameters of blood were not influenced by additives in prebiotics and probiotics in feed and all morphometric indices were not different ($P>0.05$).

Introduction

In the last decade, several carbohydrates such as mannan oligosaccharides (MOS), oligofructose and inulin (Teitelbaum and Walker, 2002; White *et al.*, 2002) and compounds such as peptides, proteins and some lipids have been incorporated in prebiotics (Gibson, 1998; Fooks *et al.*, 1999). Effects of different prebiotics and their levels on fish growth rate, feed efficiency, digestibility, immune responses and survival have been investigated in a number of studies (Staykov *et al.*, 2007; Burr *et al.*, 2008; Salze *et al.*, 2008; Dimitroglou *et al.*, 2010).

Because of the rapid industrialization of carp production, attention must be paid to develop diet formulation to support the economical and environmental problems facing the fish culture (Balcazar *et al.*, 2006).

The aim of this work is to evaluate the influence of prebiotics (mannanoligosaccharides) and probiotics (*Pediococcus pentosaceus* and *Lactobacillus sakei*), growth intensity of carp grown in a closed recirculation system, intestinal absorption surface and morphological parameters of blood.

Material and methods

Fish were living in three different basins for nine months.The first (I) study group of carp with commercial diet feed get 0.2% prebiotic mannanoligosaccharides, the second (II) study group received an additional 0.2% mannanoligosaccharides and 3% probiotics (*Pediococcus pentosaceus* and *Lactobacillus sakei*), and the control group received only commercial feed. Prebiotics are extracted from yeast cell walls of Saccharomyces cerevisiae that have been exposed to high pH, pH control and autolysed. After autolysis, yeast cell wall and yeast extract are separated. Prebiotics use dried yeast cell walls. Prebiotics "Agrimos" are in powder form, light yellowish-brown color. These prebiotics, in all types of feed, in granules, powders, liquids, maintain a stable form and are uniformly distributed in premixes. The second group of investigated carps in addition received probiotics Pediococcus pentosaceus and Lactobacillus sakei (MOS + PRO) which were sprayed on the feed by 3%. from the total weight of the feed. These probiotic cultures have been expanded at the Department of Food Safety and Quality of the Lithuanian University of Health Sciences. Probiotics are grown at pH = 6.2 ± 0.2, at 25 ° C on M.R.S. broth (de man, Rogosa, Sharpe) and treated with lactic acid bacteria. At the beggining of the trial carp weight was ~ 240 g. They were grown for 9 months.

After the end of the experiment, the fish were weighed, the lengths of the anatomical parts of the body were measured, weights were calculated, body, intestinal samples were taken and histology performed by www.aquaticnation.org which made it easy to measure and record the length of the intestinal tract with micrometres (µm).

Post-mortem and histopathological (*Duodenum*) mucosal histomorphometry. Samples were fixed with 10 % neutral formalin solution. Using standard procedures for histologic evaluation the tissues were embedded into paraffin, cut with rotary microtome by 4 µm thick tissue sections, which were painted with hematoxylin and eosin. Prepared histological samples were examined using an "Olympus BX63" microscope, "Olympus DP72" video camera and the computer "Image Pro Plus" program (Olympus) system, control and test groups of broiler duodenum, ileum villi height and crypt depth, caecum crypt depth were morphometrically measured and microscopically. Villi height measurement were carried out from top of the villi to villi-crypt connector and total mucosal thickness was measured from the top of the papillae to the muscularis mucosa (caecum samples was carried out only one – the latter measurement). Each sample was subjected to 10 intestinal villi measurements (best expressed) to measure the height and in the same places for 10 measurements to measure the thickness of the lining. The thickness of the lining of the villi height was obtained by subtracting the crypt depth ratio (Pockevičius *et al.*, 2014).

Blood samples were also taken three times. Blood samples were collected from the caudal tail vein of carp for haematological analysis. Blood samples were received in plastic tubes. Red blood cell count (RBC) and white blood cells (WBC) were counted by using a haemocytometer. Haematocrit (HCT) was determined using the micro-HCT methods, and HCT value are reportes as packed cell volume percentage. Hb levels were estimated using Sahli's method. Differential WBC (neutrophils, lymphocytes and monocytes) were performed using May-Grunwald_Giemsa- stained blood smears.

The results of the experiment were analyzed using the 1-way ANOVA test, and significant differences between groups were determined by Duncan's multiple range test. Statistica 8.0. for Windows™ software was used. Differences were considered significant at $P<0.05$.

Results and discussions

The application of probiotic bacteria in aquaculture has demonstrated many positive effects on the host. Guardiola *et al.*, (2016) reported that dietary supplementation of date palm fruit extracts and probiotic Shewanella putrefaciens Pdp11 administered alone or in combination had beneficial effects on antioxidant status and innate immune parameters of European seabass (Dicentrarchus labrax). Bacillus sp. NP5 which was isolated from the gastrointestinal tract of tilapia hasbeen proven to increase the activity of digestive enzymes, induce natural immune system, improve the pathogen inhibition activity, increase growth rate and increase survival in several fish species (Putra and Widanarni, 2015; Djauhari *et al.*, 2016). If probiotic and prebiotic are combined in a single product (synbiotic), the benefits will increase (Lisal, 2005).

Table 1. Effects of prebiotics (mannan-oligosaccharides) and probiotics (*Pediococcus pentosaceus* ir *Lactobacillus sakei*) on carp morphological body composition

Parameter	Groups		
	Control	I experimental	II experimetal
Fish weight, g	854.9±170.58	1042.70±186.29*	714.20±243.68*
Fish lengt, mm	367.44±30.50	400.33±24.57	338.00±22.76
Part cranial lenth, mm	82.22±5.45	88.67±7.43	76.33±5.32
Length of torso section, mm	145.44±10.51	153.22±10.26	131.33±10.06
Part of the tail lenght, mm	74.00±8.89	81.56±7.40	68.56±9.62
Height of body, mm	93.78±31.91	116.00±9.41	100.44±8.66*
Weight of internal organs, g	121.33±23.19	127.00±37.54	90.22±21.03
Gut weight, g	23.33±7.84	33.78±9.51*	21.11±8.05
Gut lenght, cm	644.77±80.51	736.11±161.42*	624.44±90.67
Fish coverage, mm	247.33±86.52	296.33±18.89	251.00±24.29
Head weight, g	193.44±39.58	240.66±51.02	156.89±23.53*
Fish weight without internal organs, g	729.56±161.52	910.44±179.83*	558.33±125.23*

*-data statistically signifficant at P<0.05

Probiotics live in microbial feed additives that modulate gastrointestinal microbial communities whereas Prebiotics, non-digestible forage additives stimulate the activity or abundance of beneficial gastrointestinal bacteria. Both of these have received widespread attention, showing the improved production, health and disease resistance of aquatic animals (Daniels *et al.*, 2011). In our results fish weight in experimental group I increased 22% (P<0.05) in experimental group II decreased 16% (P<0.05) compared to the control group. When commercial diet feed get 0.2% prebiotic mannanoligosaccharides, statisticaly signifficant results were get on height of body increased 24 % (P<0.05), weight of gut – 45% (P<0.05), lenght of gut – 14% (P<0.05), fish coverage – 20% (P<0.05) and fish weight without internal organs -25% (P<0.05) comapared with a control group. When commercial diet was supplemented with 0.2% mannanoligosaccharides and 3% probiotics (*Pediococcus pentosaceus* and *Lactobacillus sakei*) (experimental group II) decreased there parameters: fish weight without internal organs – 23% (P<0.05), head weight – 19% (P<0.05), weight of internal organs – 26% (P<0.05) compared to the control group.

Table 2. Effects of prebiotics (mannan-oligosaccharides) and probiotics (*Pediococcus pentosaceus* ir *Lactobacillus sakei*) on carp gut villi lenght

Parameter	Group		
	Control	I experimental	II experimental
Villi lenght, µm	1353.03±101.90	1395.36±101.71	1323.17±126.06

Effects of prebiotics (mannan-oligosaccharides) and probiotics (*Pediococcus pentosaceus* ir *Lactobacillus sakei*) on carp gut villi lenght is presented in Table 2. In experimental group I this parameter increased

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3% (P>0.05), IN experimental group II it decreased by 2% (P>0.05) compared to the control group. The measurement of hematological parameters of cultured fish is a prevent ative effort that is commonly performed to observe the fish health status (Fazio *et al.*, 2013). The use of immunostimulants may enhance activities in a nonspecific defense mechanism, the increase resistance to infectious disease by enhancing innate humoral and cellular defense mechanisms and indirectly cause increased growth of aquatic animals (Amlashi *et al.*, 2011).

Table 3. Effects of prebiotics (mannan-oligosaccharides) and probiotics (*Pediococcus pentosaceus* ir *Lactobacillus sakei*) on carp blood morphological composition

Parameter	Groups		
	Control	I experimental	II experimental
Red Blood Cell (RBC), 10^{12}/l	1.64±0.22	1.68±0.40	1.46±026
White Blood Cell (WBC), 10^{9}/l	4.35±0.56	4.46±1.05	3.87±0.70
Hematocrit (HCT), %	36.20±4.76	36.80±6.11	36.30±3.40
Mean Corpuscular Volume (MCV), fl	221.65±25.92	227.28±57.17	254.34±35.98*

*-data statistically signifficant at P<0.05

In our results, red blood cellsin experimental group II decreased 11% (P>0.05), white blood cell – also 11% (P>0.05), mean corpuscular volume increased – 15% (P<0.05) compared to the control group. In i experemental group all morphological blood indices variuos very low, compared to the control group.

Conclusions

The results of this study clearly demonstrate that combination of synergistically acting probiotic and prebiotic cultures, had negative effect on carps productivity, development of morphological carp body. The positive effect on carps productivity and development of carps body part, was determine when carps compound feed was supplemented with 0.2% of mannanoligosaccharides.

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Beta-(1,3)-glucan, a functional carbohydrate, its role as an immunomodulator

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Abstract

Non-digestible carbohydrates are mainly used for their prebiotic effect in animal nutrition, as they are complex structures which are not digested enzymatically in the animal's digestive tract, but fermented by the intestinal microbiota providing beneficial effects to the animal. Another, not to be neglected, role of some specific carbohydrates is their immune modulating effect, as several immune cells in the animal's body are able to recognize beta-(1,3)-glucan carbohydrate structures. A solid source of beta-(1,3)-glucan is the alga *Euglena gracilis,* as this organism stores this carbohydrate product in its cytoplasm as an energy source. The objectives of this research were, firstly, to test, whether this beta-(1,3)-glucan is readily phagocytosed by antigen presenting cells and is consequently inducing a release of immune modulating cytokines, and secondly, to test, whether those first results could be confirmed during applied research, resulting in a higher resistance against disease. The results of the basic research showed that beta-(1,3)-glucans were readily phagocytosed by antigen presenting cells resulting in a release of immune modulating cytokines such as IL-10, IL-6 and IL-18. In the second phase, the immune modulating effect of a beta-(1,3)-glucan (Aleta™) was tested *in vivo*: broilers challenged with different *Eimeria* strains showed significantly less intestinal lesions due to an increased intestinal presence and activation of antigen presenting cells. Based on this research, we could conclude that specific carbohydrates, namely beta-(1,3)-glucans, serve as immune modulating molecules enhancing the natural defense mechanisms of the animal and consequently contribute to an improved animal health and performance.

Introduction

Specific non-digestible carbohydrates are mainly used for their prebiotic effect in animal nutrition, as they are complex structures which are not digested enzymatically in the animal's digestive tract, but fermented by the intestinal microbiota providing beneficial effects to the animal (Pourabedin and Zhao, 2015). Another, not to be neglected role of some specific carbohydrates is their immune modulating effect. Several immune cells in the animal's body, like the antigen presenting cells (such as macrophages and dendritic cells), can recognize beta-(1,3)-glucan carbohydrate structures by specific receptors on their surface (such as the dectin-1 receptor) (Goodridge *et al.*, 2009; Soltanian *et al.*, 2009). One organism effectively producing beta-(1,3)-glucans is the alga, *Euglena gracilis,* as this organism stores the molecule as a carbohydrate product in its cytoplasm (Krajcovic *et al.*, 2015). This new algal derived beta-(1,3)-glucan is available as an in-feed solution for all animal species. The objective of this research was, firstly, to test whether this beta-(1,3)-glucan is readily phagocytosed by antigen presenting cells and is consequently inducing a release of immune modulating cytokines. Secondly, the objective was to test whether those previous results could be confirmed by an *in vivo* trial in broilers challenged with several coccidiosis strains, evaluating the effect of disease and beta-glucan supplementation on intestinal morphology and immune cell recruitment and activation.

Materials and methods

Firstly, 2 experiments, investigating the mode of action of beta-(1,3)-glucan, were performed. Mouse macrophages were collected from mouse intestine and spleen and distributed into chamber slides ($5x10^5$ cells/chamber). Triplicate wells were incubated with fluorescein isothiocyanate (FITC)-linked beta-(1,3)-glucan preparations (5 µg/ml) for 2 hours and stained for macrophage marker (CD11b) and nucleus marker (4',6-diamidino-2-phenylindole, DAPI). Cells were imaged using confocal microscopy. For the second trial, mice were given FITC-linked beta-(1,3)-glucan at 125, 500, 5000 µg per day by oral gavage, equivalent to 50, 200 and 2000 g per ton of feed respectively, for 7 consecutive days. On day 8, macrophages (F4/80 cells) were isolated from collagenase digested small intestine by magnetic sorting, cultured overnight, and the supernatants were tested for the expression of various cytokines, IL-6, IL-10 and IL-18 by luminex multiplex assay.

A broiler trial was performed at Southern Poultry Research in Georgia, USA. 320 one day old COBB 500 male broilers were divided over 8 cages per treatment. The experiment consisted of 4 treatments: a non-infected, non-supplemented group (1), an infected, non-supplemented group (2), and 2 infected, supplemented groups (3,4). The algal derived beta-(1,3)-glucan product (Aleta™, Kemin Europa NV) was added at 100 and 150 g/ton, in groups 3 and 4 respectively, to a standard starter diet. This ration was fed in mash form ad libitum for 28 days. On day 14, all birds except those in group 1, were orally challenged with a coccidial inoculum (1 ml of distilled water mixed with the inoculum) consisting of mixed cultures of three species of *Eimeria*: *E. acervulina* (75,000 oocysts/ bird), *E. maxima* (40,000 oocysts/ bird), and *E. tenella* (50,000 oocysts/bird). The non-infected control birds received an oral dose of 1 mL distilled water. On day 20, birds from each cage were sacrificed and lesion scored. The upper, middle, and cecal regions of the intestine were lesion scored wherein 0 was normal and 1, 2, 3, or 4 indicate increasing severity of infection (Johnson and Reid, 1970) and reported as an average score. On day 28, 1 cm sections of the distal portion of the ileum were taken from 4 birds per group for analysis of immune cell density and activity. Total antigen presenting cell (APC) frequency was calculated by counting PE-positive cells in two randomly selected, equally sized areas per section stained with macrophage marker or MHC-II (Major Histocompatibility Complex) marker, respectively. Average MHC-II expression levels were determined by measuring the fluorescence intensity of two randomly selected, equally sized areas per section under the green and red fluorescent channels, respectively. All experiments were conducted in accordance with an approved animal use protocol (IACUC).

Results

The results of the confocal imaging showed that beta-(1,3)-glucan was readily phagocytosed by mice macrophages (Fig.1), as the image shows a clear presence of the FITC-labelled beta-glucan in the cytoplasm of the macrophage.

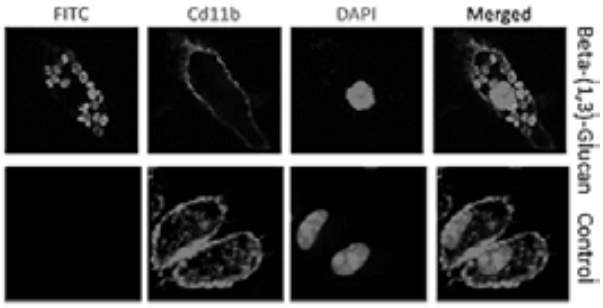

Fig. 1. FITC-labelled beta-(1,3)-glucan (FITC), Mouse macrophages stained for surface (CD11b) and nucleus (DAPI) for beta-glucan (row 1) and non-supplemented macrophages (row 2). Merged picture (Merged) of FITC, DAPI and CD11b staining showing the uptake of beta-(1,3)-glucan by mouse macrophages.

Further research demonstrated a dose responsive release of immune modulating cytokines such as IL-10, IL-6 and IL-18 by macrophages (Fig. 2) harvested from the small intestine of mice, in response to increased supplementation with beta-(1,3)-glucan.

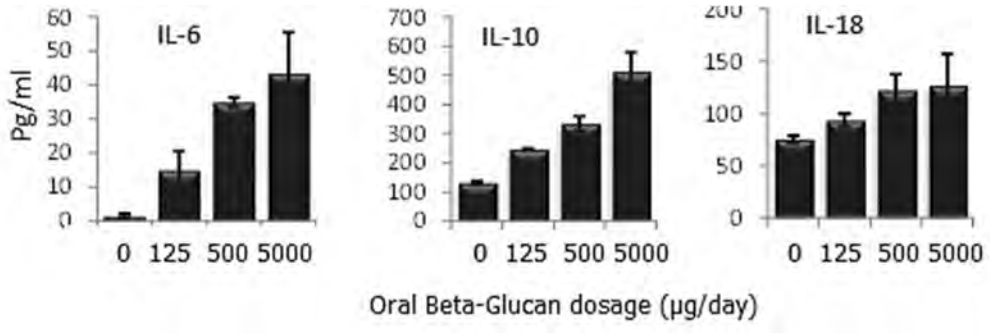

Fig. 2. IL-6, IL-10, and IL-18 secretion by macrophages harvested from the small intestine of mice orally fed with different doses of beta-(1,3)-glucan for 7 days.

The results of the broiler trial show significant effects of beta-(1,3)-glucan on intestinal lesions and immune cell recruitment and activation. The lesion scores on day 20 of the trial are shown in Table 1. Compared to the infected but non-supplemented group (2), all Aleta™-treated groups (3,4) had significantly lower lesion scores throughout the intestine.

Table 1. Average lesion scores (d 20) in male broilers challenged with different *Eimeria* strains and fed increasing levels of beta-(1,3)-glucan. Means (8 replicates) with different superscripts are statistically different (P<0.05).

Group	treatment	Average lesion score
1	Not challenged, not supplemented	0.00^{c}
2	Challenged, not supplemented	1.73^{a}
3	Challenged and supplemented at 100 g/ton	1.54^{b}
4	Challenged and supplemented at 150g/ton	1.50^{b}
P-value		<0.0001

Results of the analysis of intestinal immune cell density and activity in the broiler trial are shown in Fig. 3. A significant increase in APC frequency and MHC II was observed in both beta-(1,3)-glucan groups (3, 4) compared to both control groups (1, 2).

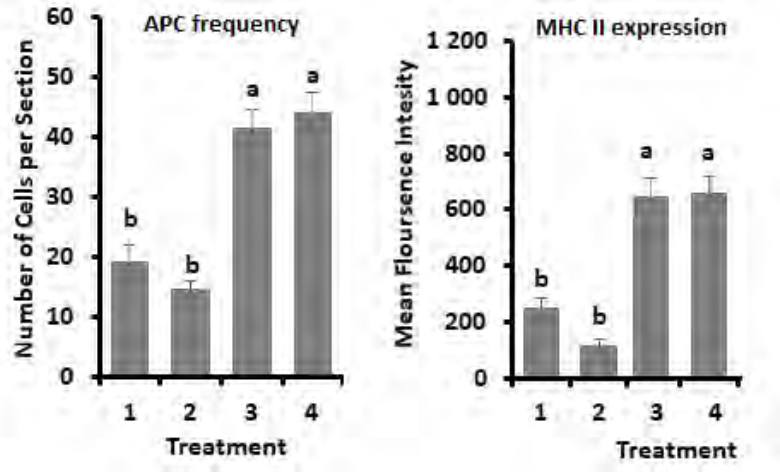

Fig. 3. Immune cell quantification (APC frequency and MHC expression) in the distal ileum of broilers at d28. Data are means ± standard error (n=32 per treatment). Bars with different superscripts are statistically different (P<0.05).

Discussion and conclusion

An ideal immune modulating substance needs to help induce an immune response to pathogens entering the animal's body. Therefore, the immune response cascade needs to be respected and the following characteristics are required: the candidate ingredient must be recognized by antigen presenting cells (macrophages) and induce a secretion of cytokines (responsible for intercell communication), resulting in recruitment and activation of other immune cells combatting the pathogen (Medzhitov, 2007; Soltanian *et al.*, 2009). In this paper, data are summarized showing the potential immune modulating capabilities of an algal derived beta-(1,3)-glucan. During a first trial, it was demonstrated that this beta-(1,3)-glucan is recognized and phagocytosed by antigen presenting cells (macrophages) and is consequently inducing a release of immune modulating cytokines. The production of IL-6 (a pro- and anti-inflammatory cytokine), IL-10 (an anti-inflammatory cytokine), and IL-18 (a pro-inflammatory cytokine) was shown in this paper. As both pro-inflammatory and anti-inflammatory cytokines are secreted in response to this specific beta-glucan, we can conclude that the algal derived beta-glucan triggers a balanced immune response: activation but no overstimulation of the immune system (which would result in inflammation).

