Intensity of feed processing: Influence on nutrient digestibility, performance and health of growing-finishing pigs

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I Abbreviations

(Used in Introduction, Background, General Discussion and Conclusion)

ADG	Average daily gain
BW	Body weight
CD	Cluster of differentiation
CP	Crude protein
DDGS	Distillers´ dried grain with soluble
DFI	Daily feed intake
DM	Dry matter
FGR	Feed to gain ratio
LSMeans	Least square means
MSC	Macroscopic stomach score
MFI	Mean fluorescence intensity
p	Significance level
PC	Principal component
PCA	Principal component analysis
PP	Peyer´s patches
r	Correlation coefficient
SE	Standard error
SEM	Scanning electron microscopy

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1 Introduction

The livestock sector has already undergone and will undergo even more radical changes in the future. The world population is projected to increase further up to 9.7 billion people in 2050 and 11.2 billion people in 2100 (UNDESA, 2015). Simultaneously, the global consumption of animal protein will increase by 60 % from 2003 - 2030 (PBL, 2009) which is related to urbanisation and increasing incomes beside the growth of human population (Steinfeld et al., 2006; Thornton, 2010). To maintain the nutritional standard of the present level and with regard to the current developments in global consumption an amount of food of animal origin has to be provided for ~ 13 billion people in 2050 (Steinfeld et al., 2006). However, the increasing demand for livestock products will intensify the rising competition for arable land between food and feed production. Furthermore, the request of bioenergy is projected to exacerbate this competition (Thornton, 2010). To meet the increased demand of livestock products in the future the livestock production and productivity has to be increased and a resource conserving production is necessary. This includes on the one hand the improvement of the exploitation of the animal performance potential and on the other hand the reduction of animal losses. These facts, in turn, strongly depend on the availability and quality of feed.

The feeding of pigs is mainly based on mixed feedstuffs and on complete feedstuffs, respectively. Therefore, the feed is the major item of cost in pig production and pig fattening besides the costs for the purchase of the animals (Ziron, 2008). Thus, it is also a key factor to save the existence especially of pig farmers with regard to the volatile price fluctuation.

Because of a better nutritional and physiological understanding and the knowledge about the environmental impact on animal performance a number of technical feed treatments were developed in the recent years to improve the product quality of feedstuffs and their efficiency. Furthermore, the increased demand for special feed in the sector of pets, aquaculture, ratites and weaning diets for nursey pigs have pushed the development of sophisticated feed processing methods. These processing methods also permit a higher flexibility in component selection. Especially hydro-thermal treatment of feedstuffs could be useful with regard to the new challenge for replacement of soybean meal because of its social unacceptability in central Europe (Lucht, 2003).

However, besides positive effects of technical feed treatment on feed quality and nutrient digestibility as well as on animal performance there has to be observed also a development of negative impacts especially on health aspects of the digestive tract of pigs and broilers (Betscher et al., 2010). Animal losses caused by health problems of the digestive tract are one of the main reasons of premature animal losses in the fattening period (Jurkschat et al., 2015) which in turn have a main impact on economic aspects. Moreover, the sensitisation of the consumer to the animal health and welfare has steadily increased in the last few years and grown to a global concern (Thornton, 2010).

The possibilities to treat feed by processing methods are versatile; however, the beneficial effects and the disadvantages have to balanced carefully.

2 Background

2.1 Types of technical feed treatments and their consequences for technological properties of feed

There are various technical feed treatments which can be divided into mechanical, thermal and hydro-thermal processing methods. Hydro-thermal processing methods can further be subdivided into hydro-thermal methods; with and without the application of pressure (Figure 1).

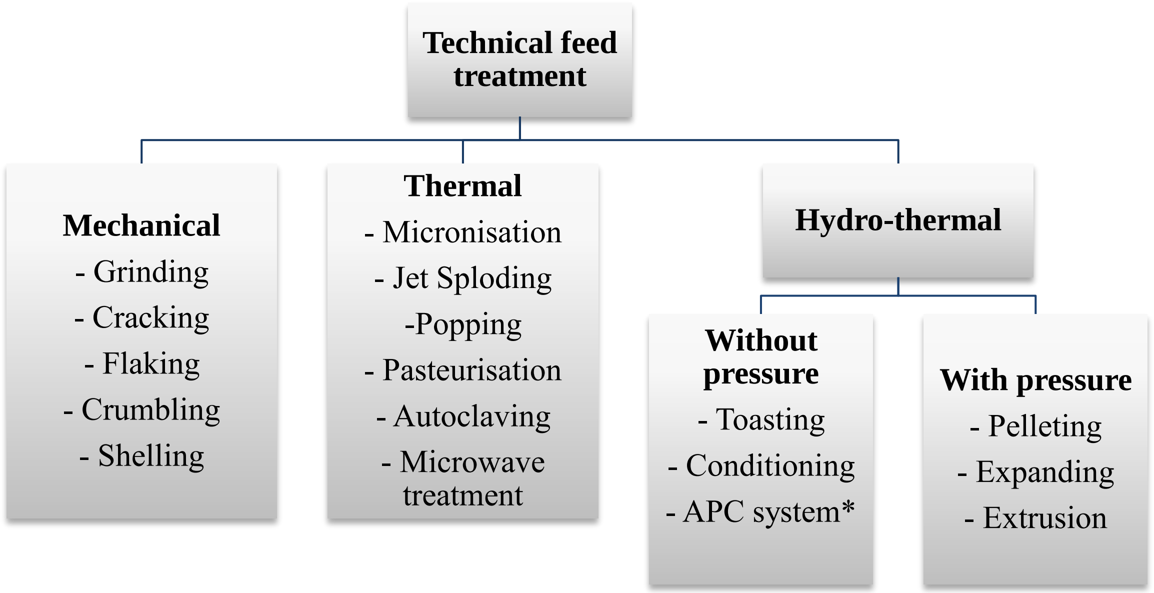

Figure 1 Types of technical feed treatment according to Kamphues et al. (2009)
Notes: * APC system, anaerobic pasteurising conditioning system

Besides technical feed processing methods, there are also chemical treatments, ensiling methods or combinations of different methods of feed processing. Because of the practical relevance of mechanical feed treatments and hydro-thermal processing methods with pressure application in processing of mixed fattening pig feed only those procedures will be considered hereinafter.

2.1.1 Cracking, dry rolling, grinding/milling

The ability of pigs to chew is limited, especially that to chew small grains. Therefore it is unavoidable to break the seed coat by mechanical treatments before feeding. The simplest form of technical feed treatment is the cracking, dry rolling or grinding of feedstuffs for example by hammer mill or roller mill. This kind of technical feed treatment aims to expose the endosperm and reduce the particle size besides breaking the seed coat. While rolling is mostly used to crack the seed coat grinding or milling can be used to produce considerably fine particles (Rowe et al., 1999).

The particle size distribution after grinding is strongly depended on the type of cereal (Wolf et al., 2010). Furthermore, the damage of the grain during grinding is related to the hardness of the processed grain. A higher hardness results in a higher degree of damage, shearing and shattering of the grain compared to softer grain (Rowe et al., 1999).

Concerning technological aspects the importance of grinding is based on the unification of particle sizes of the different components of a mixed feed. A uniform distribution of particles of feedstuffs improves the mixing of components, increases the bulk density and inhibits particle segregation (Behnke, 1996). The degree of fineness also influences the flow characteristics of feeds. Furthermore, the reduction of particle size facilitates further processes such as pelleting, expander treatment and extrusion (Behnke, 1996). However, it should be noted that a high degree of fineness also increases dust pollution caused by feed.

2.1.2 Hydro-thermal treatment

According to DVT (2016) 80 % of mixed feedstuffs are in demand and traded in a pelleted form. Only in case of laying hens pelleted feed is less frequently used. Expander treatment and extrusion are special forms of the feed technology and are referred to as typical High-Intensive-Short-Term processes. In general, all expanders can be defined as a simplified form of an extruder (Heidenreich, 1994; Nahm, 2002). Usually, the boundary between expander treatment and extrusion is blurred. These two hydro-thermal processing methods can be used in independent processes to treat feed and feedstuffs or in combination with pelleting as a pressure conditioner (Nahm, 2002). Expander treatment is often used for pre-conditioning of feed mixtures especially with high proportions of ingredients which are difficult to pelletise (Thomas et al., 1997). This aspect allows a high flexibility in component selection. In general expander treatment increases the pellet-stability (Thomas et al., 1997). Because of its high investment and operating costs extrusion is only profitable in processing of particular compound feedstuffs (Heidenreich, 1994). Therefore, extrusion is often used in human-food industry. Recently, the importance of this processing method has increased in pet-feed-production or in feed-production for piglets. Furthermore, extrusion plays a key role in feeding of aquaculture because of the resulting modification of the density of feeds which influences the sedimentation rate (Kersten et al., 2010).