During a consecutive broiler trial, we could observe an increased APC frequency and MHC II expression in all beta-(1,3)-glucan supplemented groups. The latter is an indicator of the activation of APC (Sonck *et al.*, 2011). These results prove an enhanced recruitment and activation of antigen presenting cells in the ileum by the beta-(1,3)-glucan supplementation, increasing the animal's resistance to disease. This may explain the significant reduction in intestinal lesions in response to the *Eimeria* challenge in the beta-glucan supplemented groups. Notably, the infected control had many fewer APCs and less MHC II expression compared to all other groups, possibly due to the parasite using the Th2/IL10 pathway to suppress immune function and preclude attack (Cornelissen *et al.*, 2009; Haritova and Stanilova, 2012).

Based on this research, we could conclude that specific carbohydrates, namely beta-(1,3)-glucans, are serving as immune modulating molecules enhancing the natural defense mechanisms of the animal against diseases and consequently contributing to an improved animal health and performance.

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Traubenpolyphenole, ein effizientes Instrument zur Optimierung von Vitamin E in der Tierernährung

Grape polyphenols, an efficient tool to optimise vitamin E

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Abstract

Vitamin E is one of the reference dietary antioxidant compounds used in animal nutrition. Its nutritional needs from all species associated to its antioxidant activity have led to its generic utilisation. However, recent economical context of vitamin E prices and recurrent crisis have led the feed industry to try and find stable, reliable and efficient alternatives to partially replace vitamin E in the diet and optimise for the animal to optimise it.

Polyphenols are active secondary metabolites from plants, and have a largely documented antioxidant activity. Many researches have been conducted on their efficacy to work in synergy with vitamin E, either *in vitro*, *ex vivo* or *in vivo*.

This work reviews research conducted *in vitro*, *ex vivo* and *in vivo* which evidences the possibility to partially replace vitamin E by grape polyphenols in the diet of livestock raised in intensive farming systems.

Einleitung

Alle höheren Organismen, welche in der intensiven Landwirtschaft gehalten werden, haben einen oxidativen Metabolismus, was bedeutet, dass Sauerstoff der Schlüssel für einen gut funktionierenden Organismus ist [1].
Phänomene der freien Radikale, die von dieser Art des Metabolismus herrühren, sind in allen Nutztier-Spezies (terrestrisch ebenso wie Aquakultur) vorhanden und können schädliche Auswirkungen auf zootechnische Leistungen und / oder die Gesundheit von Tieren hervorrufen [1-3].
Um gegen diese aggressiven Moleküle anzukämpfen, macht sich der Organismus antioxidative Verbindungen als Abwehrmechanismus zu nutze. Die Abwehrmechanismen können endogener Natur sein, synthetisiert durch die Zellen (Enzyme wie Superoxiddismutase, Glutathionperoxidase, Katalase oder Vitamin C für einige Spezies) und exogen, durch die Nahrung aufgenommen (Vitamin E, Selen und Vitamin C für einige Spezies) [1].
Polyphenole sind sekundäre Metaboliten, die von Pflanzen synthetisiert werden. In einer Vielzahl von Pflanzen untersucht, können ihnen viele interessante biologische Aktivitäten zugesprochen werden. Das starke antioxidative Potenzial ist eines ihrer wichtigsten Vorzüge [1, 4]. Polyphenole aus Trauben werden aufgrund ihrer natürlichen antioxidativen Eigenschaften, die insbesondere im Rahmen der "French Paradox" -Studie entdeckt wurden, seit vielen Jahren intensiv erforscht. Bis dato existieren zahlreiche Studien, in denen ihre antioxidativen Eigenschaften mit denen des bekannten Antioxidans Vitamin E verglichen wurden.
Jüngste Forschungen haben darüber hinaus gezeigt, dass Traubenpolyphenole mit anderen zellulären antioxidativen Abwehrmechanismen wie Vitamin C oder E interagieren können. Diese Entdeckung hat zu mehr und mehr Forschung in Bezug auf einer möglichen Substitution von Vitamin E, als das

Referenzantioxidans in der Tierernährung, mit Traubenpolyphenole geführt. Solche Arbeiten wurden im Folgenden kritisch hinterfragt.

In vitro

Mehrere Studien schreiben den in Trauben gefundenen Polyphenolen eine stärkere antioxidative Wirkung als Vitamin E zu (höhere antioxidative Wirkung, Neutralisationskapazität freier Radikale, Lipidschutz gegen Peroxidation, besserer Gesamtschutz gegen oxidativen Stress [5-12]). Abhängig von den experimentellen Bedingungen und der Herkunft wurde nachgewiesen, dass die antioxidative Aktivität bis zu 25-mal höher ist als die von Vitamin E.
Darüber hinaus haben einige Untersuchungen die Fähigkeit der phenolischen Verbindung aufgezeigt, oxidiertes Vitamin E zu regenerieren. Amorati *et al.* und Pazos *et al.* demonstrierten *in vitro* zweifellos die hoch effiziente Regeneration von Vitamin E durch phenolische Verbindungen [13-14].

Ex vivo

In vitro-Modelle können jedoch hinsichtlich der Darstellung komplexer Wechselwirkungen zwischen der getesteten Verbindung und der zellulären Umgebung eingeschränkt sein. So wurden in mehreren Studien komplexere *Ex vivo*-Analysen verwendet, um Interaktionen zwischen Traubenpolyphenolen und Vitamin E nachzuweisen. Insbesondere in Bezug auf die Fähigkeit der Traubenpolyphenole oxidiertes Vitamin E zu regenerieren [15-17].
Um ein repräsentatives Verständnis der *in vivo* Aktivität von Polyphenolen aus einem standardisierten Traubenextrakt zu erhalten, der als EU-Futterzusatzstoff (getrockneter Traubenextrakt, 2b485) registriert wurde, ist eine *ex vivo*-Testkampagne (KRL-Test, KIRIAL International, Frankreich) initiiert worden.
Die Ergebnisse belegten, dass die antioxidative Aktivität des Traubenextrakts durchschnittlich 7,36-mal höher war als die von α-Tocopherol, gemessen in Trolox oder Gallussäureäquivalent pro Gramm Extrakt. Des Weiteren konnte eine Mischung aus natürlichem Tocopherol und dem Traubenextrakt eine Synergie aufweisen, die dem 1,67-fachen des theoretischen Wertes entsprach. Die Kombination von 1 Gramm Traubenextrakt mit Vitamin E könnte daher als äquivalent zu 12,29 Gramm Vitamin E (100%) berechnet werden (7,36 * 1,67).

In vivo

Neueste Forschungen untersuchten ergänzend die Wirkung einer teilweisen Substitution von Vitamin E durch Traubenpolyphenole auf verschiedene Parameter. Iqbal *et al.* analysierten den Effekt einer teilweisen Substitution von Vitamin E durch einen Traubenextrakt in Broilerfutter, wobei die Substitutionsrate von 25% bis 75% im Verhältnis 1:1 reichte [18]. Die Ergebnisse zeigten keine signifikanten Unterschiede zwischen den Gruppen im Wachstum der Tiere. Indes berichteten sie von einer signifikanten Verbesserung einiger Parameter wie Antikörperspiegel durch diese Diätänderung [19].
Rodriguez-Ortega *et al.* konnten ebenfalls nachweisen, dass eine teilweise Substitution von Vitamin E (75%) durch Polyphenole der Trauben das Wachstum oder den Futterverbrauch zwischen den Gruppen nicht signifikant beeinflusste [20]. Auch bei Legehennen werden mit einer vergleichbare Substitutionsrate von Vitamin E mit Traubenpolyphenolen in der Fütterung weder deren Leistungsfähigkeit (Legeleistung) beeinflusst noch die antioxidative Kapazität der Eier beeinträchtigt, wie Olteanu *et al.* berichten [21].
Schließlich weisen Nardoia *et al.* darauf hin, dass eine teilweise Substitution von Vitamin E durch Traubenpolyphenole in der Diät von Masthähnchen genauso wirksam sein kann wie Vitamin E, um das Auftreten von sekundären Oxidationsprodukten im Fleisch zu begrenzen [22].

Ferner bekräftigten Untersuchungen von Engler, die starke Wirkung der Traubenpolyphenole als Antioxidans im Tier. In seiner Studie rief ein Ersatz von 86% des Vitamins durch Traubenpolyphenole keine signifikanten Leistungsunterschiede zwischen Legehennen hervor, die einem Ernährungsstress unterzogen wurden [1].

Substitutionsrate

Die Literatur zeigte, dass ein teilweiser Ersatz von Vitamin E durch Traubenpolyphenole erreicht werden kann, ohne die Wachstumsleistung der Tiere negativ zu beeinflussen. *Ex vivo*-Studien veranschaulichten auch eine Substitutionsrate, wonach 1 Gramm trockener Traubenextrakt etwa 12 Gramm Vitamin E entsprechen.

Ein Beleg hierfür ist u.a. die Studie von Juin *et al.*, bei dessen Ersatz von 100 ppm Vitamin E durch 10 ppm trockenen Traubenextraktes die Leistung junger Küken nicht beeinträchtigt wurde, sondern stattdessen zu einer signifikanten Zunahme des Wachstums in der Traubenextrakt-Gruppe führte [23]. Ähnliches konnte auch Engler *et al.* in Kälbern bestätigen, die teilweise Substitution von Vitamin E (25%) im Verhältnis 1:11 (1 Gramm Traubenextrakt anstelle von 11 Gramm Vitamin E) hatte keinen signifikanten Einfluss auf die Leistung der Kälber [24].

Antioxidative Abwehrsysteme sind, über alle in der intensiven Landwirtschaft gehaltenen Tierarten hinweg, gleich. Sie funktionieren in der Tat durch zelluläre Mechanismen, die zwischen diesen verschiedenen Arten nicht wesentlich differieren. Eine Beobachtung in einer Art kann daher auf zellulärer Ebene bei anderen Arten ebenso erwartet werden.

Obwohl einige Studien bewiesen, dass der Ersatz eines hohen Anteils des Vitamin E (mehr als 50%) durch Traubenpolyphenole möglich ist ohne die zootechnischen Leistungen negativ zu beeinträchtigen, sollte der vollständige Verzicht auf Vitamin E vermieden werden. Vitamin E ist durchaus für die gute Funktion des Organismus notwendig und sollte dem Tier immer in einer Menge zugeführt werden, die mindestens dem von Referenzforschungsorganisationen (z. B. NRC, INRA) festgelegten Nährstoffbedarf entspricht.

Schlussfolgerung

Die starken antioxidativen Eigenschaften von Traubenpolyphenolen, verbunden mit einer synergistischen Wirkung in Kombination mit α-Tocopherol, ermöglichen den teilweisen Ersatz von Vitamin E über die gesamte Fütterung von Nutztieren. Diese Eigenschaft wurde *in vitro* und *in vivo* nachgewiesen. Darüber hinaus ist eine teilweise Substitution von Vitamin E durch getrockneten Traubenextrakt in einem Verhältnis von 1 zu 11 realisierbar und beeinträchtigt nicht die zootechnischen Leistungen von Nutztieren.

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Autorenanschrift

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Both energy and protein balance early postpartum affect selected fertility traits in dairy cows

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Abstract

The aim of this study was to investigate the effect of nutrient intake, meeting the level of energy and protein requirements and milk production during the early-to mid-lactating period on the conception interval (CI) in dairy cows. The intake of energy and nutrients as well as milk production parameters from week 3 to 17 postpartum in 30 dairy cows differing in the CI [i.e., early (E, n=8), intermediary (I, n=11) and late (L, n=11) for cows confirmed pregnant within 10 weeks or between 11 and 17 weeks, or thereafter, respectively] were compared. The level of meeting the energy and protein demands from week 3 to 10 postpartum was instrumental in shortening the CI in cows of group E. The cows of group I showed higher milk urea nitrogen compared to L cows. In conclusion, exceeding the requirements in energy and protein in the early lactation period shortened the CI to <10 weeks. Moreover, a better protein status might contribute to improved fertility in mid-lactating cows.

Introduction

During the last decades, there has been an alarming decline in cow fertility. Improper nutrition of cows during the transition period and excessive deficiency in energy and nutrients postpartum has been suggested to play a role in the impaired fertility traits (Walsh *et al.*, 2011). Among nutrients directly linked to fertility, minerals, and vitamins, and dietary protein level and especially protein quality have been reported to play a role (Moellers and Riese, 1988). However, there is a paucity of information regarding the influence of the level of meeting the energy and nutrient demands on the reproductive performance in cows during early lactation. The aim of this study, therefore, was to compare the level of meeting the energy and protein requirements, nutrient intake and milk production parameters during the early-to mid-lactating period in dairy cows differing in fertility traits.

Material and methods

The study was conducted at the Dairy Research Facilities of the Institute of Livestock Research, Agricultural Research and Education Centre Raumberg-Gumpenstein, Austria. The experiment involved 30 Holstein and Simmental dairy cows that were monitored daily for intake of energy and nutrients from week (wk) 3 to 17 postpartum, and for reproduction traits until confirmed pregnant. Dietary ingredients were 15% hay, 19% corn silage, 16% grass silage, 39% barley grain and 11% protein-mineral-vitamin supplement (dry matter basis). Cows were fed ad libitum and milked twice daily and milk samples were analyzed for fat, protein, lactose, and milk urea N (MUN) by Milkoscan (Foss Electric, Denmark). The daily energy and protein balances were estimated according to Gruber *et al.* (2014).

After 3 wk postpartum cows were monitored for estrus and inseminated for the first time after 6 wk postpartum. The interval from parturition to the first service was recorded. The conception interval (CI) was considered the time lapse from parturition until the successful insemination. The number of services until the conception was also considered as insemination index. Based on the length of the CI, cows that were successfully inseminated within 10 wk postpartum were classified as early (E; n=8), whereas cows that got pregnant between wk 11 and 17 after parturition were considered as intermediary (I; n=11) and cows that were confirmed pregnant thereafter were classified as late (L; n=11) CI group. Statistical analysis was performed using PROC MIXED of SAS (SAS, version 9.2), considering repeated measures.

Results and discussion

The level of meeting the energy and protein demands affected the interval from parturition until first insemination ($P < 0.01$), with a longer duration in group I and L compared to E (+22 d and +29 d, respectively). Moreover, the insemination index differed among groups ($P < 0.01$), being one service for E group, while cows of group L needed almost 3 services until successful insemination.

The overall dry matter intake from wk 3 to 10 in milk did not differ among CI groups. No group effect for the intake of NEL, rumen-degradable protein (RDP), rumen-undegradable protein (RUP), utilizable crude protein at the duodenum (uCP) as well as the ruminal nitrogen balance was observed. The ratio of RDP to RUP showed a linear increase with prolongation of the CI from E to L ($P = 0.01$). Moreover, a comparison of group I to L from wk 11 to 17 after parturition revealed a tendential lower ratio of RDP to RUP for I compared to L group ($P = 0.09$). The intake of most of the investigated minerals did not differ among groups. However, E cows tended to consume more sodium compared with L cows during the first 10 wk in milk ($P = 0.07$).

The level of meeting the requirements in NEL was different among the CI groups. As shown in Fig. 1, the E cows were in lesser negative energy balance (NEB) during early lactation compared with I and L cows, showing a slight oversupply in NEL compared to group I and L from wk 5 to 10 in milk ($P < 0.01$). This is supported by previous research, showing that energy deficiency is linked with impaired reproductive performance (Butler, 2003; Santos *et al.*, 2010).

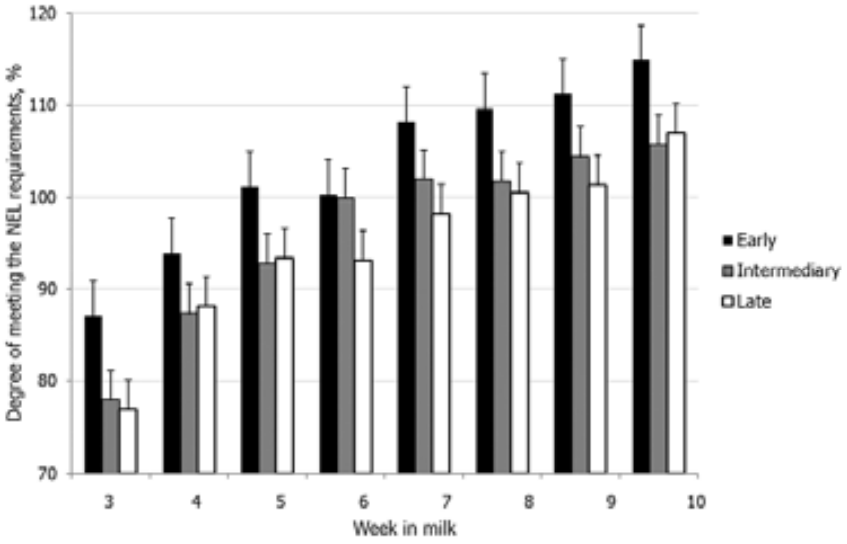

Figure 1. Degree of meeting the energy requirements in cows differing in the conception interval

A comparably trend was observed among groups with respect to meeting the requirements in utilizable crude protein at the duodenum (uCP; Fig. 2).

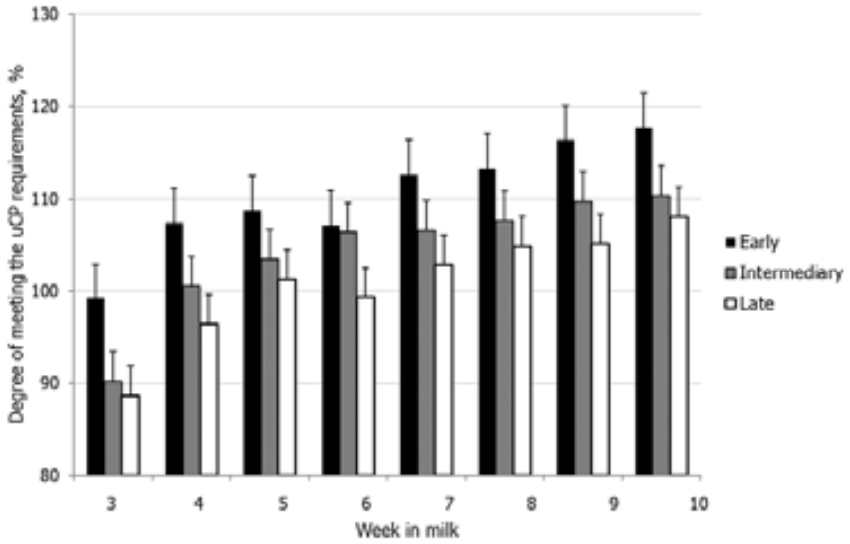

Figure 2. Degree of meeting the requirements with utilizable crude protein at the duodenum (uCP) in cows differing in the conception interval

The effects of dietary protein level and source on cow's fertility have been differently discussed. While high protein levels seem detrimental to fertility (Sinclair *et al.*, 2014), feeding high levels of RUP has been reported to improve several reproductive traits in cows (Aboozar *et al.*, 2012). The present results suggest a role for quantitative (uCP supply) as well as qualitative (RDP:RUP ratio) protein supply on fertility traits in cows.

The milk yield from wk 3 to 17 showed only a numerical increase from cows with short to long CI. The milk fat and protein concentration did not differ between groups from wk 3 to 10 after parturition. A trend towards a linear increase of lactose concentrations from group E to L was detected ($P = 0.10$). During wk 11 to 17, a lower milk protein concentration of the cows of group I compared to group L was observed ($P = 0.04$). The cows of I group showed higher MUN concentrations compared to cows of L group (+27%; $P = 0.02$), which was also observed from wk 11-17 (+31%; $P < 0.01$). Concentrations of MUN of >19 mg/dL have been demonstrated to impair reproduction rates in dairy cows (Butler *et al.*, 1996), whereby this concentration was not reached in I cows. As lower MUN concentrations were found in the group with the longest CI compared to group I, it seems that greater MUN in I group can be interpreted as improved protein status in those cows, suggesting that this condition can even improve the fertility, if it does not exceed a level that might lead to damage of the liver and the uterine environment.

Conclusions

Taken together, timely meeting of the requirements in NEL and uCP during the early lactation is instrumental for shortening CI length in E cows. Further research is required to identify possible reasons for the differences in fertility in mid-lactating cows.

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The effect of wheat replacement by wheat bran on the growth performance and carcass quality of fattening pigs

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Abstract

The properly balanced composition of the feed ensures the growth performance of fattening pigs, reduces the feed costs and the production prime cost. Moreover, various feed components affect the quality of pig meat and carcasses. Therefore, when optimizing the composition of the feed, it is necessary to take into account not only the price of the feed but also its impact on the quality of products. Wheat bran is a raw material having a lot of fiber and being cheaper than wheat. In order to reduce the price of the feeds for fattening pigs, it is necessary to use as much wheat bran as possible. It is considered that the use of a great amount of wheat bran will worsen the growth results of fattening pigs and the quality of their carcasses. Therefore, we tried to determine the effect of partial wheat replacement by 20 percent of wheat bran on the growth of fattening pigs and the quality of carcass.