During hydro-thermal processes such as pelleting, expanding or extrusion the three parameters moisture, temperature and pressure (mechanical and steam pressure) interact with the feed. Expanders as well as extruders operate with markedly higher temperatures and pressure compared to the pellet press (Table 1).

Table 1 Processing conditions of various hydro-thermal feed treatments

Parameter	Hydro-thermal feed treatment		
	Pelleting	Expanding	Extrusion
Temperature	60 - 80 °C [1]	100 - 130 °C [1]	125 – 170 °C[2] 110 - 160 °C [1];[3]
Time	~ 10 - 15 s [2]	~ 5 - 10 s [3]	~ 10 - 30 s [2]
Pressure supply	Environmental pressure [3]	20 - 40 bar [3]	~ 60 bar (40 – 100 bar) [3]

Notes: according to 1) Kamphues et al. (2009); 2) Rowe et al. (1999); 3) GV-SOLAS (1996)

The hydro-thermal processes result in a wide range of physical and chemical changes of the feed which can be more or less pronounced according to specific conditions of the processing method (Table 2).

Many of these changes have a positive effect on the feed quality. The supply of heat and steam results in the development of a thin water film around the feed particles which enable the binding between them. Therefore products of hydro-thermal treatment increase agglomeration which in turn decreases dust formation and avoids segregation of feed components. The capillary sorption of water promotes coalescing of feed particles, and their binding is also supported by a wide range of other physico-chemical changes during the hydro-thermal processes (Thomas et al., 1997). These modifications include especially the thermal softening of the feeds, the denaturation of proteins and the gelatinisation of starch which also can increase the feed viscosity (Gomez and Aguilera, 1983; Chae et al., 1997a; Thomas et al., 1997; Thomas et al., 1998; Rowe et al., 1999; Svihus et al., 2005; Döll et al., 2007). Furthermore, hydro-thermal processes also influence the flow characteristics, the bulk density, the storage volume and water binding properties of the feed. For instance, extrusion can increase the water binding properties by 200 to 300 % (Kersten et al., 2010). The durability and storage quality of hydro-thermal feed is also related to the germ-reduction for example of salmonella or fungi in feeds and their hygienisation (Lucht, 2003; Wecke et al., 2006). Furthermore, anti-nutritional factors and contaminants of feedstuffs such as tannins, glucosinolates, trypsin-inhibitors and mycotoxins can be decreased (Heidenreich, 1994; Lucht, 2003; Bullerman and Bianchini, 2007; Kersten et al., 2010).

A negative effect of the high processing intensity, especially of the high processing temperatures, can be the destruction of thermosensitive feed additives such as probiotics, enzymes or proteins (Grala et al., 1994; Heidenreich, 1994; Inborr and Bedford, 1994; Kersten et al., 2010).

Table 2 Changes of physico-chemical characteristics of feed due to various technical feed treatments

Variable	Technical feed treatment				References
	Grinding	Pelleting	Expanding	Extrusion	
Disruption of seed layer; exposing endosperm	+++	+++	n.st.	+++	Rowe et al. (1999)
Reduction of particle size	+++	++	+	n.st.	Dirkzwager et al. (1998); Goelema et al. (1999); Grosse-Liesner et al. (2009); Wolf et al., (2010)
Gelatinisation of starch	n.e.	+	++	+++	Goelema et al. (1999); Johnston et al. (1999); Rowe et al. (1999); Svihus et al. (2005); Lundblad et al. (2011)
Feed viscosity	n.st.	+	++	+++	Lundblad et al. (2011)
Germ reduction; hygienisation	n.e.	+	++	+++	GV-SOLAS (1996); Lucht (2003); Wecke et al. (2006)
Reduction of mycotoxins	n.e.	n.st.	n.st.	+++	Bullerman and Bianchini (2007)
Inhibition of anti-nutritional factors	n.e.	+	++	+++	GV-SOLAS (1996); Lucht, (2003)
Protein damage	n.e.	n.e.	n.st.	++	GV-SOLAS (1996)
Vitamin damage	n.st.	++	+++	+++	Coelho (1996); Riaz et al. (2009)

Notes: n.e., no effect; +, low; ++, moderate; +++, high; n.st., not stated

The development of the named positive or negative effects and the quality of the resulting products is strongly dependent on the intensity of treatment (specific energy input, moisture, pressure and temperature), as well as on feed components, ingredients (protein, crude fibre, crude fat, starch or minerals) and structure of the feed material (Wood, 1987; Thomas et al., 1998; Briggs et al., 1999). For instance, according to Briggs et al. (1999) higher protein content results in higher pellet durability. In contrast, higher oil content decreases the pellet durability. Moreover, lipids can inhibit starch gelatinisation or increase starch gelatinisation temperature (Thomas et al., 1998). Sugar cans also support the binding of feed particles if sufficient steam is added. However, the interaction between heat, moisture and sugar promotes the risk of nutritionally undesirable Maillard products (Thomas et al., 1998). Soluble fibre increases the viscosity which can also bind particles; however, large fibres increase the risk of weak spots in the pellet. The degree of fineness has a high influence on the hardness of pellets or on avoiding a high degree of abrasion which are important features of pellet quality (Wondra et al., 1995a; Dirkzwager et al., 1998). Furthermore, the interaction between the intensity of treatment and the duration of the treatment as well as the residence time plays a key role to the manifestation of either positive or negative effects (Parsons et al., 1992; Heidenreich, 1994; Thomas et al., 1998; Bullerman and Bianchini, 2007).

2.2 Nutritional and physiological consequences of technical feed treatments

2.2.1 Influences on nutrient digestibility and animal performance

In several studies, a reduction of particle size increased the digestibility of nutrients (Owsley et al., 1981; Wondra et al., 1995a; Wondra et al., 1995b; Wondra et al., 1995c; Oryschak and Zijlstra, 2002). This effect in turn resulted in a lower feed to gain ratio (FGR) (Wondra et al., 1995a; Wondra et al., 1995b). In these investigations, no influences of particle size on the daily feed intake (DFI) or the average daily gain (ADG) were found. In contrast, Pickett et al. (1969) reported a significant reduction of ADG with increasing particle size. Differences between DFI due to varying grinding degrees were not stated in these studies.

Also, hydro-thermal treatment is associated with an improved digestibility of nutrients of fattening pig feed. Thus, a higher digestibility was achieved in pigs fed pelleted feed compared to feed without hydro-thermal treatment in previous studies (O'Doherty et al., 2001; Ohh et al., 2002; Park et al., 2003). A higher digestibility could also be achieved by expanding and extrusion of pig feed in studies of Hancock et al. (1991) and O'Doherty et al. (2001) compared to feed without hydro-thermal treatment. However, in studies of Cho et al. (2001) extrusion reduced the digestibility of lysine. Sauer et al. (1990) reported higher digestibility of dry matter (DM), energy and crude protein (CP) of extruded feed and extruded and re-pelleted feed compared to pelleted feed. The digestibility of the mentioned nutrients of extruded feed and extruded and re-pelleted feed was similar. Park et al. (2003) also observed higher digestibility of DM of expanded and re-pelleted feed compared to solely pelleted feed.

In studies of Baird (1973) and O'Doherty et al. (2001) pelleting resulted in a lower DFI compared to meal feed. Furthermore, pigs fed meal feed had a significantly higher ADG compared to animals fed the pelleted feed in grower and finisher phase in studies of O'Doherty et al. (2001). In contrast, pelleting increased the ADG of fattening pigs compared to meal feed in studies of Chamberlain et al. (1967), Flatlandsmo and Slagsvold (1971) and Baird (1973). Pellets made from finely ground meal did not result in a further increase of ADG compared to pellets made from coarsely ground meal in studies of Flatlandsmo and Slagsvold (1971). Only in studies of Dirkzwager et al. (1998) higher ADG and DFI were recorded when feeding coarsely ground and pelleted feed in the fattening phase between 25 – 45 kg body weight (BW) compared to finely ground and pelleted feed. However, over the entire fattening period no influences of grinding were found in these studies.