Introduction

A proper nutritious diet is one of the factors of successful pig production. The feeds for pigs should be balanced by well-digestible proteins, carbohydrates and fats. Moreover, the feeds should contain minerals, vitamins and growth-promoting substances. The grains of cereals - wheat, barley, triticale and their bran - are the main ingredients of compound feed for fattening pigs. The Lithuanian farmers used to grow wheat. The grown wheat is exported. Therefore, quite often the pig growers face the lack of the feed grains or their increased cost. Consequently, the pig growers are looking for alternative feed ingredients. The wheat bran is one of them. The wheat bran is got when producing the bread flour. For that reason, the quantity and price of this raw material is stable in the Lithuanian market. The wheat bran contains approximately 12-17 percent of proteins. In comparison with wheat, the wheat bran is protein-rich, contains a lot of phosphorus and B-group vitamins (Leikus *et al.*, 1999). However, it also has a lot of fiber. Consequently, its content in the compound feeds for fattening pigs is limited up to 20 per cent. Grains and their bran contain non-starch polysaccharides, which excrete the endogenous enzymes in pig's organism and which are almost indigestible. Moreover, such antinutrient materials reduce the digestion of other, well-digestible nutrients, by "closing" them. In order to eliminate such effect of this negative non-starch polysaccharides, the xylanase, glucanase and other enzymes are used (Triukas *et al.*, 1998). The use of wheat bran for fattening pigs is a good alternative to more expensive wheat. However, the effect of a greater amount of wheat bran on the fattening pigs' growth performance and carcass quality is not known. The aim of this work is to study the influence of wheat bran on the growth performance, state of health of fattening pigs and their carcass quality.

Material and methods

The experiment was conducted in the year 2017 in industrial pig farm cultivating up to 24 000 fatting pigs per year. The trial was performed with mixed Norwegian Yorkshire, Norwegian Landraces and Duroc breed pigs. Two-month-old pigs were divided into two groups by analogue approach. There were 26 pigs in each group. The experiment consisted of two periods – the growing period (20-60 kg) and the final period (61-100 kg). The first period lasted for 62 days, the second one - for 42 days. The pigs

of the first group were the control ones and were fed with a compound feed without wheat bran. The pigs of the second group were fed with a compound feed with partial replacement of wheat by 20 per cent of wheat bran. The composition and nutritive value of the compound feed are presented in Table 1. While composing the recipes of the compound feeds, the computer programme "Hybrimin Futter 5" was applied. The energy amount of the compound feeds was calculated according to the formulas (Jeroch *et al.*, 2004). During the experiments, the pigs of both groups were fully fed ad libitum by the dry powder compound feed from automatic feeders. Automatic separate water-troughs were applied in order to get enough water. Every stall was equipped with one automatic water-trough and feeder. During trial, the pigs of both groups were kept under the same conditions. At the end of experiment all pigs from control and experimental group were slaughtered. The animals were slaughtered according to the Methods of Control Pig Fattening and Slaughtering (Methods of control pig fattening and slaughtering in Lithuania). Data were analysed using the t-test (Juozaitienė and Kerzienė 2001).

Table 1: Biochemical composition and nutritive value of feeds

	Group 1		Group 2	
Ingredients, %	Growing period 20-60 kg weight	Final period 61-100 kg weight	Growing period 20-60 kg weight	Final period 61-100 kg weight
Barley	40,0	49,0	40,0	49,0
Wheat	37,22	36,7	17,22	16,7
Wheat bran	-	-	20,0	20,0
Soya bean extracted	13,0	5,0	13,0	5,0
Sunflower seed extracted	5,0	5,0	5,0	5,0
Rape seed oil	1,0	1,0	1,0	1,0
Lysine HCl	0,18	0,3	0,18	0,3
Sodium chloride	0,3	0,3	0,3	0,3
Calcium carbonate	1,4	1,4	1,4	1,4
Monocalcium phosphate	0,9	0,3	0,9	0,3
Premix	1,0	1,0	1,0	1,0
Calculated analysis / kg feed:				
Metabolizable energy, MJ	12,77	12,66	12,05	11,94
Crude protein, %	17,18	14,22	17,69	14,73
Crude fibre, %	4,46	4,73	6,12	6,38
Crude fat, %	2,76	2,81	3,17	3,22
Calcium, %	0,78	0,67	0,80	0,69
Phosphorus, %	0,61	0,45	0,80	0,64
Sodium, %	0,16	0,16	0,16	0,17
Lysine, %	0,91	0,78	0,96	0,83
Methionine, %	0,29	0,24	0,29	0,25
Threonine,%	0,62	0,49	0,65	0,52
Tryptophan, %	0,21	0,17	0,23	0,19

Compound feed content per kg: 9000 IU vit. A; 1800 IU vit D; 120 mg vit. E; 2,4 mg K3; 1,8 mg B1; 4,8 mg B2; 13,5 mg pantothenic a.; 22,5 mg niacin; 3 mg B6: 25,5 mcg B12; 60 mcg biotine; 0,9 mg folic a.; 90 mg Fe; 90 mg Zn; 45 mg Mn; 15 mg Cu; 1,2 mg Co; 1,5 mg J, 0,42 mg Se; 450 FTU Phytase

Results and discussion

During the experiment no health disorders were identified within the groups of controlled experiment and exploratory. In the course of the experiment, the pigs of the first group were fed with compound feeds, the composition of which was optimized in accordance with requirements (Jeroch *et al.*, 2004) by using wheat. The pigs of the second group were fed with compound feeds, the composition of which was changed by replacing wheat with wheat bran. The feed of the second group was not additionally optimized. After replacing wheat with wheat bran, the second group of pigs received compound feeds,

containing 50 percent more fiber than the first group and exceeded permissible limit by 6 per cent. During the experiment it was determined that such a compound feed of the second group with a high content of fiber influenced the growth of pigs on the first fattening stage. The test results are presented in Table 2. During the first fattening period, the second group pigs daily gained in weight 6.3 per cent less than the pigs of the first group. However, on the second fattening stage, after receiving the unbalanced compound feeds with a high content of fiber, the pigs of the second group grow even better than the first group pigs receiving the balanced compound feeds. In the course of the second fattening period, the second group pigs daily gained in weight 7.3 per cent more than the pigs of the first group. When calculating a daily body weight gain, it was determined that a high amount of fiber in the feed did not have any adverse effect on the growth of the fattening pigs. The pigs of both the first and the second group gained in weight daily 709 and 705 g respectively. It is sufficiently high growth rate in an industrial farm.

Table 2: Growth rate of pigs

Item	Group 1 (Control) $\bar{x} \pm se$	Group 2 (Experimental) $\bar{x} \pm se$
Average weight of pigs, kg		
at the start	21,5±0,56	21,5±0,59
at the end of the first period	64,5±0,94	61,8±1,21
at the end	95,2±1,26	94,7±1,30
Average daily gain, g:		
during the first period	694±11	650±15
during the second period	731±18	784±19
during the whole trial	709±10	705±13

During the experiment it was determined that when feeding the fattening pigs with compound feeds containing more than 6 per cent of fiber, the conversion of feed does not increase significantly. It was determined that in the first fattening period, per 1 kg gain in weight, the second group of pigs consumed 4 percent more feed than the pigs of the control group (Table 3). In the second fattening period, such content of fiber did not adversely affect the feed conversion. During this period, per 1 kg gain in weight, the pigs of the second group consumed 1.7 percent less feed than the pigs of the first group. The calculations over the whole period of fattening allow stating that the replacement of wheat with wheat bran and feeding of pigs with the feed containing more than 6 per cent of fiber are acceptable.

Table 3: Compound feed consumption per kg gain kg

Item	Group 1	Group 2
During the first period	3,04	3,16
During the second period	3,99	3,92
During the whole trial	3,44	3,48

The main method of evaluating the effective qualities of the fattening pigs is a control slaughtering of pigs and evaluation of carcass. In our experiment, after the end of fattening, a control slaughter of all pigs and an assessment of their carcasses were performed. The data obtained are presented in Table 4. During the control slaughter it was determined that the mean weight of the pigs slaughtered did not differ significantly. The pigs were slaughtered at an average weight of 95 kg. Before the slaughtering, the mean weight of the pigs from the second group was 0.5 kg lower than the weight of the control pigs. Due to this, the weight of the second group pigs' carcasses was 0.7 kg lower than the one of the first group. When fattening the pigs, it is important to achieve not only their rapid growth, but also a high yield of carcasses. The yield of carcasses and products thereof depends on a great number of factors with most significant as follows: animal species, breed, gender, age, feeding rate, fatness, conditions of growing and living, individual animal characteristics (Sudikas *et al.*, 2010). During the experiment it was determined that the feeding of pigs with wheat bran does not worsen their carcass

yield. The pigs with a big muscular tissue are the most appreciated. The lean meat content in the carcasses represents the ratio of the carcass weight to the muscle mass and is expressed in per cents. During the experiment it was found that the amount of lean meat in the carcasses of the pigs fed with compound feeds containing wheat bran was slightly (1.1 per cent) higher than in the carcasses of the control group pigs.

Table 4: Carcass quality

Item	Group 1 (n=26) $\bar{x} \pm se$	Group 2 (n=26) $\bar{x} \pm se$
Average final weight, kg	95,2±1,26	94,7±1,30
Carcass weight, kg	73,5±1,17	72,8±1,45
Dressing percentage	77,2±0,68	76,9±0,98
Lean meat, %	54,1±0,73	55,2±0,77

Summarizing the results of the experiment, we can state that quite frequent cases when in pig farms the missing feed grains are replaced with wheat bran, thus reducing the price of produced compound feeds, have no negative consequences on pigs' growth performance and quality of their carcasses. In the analyzed cases, the replacement of wheat by 20 percent of wheat bran and the feeding of fattening pigs with compound feeds containing 6 per cent more fiber, had no significant effect on the health state of fattening pigs, their growth performance, conversion of feeds and quality of their carcasses, in comparison with fattening results of the pigs who were fed with the balanced compound feeds conforming with norms.

Conclusion

When feeding the fattening pigs and in the case of feed grains absence, it is possible to replace the wheat of compound feed by 20 percent of wheat bran. The presence of more than 6 percent of fiber in compound feeds of fattening pigs did not have any negative effect on the health state of fattening pigs, growth rates, feed conversion and carcass quality.

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Effect of protein source and treatment on performance and oxidative status in piglets

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Abstract

The present study hypothesized that dietary protein sources with low ANF and allergen concentration positively stimulate weaned piglets to higher growth due to lower inflammatory and diarrhea response. In total 216 weaned pigs (initial BW: 6.69kg) were randomly assigned to 6 dietary treatments with 6 replicates of 6 pigs per pen. Experimental period was divided into phase 1 (d1-14) and phase 2 (d15-28). The 6 dietary treatments were a control diet consisting of corn-wheat-soybean meal basal diet (CON) and test diets were CON were soybean meal was replaced with either soy protein concentrate (2), fermented soybean meal (3), fish meal (4), enzyme-treated soybean meal (HP 300) (5) in phase 1. In diet 6 enzyme-treated soybean meal (HP 300) replaced SBM in both phase 1 and 2 (6). All diets were formulated to be iso-nitrogenous. During d1-14, pigs in ESBM1 or ESBM2 had greater (P<0.05) average daily gain (ADG) than CON, SPC or FSBM. There were no differences in ADG and feed efficiency (ADG/ADFI) between FM and ESBM1. ESBM2 had highest daily gain compared to all other groups from d15-28 and during the total period. Diarrhea rate was lower (P<0.05) for pigs fed ESBM1 or ESBM2 compared to CON. Pigs fed ESBM1 or ESBM2 had higher (P<0.05) SOD, and lower (P< 0.05) MDA concentration compared to CON. Pigs fed ESBM2 had higher (P<0.05) T-AOC values compared to CON and FSBM. Supplementation of ESBM2 increased (P< 0.05) the concentrations of SOD, GSH-PX and T-AOC compared with CON, SPC and FSBM, and decreased (P< 0.05) the concentration of MDA compared to CON. In conclusion, the study confirmed that ESBM low in ANF's and antigen results in increased growth performance, reduced diarrhea and improved oxidative status in piglets.

Introduction

At weaning, piglets have to cope with the sudden withdrawal of sow milk and adapt to solid feed based on animal and plant protein sources. Due to the immature digestive system, weaned piglets are at risk of diarrhea when exposed to complex plant protein sources. Soybean meal (SBM) is the most widely used plant protein, despite the content of anti-nutritional factors (ANF) and antigens. ANF's includes oligosaccharides e.g. raffinose, stachyose and sucrose, which are indigestible in piglets but rapidly ferment in the lower gut and affect gut health negatively (Choct *et al.*, 2010). Trypsin inhibitor is associated with decreased protein digestibility and antigens negatively affect growth of piglets most likely through intestinal damage and gut inflammation (Lalles, 1993, Chen *et al.*, 2011). Lenehan *et al.* (2007) reported that replacing SBM with soy protein concentrate in piglet diets improved performance. Kim and Easter (2001) showed that fishmeal improved growth performance and immune function of weaned pigs. We hypothesized that dietary protein sources with low ANF and allergen concentration positively stimulate weaned piglets to higher growth due to lower inflammatory and diarrhea response. The objectives of the study was to evaluate the effect of replacing SBM with low ANF and antigen protein sources on daily gain and feed intake, diarrhea incidence and serum antioxidant parameters.

Material and methods

In total, 216 [Duroc × (Landrace × Yorkshire)] with 6.69 kg initial BW were randomly assigned to 6 dietary treatments. Each treatment had 6 replicate pens with 6 pigs per pen. Experiment diets were fed in phase 1 (d 1 to 14) and 2 (d 15 to 28). Diet formulation is shown in table 1. All diets were formulated to meet or exceed the estimated nutrient requirements for nursery pigs as recommended by NRC (2012). Pigs were housed in a temperature-controlled nursery house (25 to 27 °C). Pigs had *ad libitum* access to feed and water and were individually weighed on days 1, 14 and 28 of the experiment. Feed consumption was measured per pen. These values were used to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed efficiency (ADG/ADFI).

Table 1. Composition of experimental diets.

Item	phase 1 (1-14 d)						phase 2 (15-28 d)					
	CON	SPC	FSBM	FM	ESBM1	ESBM2	CON	SPC	FSBM	FM	ESBM1	ESBM2
Corn	36.0	41.5	40.2	42.6	41.1	41.1	41.6	41.6	41.6	41.6	41.6	49.8
Wheat	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
SBM	24.0	12.0	12.0	12.0	12.0	12.0	22.0	22.0	22.0	22.0	22.0	12.0
SPC		7.5										
FSBM			9.8									
ESBM					9.0	9.0						7.5
FM				7.4								
SDPP	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0
SB oil	6.6	5.8	5.5	6.2	5.4	5.4	5.4	5.4	5.4	5.4	5.4	4.8
Sucrose	6.0	5.8	5.1	5.6	5.1	5.1	5.0	5.0	5.0	5.0	5.0	0.0
Rest[1]	4.5	4.5	4.5	3.3	4.0	4.5	4.0	4.0	3.6	4.0	4.0	4.0
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated nutrient level												
ME, MJ/kg	14.3	14.3	14.3	14.3	14.3	14.3	14.0	14.0	14.0	14.0	14.0	14.0
CP	19.3	19.1	19.1	19.1	19.1	19.1	18.1	18.1	18.1	18.1	18.1	18.1
Dig Lys	1.35	1.35	1.35	1.35	1.35	1.35	1.23	1.23	1.23	1.23	1.23	1.23
Dig Met	0.39	0.39	0.39	0.39	0.39	0.39	0.36	0.36	0.36	0.36	0.36	0.36

[1]Rest includes minerals and amino acids, and a premix which provided the following per kg of feed: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 30 mg; vitamin B_{12}, 12 µg; riboflavin, 4 mg; pantothenic acid, 15 mg; niacin, 40 mg; choline chloride, 400 mg; folic acid, 0.7 mg; vitamin B_1, 1.5 mg; vitamin B_6, 3 mg; biotin, 0.1 mg; Mn, 40 mg; Fe, 90 mg; Zn, 100 mg; Cu, 8.8 mg; I, 0.35 mg; Se, 0.3 mg. No antibiotics in premix.

Pigs were observed for clinical signs of diarrhea every day and a scoring system was applied to indicate diarrhea as follows: 1= hard feces; 2= slightly soft feces; 3 =soft, partially formed feces; 4= loose, semi liquid feces; 5 =watery, mucous-like feces. Diarrhea rate was calculated as: diarrhea rate (%) = (number of pigs with diarrhea × diarrhea days)/(number of pigs × total observational days) ×100. On days 14 and 28, one barrow per pen was bleed via jugular vein into vacuum container tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Blood samples were allowed to clot at room temperature for 20 min and centrifuged at 3,000 ×g at 4°C for 10 min. Serum was removed and stored at −20°C until determination. Serum antioxidant parameters malonyldialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX) were determined using assay kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Company, China). Data were analyzed using SAS (version 9.2; SAS Inst. Inc., Cary, NC). Data were analyzed with pen or pig as the experimental unit using GLM procedures of SAS followed by Duncan's multiple-range tests.

Results

The effects of enzyme-treated soybean meal on growth performance and diarrhea rate of weaned piglets are shown in Table 5. During d 1-14, pigs in ESBM1 and ESBM2 had higher (P<0.05) ADG compared to

CON, SPC and FSBM. During d 15-28, the ADG in ESBM2 was higher (P<0.05) than in other treatments. During the total period (d1-28), the ADG in ESBM2 was greater (P< 0.05) than in the other treatments.

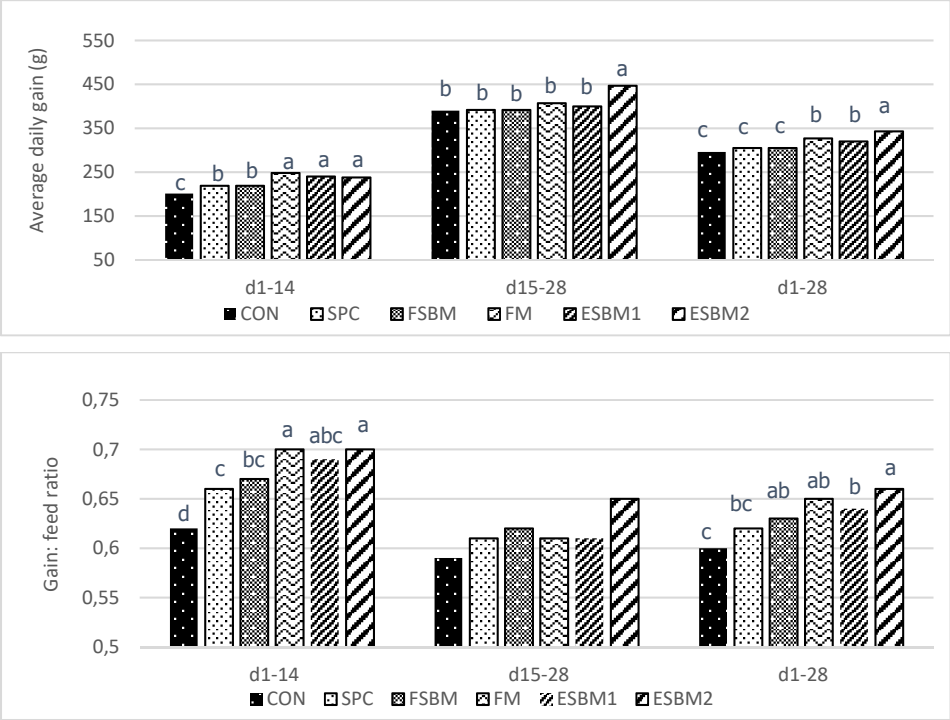

Fig. 1. Effect of protein source and soy treatment on performance

Diarrhea incidence during d 0-14 was significantly lower in ESBM1 and ESBM2 fed piglets when compared to CON (1.79 and 1.98 compared to 6.15 % respectively, P<0.05). There was a trend for a lower diarrhea rate for same groups during the whole experiment (2.38 and 1.79 compared to 5.06% respectively, P=0.08). The effects of protein source on oxidative status is shown in table 2. On d 14, Pigs fed ESBM1 or ESBM2 had higher (P<0.05) concentrations of SOD, and had lower (P<0.05) concentration of MDA compared with CON. Pigs fed ESBM2 had higher (P<0.05) concentrations of T-AOC compared with CON and FSBM. On d 28, compared with the CON, SPC and FSBM, pigs in ESBM2 had higher (P<0.05) concentrations of SOD, GSH-PX and T-AOC but MDA concentration was lower in ESBM2 than in CON.