In studies of Vande Ginste and De Schrijver (1998) expanding tended to increase the DFI and the ADG of fattening pigs compared to meal feed. However, this effect disappeared when the feed was expanded and additionally pelleted. In studies of Johnston et al. (1999) with weaning pigs, expanding of complete feed resulted in markedly lower ADG despite higher digestibility of crude nutrients compared to meal feed or pelleted feed, which was mainly caused by the considerable decreased DFI. Expanded feed also reduced the DFI of growing and finishing pigs in studies of O'Doherty et al. (2001) and Millet et al. (2012). While the ADG was not influenced by feed treatment in studies of Millet et al. (2012) it was reduced by feeding expanded feed in studies of O'Doherty et al. (2001), considering the entire fattening period. In both studies no influence on FGR was recorded.

Chae et al. (1997b) could reach higher digestibility of crude nutrients by extrusion and pelleting compared to solely pelleted feed or meal feed in studies with growing-finishing pigs. However, the ADG was markedly reduced by solely pelleted feed, which was also caused by a significantly lower DFI.

In studies of Sauer et al. (1990) ADG of animals fed extruded feed was higher compared to animals fed pelleted feed. However, ADG was not increased when the extruded feed was additionally re-pelleted.

In several studies with fattening pigs hydro-thermal treatment such as pelleting, expanding and extrusion also resulted in lower FGR compared to feed without hydro-thermal treatment (Chamberlain et al., 1967; Chae et al., 1997b; Park et al., 2003; Mikkelsen et al., 2004; Hedemann et al., 2005). Furthermore, in studies of Park et al. (2003) feeding expanded and re-pelleted feed decreased FGR compared to solely pelleted feed. While extrusion of pig feed resulted in significantly reduced FGR compared to pelleted feed, FGR of pigs fed extruded and re-pelleted feed was similar to those fed solely pelleted feed in studies of Sauer et al. (1990).

Many previous studies observed no effects of technical feed treatment on slaughter performance (Dirkzwager et al., 1998; Laurinen et al., 2000; Millet et al., 2012). In studies of Wondra et al. (1995a) no influence on slaughter performance was determined between animals fed pelleted feed and animals fed meal; however, a reduction of particle size resulted in an increased dressing percentage. In studies of Park et al. (2003) a higher back fat thickness and a lower fat-free lean index were determined when feeding pellets versus meal feed.

2.2.2 Influences on the gastrointestinal tract

Chyme

Feeds treated with different technical methods lead to changes in structure and composition of the gastrointestinal content corresponding to their physico-chemical modifications. Alterations range from differences in physico-chemical properties of the chyme to microbial colonisation or changes in the passage rate of the chyme. Table 3 summarises changes of selected parameters of the stomach content of fattening pigs dependent on feeding of various technically treated feeds examined by previous studies.

Table 3 Changes in parameters of stomach content caused by various technical feed treatments compared to coarsely ground feed (finely ground feed) or meal feed (pelleting; expanding)

Variable	Technical feed treatment		
	Fine grinding	Pelleting	Expanding
Fluidity (according to a macroscopic score)	↑ (Reimann et al., 1968; Maxwell et al., 1970; Healy et al., 1994)	↑ (Flatlandsmo and Slagsvold, 1971)	↑ (Nuwer et al., 1967)
DM content	↓ (Reimann et al., 1968; Maxwell et al., 1970; Mikkelsen et al., 2004)	↓ (Mikkelsen et al., 2004; Große Liesner, 2008) n.e. (Betscher, 2010)	n.st.
pH value	↑ (Maxwell et al., 1970) n.e. (Reimann et al., 1968; Mikkelsen et al., 2004) ↓ (Reimann et al., 1968)	n.e. (Mikkelsen et al., 2004) ↑ (Betscher, 2010)	n.e. (Mahan et al., 1966; Nuwer et al., 1967)

Notes: DM, dry matter; n.e., no effect; n.st., not stated; ↑, increased compared to coarsely ground feed (finely ground feed) or meal feed (pelleting; expanding); ↓, decreased compared to coarsely ground feed (finely ground feed) or meal feed (pelleting; expanding)

In several studies it was suggested that coarsely ground meal supports the development of a pH gradient between the *Pars nonglandularis* and the pyloric and fundic region (Maxwell et al., 1970; Mößeler et al., 2010). While higher pH value dominated in the esophagastric region, the pH value of the pyloric region was lower. In contrast, in stomachs of animals fed finely ground feed or pelleted feed this effect was inhibited (Maxwell et al., 1970).

Mößeler et al. (2010) also described the development of a chloride-gradient after feeding meal feed which was lacking in stomachs of animals fed pelleted feed, too. Moreover, a higher chloride-secretion rate was determined in pigs fed pelleted feed compared to animals fed meal. Furthermore, Maxwell et al. (1970) found a trend towards a higher pepsin activity in stomach content of pigs fed cracked maize compared to animals fed finely ground maize. In the same investigation the development of a gradient in pepsin activity between the esophagastric region and the pyloric and fundic region was observed when feeding cracked maize. In contrast Reimann et al. (1968) detected significant increases of pepsin activity in stomach content when reducing particle size of maize. Regina et al. (1999) also observed a formation of a pepsin gradient in stomachs of animals fed coarsely ground meal which in this case, too, was not found in animals fed finely ground and pelleted feed. Additionally, Mikkelsen et al. (2004) found significant influences of fineness of feed on acetic acid, propionic acid, butyric acid and lactic acid concentrations in the stomach content of pigs. Furthermore, significant differences were detected in these studies between acetic acid and propionic acid concentration comparing pelleted and non-pelleted feed.

Differences in the microbial community in different sections of the gastrointestinal tract were found in studies of Sander et al. (2012) with young pigs fed different technically treated feeds. For instance, feeding coarsely ground meal resulted in significantly higher counts of lactobacilli in stomach and caecum and significantly higher counts of coliform bacteria in stomach content compared to finely ground and pelleted feed. In contrast, the number of enterococci was significantly decreased in small intestine of pigs fed coarsely ground feed compared to animals fed pelleted or extruded feed. Kamphues et al. (2007) found higher lactobacilli counts and counts of gram positive cocci in colon content of piglets fed coarsely ground and pelleted feed compared to animals fed normally ground and pelleted feed. Influences of fineness of feed and pelleting on microbial counts in different parts of the gastrointestinal tract were also described in studies of Mikkelsen et al. (2004).

Besides these effects changes in viscosity of the intestinal chyme were observed when feeding different technically treated feeds in various studies with broilers (Engberg et al., 2002; Yasar, 2003). This is an important aspect, especially considering the feed digestibility or the excrement management in broiler fattening. However, in pig nutrition this aspect did not receive much attention in the past.

The influence on the passage rate of the chyme was only examined in a few studies. Maxwell et al. (1970) determined a higher stomach passage rate in gastric fistulised pigs fed finely ground feed compared to coarsely ground feed. In studies of Seerley et al. (1962) a faster passage rate of particles through the alimentary tract was determined in pigs fed pelleted feed compared to meal feed.

Anatomical and morphological parameters of the gastrointestinal tract

On the one hand technically treated feed can directly influence morphological parameters of the gastrointestinal tract via its structural characteristics. On the other hand an indirect impact can result by alterations of the microbial digestion of the feed and released metabolic substances as well as alterations in microclimatic conditions and a resulting substrate adapted microflora. Furthermore, technically treated feed influences physiological processes and secondary physiological processes such as the secretion of the pancreatic substrates or the motility of the small intestine (Betscher et al., 2010).

In several previous studies anatomical and morphological alterations of the stomach were described. Thus, Hedemann et al. (2005) determined higher stomach weights in animals fed coarsely ground feed compared to animals fed finely ground feed. Furthermore, Eisemann and Argenzio (1999) and Hedemann et al. (2005) reported lower stomach weights in fattening pigs fed pelleted feed compared to animals fed meal feed.

It is also known that a reduction of particle size promotes incidences of stomach lesions (Mahan et al., 1966; Reimann et al., 1968; Pickett et al., 1969; Maxwell et al., 1970; Flatlandsmo and Slagsvold, 1971; Wondra et al., 1995a; Mikkelsen et al., 2004). Likewise pelleting or expanding is often associated with a higher risk of stomach lesions (Mahan et al., 1966; Chamberlain et al., 1967; Nuwer et al., 1967; Riker et al., 1967; Pickett et al., 1969; Flatlandsmo and Slagsvold, 1971; Eisemann and Argenzio, 1999; Mikkelsen et al., 2004; Millet et al., 2012). Moreover, in studies of Dirkzwager et al. (1998) the development of stomach lesions increased when the particle size of feed was decreased before pelleting.