Table 2: Effect of treatment on oxidative parameters

Item	CON	SPC	FSBM	FM	ESBM1	ESBM2	SEM	*P*-value
d 0-14								
T-SOD(U/ml)	102.76^{d}	125.32c	127.90^{bc}	141.54^{a}	137.08^{abc}	139.36^{ab}	3.90	0.01
MDA(nmol/ml)	5.84^{a}	3.80 ^{b}	3.68 ^{b}	2.96 ^{b}	3.12 ^{b}	3.05 ^{b}	0.32	0.01
GSH-PX(U/ml)	723.14	764.56	742.92	750.87	808.09	838.26	28.76	0.08
T-AOC(U/ml)	7.27^{d}	9.34^{bc}	8.96 ^{cd}	11.36 ^{a}	10.84 ^{ab}	11.01^{ab}	0.61	0.01
d 15-28								
T-SOD(U/ml)	113.52^{c}	125.80^{bc}	128.35^{b}	134.25^{ab}	136.41^{ab}	146.33^{a}	4.71	0.01
MDA(nmol/ml)	4.46^{a}	3.69^{ab}	3.77^{ab}	2.99^{b}	3.07^{b}	2.75^{b}	0.34	0.02
GSH-PX(U/ml)	692.63^{c}	739.17^{bc}	792.65^{b}	871.52^{a}	877.61^{a}	895.31^{a}	23.12	0.01
T-AOC(U/ml)	5.84^{d}	8.58^{c}	9.45^{bc}	10.48^{ab}	9.55^{bc}	10.94^{a}	0.36	0.01

Discussion

The significantly higher growth performance of ESBM1, ESBM2 and FM compared to SBM is in agreement with Cervantes-Pahm and Stein (2014) and is explained by higher amino acids digestibility due to lower concentration of trypsin inhibitor and consequently more protein deposition in body tissue. The immediate derived effect of high digestibility is less amino acids bypassing the small intestine and become available for pathogen bacteria proliferation and toxins production in the lower gut (Apajalahti and Vienola, 2016). This corresponds with the low diarrhea score for the ESBM1, ESBM2 diets. Soy allergens causes inflammatory responses (Chen *et al.*, 2011) and create oxidative stress (Lugrin *et al.*, 2014), which is in agreement with the present study. A significantly higher incidence of oxidative stress and lower anti-oxidant status in CON piglets compared to ESBM1, ESBM2 and FM piglets were measured, supposedly due to lower content of allergens. The high anti-oxidant status in piglets fed FM could also be explained by added preservatives to FM. The inflammatory response of SBM leads to changes in the intestinal morphology (Lalles, 1993). Consequently, nutrient absorption is reduced due to lower intestinal surface area, which also contribute to the overall lower growth performance of CON fed piglets as measured in the present experiment.

Conclusion

In conclusion, the study confirmed that ESBM low in ANF's and antigen results in increased growth performance, reduced diarrhea and improved oxidative status in piglets. Based on these data it can be recommended to feed weanlings diets low in ANF's and allergens to reduce the incidence of diarrhea for optimal growth performance.

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Einfluss löslicher und unlöslicher Faser auf die Gesamt-traktverdaulichkeit, sowie die Konzentration an mikrobiellen Metaboliten im Ileum und Colon von Absetzferkel

Effect of soluble and insoluble dietary fibre on apparent total tract digestibility and concentration of microbial metabolites in ileum and colon of piglets

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Abstract

Dietary fibre represents the part of carbohydrates, which is not digested by the mammalian enzymes but partly fermented to e.g. SCFAs in the hindgut. Regarding this, fibre passes unaffected through the whole digestive tract. The soluble and insoluble proportion might influence the absorption of nutrients and alter the concentration of microbial metabolites in the colon in dependence of the substrate available. These processes are related to the age of animals, due to intestinal development, and influencing the ability to use fibre. The current study hypothesized that different proportions of soluble and insoluble fibre influence the apparent total tract digestibility (ATTD) and amount of microbial metabolites considering a balanced diet for piglets. Two consecutive feeding trials were conducted with 48 piglets each, separated in four treatments with the same analyzed levels of total dietary fibre (T1: no additional fibre; T2: soybean hulls, T3: lignocellulose I, T4: lignocellulose II). The results showed that the inclusion of soybean hulls resulted in 20% higher aNDFom ATTD ($p<0.05$). Furthermore soybean hulls resulted in increased concentration of cadaverin in ileal digesta while methylamin and putrescin were higher in colonal digesta. Lignocellulose I expressed higher amounts of tyramin in tendency ($p<0.10$). We conclude that higher amounts of ATTD of aNDF with soybean hulls were obviously attended by higher concentrations of biogenic amines. Further investigation is necessary.

Einleitung

Der adäquate Einsatz von Faserquellen und deren physiologische Auswirkung stellen nicht zuletzt auch in der Rationsgestaltung von Ferkel einen viel diskutierten und mit Widersprüchen behafteten Bereich innerhalb der Nutztierwissenschaft dar. Faser ist physiologisch als jener Kohlenhydratgehalt eines Futtermittels definiert, welcher von körpereigenen Verdauungsenzymen nicht hydrolysierbar ist und teilweise im Dickdarm z.B. zu kurzkettigen Fettsäuren (SCFA) fermentiert werden kann (Cummings, *et al.* 2009). Anteilig an der Ration verbleiben somit die Faserbestandteile am längsten unverändert im Chymus und üben auf Grund ihrer chemisch-physikalischen Eigenschaften möglicherweise Einfluss auf die Verdaulichkeit von Rohnährstoffen sowie die Darmmikrobiota. Diese heterogene Stoffgruppe der Faser, bestehend je nach Futtermittel hauptsächlich aus Cellulose, Hemicellulose, Pektin, resistenter Stärke, etc. ist dabei unterschiedlich löslich sowie fermentierbar und kann damit in Abhängigkeit des Alters des Tieres und Entwicklungsstadiums des Gastrointestinaltraktes (GIT) unterschiedlich wirken und vom Tier genutzt werden. Je nach Zusammensetzung der Faser werden Einflüsse auf die

zootechnischen Leistungen, die Darmmikrobiota, verbesserte Gesundheit des GIT bis hin zu gesteigertem Tierwohl beschrieben (Gerritsen *et al*. 2012; Li *et al*. 1994; Schedle *et al*. 2008 a/b; Kraler *et al*. 2015). Es wird erwartet, dass Faserquellen mit unterschiedlichen Anteilen an löslicher und unlöslicher Faser bei gleichbleibendem Gesamtfasergehalt (TDF) der Ration unterschiedlich auf die Gesamttraktverdaulichkeit, die Bildung von kurzkettigen Fettsäuren (SCFAs) und biogenen Aminen wirken. Daher bestand die vorliegende Forschungsarbeit darin den Einfluss einer unterschiedlich zusammengesetzten Gesamtfaser auf die ATTD ausgewählter Rohnährstoffe sowie mikrobieller Metaboliten im Ileum und Colon in der Ferkelaufzucht zu untersuchen.

Material und Methoden

In zwei aufeinanderfolgenden Fütterungsversuchen an der landwirtschaftlichen Fachschule Hatzendorf (Steiermark) wurden jeweils 48 Absetzferkel (7,33 ± 0,12 kg) in vier Versuchsgruppen mit Rationen welche mit unterschiedlichen Anteilen an löslicher und unlöslicher Faser (durch Sojaschalen vs. Lignocellulosen) angeboten (Tabelle 1). Die Rationen setzten sich aus den Hauptkomponenten Mais, Sojaextraktionsschrot und Gerste zusammen, waren isonitrogen und isokalorisch sowie auf identen Fasergehalt (TDF, total dietary fibre) kalkuliert. Die männlichen und weiblichen Tiere wurden randomisiert in acht Boxen zu je sechs Tieren aufgestallt. Die Versuchsdauer je Durchgang war in eine zweiwöchige Absetzphase sowie eine darauffolgende Aufzuchtphase (sechs Wochen) unterteilt und ergab somit einen Gesamtzeitraum von acht Wochen.
Zur Bestimmung der Gesamttraktverdaulichkeit mittels Markermethode (Titandioxid nach Jagger *et al.* 1992) wurde in der siebten Versuchswoche eine tierindividuelle Kotsammlung durchgeführt. Die gewonnen Proben sowie die Futtermischungen wurden laut Standardmethoden der VDLUFA (Naumann und Bassler, 2012) auf die Inhaltstoffe (TM, XP, aNDFOM, GXL) untersucht und für die Verrechnung der Verdaulichkeit herangezogen. Die Tiere des ersten Versuches wurden nach Versuchsende fachgerecht geschlachtet, Chymusproben (Ileum und Colon) gewonnen, welche bei -20°C bis zur weiteren Analyse gelagert wurden. Die Ermittlung der Gehalte an SCFAs erfolgte nach Zhao *et al.* (2006) (Gaschromatograph: Agilent Technologies 7890A-G3440AGC System, Agilent Technologies, Santa Clara, USA, NUKOL Silica Capillary column 15 m × 0.53 mm, 0.5 µm, Supelco, Bellefonte, PA, USA). Die biogene Amine wurden nach von Saarinen (2002) mittels HPLC (Waters 2695 Separations Module, Waters, Massachusetts, USA) bestimmt. Die statistische Auswertung erfolgte mittels GLM Prozedur von SAS (SAS Inst., Inc., Cary, NC, USA). Es wurde eine Varianzanalyse (ANOVA) mit den Faktoren Versuchsgruppe und Geschlecht sowie Interaktionen mit anschließendem Tukey-Kramer-Test durchgeführt. Signifikanzen wurde bei $p<0,05$ deklariert.

Tabelle 1: Versuchsdesign und Rationszusammensetzung

	Versuchsgruppe			
	VG 1	VG 2	VG 3	VG 4
Versuchsdesign				
Tiere pro Box, n	6	6	6	6
Anzahl Boxen, n	2	2	2	2
Durchgang, n	2	2	2	2
Anzahl Tiere, n	24	24	24	24
Rationszusammensetzung				
Faserquelle, in %	-	2,5	1,5	1,5
Zusammensetzung d. Faserquellen, in g/kg TM				
Neutral Detergenzien Faser (NDF)		562	919	926
lösliche Faserbestandteile (SDF)	-	70	13	11
unlösliche Faserbestandteile (IDF)	-	585	932	942
Gesamtfaser (TDF)	-	654	945	953

VG 1, Versuchsgruppe ohne spezielle Faserzulage; VG 2, Versuchsgruppe mit Sojaschalen; VG 3, Versuchsgruppe mit Lignocellulose I, VG 4; Versuchsgruppe mit Lignocellulose II; TM, Trockenmasse

Ergebnisse und Diskussion

Die Auswertung der ATTD ergab für aNDFom einen hoch signifikanten Unterschied ($p<0,0001$) der VG 2 (Sojaschale) von 20% höhere Verdaulichkeit im Vergleich zur den VG 1 sowie VG 3 und 4. Für alle weiteren Parameter (TM, OM, XP, GXL) wurden keine Unterschiede ermittelt. Die Bestimmung der biogenen Amine (Tabelle 2) ergab Unterschiede für Cadaverin im Ileum (VG 2 zu VG 3 und VG 4; $p<0,05$). Des Weitern unterschieden sich im Colon Methylamin signifikant (VG 2 zu VG 3; $p<0,05$) und Putrescin sowie Tyramin (VG 2 zu VG 3; $p<0,1$).

Tabelle 2: Konzentration an biogenen Aminen im Ileum und Colon (in mg/kg TM)

	Ethanolamin	Methylamin	Isopropylamin	Pyrrolidin	Putrescin	Cadaverin	Histamin	Tyramin	Spermidin	Spermin	Summe
Ileum											
VG 1	132	43	8	100	168	329 ^{ab}	135	12	420	641	1915
VG 2	131	51	12	102	165	370 ^{a}	133	47	441	537	1890
VG 3	118	60	9	101	133	146 ^{bc}	157	39	435	521	1572
VG 4	112	54	11	107	92	53 ^{c}	315	23	392	536	1575
m	110	49	11	105	130	212	166	18	434	580	1739
w	143	56	9	98	151	252	237	42	406	531	1737
SEM	11	4	1	4	11	33	43	9	18	25	64
VG	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.
sex	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
VG*sex	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Colon											
VG 1	272	213 $^{(ab)}$	22	48	301 ^{b}	485	194	44 $^{(ab)}$	631	20	2191
VG 2	312	220 $^{(a)}$	21	56	430 ^{a}	595	200	47 $^{(ab)}$	701	23	2400
VG 3	241	145 $^{(b)}$	21	48	315 ^{b}	516	193	81 $^{(a)}$	577	30	2063
VG 4	269	210 $^{(ab)}$	20	55	339 ^{a}	369	274	34 $^{(b)}$	610	20	2083
m	293	196	19	53	391	544	235	49	645	19	2261
w	248	201	23	50	300	434	194	57	611	29	2092
SEM	11	12	2	3	18	52	14	6	21	2	72
VG	n.s.	<0.1	n.s.	n.s.	<0.1	n.s.	n.s.	<0.1	n.s.	n.s.	n.s.
sex	<0.1	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.
VG*sex	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

VG 1, Versuchsgruppe ohne spezielle Faser; VG 2, Versuchsgruppe mit Sojaschalen; VG 3, Versuchsgruppe mit Lignocellulose I, VG 4; Versuchsgruppe mit Lignocellulose II; ^{a,b} Mittelwerte in der gleichen Spalte unterschieden sich signifikant ($p<0,05$); $^{(a),(b)}$ Mittelwerte in der gleichen Spalte unterschieden sich tendenziell ($p<0,1$); n.s., nicht signifikant; SEM, Standardfehler; m: männlich; w: weiblich.

Bei Betrachtung der Analysewerte der einzelnen Faserkomponenten wiesen die Sojaschalen einen geringeren Anteil an aNDFom als Lignocellulosen (ca. 56 % zu 90 %) auf. Auch bei einem Einsatz von 2,5 % in der Ration scheint der höheren Anteil an löslicher Faser der Sojaschale (SDF 7,0 % zu 1-1,3 %) entscheidend für die Verdaulichkeit des aNDFom zu sein. Als verdauliche Faser kann laut Definition ausschließlich jener Anteil angesehen werden der fermentierbar ist, im Gastro-intestinaltrakt umgewandelt wird und bei der Bestimmung der Gesamttraktverdaulichkeit folglich Berücksichtigung findet. Diese Eigenschaft ist zu hohem Maße der löslichen Faser zugeschrieben, wenn auch im unlöslichen Anteil nicht unbeachtliche Mengen in Abhängigkeit des Lignifizierungsgrades fermentierbar sein können (Bach Knudsen 2001). Zu übereinstimmenden Ergebnissen gelangten auch Kraler *et al.* (2014), die mit speziell aufgeschlossener (Fermentation und Extrusion) und damit leichter fermentierbarer Weizenkleie gegenüber nativer Kleie eine gesteigerte Faserverdaulichkeit nachwiesen. Die gesteigerten Werte der biogenen Amine lassen sich teilweise durch den erhöhten Anteil löslicher

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Faser der Sojaschale sowie durch einen möglichen vermehrten mikrobiellen Proteinabbau durch fasergebundenes Protein der Sojaschalen erklären.

Schlussfolgerung

In der Gesamtbeurteilung einer Ration sollte die Zusammensetzung der Faser in der Ration und mögliche Wechselwirkungen dieser in Zusammenhang mit Nährstoffverdaulichkeit nicht außer Acht gelassen werden. Neben der positiv gesteigerten Verdaulichkeit der aNDFom in der Sojaschalengruppe und folglich des höheren Fermentationspotentials dieser Ration, sollte die vermehrte Bildung von biogenen Aminen gegenüber gestellt werden. Auf Grund der dargestellten Ergebnisse kann darauf verwiesen werden, dass in diesem Bereich weiterer Forschungbedarf besteht.

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Effects of using different dietary copper sources in piglets on performance and tissue copper concentration

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Abstract

Copper is an essential trace mineral needed for numerous metabolic functions. Dietary levels above nutritional requirement can increase growth performance in particular for piglets. Little information exists however comparing different Cu-Sources at EU maximum levels with regard to performance and bioavailability. Two studies investigated the effects of using a chelated copper with hydroxyl analog of methionine as ligand ($Cu(HMTBa)_2$) against copper sulfate or $Cu_2(OH)_3Cl$ (tri-basic copper chloride, TBCC). Treatments for trial 1 were 6 mg/kg Cu from $CuSO_4$ (T1), 170 mg/kg Cu from $CuSO_4$ (T2) or $Cu(HMTBa)_2$ (T3). Each group contained 70 weaned (26d) piglets with 8 replicates and 10 piglets/pen. After 42d the animals fed T3 had a significant higher daily gain ($p=0.03$) together with an improved feed efficiency than pigs fed the T1 or the T2 diets. At the same time piglets receiving T3 had greater liver Cu contents than pigs fed T2 (30.46 vs. 18.37 mg/kg; $p=0.06$) and T1 (6.83 mg/kg; $p<0.01$). In trial 2, four treatments (7 replicates with 22 piglets/pen) were used: a control diet without additional Cu (T1) and 3 diets supplemented with 150 mg/kg Cu from either $CuSO_4$ (T2), TBCC (T3) or $Cu(HMTBa)_2$ (T4). Feed conversion rate was significantly reduced from 1.35 (T1) to 1.22 (T4, $p=0.01$) followed numerically by T2 (1.26) and T3 (1.31). Liver copper levels were significantly higher for piglets receiving T4 ($p=0.01$) compared to group T1 or T3, while T2 was intermediate. Conclusively the type of copper source will result in a different effect on performance and liver Cu content (availability).

Introduction

Requirement of Cu as a nutrient is low and NRC (2012) recommends 3 to 6 mg/kg Cu for nursery pigs. On the other site, increased levels such as 150 mg/kg of Cu under commercial conditions are known to reduce scouring together with improved weight gain and feed intake. The growth-stimulation effect of dietary copper was linked to its antimicrobial actions in the gut; for example, copper inhibits the growth of enteric disease causing microorganisms, thus eliciting an antibiotic-like effect (Fuller *et al.*, 1960). Højberg et al (2005) showed that high dietary $CuSO_4$ inhibited the Coliforms and thus potential pathogens. However, high dietary Cu presents an environmental concern when excess Cu is excreted in feces (Kornegay and Harper, 1997). Due to the negative impact of high dietary $CuSO_4$, the Commission of the European Communities regulate maximum allowed total Cu in feed with 170 mg/kg in piglets up to 12 weeks of age and 25 mg/kg in all other pigs. Limited information is available comparing different Cu-Sources at EU maximum levels in diets for piglets. Two studies investigated the effects of using a chelated copper with hydroxyl analog of methionine as ligand ($Cu(HMTBa)_2$) against copper sulfate or tri-basic copper chloride (TBCC). Compared to the inorganic copper sources, the Copper-Methionine-Hydroxy-Analogue Chelate (MINTREX® Cu, Novus Int., USA) is surrounded by a ring structure with two molecules of the hydroxy analogue of methionine (2-hydroxy-4 methylthiobutanoic acid, HMTBa). This sophisticated ring structure protects the copper from potential interactions in the feed and digestive tract ensuring highest bioavailability of copper for the animal.

Material und method

In experiment 1 the performance in post-weaning piglets was compared by using either $CuSO_4$ or $Cu(HMTBa)_2$ as a copper source. The trial was conducted in Segovia, Spain and met appropriate current quality standards and guidance of the European Food Safety Authority (EFSA). A total of 240 crossbred piglets (Large White×Landrace, 26 d of age, 7.36±0.10 kg) were randomly allocated to 1 of 3 experimental treatments based on sex, litter of origin, and BW with 8 replicates for each treatment and 10 piglets per pen. All pigs were fed a common creep feed from 21 to 25 days of age, then initiated treatments at 26 days of age. In table 1 the three different treatments are described.

Table 1: Treatment design (Exp. 1)

Treatment		Phase I (d 0 to 14)	Phase II (d 15 to 42)
1	NRC control ($CuSO_4$ at 6 mg/kg)	Cu 6 mg/kg, Mn 4 mg/kg (as Oxide), Zn 100 mg/kg (as sulfate)	Cu 5 mg/kg, Mn 3 mg/kg, Zn 80 mg/kg from sulfates
2	$CuSO_4$ at 170 mg/kg	Cu 170 mg/kg, Mn 150 mg/kg (as oxide), Zn 150 mg/kg from sulfates	Same as phase I
3	$Cu(HMTBa)_2$ at 170 mg/kg	$Cu(HMTBa)_2$ 170 mg/kg, Mn 150 mg/kg (as oxide), Zn 150 mg/kg (as sulfates)	Same as phase I

Methionine activity from $Cu(HMTBa)_2$ was accounted for and all diets were adjusted to be equal in methionine

Typical European mixed-grain diets (barley, wheat, corn, and soybean meal) were formulated to meet or exceed NRC nutrient recommendation for piglets. Pigs were fed two phase diets (d 0 to 14 and d 15 to 42). Weight gains, feed intakes and feed conversion were determined at the end of each feeding period. At the end of the experiment, one medium-size piglet per pen was slaughtered and tissue sampled (muscle, liver, kidney, skin and fat, and tibia bone) for Cu deposition analysis (AOAC 968.08).

Exp. 2 was conducted at the University of Missouri (Columbia, MO). All animal procedures were approved by the University of Missouri Animal Care and Use Committee. A total of 616 crossbred piglets (PIC, 20 days of age, 5.02±0.25 kg) were randomly allocated to 4 experimental treatments based on sex and BW with 7 replicates for each treatment and 22 piglets per pen. Table 2 listed detailed treatment descriptions.