Some studies indicated that feeding different technically treated feeds can also influence the architecture of the gut wall. Hedemann et al. (2005) observed significantly higher villus length in fattening pigs fed coarsely ground meal compared to animals fed coarsely ground pelleted feed. Also, a trend to influence the crypt depth by feeds treated by different technical methods was detected in the proximal small intestine. Brunsgaard (1998) reported higher crypt volume by coarsely ground feed compared to finely ground feed. These studies also point out that feeding of different technically treated feeds affects the production and the composition of mucin in the small intestine of fattening pigs (Brunsgaard, 1998; Hedemann et al., 2005).

2.2.3 Influences on the immune system

The impact of technical feed treatments on the immune system of farm animals is still underresearched. However, studies of Liu et al. (2006) with broiler chickens indicated that particle size affect immune cells of the intestine. They demonstrated that coarsely ground feed significantly reduced the number of mast cells in the intestine of broilers compared to finely ground feed and contributed to develop mast-cell-cluster in the apical region of the villi. Döll et al. (2007) found a trend to increased IgA stimulation index of peripheral blood lymphocytes of pigs fed pelleted feed compared to pigs fed mash.

Furthermore, it is suggested that especially the allergenicity of feeds could be inhibited or increased by processing methods such as grinding or heating. It is likely that technical feed treatment induces a higher release rate or a development of new epitopes or allergens from the feed which can in turn modulate the immune system of animals (Betscher et al., 2010). Fiocchi et al. (2004) reported in a meta-analysis of human studies that processing of cereals increased their allergenicity.

3 Scope of the thesis

The requirement of further studies concerning the impact of technical feed treatment on digestibility of feed and performance of pigs was demonstrated by the mentioned heterogenic results of previous studies. Furthermore, in previous studies different experimental designs and feed components were used. Therefore, comparisons between the different common feed treatments are not appropriate to assess their benefits and disadvantages. Also health aspects have to be considered in further studies with regard to economic considerations as well as regarding the importance of animal welfare. Moreover, there are indications that feeding different technically treated feeds has an important influence on further segments of the gastrointestinal tract besides the stomach. Because of the key role of the intestine in nutrient-absorption and protection against potential pathogens a possible impact on this digestive organ has to be studied in more detail.

Therefore, two experiments with fattening pigs were conducted which aimed to balance the benefits and disadvantages of different processing degrees of feed on nutrient digestibility, performance of animals and health aspects. The first experiment compared the common technical treatments of pig feed and combinations between them in one common basal feed (**Paper I**). The two technical feed treatments used in the following experiment were chosen after considering their beneficial effects on digestibility, performance and/ or stomach lesions based on the first experiment´s results. This experiment focused on the influence of different technical feed treatments on two different diets (Diet 1, with soybean meal vs. Diet 2, rapeseed meal/ distillers´ dried grain with soluble (DDGS)/ soybeans) and their impact on digestibility and performance of fattening pigs (**Paper II**). Furthermore, this study also concentrated on the effects of different technically treated diets on animal health and immunological aspects of the porcine gastrointestinal tract (**Paper II; Paper III**).

The following hypotheses were verified during the described experiments:

- The higher extent of feed processing, the higher nutrient digestibility and the higher performance of fattening pigs
- The higher extent of feed processing, the higher risk of stomach lesions
- Feeding different technically treated feeds influence the intestinal morphology and the intestinal immune system of fattening pigs which in turn affect the peripheral immune system
- Different feed composition alter the local and peripheral immune system in a technical feed treatment related manner

4 Paper I

Effects of particle size and hydro-thermal treatment of feed on performance and stomach health in fattening pigs

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Abstract

Effects of grinding and hydro-thermal treatment of feed on growth performance, slaughter traits, nutrient digestibility, stomach content and stomach health were examined by using 96 crossbred fattening pigs. Pigs were fed a grain-soybean meal-based diet processed by various technical treatments. Feeding groups differed in particle size after grinding (finely vs. coarsely ground feed) and hydro-thermal treatment (without hydro-thermal treatment, pelleting, expanding, expanding and pelleting). Fine grinding and hydro-thermal treatment showed significant improvements on the digestibility of crude nutrients and content of metabolisable energy. Hydro-thermal treatment influenced average daily gain (ADG) and average daily feed intake (DFI) significantly. Finely ground pelleted feed without expanding enhanced performances by increasing ADG and decreasing feed-to-gain ratio (FGR) of fattening pigs. Coarsely ground feed without hydro-thermal treatment resulted in the highest average daily gain and feed intake, however also in the highest FGR. Expanded feed decreased feed consumption and ADG. Slaughter traits was unaffected by treatment. Coarsely ground feed without hydro-thermal treatment had protective effects on health of gastric *pars nonglandularis*. However, pelleting increased gastric lesions. Hydro-thermal treatment, especially expanding, resulted in clumping of stomach content which possibly induced satiety by slower ingesta passage rate and thus decreased feed intake. Pigs fed pelleted feed showed less pronounced development of clumps in stomach content compared to expanded feed.

Keywords: pigs; particle size; expansion; pelleting; stomach

1. Introduction

Feed processing aims at an improvement of nutrient and feed efficiency. Therefore many processing methods were developed. The grinding of feed is the simplest method to enhance digestibility. It is well known that fine grinding improves nutrient digestibility by enhancing the accessibility of digestive enzymes (Healy et al. 1994; Wondra et al. 1995; Oryschak and Zijlstra 2002). Thus, many studies revealed increased average daily weight gain (ADG) and/or feed efficiency as a result of enhanced nutrient digestibility (Kirchgessner et al. 1985; Healy et al. 1994; Döll et al. 2007). Thermal treatments, such as pelleting and expanding or extrusion, were also developed to improve storability, prevent demixing, increase the digestibility of crude nutrients and inactivate anti-nutritive constituents of animal feed, especially for pigs and poultry (Traylor et al. 1999). It has to be emphasised that all extruders might be defined as expanders. Operating differences between expanders and extruders are mainly based on pressure, temperature and moisture levels during the production process. It is often difficult to make clear distinctions in literature. Previous studies could confirm higher animal weight gain or decreased feed-to-gain ratio (FGR) by using pelleting or expanding methods (Lawrence 1983; Eisemann and Argenzio 1999a; Hedemann 2005). However, there are also studies in which extruded or expanded diets showed reduced ADG and daily feed intake (DFI) despite higher digestibility (Chae et al. 1997b; Johnston et al. 1999).

Moreover, there is evidence that increased extent of technical feed treatment might also adversely affect animal health. Earlier studies showed that fine grinding induces stomach lesions (Maxwell et al. 1967; Healy et al. 1994; Dirkzwager et al. 1998). Further, treatments such as pelleting or expanding of grain resulted in a secondary decrease of particle size, which appeared to be strongly associated to keratinisation and ulceration in the stomach (Grosse Liesner et al. 2009; Millet et al. 2012). Likewise pelleting and expanding per se could be identified as a precursor of ulceration (Chamberlain et al. 1967; Nuwer et al. 1967; Grosse Liesner et al. 2009). Furthermore, extensive treatments enhance energy input and processing costs (Traylor et al. 1999).

Comparisons of processing methods based on the results of previous studies are difficult because ingredients or processing conditions differ. Thus, the aim of the present study was to balance the benefits and disadvantages of selected conventional processing methods by using one common basal diet divided in three feeding phases for fattening pigs with regard to economic and health aspects. Processing methods were to a great extent standardised and well documented.

2. Material and methods

2.1. Experimental diets and design

Feeding groups differed only in the extent of grinding (finely ground vs. coarsely ground) and in hydro-thermal treatment (meal without hydro-thermal treatment, pelleting, expanding, expanding and pelleting), resulting in eight feeding groups (Table 1). Each feed was ground in a hammer mill through a 6.0 mm (coarsely ground feed) or a 3.0 mm (finely ground feed) screen. Pellets were pressed at 74 °C and 3% steam. Expandats were produced at a processing temperature of 113 °C and 3% steam. Re-pelleting of feed for Groups CgExP and FgExP occurred under processing temperatures of 87 °C and steam conditions of 3%. Pellets had a diameter of 3 mm.

Table 1. Combination of technical feed treatments.

	Grinding		
Feeding group	Intensity	Screen size of sieve	Hydro-thermal treatment
CgM	Coarsely ground (Cg)	6 mm	Without (meal) (M)
CgP	Coarsely ground (Cg)	6 mm	Pelleted (P)
CgEx	Coarsely ground (Cg)	6 mm	Expanded (Ex)
CgExP	Coarsely ground (Cg)	6 mm	Expanded + pelleted (ExP)
FgM	Finely ground (Fg)	3 mm	Without (meal) (M)
FgP	Finely ground (Fg)	3 mm	Pelleted (P)
FgEx	Finely ground (Fg)	3 mm	Expanded (Ex)
FgExP	Finely ground (Fg)	3 mm	Expanded + pelleted (ExP)

Table 2. Ingredients [g/kg diet] of basal diets in the starter, grower and finisher phase.

	Starter (23 – 45 kg BW)	Grower (45 – 72 kg BW)	Finisher (72 – 120 kg BW)
Wheat	250.0	-	-
Barley	324.1	200.0	250.0
Rye	125.0	250.0	250.0
Triticale	-	261.0	246.0
Soybean meal	111.1	97.7	30.0
Wheat bran	72.0	80.0	90.0
Soy beans full fat, toasted	47.5	-	-
Rapeseed meal	-	50.0	70.0
Bread flour, toasted	-	15.0	-
Oats	-	10.0	20.0
Malt culms	15.0	-	-
Linseed	5.0	-	-
Mid-chain fatty acids	-	10.0	5.0
Calcium carbonate	12.0	10.0	13.0
Sodium chloride	5.0	4.3	4.7
Soybean oil	11.0	-	-
Molasses	-	-	10.0
Carbon sodium phosphate	5.9	-	-
Acid mixture[◊]	5.0	-	-
Lysine hydrochloric acid	5.4	3.0	3.5
DL-Methionine	1.4	0.5	0.5
L-Threonin	1.9	-	-
Premix[*]	2.7	8.5	-
Premix[†]	-	-	7.3

Notes: [◊]Contained: sodium formate, 18%; formic acid, 38%; propionic acid, 23%; *Delivers per kg feed: 8000 IU vitamin A; 1000 IU vitamin D3; 70 mg vitamin E; 8 mg copper; 80 mg iron; 16 mg manganese; 54 mg zinc; 0.24 mg selenium; 2 mg iodine; 1500 FYT 6-phytase; [†]Delivers per kg feed: 6000 IU vitamin A; 750 IU vitamin D3; 50 mg vitamin E; 6 mg copper; 60 mg iron; 12 mg manganese; 48 mg zinc; 0.18 mg selenium; 2 mg iodine; 1500 FYT 6-phytase.

The basal diet was created according to commercial rations and the requirements of growing and finishing pigs (GfE 2008), respectively (Tables 2 and 3). Fattening was subdivided into three phases for energy and nutrient requirement adjustment.

2.2. Balance study

Balance studies underlay a granted animal care statement of Lower Saxony State Office for Consumer Protection and Food Safety which was in accordance to the Directive 2010/63/EU (2010).

Eight crossbred barrows ((German Landrace × German Large White) × Piétrain) were used per fattening phase for four balance studies according to the total collection method described by Schiemann (1981) covering the starter and the grower phase. The average body weight (BW) of the animals was 45 kg in the first balance study and 83 kg in the last one. Pigs were randomised by a Latin square design to the eight feeding groups. Animals were housed individually in balance cages (Farries and Oslage 1961) during the 5-days- sampling period to allow quantitative collection of faeces and urine. Between sampling periods pigs were individually penned in concrete-floored boxes without bedding. They were restrictedly fed twice a day. In the first trial, pigs received 1600 g feed per day, which was mixed with 1 l tap water. The daily rations were increased by 200 g feed per day per period and the volume of added water was also enhanced until a total amount of 1.5 l. After feeding, animals received additionally 1 l water. Animals were weighed at the beginning and ending of sampling periods. Faeces were collected twice daily during feeding at 6:30 h and 14:30 h and urine once daily. The aliquot samples of acidified urine were pooled for each individual pig and kept frozen. Total amount of faeces were also pooled and kept frozen.

Table 3. Calculated metabolisable energy (ME) and analysed dietary nutrients (on dry matter basis).

Phases	Grinding	Hydro-thermal treatment	ME[◊] [MJ/kg]	Dry matter [%]	Crude protein [g/kg]	Ether extract [g/kg]	Crude fibre [g/kg]	Crude ash [g/kg]	Starch [g/kg]	Starch disintegration [%]
Starter (23-45 kg BW)	Cg[*]	M[∇]	14.8	89.1	182	43.8	47.0	52.5	479	-
		P[‡]	14.7	87.8	188	44.8	50.5	58.8	446	-
		Ex[□]	14.8	89.2	187	48.7	47.9	58.3	456	-
		ExP[•]	15.2	89.0	185	46.8	46.0	51.3	474	-
	Fg[Δ]	M	15.0	88.2	185	51.2	50.0	61.3	435	-
		P	15.1	88.4	186	49.1	47.1	59.8	453	-
		Ex	15.3	89.3	187	48.6	51.9	60.0	449	-
		ExP	15.4	89.3	188	51.7	53.0	61.3	444	-
Grower (45-72 kg BW)	Cg	M	14.7	88.4	173	39.0	43.7	49.2	494	-
		P	14.7	87.6	175	36.6	47.2	46.7	494	-
		Ex	14.4	89.1	171	35.5	47.8	47.9	490	-
		ExP	14.7	89.5	173	33.2	46.0	48.3	487	-
	Fg	M	14.4	87.8	175	34.5	43.6	47.9	372	-
		P	14.7	87.2	173	35.7	45.7	49.6	484	-
		Ex	14.8	88.4	165	33.6	47.4	48.1	497	-
		ExP	14.9	89.4	167	35.0	44.9	46.3	479	-
Finisher (72-120 kg BW)	Cg	M	14.5	88.1	152	33.8	50.3	53.9	500	10.8
		P	14.7	88.1	152	34.0	46.9	49.3	497	21.6
		Ex	14.5	89.5	145	30.6	50.3	51.5	493	32.9
		ExP	14.7	89.7	148	31.1	47.5	48.6	504	47.7
	Fg	M	14.5	88.0	148	31.0	50.2	48.8	494	7.80
		P	14.7	88.0	150	36.1	49.2	48.5	499	21.6
		Ex	14.7	89.4	149	35.5	47.8	49.1	500	40.7
		ExP	14.9	89.6	150	41.9	46.6	50.0	502	50.2

Notes: [◊]Calculated on base of digestible crude nutrients (starter and grower phase) and on basis of crude nutrients (finisher phase) according to GfE (2008); [*]Cg, Coarsely ground; [Δ]Fg, Finely ground; [∇]M, Meal; [‡]P, Pelleted; [□]Ex, Expanded; [•]ExP, Expanded and re-pelleted.

2.3. Growth experiment

A total of 96 crossbred barrows ((German Landrace × German Large White) × Piétrain) were fattened for 10 weeks between November and February. During the experimental period the animals were housed individually in concrete-floored pens without bedding, which was equipped with an individual feeding trough. Pens were integrated in a heat controlled building. Environmental temperatures were maintained at 20–18 °C. The initial average BW of the animals was 23.8 ± 2.7 kg. Animals were randomly assigned on the basis of BW to 12 pens per feeding group. Pigs were fed ad libitum with dry feed until immediately before slaughtering. Feeding occurred according to trough fill-level. Water was also available ad libitum by nipple drinkers. BW and feed intake were recorded weekly.

Animals were slaughtered under conventional conditions after reaching 120 kg BW (121.6 kg ± 5.9 kg). After bleeding and scalding, dissection of stomach followed. This organ was cut longitudinally along the *curvatura major*. Mixed stomach content was collected for determination of dry matter and pH-value. Subsequently the stomach was rinsed with water. The mucosa of pars nonglandularis was assessed according to a macroscopic scoring system (Score 0 – intact epithelium, smooth and glistening white surface; Score 1 – mild changes, partially bile staining and hyperkeratosis; Score 2 – moderate degree of hyperkeratosis and bile staining over entire surface; Score 3 – high-degree hyperkeratosis; Score 4 – severe lesions and scarring). Each stomach was scored blind to the treatment by the same person. The organs liver without bile, bladder, kidneys and spleen were also dissected and weighed.