Table 2: Treatment description (Exp. 2)

Treatment	Phase I (d 0 to 7)	Phase II (d 7 to 21)
1	NRC control ($CuSO_4$ at 6 mg/kg)	NRC control ($CuSO_4$ at 6 mg/kg)
2	$CuSO_4$ at 150 mg/kg	$CuSO_4$ at 150 mg/kg
3	$Cu_2(OH)_3Cl$ at 150 mg/kg	$Cu_2(OH)_3Cl$ at 150 mg/kg
4	$Cu(HMTBa)_2$ at 150 mg/kg	$Cu(HMTBa)_2$ at 150 mg/kg

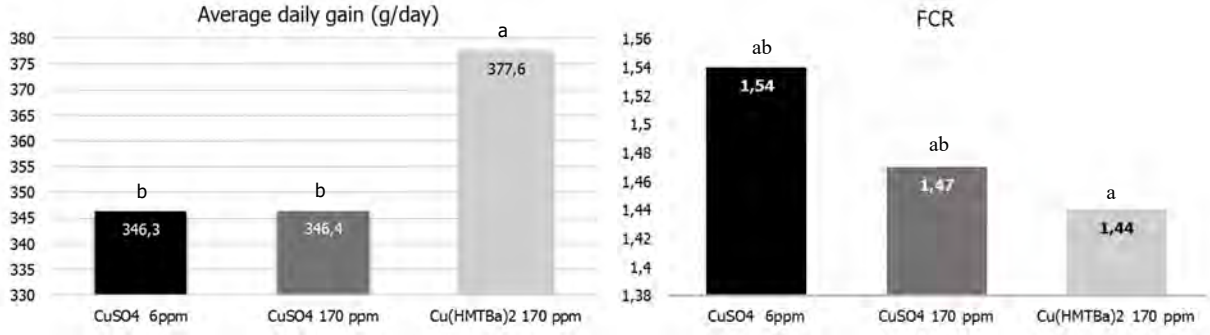
Methionine activity from $Cu(HMTBa)_2$ was accounted for and all diets were adjusted to be equal in methionine

Diets were formulated to meet or exceed NRC requirement and analyzed for Cu content using atomic absorption spectrometry (AOAC 968.08). Weight gain, feed intake and feed efficiency were determined for the whole experimental period (21 days post-weaning). At the end of the trial, one medium-size piglet per pen was slaughtered for liver mineral analysis.

Results

In experiment 1 (Figure 1) it was found that piglets fed the chelated source of copper grew 9% faster over the 42 day period than those fed the diets containing the inorganic copper source with ADG values (g/d) of 346, 346 and 378 for treatments T1, T2 ,T3, respectively ($P = 0.032$). In the same period, feed intake was greater for piglets fed the chelated copper source when compared to piglets fed T2, 544

vs.510 g/d, respectively (P = 0.034). Feed conversion rate tended to be improved with $Cu(HMTBa)_2$ supplementation compared with the other two treatments (p = 0.06).



[a,b]Means in a row with no common superscripts significantly differ (p<0.05).

Figure 1: Effects of $Cu(HMTBa)_2$ and $CuSO_4$ on average daily gain and FCR in weaned piglets

Piglets in the $Cu(HMTBa)_2$ group had 2.7 times higher liver copper (Figure 2) than pigs fed 170 mg/kg $CuSO_4$ (p=0.06) and significantly higher liver copper than pigs receiving 6 mg/kg $CuSO_4$ (p<0.01).

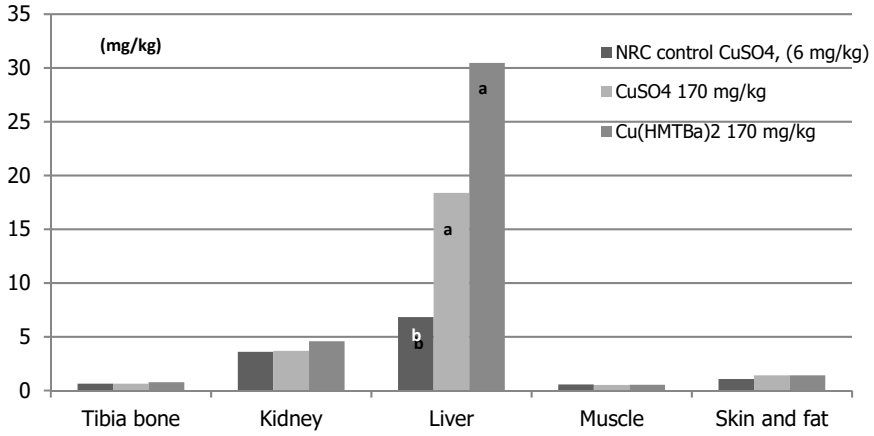


Figure 2: Effects of $Cu(HMTBa)_2$ and $CuSO_4$ on tissue Cu concentration (DM basis)

In experiment 2, pigs fed 150 ppm of the copper sources tended to have better ADG (p = 0.05) being the highest when supplementing the diet with $Cu(HMTBa)_2$. Feed conversion improvements with Cu supplementation ranged from 2.9% for pigs fed TBCC to 13.5% (p<0.05) when Cu was supplied as $Cu(HMTBa)_2$. Liver Cu on a dry liver weight basis was significantly increased for piglets receiving $Cu(HMTBa)_2$ compared to the control or TBCC group (p>0.01, Table 3)

Table 3: Effect of copper source on weight gain, feed conversion and liver cupper levels

	Control	$CuSO_4$ 150	TBCC 150	$Cu(HMTBa)_2$ 150	SE	P<
Weight						
Day 21	10.0	10.677	10.618	10.904	0.34	0.01
Day 0-21						
Gain g/d	290.3	326.6	317.5	340.2	0.02	0.05
Intake g/g	390.1	417.3	413.3	417.3	0.03	0.63
Feed/Gain	1.35^{a}	1.26^{abc}	1.31^{ab}	1.22^{c}	0.02	0.001
Liver dry wt. basis Cu ppm	39.8^{b}	52.1^{b}	48.8^{b}	73.3^{a}	8.1	0.01
Liver ash Cu ppm	806.8^{b}	993.4^{ab}	880.9^{b}	$1,329,0^{a}$	394	0.05

Discussion

Feeding chelated copper in the form of $Cu(HMTBa)_2$ increased copper levels in the liver tissue, improved growth and feed conversion rate compared to $CuSO_4$ or TBCC. Such a different response to the copper sources can be linked to the higher bioavailability when feeding HMTBa-Chelates (Richards *et al.*, 2012) and its indirect possible effect to a higher antibacterial effect in the gut. Early research in piglet showed that the growth-stimulation action of dietary copper was linked to its antimicrobial actions in the gut; i.e. copper inhibits the growth of enteric disease causing microorganisms, thus eliciting an antibiotic-like effect (Fuller *et al.*, 1960). Højberg et al (2005) showed high dietary $CuSO_4$ inhibited the Coliforms and thus potential pathogens. At the same time, Bowland (1961) stated that the growth promoting effect of copper was more important for the most digestible sources of copper. Zhou et al (1994) demonstrated that intravenous injection of copper at 250 ppm to weaned piglets was able to generate weight gain similarly to copper sulfate in the diet even when bypassing the intestine. Based on the copper metabolism including the intestinal uptake, storage (mainly liver) and excretion via the bile it could be demonstrated that higher copper levels in the liver are resulting in higher excretion in the bile (Hamza and Gitlin, 1996) where biliary salts and other copper-related compounds are exhibiting their antimicrobial activities in the intestinal tract. The link between a higher copper availability, storage in the liver and its higher antibacterial effect is supported by an experimental test in weaning piglet (Arnold, 2011) showing similar antimicrobial activity of the bile exudate when piglets received either 250 ppm of Cu as sulfate or 75 ppm only of copper as $Cu(HMTBa)_2$. These elements together with the feeding trials support the theory about the Cu recycle pathway (Zhao *et al.*, 2014) demonstrating that copper needs to be absorbed and stored in the liver, to exert such growth stimulation.

Conclusion

Chelated Cu as $Cu(HMTBa)_2$ is more available based on performance and liver Cu concentration. It avoids mineral antagonisms and ensures optimal supply of copper essential for health and growth of the animal. Studies are indicating that the improved antimicrobial action may be indirectly caused by a higher bioavailability of copper through an increased uptake of Cu by the liver, secretion to the bile and then back to the intestinal lumen (Cu recycle pathway).

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Relationship between feed processing with expander, particle size and mill type on growth performance of broilers

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Abstract

Indifferent to diet composition, different feed processing steps affect broiler performance. In the present study, three factors in feed processing were applied before pelleting to investigate their influence on broiler performance. The factors were two mill types, i.e. hammer (HM) and roller (RM) mill; three particle sizes, i.e. 800, 1200 and 1600 µm; and two conditionings, i.e. with and without expander, which were applied to produce grower and finisher diets. In total 864 unsexed Ross-308 chickens were randomly distributed in a 2x3x2 factorial arrangement based on a block design. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured on day 22 and 38. The results showed that FI was higher in broilers fed pellets without expander processing in the finishing (P=0.0330) and in the overall period (P=0.0496). In the grower phase, mill type and conditioner interacted (P=0.0336), i.e. FI of broilers was higher when diets were milled with RM and pelleted without expander. BWG was not affected by treatments. For the grower phase (P=0.0315) and the overall period (P=0.0130) the best FCR was obtained when 1600 µm of particle size was obtained after RM grinding and expander processing before pelleting. In conclusion, feed processing improved broilers FCR from 8 to 38 d of age when using RM, ground coarsely and expanding before pelleting.

Introduction

Grinding is the first processing step applied to the raw ingredients in a feed mill processing line (Amerah *et al.*, 2007). In a diet based on corn-soybean for broilers, corn has quantitatively the biggest effect on the particle size regulation of the compound diet. In broilers, coarse particle size is associated to higher digestibility of the organic matter by promoting a higher development and activities of the gizzard (Naderinejad *et al.*, 2016). This results in longer retention time (Xu *et al.*, 2015), lower pH, more refluxes cycles, and consequently improved digestive enzymatic hydrolyzes reactions, resulting in higher absorption rates of nutrients. Typically two mills are used for grain grinding, i.e. hammer mill (HM) and roller mill (RM). HM accomplishes size reduction by impacting a slow moving grain with a rapidly moving hammer. Sizing is a function of hammer type, speed, screen design and hole size. RM accomplishes size reduction through a combination of forces and design features. If the rolls rotate at the same speed, compression is the primary force used. If rolls rotate at different speeds, shearing and compression are the primary forces used. If the rolls are grooved, a tearing or grinding component is introduced (Koch, 2002). Equipment adjustments are used to obtain specific particle sizes, which is possible through screen hole diameter or shaft speed; in RM, this is made by a gap between rollers and feeding rate (Goodband *et al.*, 2002). In addition, adjustments in the feed processing line such as the hydrothermic treatment prior to pelleting with expander, called high-temperature-short-time (HTST) technology, may improve feed hygienization, nutrient digestibility and reduce thermo-labile anti-nutritional factors in compound feeds (Puntigam *et al.*, 2017). However, studies establishing relationships between mill type, particle

size and conditioning prior pelleting are scarce. Therefore, the objective of the current study was to evaluate the effect of using three adjustments in the feed processing line on broiler performance. We hypothesized that the combination of coarse grinding with the expander processing prior to pelleting improves the performance of broilers.

Material and methods

A total of 864 one-day-old unsexed broiler chicks Ross 308 were randomly allocated to 36 pens with 12 birds each in a two periods trial, at the poultry research station 'Wimitz' in Carinthia, Austria. Each pen was equipped with a tube feeder and bell drinker and wood shaves as litter. Water and feed were available *ad libitum*. FI, BWG and FCR were measured at 22 and 38 days of age. Corn-soybean meal diets were formulated based on Austrian commercial practices. Until day 8, bird received a common diet (AMEn: 12.35 MJ/kg and Lys dig.: 1.16%). Two feeding phases were used, the grower was from 8 to 22 days (AMEn: 12.8 MJ/kg and Lys dig.: 1.05%) and the finisher from 23 to 38 days (AMEn: 12.7 MJ/kg and Lys dig.: 0.83%). Experimental diets were processed with different adjustments to obtain the treatments, as described in Table 1. A HM (Awila Typ AWF 20) and a RM (LWM 400-1) were used. The diets were mixed in a horizontal mixer (FKM 1200 D) for 5 min. Half of each batch was conditioned and pelleted and the other half was conditioned and expanded (Expander OE 15) prior pelleting. All pellets were obtained with a pellet press, model Typ 33-390. Pellets were cooled in a belt cooler and crumbled in a LWM 100-1 crumbler. The trial was performed as a 2x3x2 factorial arrangement with two mills (HM and RM), three particle sizes of major mixed ingredients (800, 1200 and 1600 µm) and two conditioners (with and without expander) prior pelleting. Analysis of variance was performed with PROC mixed and differences between means were compared with Tukey test at 5% using SAS. Interactions were compared with Bonferroni Holm test (Holm, 1979).

Table 1. Technical data applied in the feed processing line

Item	Hammer mill				Roller mill			
	A[2]	t/h[3]	Speed, Hz	Sieve, mm	A	t/h	Hz[5]	Gap, mm
Particle size[1]								
800 µm	18.0	1.76	50	8	17.2[4]	0.25[4]	12/8	0.2[4]
1200 µm	16.0	2.00	46	15	9.8	0.56	12/8	0.3
1600 µm	12.5	0.88	33	15	8.7	0.85	12/8	1.0
	Conditioner				Pellet press			
	Water input, L/H	Steam input, %	Bulk, ºC	Moisture, %	Press ratio[7]	kWh/t	Bulk, ºC	
Expander[6]								
With	0.7	3.80	84.58	15.41	1/6	5.83	66.33	
Without	0.0	3.58	75.83	14.10	1/10	4.25	75.00	

[1]Mean values, n=4. [2]Ampères. [3]Tons per hour. [4]Two pairs of rollers, first pair worked with, 8.7A, 0.855 t/h and 1 mm of the gap. [5]Relative speed between rollers. [6]Expanded conditions: 17 bar of pressure, 103.16ºC, 6 kWh/t, 1 t/h of throughput, bulk temperature 71.83ºC and 13.93% of moisture, n=12. [7]Press die with 4 mm hole.

Results

Broiler performance was influenced by treatment factors as shown in Table 2. In the finisher and overall period there was a conditioning effect as broilers fed pellets without prior expansion had increased FI by 2.48% and 2.08%, respectively. In the grower phase, mill type and conditioning interacted, resulting in lower FI for broilers fed diets ground with hammer mill and pelleted with expansion (Tables 2 and 3). BWG was not affected by treatments. Feed conversion ratio of broilers was affected by the three-way interaction in the grower and overall period (Table 3). Best FCR were obtained in the finisher

(from 2.07% to 10.23%) and overall period (from 1.12% to 7.27%) when 1600 µm of particle sizes was performed in a roller mill and expanded before pelleting. Particle size did not show main effect.

Table 2. Mean values for main effects for feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers feed corn-soybean diets with different feed processing line arrangement from 8 to 38 d of age

Item	FI, g			BWG, g			FCR, g		
	Grower[2]	Finisher[2]	Overall[2]	Grower	Finisher	Overall	Grower	Finisher	Overall
Mill									
Hammer	1,093[b]	2,744	3,841[(b)]	807	1,622[(b)]	2,429[(b)]	1.348	1.690	1.577
Roller	1,117[a]	2,795	3,915[(a)]	814	1,661[(a)]	2,475[(a)]	1.355	1.679	1.571
Particle size, PS									
800 µm	1,105	2,786	3,896	810	1,648	2,459	1.347	1.689	1.577
1200 µm	1,094	2,749	3,843	806	1,644	2,451	1.347	1.670	1.565
1600 µm	1,115	2,774	3,894	815	1,632	2,447	1.360	1.694	1.582
Conditioner, CD									
Without[3]	1,110	2,803[a]	3,917[a]	813	1,646	2,459	1.359	1.705[(a)]	1.590[a]
With[3]	1,100	2,736[b]	3,839[b]	808	1,636	2,445	1.344	1.664[(b)]	1.559[b]
Mean	1,105	2,769	3,878	811	1,641	2,452	1.351	1.684	1.574
SEM	39.20	131.96	165.49	34.07	94.09	108.89	0.052	0.101	0.065
P-value[1]									
Mill	0.0126		0.0627		0.0855	0.0763			
CD		0.0330	0.0496					0.0963	0.0471
Mill x CD	0.0336								
Mill x PS x CD								0.0315	0.0130

[ab]Means within the same column with different superscripts differ by Tukey test (P≤0.05; within brackets P<0.10). [1]Only when P<0.05. [2]grower (8-22 d), finisher (23-38 d) and Overall (8-38 d). [3]With expander and without expander.

Table 3. Mean values for interactions, i.e. mill *vs* conditioner, mill *vs* particle size (PS) *vs* conditioner, on feed conversion rate (FCR) and feed intake (FI) of broilers feed corn-soybean diets with different feed processing line arrangements, 8 to 38 d

Item		FCR 23-38 d, g:g		FCR 8-38 d, g:g		FI 8-22 d, g	
		Conditioner[1]					
		With	Without	With	Without	With	Without
Mill							
Hammer						1,078[hW]	1,108[gV]
Roller						1,112[gV]	1,112[gV]
	PS, µm						
Hammer	800	1.673[A(ab)X]	1.700[AaX]	1.553[AaX]	1.588[AaX]		
	1200	1.626[A(b)X]	1.713[AaX]	1.528[AaX]	1.600[AaX]		
	1600	1.745[A(a)X]	1.681[AaX]	1.621[AaX]	1.575[AaX]		
Roller	800	1.718[A(a)X]	1.666[AaX]	1.596[AaX]	1.570[AaX]		
	1200	1.630[A(ab)X]	1.711[AaX]	1.543[AaX]	1.590[AaX]		
	1600	1.593[B(b)Y]	1.756[AaX]	1.511[BbX]	1.620[AaX]		

[AB]LSmeans differ between processes within mills and particle sizes. [ab]LSmeans differ between particle size within mill and conditioners. [XY]LSmeans differ between mills within particle size and conditioners. [gh]LSmeans differ between processes within mills. [VW]LSmeans differ between mills within conditioners. All comparisons were obtained by Bonferroni Holm test, (P<0.05; within brackets P<0.10). Exp-pellet: expander conditioning before pelleting. [1]With and without expander.

Discussion

Similar studies using these three factors at the same time in a feeding trial are scarce. Therefore, results are discussed individually. Svihus *et al.* (2004) obtained similar results as in the present study for FI,

BWG and FCR of broilers from 11 to 30 days of age when diets were ground in hammer mill or roller mill. These authors did not show data in phases for comparison with current grower phase. Parsons *et al.* (2006) found linear increasing of FI and FCR when increasing coarse corn particle size was used. This is in contrast to the present study, where particle size did not affect FI and FCR. It is possible that the absence of responses, which are directly related to particle size, are associated to pelleting. It is recognized that particle size is more important in mash diets (Amerah *et al.*, 2007) compared to pelleted ones. In contrast to current results, Campbell *et al.* (2006) did not find differences in FI between diets, expanded or not prior the pelleting, neither for BWG and FCR, similar to this study. In summary, it is difficult to compare published literature with the results of the present trial, because three factors were applied on each diet and it possibly alters their individually effects on broiler´s performance. It is imperative that more research is conducted to better understand the interactive effects of different feed processing conditions on feed quality, nutrient utilization and growth performance of broilers.

Conclusion

Feed processing affected broiler performance using the same diet formulation. Using roller mill with coarse grinding and expanding before pelleting is an option for the feed processing line aiming to reduce FCR.

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Fermentation and enzymatic treatment of pea for turkey nutrition

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Abstract

The present study was conducted to investigate how fermentation (FE) and enzymatic treatment (ET) of pea affect growth performance and turkey health. 960 turkeys were allocated into 60 pens and received 4 different diets consisted of a basal mash wheat-SBM diet (CON) and there experimental diets which were prepared by inclusion of each pea products NP (NPD), FEP (FEPD) and ETP (ETPD) in the basal diet at the rate of 100 g/kg. The experiment lasted 105 d. In general, in the most time periods of the performance trial, birds received ETPD or FEPD diets showed better growth performance than those fed NPD diet, while birds in ETPD group displayed similar performance to those fed CON diet. At the end of the trial, birds fed CON and ETPD diets had the best FCR and birds which received NPD diet had the worst one ($P \leq 0.05$). Birds in ETPD group showed the best footpad dermatitis score and turkeys in the NPD group had the worst score ($P \leq 0.05$). The footpad dermatitis scores for turkeys in CON and FEPD groups were identical and considerably different from those in ETPD and NPD groups ($P \leq 0.05$). The present data shows the feasibility of these processes, particularly ET, for improving the nutritional quality of pea as a protein source for turkey diets.

Introduction

Due to growing demand for alternative high-protein feed components, in particular those with health promoting properties, and increasing public awareness of environmental and ethical issues in meat production, alternative protein sources are urgently needed in poultry nutrition. Soybean meal is the most common plant protein source in poultry nutrition globally, but environmental factors remarkably limit their cultivation in the major part of the world, especially Europe, where alternative plants (eg. domestic legumes peas, beans, lupins or rapeseed) can be grown as protein sources (Wiseman *et al.*, 2003). Peas are legume plants with a moderately high protein content. In the previous study, fermentation of pea with *Bacillus subtilis* and enzymatic treatment of pea with α-galactosidase, protease and pectinases could effectively reduce α-galactosides, phytate, trypsin inhibitor activity (TIA) and RS in peas (Goodarzi Boroojeni *et al.*, 2017). Thus, it can be hypothesized that FE and ET can improve the nutritional quality of pea in turkey diets (by reduction in ANF) and through this improvement may reduce incidence of footpad dermatitis and positively impact the growth performance of turkeys. Furthermore,

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processed peas with reduced levels of ANF may be promising alternatives for partial replacement of SBM in turkey nutrition.

Therefore, the present study was conducted to investigate if fermentation and enzymatic treatment of pea affect growth performance and bird health, in case of 10% inclusion of different pea products in turkey diets.

Materials and methods

In this experiment were tested pea meal, fermented pea meal with probiotic (*Bacillus subtilis and Bacillus licheniformis*) and predigested pea meal with enzyme mixture (alphagalactosidase, hemicellulases, pectinases, protease). A commercial probiotic, BioPlus 2B® (Chr. Hansen, Denmark) was employed for the solid state fermentation (SSF) process. In the fermentation process the used water for dough production was inoculated with the probiotic spore, resulting in final concentration of approximately 2.57 x 10^8 *Bacillus subtilis* spores/kg pea. The dough was pumped in internal cathodic protected (ICP) tanks and incubated in temperature controlled containers for 48 h. For enzymatic treatment, the used water for dough production contained three different commercial exogenous AlphaGal™ (Kerry EMEA, USA), RONOZYME® ProAct and RONOZYME® VP (DSM, Switzerland). After filling in ICP tanks, the prepared dough was incubated for 24 h. Nine hundred and sixty one-d-old female turkeys (Hybrid Converter) were obtained from a local hatchery and, using completely randomized design, were assigned to 4 groups each with 15 replicate-pens (floor pens) and 16 birds per pen. The experimental diets consisted of a basal mash (produced in Agrocentrum feed mill, Kałęczyn, Poland) wheat-SBM diet (CON) and three experimental diets which formulated by inclusion of each pea products NP (NPD), fermented (FEPD) and ETP (ETPD) in the basal diet at the rate of 100 g/kg. The experiment lasted 105 d and a four-phase feeding schedule was used with formulation of a pre-starter (d 1–28), starter (d 29–56), a grower (d 57–84) and a finisher (d 85–105) diet. The diets were formulated to be isocaloric and isonitrogenous for each phase and meet or exceed the recommendations of Hybrid Converter (Hybrid Turkeys, 2016). Body weights (BW) of the birds were recorded at d 1 and every 4 (or 3 - last period) weeks during the experiment. Feed intake (FI) was recorded weekly and feed conversion ratio (FCR) was calculated. At the end of the experiment footpad dermatitis scores for all birds were determined by trained personnel according to the method described by Hocking *et al.* (2008). The method is based on a subjective scoring system from 0 to 4; 0 representing no external signs of footpad dermatitis and 4 representing swelling footpad with more than a half covered by necrotic cells.

Results

Table 1: Effect of the experimental diets on performance and Foot Pad Dermatitis (mean[1] values)

Period/age		Experimental groups			
		CON	NPD	FEPD	ETPD
D 1-28	dBWG (g)	38.8^{c}	41.3^{a}	40.7^{a}	39.8^{b}
	dFI (g)	53.1^{c}	55.7^{a}	54.5^{b}	54.1^{bc}
	FCR	1.37^{a}	1.35^{ab}	1.34^{b}	1.37^{a}
D 29-56	dBWG (g)	100.9^{b}	104.1^{ab}	107.3^{a}	107.1^{a}
	dFI (g)	168.7^{b}	178.5^{a}	181.1^{a}	176.7^{ab}
	FCR	1.66^{c}	1.72^{a}	1.69^{b}	1.65^{c}
D 57-84	dBWG (g)	135.2^{b}	133.3^{bc}	132.0^{c}	139.8^{a}
	dFI (g)	310.6	319.1	309.8	319.6
	FCR	2.27^{b}	2.40^{a}	2.35^{a}	2.25^{b}
D 85-105	dBWG (g)	131.0^{a}	128.9^{a}	127.4^{ab}	122.7^{b}
	dFI (g)	406.9	409.2	405.1	408.3
	FCR	3.11^{c}	3.23^{ab}	3.20^{bc}	3.31^{a}
D 1-105	dBWG (g)	99.5	100.1	100.0	101.0
	dFI (g)	246.1	239	238.3	240.1
	FCR	2.21^{c}	2.28^{a}	2.25^{b}	2.21^{c}
D 105	FPD	1.37^{b}	1.94^{c}	1.52^{b}	0.36^{a}

[1] Data are means of 15 replicate pens with 16 birds per pen.
[2] CON = Control wheat-soybean diet without pea products, NPD = Wheat-soybean diet with 100 g/kg native pea, FEPD = Wheat-soybean diet with 100 g/kg fermented pea, ETPD = Wheat-soybean diet with 100 g/kg enzymatically treated pea.
[3] Values in same rows with no common superscript are significantly different ($P \leq 0.05$)

Conclusions

Inclusion of ETP (100g/kg) in turkey diets demonstrated neither positive nor negative impact on growth performance, while it remarkably improved footpad dermatitis score and animal welfare.

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Effect of different components of dietary fibre and starch levels on the performance and health of fattening rabbits

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Abstract

The aim of this study was to evaluate the effects of different agro-industrial by-products in diets and to determine the nutritive value and energy contained in feeds for meat production and the biochemical parameters in the digestive tract of rabbits. Complete mixture must fully satisfy the nutritional needs of individual species and categories of animals as well as energy requirements.
Lucerne hay, wheat and barley middlings, malt sprouts, dried sugar beet pulp, pomace, peas and bean hulls, extracted sunflower and rapeseed meal are the main sources of nutrients currently used in diets for rabbits. There is a continuous search for new sources of fibre to reduce feed cost.
A total of 96 rabbits were divided into 4 similar groups, 24 animals in each group, and kept in standard metal cages, two animals per cage. In fattening experiment the growth of live weight and feed intake per unit of live weight growth were studied. Animals were given granulated diets ad libitum. Feed mixture (by-products) for rabbit fattening did not negatively influenced biochemical parameters and the quality of rabbit meat.

Introduction

Dietary energy level and with digestible protein greatly affects growth performance in the intensive rabbit system. However, a carbohydrate overload of the hind gut may occur due to a diet with high starch level, resulting in a high percentage of digestive disorders and mortality. Digestion and absorption of nutrients in the stomach and small intestine are similar to other monogastric animals. Lucerne hay, wheat and barley middlings, oats, beet pulp, pomace and sunflower meal are the main sources of fibre currently used in diets for rabbits.
Rabbit with its particular digestion equipment – by way of ceacotrophy – is able to utilize feeds of poorer nutritive matter and richer crude fiber. From feed industry processing in the agricultural production originate numerous by-products, which can be taken into account in rabbit feeding. Many by-products have a potential value as animal feedstuffs. The utilization of agro-industrial by-products may be economically effective, since conventional feedstuffs are often expensive.
The aim of this study was to evaluate the effects of different agro-industrial by-products in diets and to determine the nutritive value and energy contained in feeds for meat production and the biochemical parameters in the digestive tract of rabbits.

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Material and methods

A total of 96 rabbits (meat line M91 a P91 hybrid rabbits), reared in an intensive rabbitry in the Slovak Republic were used in the experiment. The rabbits were divided into 4 similar groups, 24 animals in each group, and kept in standard metal cages, two animals per cage. In the fattening experiment the growth of live weight and feed intake per unit of live weight growth were studied. Animals were given granulated diets ad libitum. The ingredients and chemical composition of diets are reported in table 1 according to procedures of the AOAC (2000) and Van Soest *et al.* (1991).
The experiment lasted for 42 days until the animals attained the slaughtering weight 2.5 kg. Body weight and feed consumption were registered weekly. Three animals from each group were slaughtered at 42nd day by cutting the jugularis and the carotid artery after electroanaesthesia (90 V for 5 sec). Samples of *Musculus longissimus dorsi* (MLD) were collected immediately after death and stored for 24 h at 4°C. Then standard physico-chemical analysis (according STN 57 0185) were made. The gross energy value of meat was calculated:
Energy value (kJ.100g^{-1}) = (16.75 * total protein content + 37.68 * total fat content).
Caecal samples from each of the three slaughtered rabbits were collected for caecal bacteria analyses. Digesta was analyzed for pH and VFA, ammonia-N and content lactic acid.
Data were subjected to ANOVA (analysis of variance). The means and standard deviation of the generated data per group were determined. For the subsequent statistical the data were examined for significant differences using the t-test.

Results and discussion

The experimental diets were balanced on nutrients content and energy value (Table I).
The study was carried out at the National Agricultural and Food Centre - Research Institute for Animal Production, Nitra, in the Slovak Republic. We did not find large differences among experimental groups in feed intake, body weight, mean daily gain and carcass value in the fattening experiment (Table III).

Table I: Ingredients and chemical analysis of the granulated diets for growing rabbits

Ingredients in %	1EG	2EG	3EG	4EG	Item in g/ kg	1EG	2EG	3EG	4EG
Lucerne meal	41	37	20	15.2	Dry matter	901.8	911	901.9	904.8
Maize	11	4	7	0	Crude protein	164.7	174.3	186.4	171.4
Oats	3	15	5	8	Crude fibre	218.2	161.4	140.5	173.6
Barley	0	9	0	4.5	Fat	22.1	27.8	33.7	41.3
Malt sprouts	0	12	5	6	Ash	65.4	64	71.9	70.4
Wheat bran	0	0	24	15	Starch	139.4	206.3	157.2	147.4
Wheat	0	5	0	0	Organic matter	836.4	847.4	830	834.4
Dried sugar beet pulp	19	0	15	10	Nitrogen free extract	427.4	483.9	469.4	448
Sunflower meal	10	0	10	10	ADF	202.2	204.5	196.2	210.3
Apple pomace	0	0	0	10	NDF	293.1	370.9	313.5	365.1
Sodium chloride	0.3	0.3	0.3	0.3	Calcium	10.7	8.8	6.7	6.7
Limestone, pulverized	1	1	1	1	Phosphorus	4.1	4.2	4.8	4.1
Rapeseed meal	10	12	8.5	10	Zinc	135.9	167.5	136.2	143.7
Mineral - vitamin mixture*	1.7	1.7	1.5	2	Lysine	7.7	7.8	7.7	9.1
DDGS	0	0	0	5	MET+CYS	6.5	5.2	5.65	5.8
Molasse	3	3	2.7	3	ME (MJ.kg^{-1})	8.15	9.64	11.5	10.8

*Provided per kg of diet: vit. A 12000 IU; vit. D_3 2500; vit. E 20 mg; vit. B_1 1.5 mg;
B_2 7.5 mg; B_6 4.5 mg; vit. B_{12} 30 µg; vit. K 3 mg ; nicotinic acid 45 mg; folic acid 0.8 mg; biotin 0.08 mg;
Choline chloride 450 mg; DDGS: dried distillers grains with solubles; ADF: Acidodetergent fibre; NDF: Neutraldetergent fibre; ME – metabolisable energy;

Age of the rabbit, dietary levels and type of starch influence digestion and absorption in the small intestine. Feed manufacturing processes and exogenous enzyme supplements also affect starch digestibility (De Blas and Gidenne, 1998). Starch that is not digested and absorbed in the small intestine passes into the caecum as a substrate for bacterial fermentation. Residual starch is thought to be a predisposing factor in the development of enterotoxaemia in young rabbits. Indigestible fibre is important to stimulate gut motility that moves digesta and fluid into the caecum for fermentation. Stimulate appetite and ingestion of caecotrophs. Fermentable fibre is important to provide a substrate for caecal microflora, provide optimal caecal pH and volatile fatty acid production (Piattoni *et al.*, 1996), and prevent proliferation of pathogenic bacteria in the caecum (Table V). Concentration of observed VFA shows, that most intensive process was in the caecum of rabbits in experimental groups (Table IV). The lower concentration of ammonia-N leads to increased digesta caecal of pH value.

Table II: The nutrient contents and energetic value of agro-industrial by-products

Ingredients Nutrients	Wheat bran	Sugar beet pulp	Dried malt sprouts	Sunflower meal	Rapeseed meal	DDGS from maize
Crude protein (g)	172.1	92.4	294.7	472.1	388.8	350.6
Crude fibre (g)	130.4	200.8	145.0	165.0	129.1	84.4
Crude fat (g)	34.7	8.5	20.5	17.1	35.9	118
Ash (g)	56.0	60.0	63.0	74.0	73.0	73.1
Starch (g)	162.5	3.9	59.3	35.9	38.3	2.0
ADF (g)	122.0	244.6	54.6	219.8	168.6	171.6
NDF (g)	386.0	412.2	88.2	321.4	240.4	297.5
Lys (g)	6.4	4.9	16.5	17.4	21.1	9.9
Met + Cys (g)	6.3	2.9	6.8	18.4	17.5	8.9
Calcium (g)	1.6	9.1	2.3	3.4	7.2	1.7
Phosphorus (g)	11.2	1.1	7.4	11.9	10.9	6.4
Sodium (g)	0.1	1.4	0.4	0.3	0.4	2.2
Zinc (mg)	88.5	10.4	103.4	79.1	63.4	95.5
ME (MJ)	8.27	12.61	8.82	5.26	9.37	13.84

Table III: Effect of treatment on performance of rabbits

Parameter (n=24)	1EG	2EG	3EG	4EG
Initial weight in g	905	920	970	845
Live weight on 56th day of age in g	1595	1734	1824	1734
Final weight in g	2500	2521	2500	2504
Daily gain in g	38.0	38.9	36.4	39.5
Mortality (n)	1	2	2	1
Feed conversion per kg gain	3.49	3.51	3.5	3.59
Carcass value (%)	58.37^{bc}	55.02	55.25	59.44^{bc}

bc P≤0.05

Table IV: Qualitative parameters in caecum (in 77th. day of age)

Parameter (n=3)	1EG	2EG	3EG	4EG
pH_{24}	6.12	5.72	5.99	5.76
N-NH3 (mmol/l)	10.36±0.2	13.26±1.7	22.78±4.32^{abd}	10.13
Lactic acid (g/100g)	0.056	0.98^{ad}	0.98 ^{ad}	0.036
Acetic acid (mmol/100g)	5.196±1.9	3.773±0.8	6.007±0.4^{b}	4.075±0.8
Propionic acid (mmol/100g)	0.331±0.2	0.413±0.12	0.606±0.13^{ad}	0.257±0.2
Buturic acid (mmol/100g)	1.58±0.17	1.032±0.04	1.658±0.26 ^{bd}	1.028±0.4
Other VFA (mmol/100g)	0.27±0.06 ^{bd}	0.13±0.04	0.16±0.03	0.14±0.04

abcd P<0.05

Table V: Counts of bacteria in faeces of rabbits (log 10 CFU.g^{-1})

Bacterial strains	in caecum				in faeces			
(log 10 CFU.g^{-1})	1EG	2EG	3EG	4EG	1EG	2EG	3EG	4EG
Enterococcus sp.	1.38	1.37	$<10^1$	$<10^1$	3.56	2.97	$<10^1$	$<10^2$
Lactic acid bacteria	1.42	1.04	1.54	1.29	4.65	2.40	5.44	4.63
(CNS)	3.35	3.07	3.67	2.34	3.65	3.81	3.50	3.17
(CPS)	1.52	1.81	2.79	2.28	2.54	3.36	3.14	2.43
Clostridim-like sp.	4.38	4.37	2.10	2.24	3.98	4.99	4.08	4.43
Pseudomonas-like sp.	4.95	3.43	3.42	4.55	4.88	4.54	5.23	5.37

Table VI: Physico-chemical characteristics of rabbit meat (MLD)

Parameter (g.100g^{-1})	1EG	2EG	3EG	4EG
pH_{24}	5.47	5.72	5.71	5.59
Content of water	75.5±0.2	74.9±0.7	76.07±0.36	75.1±0.13
Water holding capacity	31.7±1.9	33.3±1.1	32.8±2.9	34.15±0.8
Content of fat	1.43±0.2	2.33±1.1	$3.53±0.8^{aD}$	1.38±0.2
Total proteins	22.95±0.17	23.2±0.17	22.37±0.5	22.51±0.4
Energetic value (kJ.100g^{-1})	438.1±7.6	451.4±3.4	$491.27±8.3^{abd}$	430.9±4.2

abd P≤0.05; D P≤0.01 Significantly different

Results of selected meat quality parameters (content of water, content of proteins, fat and pH_{24} and water holding capacity) are presented in Table VI. A statistically significant difference ($P \leq 0.01$) was recorded in the case of fat content and ($P \leq 0.05$) and in the energetic value in the sample MLD in 3EG (Rafay *et al.*, 1999).

Conclusion

Based on our results we conclude:

- In all groups of the growth intensity and health status (morbidity, mortality) was compared. The aim of this work was to study fibre at three different levels of diets for growing rabbits (14.6 %, 17.4% and 21.8%).
- - with increased nutrient content and energy in feed mixture the time for fattening period is shortened and conversion (the feed consumption per unit of live weight) decreased.
- - higher content of crude fiber decreases index digestibility of organic matter, crude protein and fat in the mixture.
- - higher crude protein and amino acid ratio have positive effect on feed intake and growth rate. DDGS from maize are a good source of protein and energy of rabbits.
- Total caecal VFA concentration was significantly higher (P≤0.05) and the pH was lower in rabbits fed with dried sugar beet pulp, wheat bran and dried apple pomace diet than in the caecum of other rabbits (P≤0.05) in experimental group 3EG (Table IV). Similarly, caecal N-NH3 concentration was decreased when rabbits received diet with dried sugar beet pulp (P≤0.05). High concentrations of acetic acid, propionic acid, butyric acid and lactic acid were determined in the caecum of rabbits with the supplements of different levels of agricultural by-products, while total volatile fatty acid production, during a period of starch degradation process, was highest in 3EG (supplemented with wheat bran).
- The inclusion of different agriculture by-products into feed mixture for rabbit fattening did not negatively influence biochemical parameters and the quality of rabbit meat in MLD.

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Steirischer Kürbiskernpresskuchen als teilweiser Fisch-mehlersatz: Einfluss auf die Darmhistologie von Salmoniden und Welsen

Pumpkin seed cake as partial fishmeal substitute in fish nutrition: Effects on intestinal morphology of salmonids and catfish

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Abstract

In comparison to the numerous studies that focus on the performance traits of fishmeal replacers in fish nutrition, very few have investigated the effects of said replacers on the intestinal morphology of fish. In four feeding trials the effects on intestinal morphology of rainbow trout (*Oncorhynchus mykiss* W.), brook trout (*Salvelinus fontinalis* M.), African sharptooth catfish (*Clarias gariepinus* B.) and wels catfish (*Silurus glanis* L.) were evaluated with a diet in which 60% of the fishmeal protein of a reference diet were substituted by pumpkin seed cake protein (PSC). Results show no significant changes in histomorphology when substituting 60% of fishmeal in diets with PSC. No interactions of the factors feed and fish production system were observed. However, the biggest differences were observed comparing the production systems. In conclusion, PSC can partly substitute fish meal in diets of salmonids and catfish without negative effects on histomorphology.

Einleitung

Weltweit spielt Fisch eine sehr wichtige Rolle für die Ernährung des Menschen. Der Anteil an Fisch aus Aquakulturen nimmt dabei deutlich zu (FAO, 2016a). Eine wichtige Proteinquelle in Fischfuttermitteln ist Fischmehl, welches sich Großteiles aus Fischen aus dem Wildfang zusammensetzt und damit den Druck auf Wildbestände weiter erhöht (FAO, 2016b). Dadurch ergeben sich sowohl ökonomische als auch ökologische Probleme. Ein Fokus auf pflanzliche Alternativen wird für tragfähige Produktionssysteme in der Aquakultur von wachsender Bedeutung sein. Bei deren Evaluierung stehen jedoch größtenteils verschiedene Leistungsparameter als Kenngrößen im Vordergrund. Dabei ist eine Evaluierung der Verträglichkeit von Futtermitteln für die jeweilige Spezies bedeutsam, um mit alternativen Proteinquellen eine erfolgreiche Produktion von tierischen Nahrungsmitteln ohne die Einschränkung der Tiergesundheit zu sichern. Wichtige Zusatzinformationen kann eine histologische Untersuchung des Darmes liefern. Dabei ist zu erwarten, dass der partielle Ersatz von Fischmehl durch Steirischen Kürbiskernpresskuchen (KPK) unterschiedliche Auswirkungen je nach Produktionssystem (spezifisch für die Fischart) zeigt. Ziel dieser Arbeit ist es, den Einfluss von KPK auf die Darmmorphologie von zwei Kaltwasserfischen und zwei Warmwasserfischen zu betrachten, um dessen Eignung als heimische und nachhaltige Alternative zu Fischmehl zu evaluieren.