To estimate the carcass lean meat percentage, incisions of the cutlet were made from dorsal to ventral, between the 13 and 14 thoracic vertebras, in a chilled, left half of a carcass. Conditions of cutlet were captured photographically according to standards for calculations of lean meat percentage according to Bonner Equation (ZDS 2007).

2.4. Analytical methods

Particle size distribution of coarsely and finely ground feed before hydro-thermal treat- ment was calculated by dry sieve analysis according to DIN 66165-1:1987-04 (DIN 1987a) and DIN 66165-2:1987-04 (DIN 1987b). Briefly, 72.2 g of feed were riddled trough sieves corresponding to DIN ISO 3310/1 (DIN 2001). Thereafter, remaining fractions of each sieve were weighed and expressed relative to the weight of the inserted sample of feed. Sieving related losses amounted to 0.18% on average.

Samples of feeds and faeces were prepared and analysed in accordance with the methods of the Association of German Agricultural Analysis and Research Centres (VDLUFA). Thus, the methods by Naumann and Bassler (1976) were used to determine dry matter and additional crude nutrients, while crude protein (CP) was analysed with a combustion-method according to Dumas. Ether extract (EE), crude fibre (CF) and crude ash were analysed using methods of VDLUFA 5.1.1, 6.1.1 and 8.1. Furthermore, the proportion of sugar was determined according to Luff-Schoorl and the proportion of starch polarimetrically. Additionally, to determine the degree of starch disintegration in feeds of finisher phases, starch was analysed enzymatically by using amyloglucosidase-method of VDLUFA 7.2.6.

Stomach content was also analysed for dry matter content according to Naumann and Bassler (1976). The pH-values of stomach content were measured by using pH-meter (WTW pH 530, BLB, Brunswick, Germany).

2.5. Calculation and statistics

Metabolisable energy (ME) of feed of starter and grower phase was calculated on base of digestible (d) crude nutrients (as analysed) according to the equation of the GfE (2008) as follows:

$$\text{ME [MJ/kg]} = 0.0205 \cdot \text{dCP} + 0.0398 \cdot \text{dEE} + 0.0173 \cdot \text{Starch} + 0.0160 \cdot \text{Sugar} + 0.0147 \cdot (\text{dOM} - \text{dCP} - \text{dEE} - \text{Starch} - \text{Sugar}),$$

where dOM represents digestible organic matter and all nutrients are given in g.ME of feed of finisher phase was calculated on base of crude nutrients (as analysed) as follows (GfE 2008):

$$\text{ME [MJ/kg]} = 0.021503 \cdot \text{CP} + 0.032497 \cdot \text{EE} - 0.021071 \cdot \text{CF} + 0.016309 \cdot \text{Starch} + 0.014701 \cdot (\text{OM} - \text{CP} - \text{EE} - \text{CF} - \text{Starch}),$$

where all nutrients are given in g.

The degree of starch disintegration [%] is based on the ratio of hydrolysed starch and crude starch multiplied by 100.

ADG was calculated as the quotient of difference between final BW and initial BW and the length [d] of the feeding period. DFI was defined as the quotient of feed consumption and length [d] of the feeding period. FGR was calculated by dividing the feed intake by BW gain.

Carcass lean meat percentage was calculated according to Bonner Equation (ZDS 2007) as follows:

Lean meat percentage [%] = 59.704 – 0.147 · Fat surface [cm2] + 0.222 · Meat surface [cm2] – 1.744 · Back fat, loin [cm] – 1.175 · Back fat, middle – 0.809 · Back fat, withers [cm] – 0.378 · Side fat thickness [cm] – Fat thickness over surface of back muscle [cm].

Procedure MIXED was used for statistical analyses (SAS Enterprise Guide 4.3.). The model included the fixed effects of grinding and thermal treatment as well as their interactions. In the case of ADG, DFI, FGR and digestibility of crude nutrients, animals were considered as a repeated factor. Differences were classified as significant when $p \leq 0.05$.

3. Results

3.1. Feed analysis

Results of feed analyses of crude nutrients are summarised in Table 3. Results of particle size distribution are presented in Table 4. Coarse meal showed clearly more particles greater than 1005 µm. In contrast, more particles smaller than 125 µm and more particles between 125 and 355 µm were detected in finely ground meal.

Table 4. Particle size distribution of coarsely and finely ground meal before compaction.

	Particle size distribution [%]				
	< 125 µm	125 – 355 µm	360 – 1000 µm	1005 – 2000 µm	> 2000 µm
Starter phase (23 – 45 kg BW)					
Coarsely ground meal	3.7	11.1	21.4	40.1	23.7
Finely ground meal	15.0	25.6	44.6	14.8	0.0
Grower phase (45-72 kg BW)					
Coarsely ground meal	11.0	14.5	31.4	36.0	7.1
Finely ground meal	21.6	22.6	39.5	16.2	0.1
Feed finisher phase (72-120 kg BW)					
Coarsely ground meal	15.2	15.9	34.2	30.5	4.2
Finely ground meal	28.5	22.1	37.2	12.3	0.0

The disintegration of starch was markedly influenced by the hydro-thermal treatment of feed (Table 3). When analysing coarsely ground feed without hydro-thermal treatment a degree of starch disintegration of only 10.8% was detected. Finely ground feed without hydro-thermal treatment also showed a low starch disintegration of 7.8%. An analysis of pelleted feed by testing both grinding intensities revealed values of 21.6%. However, expanded feed without pelleting had a degree of starch disintegration of 32.9% in coarsely ground feed and 40.7% in finely ground feed. Highest values were detected in expanded and re-pelleted feeds (coarsely and finely ground feed, 47.7% and 50.2%, respectively). Finely ground expanded feeds, with and without pelleting, showed higher levels of starch disintegration compared to coarsely ground expanded feeds.

3.2. Balance trials

Analyses of variance showed a significant effect forward to increase digestibility of all crude nutrients by fine grinding. The same occurred with regard to the grower phase with exception of CF. Furthermore, hydro-thermal treatment significantly affected ME and the digestibility of EE and organic matter ($p < 0.05$) in case of the starter phase and ME, CP and EE in the grower phase, respectively. There was a significant interaction between grinding and hydro-thermal treatment in the case of CF ($p = 0.046$). Significant differences between the mean values of the eight feeding groups were observed between FgExP and CgEx in most cases (Table 5).

3.3. Growth experiment

One animal from Group CgExP had to be excluded from the growth experiment due to rectal prolapse.

ADG and DFI were significantly influenced by hydro-thermal treatment, but not by grinding (Table 6). During the fattening period the highest ADG was observed when coarsely ground feed without hydro-thermal treatment (Group CgM) and finely ground, pelleted feed (Group FgP) were fed. The lowest ADG was recorded in animals which received expanded diets. Group CgM also showed enhanced DFI compared to the other groups, while the difference to Group FgEx was significant ($p = 0.016$). Group FgEx showed the lowest DFI.

Both grinding ($p = 0.009$) and hydro-thermal treatment ($p = 0.011$) affected FGR significantly during fattening phases. The FGR was lowest in feeding groups receiving pelleted feed without expanding. Significantly higher FGR was determined when feeding coarsely ground feed without thermal treatment in comparison to pelleted feed without expanding (Group CgM vs. CgP: $p = 0.022$; Group CgM vs. Group FgP: $p = 0.005$). Carcass yield and carcass lean meat percentage remained unaffected by the treatment of feed.

Table 5. Digestibility of selected crude nutrients and metabolisable energy (ME) content of diets of starter and grower period examined in balance trials (LSMeans, n = 4).