Material und Methoden

Die Fütterungsversuche wurden vom Institut für Fischerei der Bayerischen Landesanstalt für Landwirtschaft in Starnberg (Deutschland) durchgeführt. Die Versuchsgruppen (jeweils acht Becken bei Regenbogenforelle, Bachsaibling und Afrikanischem Wels – entsprechend vier Wiederholungen und sechs Becken bei Europäischem Wels – entsprechend drei Wiederholungen) wurden randomisiert angeordnet. Im Vergleich zu einer Kontrollration (KON) wurde in der Versuchsration KPK 60% des Fischmehlproteins in KON durch KPK-Protein auf Basis des verdaulichen Proteins ersetzt. Die Fische wurden händisch einmal täglich bis zur scheinbaren Sättigung gefüttert (Tabelle 1). Details zu Haltungssystem und Fütterung sind in der Publikation von Greiling *et al.* (2018) nachzulesen.

Tabelle 1: Rationszusammenstellung der Salmoniden und Welse

	Produktionssystem			
	Regenbogenforelle, Bachsaibling		Afrikanischer bzw. Europäischer Wels	
	KON	KPK	KON	KPK
Komponenten [g kg^{-1}]				
Fischöl	110,0	110,0	115,8	115,8
Sojaschrot	247,4	247,4	260,4	260,4
Blutmehl	90,0	90,0	94,7	94,7
Weizengluten	90,0	90,0	94,7	94,7
Fischmehl (Anchovis)	200,0	80,0	210,5	84,2
Kürbiskernpresskuchen	-	141,0	-	148,5
Rapsöl	50,0	46,0	-	-
Weizen	200,0	183,0	210,5	188,3
L-Lys HCl	2,0	2,0	2,1	2,1
DL-Met	5,6	5,6	5,9	5,9
Mono-Calcium-Phosphat	3,0	3,0	3,2	3,2
Vit-Min-Prämix[2]	2,0	2,0	2,1	2,1
Chemische Analysen [g kg^{-1} TM]				
Trockenmasse [g kg^{-1}]	928	916	921	924
Rohprotein	514	488	515	503
Rohfett (hydrolysiert)	138	148	109	116
Rohfaser	18,1	22,1	18,9	22,4
Asche	67,5	61,4	69,8	63,9
N-freie Extraktstoffe	262	281	287	295
Bruttoenergie [MJ kg^{-1} TM]	22,6	22,9	22,3	22,5

[1]KON = Kontrolle; KPK = Ration für Salmoniden und Welse in der 60% des Fischmehlproteins in KON durch Kürbiskernpresskuchenprotein auf Basis des verdaulichen Proteins ersetzt wurde

[2] Vit-Min-Prämix beinhaltet (IU/kg oder g/kg Prämix): Vitamin A, 0,8 MIU; Vitamin D_3, 0,5 MIU; Vitamin E, 40 g; Vitamin K_3, 2 g; Vitamin C, 30 g; Biotin, 0,16 g; Folsäure 1,6 g; Vitamin B_1, 3 g; Vitamin B_2, 4 g; Vitamin B_6, 3 g; Vitamin B_{12}, 0,01 g; Nicotinamid, 30 g; Inositol, 80 g; Betain, 84 g; Eisensulfat, 30 g; Kupfersulfat, 0,6 g; Mangansulfat, 5 g; Zinksulfat, 12 g; Calciumjodat 0,8 g; Natriumselenit 0,04 g

Am Ende des Fütterungsversuches wurden die Fische nach 24 stündiger Nüchterung fachgerecht betäubt, getötet und zerlegt. Die Darmlänge wurde von Pylorus bis Rectum gemessen. Für die Histologie wurden proximal (10 mm hinter dem Pylorus bzw. den Pylorusanhängen) und distal (10 mm vor dem Rectum) Darmproben von circa 20 mm Länge gewonnen. Die Proben wurden mit 0,9% Kochsalzlösung gespült, in 10% Formalin fixiert, entwässert und in Paraffin eingebettet. Die Herstellung der 5 µm Gewebeschnitte erfolgte mit einem Rotationsmikrotom (Leica, Darmstadt, Deutschland). Eine AB-PAS-Färbung zur Darstellung der Becherzellen wurde durchgeführt. Die Auswertung der Gewebepräparate erfolgte mit einem Lichtmikroskop (Leica, Darmstadt, Deutschland). Folgende Parameter wurden proximal und distal vermessen: Zottenlänge, Zottenbreite, Höhe der Mucosa, Dicke der Muscularis sowie die Anzahl an Becherzellen.

Die statistische Auswertung erfolgte mit SAS 9.4 (SAS Institute Inc., Cary, USA). Gewählt wurde ein 2*4 faktorielles Design mit zwei Futtermitteln und vier Produktionssystemen.

Tabelle 2: Proximale und distale Morphologie der Fischarten im jeweiligen Produktionssystem

	Futter		Produktionssystem				SEM	*p*-Wert[2]		
	KON	KPK	Regenbogen-forelle	Bach-saibling	Afrik. Wels	Europ. Wels		FM	FI	FM*FI
Darmlänge, cm	15,7	15,6	10,3^{c}	10,4^{c}	26,6^{a}	15,2^{b}	0,8	NS	***	NS
Darm, proximal										
Zottenlänge, µm	857	911	706^{c}	878^{b}	1069^{a}	n.a.[2]	22	(*)	***	NS
Zottenbreite, µm	193	184	217^{a}	162^{c}	187^{b}	n.a.	4,1	NS	***	NS
Mucosa, µm	989	1038	842^{c}	1032^{b}	1166^{a}	n.a.	20,8	NS	***	NS
Muscularis, µm	136	134	157^{b}	76^{d}	123^{c}	184^{a}	4,8	NS	***	NS
Becherzellen, n/200 µm	5,59	5,59	7,24^{a}	3,84^{b}	4,09^{b}	7,20^{a}	0,25	NS	***	NS
Darm, distal										
Zottenlänge, µm	890	878	n.a.	n.a.	818	951	24,7	NS	***	NS
Zottenbreite, µm	193	176	n.a.	n.a.	212	157	6,5	(*)	***	NS
Mucosa, µm	1082	1075	n.a.	n.a.	999	1157	28	NS	***	NS
Muscularis, µm	236	226	145^{bc}	114^{c}	178^{b}	486^{a}	14,7	NS	***	NS
Becherzellen, n/200 µm	8,70	8,38	6,50^{b}	6,31^{b}	10,44^{a}	10,92^{a}	0,30	NS	***	NS

[1]KON = Kontrolle; KPK = Ration für Salmoniden und Welse in der 60% des Fischmehlproteins in KON durch Kürbiskernpresskuchenprotein auf Basis des verdaulichen Proteins ersetzt wurde.

[2]FM=Futtermittel; FI=Fischart; n.a. = nicht analysierbar; $p<0,10$(*); $p<0,05$*; $p<0,01$***; NS=nicht signifikant

Ergebnisse und Diskussion

Details zur zootechnischen Leistung der jeweiligen Fischarten innerhalb des Produktionssystems wurden von Greiling *et al.* (2018) beschrieben.

In Bezug auf den Haupteffekt Futtermittel – wenn 60% des Fischmehlproteins durch KPK-Protein ersetzt wurde – oder die Wechselwirkung (Futtermittel*Produktionssystem), wurden keine signifikanten Unterschiede bei den histomorphologischen Parametern gefunden (Tabelle 2). Lediglich ein Trend zu längeren Zotten im proximalen Intestinaltrakt wurde durch die Fütterung mit dem KPK bewirkt ($p<0,10$). Die Veränderung des Fasergehaltes in der Ration und im Speziellen der Anteil löslicher Faser kann dabei für die Verlängerung der Zotten verantwortlich sein, wie auch in anderen Spezies gezeigt werden konnte (Gomez-Conde *et al.* 2007; Ngoc *et al.* 2012; Sarikhan et al 2010). Auch wenn die tendenzielle Verlängerung der Zotten mit dem Fasergehalt erklärt werden kann, so kommt diese Änderung durchaus überraschend, da Studien mit Regenbogenforelle oder Atlantischem Lachs gezeigt haben, dass beispielsweise der Ersatz von Fischmehl durch Sojaextraktionsschrot in der Ration eine Beeinträchtigung der Zottenarchitektur bewirkte (Heikkinen *et al.* 2006; Krogdahl *et al.* 2003).

Die Mucusproduktion der Schleimhaut wird durch Stressoren, wie beispielsweise antinutritive Faktoren, angeregt und soll zum Schutz und zur Gleitfähigkeit beitragen (Baeverfjord und Krogdahl, 1996). So zeigten Ostaszewska *et al.* (2005), dass der Einsatz pflanzlicher Proteinquellen (Sojaextraktionsschrot) bei Regenbogenforellen eine höhere Becherzellenanzahl bewirkte. Da im vorliegenden Versuch die Becherzellenzahl durch die Fütterungsintervention nicht verändert wurde, scheint keine Beeinträchtigung der Mucosa durch den KPK aufgetreten zu sein.

Wie zu erwarten waren allerdings zwischen den verschiedenen Fischarten in ihren jeweiligen Produktionssystemen große Unterschiede zu erkennen. Diese Unterschiede lassen sich auf eine erhöhte Aktivität der Welse zurückführen, welche durch das Haltungssystem bedingt ist. Dabei lassen sich vor allem zwischen den Systemen Kaltwasser (Salmoniden) und Warmwasser (Welse) die größten Unterschiede finden. Das Produktionssystem mit Afrikanischem Wels zeigte mit 26,6 cm die längste durchschnittliche Darmlänge. Bemerkenswert ist, dass die Anzahl der Becherzellen bei der Regenbogenforelle von proximal nach distal geringfügig abnehmen, wohingegen sie bei den anderen Arten stark zunehmen. Ob dies jedoch physiologisch oder methodisch (Ermittlung der Becherzellen bei fragmentierten Zotten) zu begründen ist muss in weiterführenden Studien ermittelt werden.

Schlussfolgerung

Die Ergebnisse dieser Arbeit zeigen, dass keine Unterschiede bei der quantitativen histologischen Untersuchung durch die eingesetzte Menge an Kürbiskernpresskuchen als partiellem Fischmehlersatz festgestellt werden konnten.

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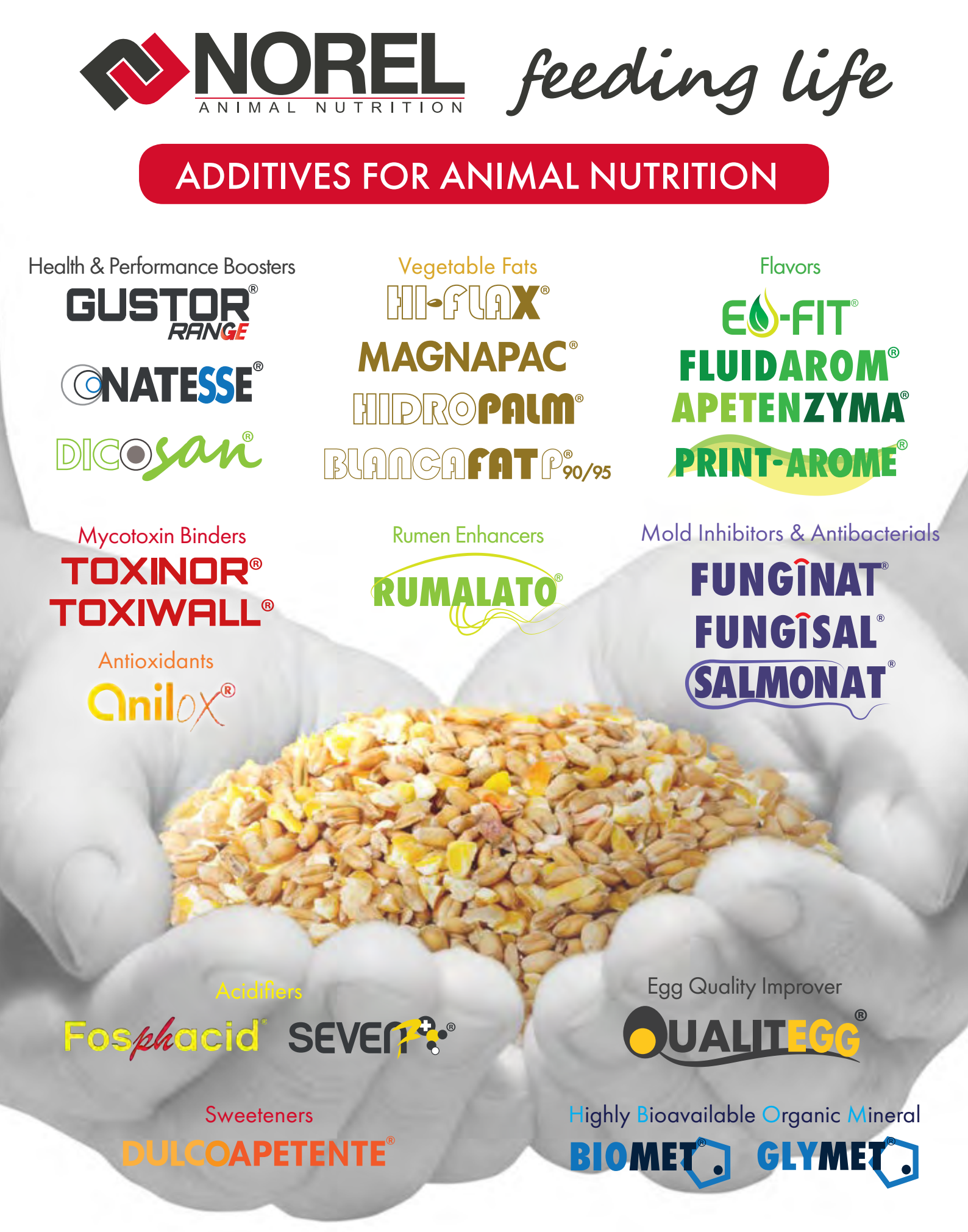

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Effective degradability and parameters of degradability of starch in different type of maize

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Abstract

In sacco experiment was conducted to determine degradation of starch in maize hybrids with dent and dent x flint type of grains. The samples of grains were harvested in four stages of maturity. The first harvesting was done in milk-waxy stage and than fornightly at intervals until full grain ripeness. Used dent x flint hybrids were Mesnil, Chambord, Queen, while Aude, Meridien, KX 13 93, Omero belonged to dent. *In sacco* experiment was carried out on three rumen canullated cows. The cows were fed a ration at maintenance level twice daily. The effective degradability of maize starch grains have decreasing tendency with increasing maturity stage. The maize hybrids of dent type had higher effective starch degradability. The degree of maturity had a significant ($P < 0.01$) effect on effective degradability of starch. The maize hybrids KX 1393 and Meridien had the highest rate of starch degradability (parameter c: 0.075 $\%.h^{-1}$ and $0.074.h^{-1}$, respectively). Starch in the hybrids dent x flint were less rapidly degraded than dent hybrids.

Introduction

Maize grain is a primary energy supplement in dairy diets and can contribute up to 30, 60, and 98 % of the diet's protein, net energy, and starch, respectively. Specialty maize hybrids are one result of efforts to select corn based on nutrient content (Dado, 1999). Maize kernel is divided into five types: flint, popcorn, floury, dent and sweet corn (Corona *et al.*, 2006). The endosperm of flint corn consists of hard-textured vitreous starch and has a greater proportion of vitreous endosperm than dent maize (Philippeau *et al.*, 1999, Kotarski et al, 1992). With advancing maturity, kernel vitreousness and density increases while ruminal starch availability and total tract starch digestibility decreases (Philippeau and Michalet-Doureau, 1997; Johnson *et al.*, 2002). Dry corn is more extensively encapsulated by prolamins and is less degradable in the rumen as compared to floury or opaque maize grains (Kotarski *et al.*, 1992). Since maize grain often accounts for a large part of the diet of beef cattle, information on the pattern of degradation of maize protein in rumen contents is important in order to improve the precision with which diets are compounded (Níkolić and Filipović, 1981). The aim of our study was to determine the effect of grain type and maturity stage on ruminal starch degradability *in situ* from selected corn hybrids of dent and flint x dent types.

Material and methods

The grains of maize hybrids of the type dent (Aude, Meridien, KX 1393, Omero) and dent x flint (Mesnil, Chambord, Queen) were used in the experiment. The maize grains were sampled at fornightly intervals, first at the time of milk-waxy maturity (13.8., at harvest maize for silage), until full ripeness, i.e. 26.8, 10.9 and 23.9. Maize grains were shelled from collected cobs, than dried at room temperature, and ground to pass through a 3 mm sieve. Starch degradability in the maize grains was determined *in sacco* (Harazim and Pavelek, 1999). *In sacco* experiments were carried out in three nonlactating cows with

large rumen cannulae (an average of 10 cm). The animals were fed twice a day with a diet consisting of 70 % forage and 30 % concentrate on a dry matter basis at maintenance level. The ration consisted of maize silage, alfalfa hay, wheat, barley meal (1:1) and Vitamix S1. Access to water was *ad libitum*. Ground samples were weighed (approx. 2.50 g dry matter) into bags made of Uhelon 130T (HEDVA, "Moravská Třebová", the Czech Republic) with pore size of 47 μm. Minimum of three separate bags for hybrids, incubation time and animals were used. The bags with samples were incubated for 2, 3, 4, 6, 9, 16, 24, and 48 hours. The 0 h time bags were only washed in water to determine washing losses. The bags were inserted into the rumen just before morning feeding. The content of starch was determined in the maize hybrids and in the residues after all incubation times. The content of starch was determined acoording to modified enzymatic method (Salomonsson *et al.*, 1984). The parameters of degradability (a, b, c, and "effective degradability") were calculated using the equations by Ørskov and McDonald (1979) with outflow rate of 0.06.h^{-1}. The obtained data on nutrients and degradability of starch in maize hybrids were evaluated statistically using models in statistical package Statistics 8.0.

Results and discussion

Due to lack of information on the grain quality of maize hybrids grown in Slovakia this study emphasized on chemical composition and *in situ* ruminal degradability of starch in dent and dent x flint hybrids. Dent x flint maize (*Zea mays L.*) hybrids are commonly planted in the early maize growing regions of Europe (Moreno-Gonzales *et al.*, 1997; Moreno-Gonzales *et al.*, 2000). Table 1 shows the results of starch on the grounds that this nutrient could most affect grain degradability in the rumen. The endosperm contains primarily starch and abundant storage proteins. The main maize proteins are prolamins, named zein, that comprise 50-60 % of total protein in maize grain. Results from the tested hybrids showed that the content of starch depends on the type of grain.

Table 1: Content of starch in grains of maize hybrids (g.kg^{-1} DM)

Nutrient		Hybrids						
Starch	Sampling	dent x flint			dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
	1.	689,4	680.8	676.5	651.2	762.2	671.8	673.6
	2.	693.9	715.7	689.7	718.7	742.7	717.9	679.4
	3.	658.3	659.6	632.8	692.3	708.4	681.2	675.8
	4.	702.4	722.5	678.3	694.3	688.9	722.7	721.5
	$\overline{x}$	686.0a*	694.7	669.3a,b	689.1a	716.6a*	698.4b	687.6a
	SEM	17.80	27.47	23.18	25.93	21.41	23.74	21.05

Means with the same letters in the same row are significantly different at P<0.05 and P<0.01* , SEM = Standard Error of Mean

The mean starch content was higher in dent hybrids, and kept on changing with ripening of grain. The maximum starch concentration was determined in hybrid KX 1393 (716.6 g.kg^{-1} DM) and the lowest in hybrid Queen (669.3 g.kg-1 DM). The differences between individual hybrids were significant (P<0.05), and while between KX 1393 and Queen were highly significant (P<0.01). The results also confirm the changes in the starch content in corn grain during the ripening process. Highly significant differences were noted between 1st and 2nd, 3rd and 4th samplings (P<0.01) and between 3rd and 4th samplings (P<0.05). Maize grain differs from other cereals in terms of degradation in the rumen and has high content of starch and unique structure and characteristics of grain (Kopčeková *et al.*, 2008). Degradation of maize starch is about 60 %, while for barley and wheat it is more than 90 %. Kopčeková *et al.* (2010) and Šimko *et al.* (2006) showed resistance to degraded maize starch in the rumen. Similar results were also documented by Lebzien *et al.* (1997), who found the effective degradability of maize starch at 55 % and wheat starch at 95 %. According to Kotarski *et al.* (1992) better understanding of chemical factors that potentially influence starch digestibility in ruminants required understanding of anatomy and physiology of maize seed. From three basic morphological parts, the endosperm represents approximately 75 % to 80 % of the corn kernel by weight and is the morphological structure which

contains starch. Prolamin proteins cross-link encapsulating starch into a water tight (hydrophobic) matrix. Starch kernels in vitreous maize are more extensively encapsulated by prolamins (zein) and therfore are less degraded in the rumen as compared to floury maize. Also, according to Michalet-Doreau *et al.* (1995) differences in the degradation of starch in maize grain among hybrids are primarily linked to the structure of the endosperm. The vitreous endosperm is hard, and abundant in protein matrix, with larger and more numerous protein bodies, and compact and polygonal starch granules. In the floury endosperm, the protein matrix is discontinuous and has few protein bodies, and the starch granules are spherical, larger, less aggregated and surrounded by air spaces (Gibbon *et al.*, 2003). Proportions of vitreous and floury endosperm vary among corn hybrids and maturity at harvest. Corn hybrids with kernels containing high proportions of vitreous (or horney) endosperm are flinty and those containing high proportions of floury endosperm are called floury, or dent (Kotarski *et al.*, 1992). Dent type hybrids (Aude, KX 1393, Meridien and Omero) showed higher effective degradability of starch than type dent x flint in all harvestings. Averages of degradability parameters for individual hybrids from all four harvesting times confirmed lower starch degradation rate in dent x flint maize grains than dent maize grains (Table 2).

Table 2: Parameters of effective degradability of starch in maize – dent x flint and dent type

Parameters of degradability	Sampling	Hybrids						
		dent x flint			dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
a (%)	1.	21.2	19.0	22.6	18.0	33.0	31.1	33.0
	2.	22.4	22.8	23.0	18.8	17.0	18.2	17.0
	3.	30.7	22.4	27.2	23.4	14.4	15.3	14.4
	4.	27.1	27.2	21.6	22.4	26.9	27.3	26.9
	$\overline{x}$	26.9	22.8	23.6	20.7	26.2	23.0	22.8
	SEM	4.01	4.17	3.14	2.40	4.45	6.93	8.64
b (%)	1.	72.5	81.0	77.4	82.0	66.9	67.9	66.9
	2.	76.8	77.2	77.0	81.2	83.0	81.8	83.0
	3.	65.7	74.1	70.4	76.6	85.6	84.7	85.6
	4.	72.9	27.2	77.7	77.6	73.1	71.1	73.1
	$\overline{x}$	72.0	75.6	75.6	79.4	73.2	76.3	77.1
	SEM	4.75	6.41	3.78	2.40	4.47	7.86	8.75
c (%.h^{-1})	1.	0.082	0.058	0.072	0.069	0.061	0.091	0.061
	2.	0.071	0.037	0.046	0.053	0.065	0.068	0.065
	3.	0.071	0.052	0.052	0.044	0.063	0.064	0.063
	4.	0.038	0.054	0.058	0.056	0.059	0.072	0.059
	$\overline{x}$	0.066g	0.051cfg	0.057be	0.056ad	0.074def	0.075abc	0.062
	SEM	0.020	0.015	0.016	0.014	0.021	0.015	0.014
EDg (%)	1.	69.4	60.0	63.2	60.9	58.7	70.5	58.7
	2.	63.2	56.4	58.0	57.5	60.4	58.7	60.4
	3.	59.8	49.9	56.5	53.4	53.9	47.4	53.9
	4.	58.4	60.8	59.7	59.2	62.2	61.0	62.2
	$\overline{x}$	62.7f	56.8ef	59.3b	57.7a	65.0 abde	59.4d	58.8e
	SEM	4.77	5.05	3.96	3.61	5.57	9.27	4.86

Means with the same letters in the same row are significantly different at P<0.05, SEM = Standard Error of Mean

Effective degradation of starch (EDS) was lower for crossbred dent x flint varieties with properties closer to dent than for flint hybrids. The differences between them were not high even though differences among hybrids were significant. The hybrids Mesnil (62.7 %) and Aude (57.7 %) were beyond the average. The rate of starch degradability was the highest in dent hybrids KX 1393 (0.075 %.h-1) and Meridien (0.074 %.h-1).

Conclusion

There were differences in starch content between dent and dent x flint maize hybrids during ripening process. Dent type maize hybrids are higher in starch content. With advancing maturity in maize grain of dent x flint type hybrids starch decrease was more than in dent hybrids. The effective degradability of starch in dent type hybrids was higher than in dent x flint type hybrids. In practical terms this means that the use of protein and starch as a source of energy from maize grains of dent x flint type hybrids is more effective in ruminants as starch is less extensively degraded in the rumen to glucose and ammonia in comparison to dent type hybrids grains.

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Carbohydrate composition of spring and winter wheat cereal grains

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Abstract

Carbohydrates – the major source of energy for poultry. Most of the carbohydrate in poultry diets is provided by cereal grains. The goal of the study were to evaluate the different wheat varieties carbohydrates concentration. Six spring and six winter wheat varieties grown up in Lithuania. Grains samples of different varieties were analysed for starch, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), arabinoxylans and beta glucans. Starch in spring wheat an average was 56.68% DM and in winter wheat was 63.89% DM. In spring wheat NDF, ADF, ADL concentrations in DM ranged as follows: 11.74-14.28%, 3.30-3.64%, 0.82-1.03% respectively, the average content of arabinoxylans (6.17% DM), and beta glucans (0.35% DM). In winter wheat NDF, ADF, ADL concentrations in DM ranged as follows: 11.59-13.79%, 3.12-3.85%, 0.91-1.26% respectively the average content of arabinoxylans (6.31% DM) and beta glucans (0.42% DM). Spring wheat cereal grains contained higher crude fibre and NDF fraction, however starch, ADF, ADL, arabinoxylans and beta glucans contents was markedly higher in winter wheat cereal grains.

Introduction

The energy necessary for maintaining the bird's general metabolism and for producing meat and eggs is provided by the energy-yielding dietary components, primarily carbohydrates and fats, but also protein. In poultry diets, carbohydrates represent the major energy component. About 70% of the bioavailable energy comes from the carbohydrates, starch being the most important fraction for animal nutrition (Klein *et al.*, 2001). Some plant sources used in poultry diets to provide carbohydrates are corn, wheat, barley, rice, and sorghum. These feedstuffs are typically high in carbohydrates and low in crude protein. The majority of the carbohydrate content in cereal grains occurs as starch, which is readily digested by poultry (Franco *et al.*, 1995; Eliasson and Gudmundsson, 1996). The majority of the carbohydrate fraction in the cell walls is composed of beta glucans and arabinoxylans (Classen, 1996). Non-digestible carbohydrates consist of resistant starch, and NSP (Bach Knudsen *et al.*, 2012). Non-starch polysaccharides include pectins, cellulose, hemicelluloses, beta glucans, arabinoxylans, fructans, oligosaccharides, and resistant starch (Bindelle *et al.*, 2008). However, the main NSP in cereal grains are arabinoxylans and beta glucans (Bach Knudsen, 2001). Thus, these dietary carbohydrates often contribute little to meeting the energy requirement of poultry, and some adversely affect the digestive processes of poultry when present in sufficient dietary concentrations. For example, the arabinoxylans and beta glucans increase digesta viscosity and reduced nutrient digestibility and absorption (Hajati and Rezaei, 2010), microbial growth and gastrointestinal tract health (Santos *et al.*, 2006), and prolonged transit time of digesta tract (Choct, 1999).

There are several studies reported in literature on feed formulation, and many focus on the influence of macronutrient ratios (protein, carbohydrate, lipid) on the live performance of broilers (Dairo *et al.*, 2010; Hosseini-Vashan *et al.*, 2010), there are fewer reports highlighting the wheat cereal grains

carbohydrates fraction. The objective of this study was to evaluate the different wheat varieties carbohydrates concentration.

Material and methods

Sample collection. Grains samples of the varieties of spring and winter wheat were collected from the Kaunas Plant Variety Testing Station (PVTS). Fertilization of spring and winter wheat was applied as $N_{11}P_{22}K_{55}$, additionally-$N_{68.8}$+$N_{68.8}$ and following parameters of the soil are: humus-1.65%, pH-7.6, P_20_5-140 mg kg^{-1}, K_20-327 mg kg^{-1}.

Carbohydrate analysis. Grains samples were taken and analysed in accordance with the Commission regulation (EU) No 691/2013 of 19 July 2013 amending Regulation (EC) No 152/2009 as regards methods of sampling and analysis. Grains samples with three subsamples for chemical analyses were ground in an Ultra Centrifugal Mill model ZM 100 (Retsch GmbH, Germany) with 1.0 mm sieve. Dry matter yield of grains, was determined by drying the sample in an oven at 105°C until a constant weight was obtained. The presence of starch was determined in spring and winter wheat by near infrared (NIRS) spectroscopy without any previous sample pretreatment except grinding. Starch were analysed using the FOSS NIRS™ DS2500 system according to the manufacturer's instructions. Crude fibre was determined as the residue after sequential treatment with hot H_2SO_4 (conc. 1.25%) and hot NaOH (1.25%) according to Weende method. The samples were subjected to the fibre component analyses for ANKOM 220 Fiber Analyzer (ANKOM Technology, USA): acid detergent fibre (ADF) and neutral detergent fibre (NDF), acid detergent lignin (ADL) using a cell wall detergent fractionation method according to van Soest (Faithfull, 2002). The beta glucans was determined using a Megazyme test kit which uses the specific enzymes and follows the method of McCleary and Glennie-Holmes (1985) and McCleary and Codd (1991). Fermented beta glucans detection kit (K-BGLU 11/07) obtained from Megazyme (Ireland). Details of the method are available at www.megazyme.com. The content of arabinoxylans was determined using a Megazyme test kit (K-Xylose 14/08) obtained from Megazyme (Ireland). Details of the method are available at www.megazyme.com.

Statistical analysis. Statistical significance was established using one-way analysis of variance (ANOVA), and the data were reported as a mean of standard deviation. Mean comparison and separation were done using Duncan's t-test ($P<0.05$). ANOVA and Pearson's correlation analysis were conducted using the statistical package SPSS 22. Means in the same column with different superscript letters are significantly different.

Results and discussion

The carbohydrates concentration of different varieties of spring and winter wheat cereal grains is presented in Table 1. The highest amount of starch in analysed spring wheat was determined in 'Cornetto' (58.67%) variety, the smallest amount was in 'Ethos' (53.29%) variety. The highest value of NDF concentration was determined in 'Diskett' variety (14.28% DM) and the lowest were determined in variety 'Tybalt' (11.74% DM) ($P<0.05$). The spring wheat varieties had accumulated less ADF concentration and it was ranged between 3.30% DM ('Ethos') and 3.65% DM ('Tybalt') ($P<0.05$). The concentrations of ADL an average was 0.90% ('Sonett' 0.82% DM, 'Diskett' 1.03% DM). The amount of arabinoxylans and beta glucans concentration in the analyzed spring wheat an average was 6.17 % DM and 0.35% DM. Rodehutscord *et al.* (2016) reported in the wheat varieties starch concentration is higher than the values observed in this study. Anjum *et al.* (2014) found that the NDF, ADF and ADL concentration in wheat varieties was higher. Mikulioniene and Balezentiene (2009) analyzed wheat and found that the NDF was 129 g kg^{-1} DM, ADF-43 g kg^{-1} DM, ADL-13 g kg^{-1} DM.

Table 1: Different carbohydrate fractions of spring wheat cereal grains (%, dry matter)

Variety denomination	Dry matter	Starch	Crude fibre	NDF	ADF	ADL	Arabinoxylans	Beta glucans
				Spring wheat				
Cornetto	88.09^{d}	58.67^{d}	1.99^{b}	12.66^{c}	3.64^{b}	0.89^{b}	5.64^{e}	0.28^{b}
Diskett	88.12^{e}	53.83^{e}	2.21^{a}	14.28^{a}	3.55^{a}	1.03^{c}	6.35^{b}	0.33^{b}
Ethos	87.96^{c}	53.29^{b}	1.76^{d}	13.92^{b}	3.30^{e}	0.83^{f}	6.16^{c}	0.36^{b}
Rospuda	87.48^{d}	58.34^{a}	1.85^{f}	12.23^{e}	3.38^{d}	0.90^{d}	6.53^{a}	0.29^{b}
Sonett	87.87^{b}	57.71^{f}	1.66^{e}	12.29^{d}	3.33^{f}	0.82^{e}	5.97^{d}	0.50^{a}
Tybalt	88.04^{a}	58.25^{c}	1.97^{c}	11.74^{f}	3.65^{c}	0.92^{a}	6.35^{b}	0.36^{b}
Mean	87.93	56.68	1.91	12.85	3.48	0.90	6.17	0.35
SD	±0.24	±2.44	±0.18	±0.95	±0.15	±0.07	±0.30	±0.10

Means in the same column with different superscript letters are significantly different, $^{a-f}$ – $P < 0.05$;

The winter wheat carbohydrates concentrations are shown in Table 2. In the winter wheat varieties starch concentration varied from 60.51 to 63.89%. The average amount of starch concentration was 61.37%. The lowest level of NDF, ADF and ADL in winter wheat content was detected for the 'Agil' variety, respectively 11.59% DM 3.12% DM and 0.91% DM). The arabinoxylans content in winter wheat between the different varieties was different and ranged between 5.47% DM ('Mariboss') and 7.09% DM ('Kovas DS'). The beta glucans content was the highest for the varieties 'Mariboss' and 'Kovas DS' (0.50% DM) and the lowest for the 'Agil' 0.27% DM (P<0.05). Arabinoxylans and beta glucans results are fairly typical for wheat are comparable to those reported by Rodehutscord *et al.* (2016).

Table 2: Different carbohydrate fractions of winter wheat cereal grains (%, dry matter)

Variety denomination	Dry matter	Starch	Crude fibre	NDF	ADF	ADL	Arabino xylans	Beta glucans
				Winter wheat				
Agil	89.06^{d}	61.13^{d}	1.66^{d}	11.59^{f}	3.12^{f}	0.91^{f}	5.51^{e}	0.27^{b}
Kovas DS	89.42^{b}	60.88^{f}	1.62^{e}	13.28^{b}	3.16^{e}	0.95^{e}	7.09^{a}	0.50^{a}
Mariboss	88.68^{a}	63.89^{b}	2.19^{a}	12.31^{d}	3.68^{b}	1.26^{a}	5.47^{f}	0.50^{a}
Mulan	89.27^{c}	60.51^{c}	1.75^{c}	13.79^{a}	3.64^{c}	1.18^{c}	6.40^{d}	0.44^{a}
Rigi	89.42^{f}	60.66^{e}	1.63^{e}	12.43^{c}	3.85^{a}	0.97^{d}	6.90^{b}	0.40^{ab}
Zentos	89.05^{e}	61.14^{a}	1.92^{b}	11.95^{e}	3.57^{d}	1.19^{b}	6.48^{c}	0.40^{ab}
Mean	89.15	61.37	1.80	12.56	3.50	1.08	6.31	0.42
SD	±0.28	±1.26	±0.22	±0.83	±0.30	±0.15	±0.64	±0.11

Means in the same column with different superscript letters are significantly different, $^{a-f}$ – $P < 0.05$;

Conclusion

Spring wheat cereal grains contained higher crude fibre and NDF fraction, however starch, ADF, ADL, arabinoxylans and beta glucans contents was markedly higher in winter wheat cereal grains. These differences are due to the different varieties, climatic and fertilization conditions.

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The effects of a biological inoculant on low dry matter maize silage quality

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Abstract

The aim was to characterize the effects of a biological silage inoculant on fermentation quality and aerobic stability of low dry matter maize silage. A mini-silo trial was conducted under controlled conditions: T1) untreated control; T2) three-strain silage inoculant (*Enterococcus faecium, Lactobacillus plantarum, Lactobacillus brevis)* at 1 x 10^5 cfu/g; T3) three-strain silage inoculant at 2 x 10^5 cfu/g. The silage inoculant had a beneficial effect on the fermentation profile (irrespective of dose) and aerobic stability (dose-dependent). Nevertheless, the observed improvement of the aerobic stability of the silage could not fully counteract the consequences of too early harvesting of immature plant material.

Introduction

Producing low dry matter maize silage (< 30 % dry matter) is not generally recommended since there is a risk of poor aerobic stability, risk of clostridial proliferation and related loss of silage palatability and quality. In some regions such as Iran, however, maize is commonly harvested early to make silage with dry matter levels as low as 23 to 25%.

In this trial, we investigated, if a three-strain biological silage inoculant could benefit farmers who produce such silage. Silos were opened after only 35 days of fermentation to create particularly challenging conditions for aerobic stability testing.

Materials and methods

Maize was harvested at milky stage of plant maturity with 25% dry matter (DM), chopped to 2cm particle lengths and immediately transported to the laboratory. Buffer capacity and fermentation coefficient were determined as described previously (Pahlow et Weissbach 1999). Three trial groups were ensiled in five replicates per sampling time point (day 7 and 35): T1: control (tap water), T2: Biomin® BioStabil Mays 1 x 10^5 cfu/g (Biomin Holding GmbH, Austria), T3: Biomin® BioStabil Mays 2 x 10^5 cfu/g. Silage was pneumatically compressed. Silage extracts in water were immediately used for pH measurement and microbiological evaluation. For HPLC measurements of organic acids, alcohols and sugars the extracts were frozen at -20 °C and then analysed in one batch, in an Agilent 1100 Series HPLC (Agilent, Santa Clara, California, US) using an ICSep ION-300 column (Transgenomic, Omaha, Nebraska, US) and a Guard column Polyspher® OA KC (Merck, Darmstadt, Germany) after Carrez precipitation. Viable lactic acid bacteria (LAB) were quantified in triplicate on MRS agar (Oxoid, Germany) and anaerobic incubation 37°C for 48 h. Yeasts and molds were quantified on Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Oxoid, Germany), incubated at 25°C for 48 h. Reinforced clostridial medium RCM (Oxoid, Germany) was used to estimate the most probable number (MPN) of clostridial spores, according to BS EN 26461-1. Microbial data were log-transformed before statistical analysis. Dry matter (DM) was determined after drying at 65°C for 24 h, followed by 3 h at 105°C. A commercial feed lab (Futtermittellabor Rosenau, Wieselburg-Land, Austria) performed Weender analysis. For the

assessment of aerobic stability, a sample of approximately 100 g silage was drawn from the center of each silo and individually exposed to air at 20 °C (ambient temperature) for 4 days. The temperature was continuously recorded with a MTR-30-2.0 temperature data logger.

Data are presented as means with the cumulative standard error of means. Statistical analysis was computed using IBM SPSS 22.0 software. The level of statistical significance was set at $P < 0.05$ for all evaluations. A P level between 0.05 and 0.10 was defined as trend.

Results

The whole-plant maize was easy to ensile with a fermentation coefficient of 102. Upon arrival at the laboratory, the maize had LAB at 6.1 log cfu/g forage, yeasts at 5.6 log cfu/g, molds at 4.3 log cfu/g and clostridia spores at 2.2 log cfu/g. All silages, including the untreated control T1, acidified equally well to a pH below 4 after seven days. The silage inoculants apparently increased the conversion of sugars into volatile organic acids (table1). Aerobic stability was significantly improved in T3 silage.

Table 1: Effect of biological inoculants on chemical composition and fermentation characteristics of maize silage after 35 days

	T1	T2	T3	cumulative	
	mean	mean	mean	SEM	P-value
DM, g/kg FM	249	250	248	1.4	0.874
Crude ash, g/kg DM	37.7	38.2	38.8	0.21	0.095
Starch, g/kg DM	259.4	233.6	236.6	5.67	0.123
Glucose, g/kg DM	16.3^{a}	2.8^{b}	2.1^{b}	1.07	0.001
Mannose, g/kg DM	14.1^{a}	3.0^{b}	2.8^{b}	1.45	0.001
Fructose, g/kg DM	129.6^{a}	97.5^{b}	84.5^{b}	6.19	0.001
aNDFom, g/kg DM	403.0^{a}	418.2^{ab}	430.6^{b}	4.69	0.042
ADFom, g/kg DM	244.2	249.2	240.4	1.83	0.143
NFC	458.0^{a}	445.8^{ab}	431.6^{b}	4.49	0.036
pH	3.70	3.69	3.69	0.005	0.745
ME, MJ/kg DM	10.73	10.64	10.60	0.026	0.112
NEL, MJ/kg DM	6.46	6.40	6.37	0.019	0.108
LAB, log cfu/g FM	6.59	6.59	6.59	0.077	1.000
Yeast, log cfu/g FM	3.43	2.21	2.64	0.270	0.182
Mold, log cfu/g FM	3.45	<LOD	<LOD	n.a.	n.a.
Clostridia, log spores/g FM	2.44	2.42	2.30	0.05	0.518
Lactic acid, g/kg DM	144.5	125.8	117.6	5.40	0.107
Acetic acid, g/kg DM	33.8^{a}	56.7^{b}	61.7^{b}	4.13	0.003
Ethanol, g/kg DM	7.4^{a}	14.5^{b}	16.0^{b}	1.12	0.001
NH_3-N, % total N	4.4	4.8	4.8	0.13	0.110
Aerobic stability, Ae St = 2°C, h	11^{a}	15^{ab}	29^{b}	3.6	0.041

SEM, standard error of the mean; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, ADF expressed exclusive of residual ash; LAB, lactic acid bacteria; LOD, limit of detection; n.a., not applicable

Discussion

Organic acids were significantly elevated after 35 days in T2 and T3 silage compared to T1. Ethanol was also higher in the inoculant treated silage but this was within the normal range for corn silage reported by Kung and Shaver (Kung et Shaver 2001). Yeast abundance was at the same time much lower in silage than in the fresh forage. Therefore the observed ethanol may originate from heterolactic hexose fermentation by *L. brevis* (McDonald *et al.* 1991) and not from yeast activity. The aerobic stability of the silage could only be significantly improved by the higher inoculant level applied in T3 silage. This

type of early harvested, highly instable maize silage nevertheless remains a feed resource at risk of aerobic deterioration.

Conclusion

The tested three-strain silage inoculant improved the fermentation profile (irrespective of dose) and the aerobic stability (dose-dependent) of low-DM whole plant maize silage. Nevertheless, the observed improvement of the aerobic stability of the silage could not fully counteract the consequences of harvesting of low-dry-matter immature plant material.

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