Ingredients	Coarsely ground feed				Finely ground feed				PSE[*]	*p*-Value		
	M[∇]	P[‡]	Ex[□]	ExP[•]	M	P	Ex	ExP		Grin-ding	Hydro-thermal treat-ment	Grinding x Hydro-thermal treatment
Starter Phase (23 - 45 kg)												
ME[◊] [MJ/kg]	14.8^{cd}	14.7^{d}	14.8^{bcd}	15.2^{abc}	15.0^{abcd}	15.0^{abcd}	15.3^{ab}	15.4^{a}	0.11	< 0.001	0.001	0.459
Digestibility [%]												
Crude protein	84.5^{b}	85.0^{b}	84.1^{b}	86.1^{ab}	87.4^{ab}	86.6^{ab}	88.5^{a}	89.2^{a}	0.69	< 0.001	0.081	0.254
Ether extract	62.6^{d}	69.1^{c}	72.9^{bc}	75.4^{bc}	71.8^{bc}	73.4^{bc}	77.3^{ab}	82.3^{a}	1.29	< 0.001	< 0.001	0.206
Crude fibre	39.9^{ab}	39.7^{ab}	34.6^{b}	41.4^{ab}	40.8^{ab}	40.3^{ab}	50.1^{ab}	52.6^{a}	2.95	0.003	0.125	0.046
NFE	90.1	89.3	89.4	91.2	90.7	90.6	91.5	91.4	0.49	0.007	0.054	0.287
Organic matter	85.3^{abc}	84.9^{bc}	84.7^{c}	87.0^{abc}	86.4^{abc}	86.5^{abc}	88.0^{ab}	88.3^{a}	0.65	< 0.001	0.030	0.373
Grower Phase (45 - 72 kg)												
ME[◊] [MJ/kg]	14.7^{ab}	14.8^{ab}	14.4^{c}	14.7^{abc}	14.4^{bc}	14.7^{ab}	14.8^{a}	14.9^{a}	0.07	0.027	0.006	< 0.001
Digestibility [%]												
Crude protein	81.9^{ab}	84.0^{a}	79.2^{b}	82.4^{a}	83.6^{a}	83.5^{a}	83.8^{a}	83.7^{a}	0.78	< 0.001	0.006	0.001
Ether extract	59.6^{bc}	61.1^{b}	55.4^{c}	60.7^{b}	58.3^{bc}	61.7^{b}	62.1^{b}	67.6^{a}	1.16	< 0.001	< 0.001	< 0.001
Crude fibre	38.9	37.4	37.2	40.4	34.8	37.0	44.2	43.1	2.23	0.391	0.155	0.105
NFE	90.8^{ab}	90.3^{ab}	89.9^{b}	90.6^{ab}	91.2^{ab}	90.8^{ab}	91.5^{ab}	91.5^{a}	0.36	0.001	0.493	0.274
Organic matter	85.5^{ab}	85.4^{ab}	84.0^{b}	85.7^{ab}	86.0^{ab}	85.8^{ab}	86.7^{a}	8.0^{a}	0.45	< 0.001	0.168	0.017

Notes: [◊]Calculated on base of digestible crude nutrients (as analysed) according to the GfE (2008); [∇]M, Meal; [‡]P, Pelleted; [□]Ex, Expanded; [•]ExP, Expanded and re-pelleted; *PSE, Pooled standard error; ^{abcd}LSMeans of the different feeding groups not sharing the same superscripts are significantly different ($p < 0.05$).

Table 6. Fattening performance and slaughter traits (LSMeans).

Variable	Coarsely ground feed				Finely ground feed				PSE*	*p*-Value		
	M▿ (n = 12)	P‡ (n = 12)	Ex□ (n = 12)	ExP• (n = 11)	M (n = 12)	P (n = 12)	Ex (n = 12)	ExP (n = 12)		Grinding	Hydro-thermal treatment	Grinding x Hydro-thermal treatment
Daily feed intake [g]	3135^{a}	2877^{ab}	2835^{ab}	2894^{ab}	2876^{ab}	2935^{ab}	2728^{b}	3016^{ab}	82.60	0.432	0.050	0.101
Average daily gain [g]	1217^{ab}	1182^{abc}	1104^{c}	1170^{abc}	1172^{abc}	1238^{a}	1131^{bc}	1231^{ab}	24.36	0.148	< 0.001	0.123
Feed to gain ratio [kg/kg]	2.68^{a}	2.45^{b}	2.61^{ab}	2.50^{ab}	2.49^{ab}	2.43^{b}	2.50^{ab}	2.48^{ab}	0.06	0.009	0.011	0.263
Carcass yield [%]	80	80	79	79	79	80	80	80	0.38	0.407	0.469	0.156
Carcass lean meat [%]	49	51	50	49	51	49	51	50	0.92	0.332	0.528	0.111

Notes: ▿M, Meal; ‡P, Pelleted; □Ex, Expanded; •ExP, Expanded and re-pelleted; *PSE, Pooled standard error; ^{abc}LSMeans of different feeding groups not sharing the same superscripts are significantly different ($p < 0.05$).

Hydro-thermal treatment significantly influenced the weight of liver and kidneys in relation to BW ($p < 0.01$) (Table 7). The weight of livers from animals receiving expanded feed was significantly increased compared with those of animals from Group CgM, which showed the lowest liver weights. Kidney weights revealed similar results. There were no effects of treatment on weight of spleen.

3.4. Stomach health

Animals fed the coarsely ground feed tended to have heavier stomachs than animals fed finely ground feed (Table 7). Hydro-thermal treatment showed no obvious effects on stomach weight.

A significant influence of hydro-thermal treatment was observed on stomach scores. The lowest average stomach score was determined in Group CgM. Feeding group CgM revealed an average stomach score of 0.42 ± 0.24, which was significantly lower compared with all other groups, excluding Group FgEx ($p < 0.05$). The highest average score was assigned to feeding groups with pelleted feed and in Group FgM. Feeding of expanded feeds with and without pelleting resulted in moderate average stomach score between 1.25 ± 0.24 (Group FgEx) and 1.91 ± 0.25 (Group CgExP).

Stomach content showed alterations particularly when feeding expanded feed, as indicated by clumping. In this case clearly demarcated clumps of undigested feed could be found, reaching weights up to 1568 g (original substance). The rest of stomach content showed a normal fluidity or mushy consistency (Figure 1A and B). Clumping of stomach content could be detected in nine animals of Group FgEx corresponding to total of 75%. In Groups CgEx, CgExP, FgP and FgExP also 6 out of 12 pigs (in Group CgExP out of 11 pigs) contained clumps in stomach. Only three pigs of Group CgP showed clumping of stomach content. Whereas all groups fed hydro-thermally treated feed demonstrated clumping of stomach content, neither animals of Group CgM nor animals of Group FgM had clumps in stomach content.

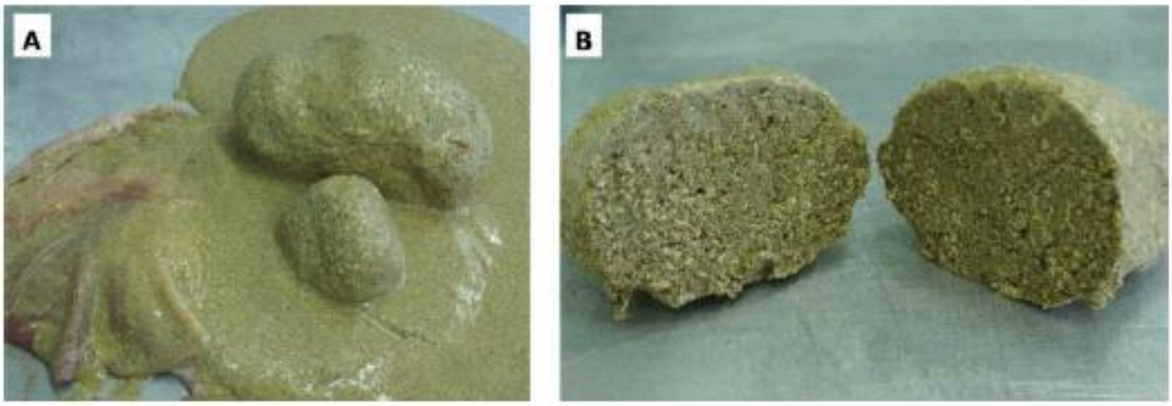

Figure 1. Alterations of consistency of stomach contents. Panel A: Example of stomach contents of Group FgEx (finely ground feed, expanded); Panel B: Consistence of clumps in stomach (here: largest diameter about 17 cm).

Hydro-thermal treatment tended to affect the pH of stomach content (Table 7). Highest pH values of stomach content were detected in the stomachs of pigs fed expanded feed with and without pelleting, however, the differences were not significant. Dry matter content of the stomach was not significantly influenced by treatment. The numerically highest proportions of dry matter content was found in stomachs of pigs fed coarsely ground feed without hydro-thermal treatment and expanded feeds without pelleting. Stomach content of animals of Groups CgP, FgM and FgP showed the lowest proportions of dry matter.

4. Discussion

The focus of the present study was to get a more detailed picture of the benefits and disadvantages of common processing methods of pig feed. Therefore, two grinding particle sizes were compared. Likewise, meal feed without hydro-thermal treatment, pellets, expandates and expanded and repelleted feed were tested.

4.1. Particle size distribution

Particle size distribution of coarsely and finely ground meal which was used in the present study corresponded to German commercial pig feeds, which are used in practice as meal feed or basic material before compaction (Neumann and Feil 2011). Only particle size distribution of coarsely ground starter feed and finely ground finisher feed had higher deviations compared to commercial pig feeds (Neumann and Feil 2011), which is made obviously in Figure 2 especially in the range of the cumulative distribution of 0.5. Compared with commercial feed for fattening pigs analysed by Wolf et al. (2010), the finely ground feed used in the present study was finer and coarsely ground feed of the starter phase was coarser.

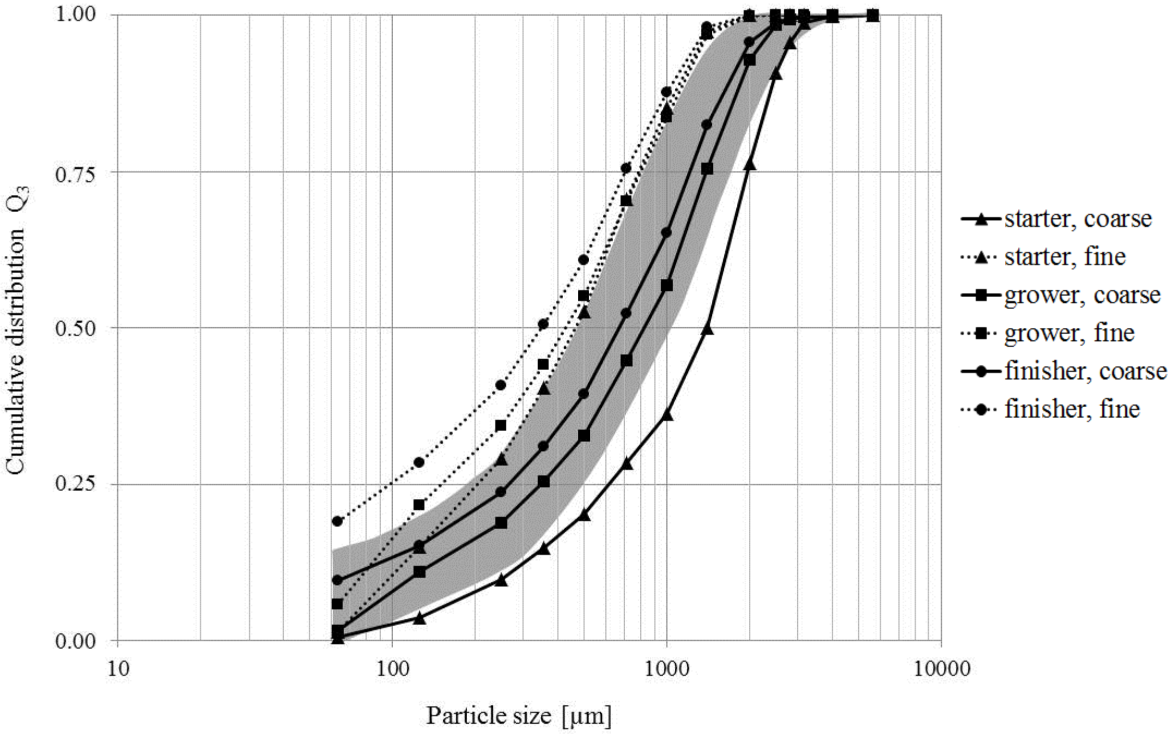

Figure 2. Cumulative distribution Q3 (partial mass, refers to whole mass = 1) of particle sizes of coarsely and finely ground meal before compaction. The grey area characterises the cumulative distribution of German commercial pig feeds according to Neumann and Feil (2011).

4.2. Digestibility and fattening performance

Results of variance analysis revealed for both treatments (finer grinding and a more extensive hydro-thermal treatment by pelleting or expanding) an improved digestibility of crude nutrients. Improvements of digestibility of feed by finer grinding were also reported in earlier studies (Healy et al. 1994; Wondra et al. 1995; Oryschak and Zijlstra 2002). Healy et al. (1994) and Oryschak and Zijlstra (2002) suggested that fine grinding increases particle surface. Therefore, crude nutrients are more accessible for digestive enzymes (Choct et al. 2004). Furthermore, studies of various authors confirm the higher digestibility after pelleting or expanding as found in the present study (Lawrence 1983; Hancock et al. 1991; Chae et al. 1997a, 1997b; O'Doherty et al. 2001). Pigs receiving finely ground and pelleted feed gained faster than animals with expanded feed and needed less feed to gain than animals of Group CgM. Beneficial effects of hydro-thermal treatment on digestibility are caused on the one hand by secondary grinding and on the other hand by gelatinisation of starch (Gomez and Aguilera 1983; Chae et al. 1997a; Traylor et al. 1999; Medel et al. 2004; Döll et al. 2007). In the present study, particle size distribution was only analysed in the basic coarsely and finely ground feed. Differences were mainly based on the particle size fractions smaller than 355 µm and greater than 1005 µm. However, it can be expected that a second grinding could occur during pelleting and expanding, which was earlier described by Wolf et al. (2010) and Grosse Liesner et al. (2009).

When the feed was coarsely ground, the highest ADG was achieved without hydro-thermal treatment (Group CgM). However, pigs needed more feed per kg weight gain, which could be partly explained by the lower digestibility of crude nutrients of this feed. However, it cannot be excluded that rummaging for feed and spillage of feed by the animals, which was observed during the trial, is a further factor for higher FGR in meal fed groups. Observation of higher feed wastage when feeding meal diets was also confirmed by O'Doherty et al. (2001), Medel et al. (2004) and Millet et al. (2012). Expanded feed provoked negative effects on feed intake which resulted in the lowest ADG. In several earlier studies, expansion of feed also decreased feed intake, especially when using high processing temperatures or pressure (Nuwer et al. 1967; Chae et al. 1997a, 1997b; Johnston et al. 1999; O'Doherty et al. 2001; Ohh et al. 2002; Lundblad et al. 2011; Millet et al. 2012). Authors assumed this was caused by the higher energy level of these diets (Millet et al. 2012) or that expanded feed had a lower palatability because of scorched ingredients when using higher processing temperature (Johnston et al. 1999; Ohh et al. 2002). Another reason for the decreased feed intake could be differences of bulk density of the feed. Expanded feed showed higher bulk density compared with the other feeds which increase the volume of the feed (data not shown). Therefore, a faster filling of stomach could result in a faster satiety. However, the present study revealed another problem of expanded feed. Although clumps in stomach content were only detected after slaughtering, it can be assumed that they were also present during the fattening period. Therefore, it is possible that clumps of stomach content evoke satiety by longer retention of ingesta in stomach and decreased feed intake. Clumps of stomach content could be induced by alteration of starch texture. Analysis of starch in finisher feeds made obvious that hydro-thermal treatment resulted in higher starch disintegration, which is considered an indicator for starch digestibility in organisms. It was assumed that disintegration of starch is in close relation with starch gelatinisation. Further, a higher starch gelatinisation is also associated with a higher feed extract viscosity (Lundblad et al. 2011). Lundblad et al. (2011) observed higher in vitro viscosity of feed extract when feed was expanded and pelleted or extruded compared with mash feed. However, steam conditioned pellets had a lower viscosity. In the present study, the viscosity of feed extract was not examined. A less pronounced formation of clumps in pelleted diets could be due to the higher fluidity of stomach content and higher water intake by these animals (unpublished results). Furthermore, in earlier studies pelleted feeds showed lower starch gelatinisation compared with expanded feed (Lundblad et al. 2011). Since all groups fed hydro-thermally treated feed showed clumps in stomach content, while no clumping was found in meal-fed pigs, it appeared that hydro-thermal processing is the main factor for the development of these alterations in stomach content. However, it cannot be excluded, that finer grinding of hydro-thermally treated feeds also contributed to these effects. Further studies are needed to explain the development of clumps in stomach content. However, it is conceivable that these alterations are due to interactions between starch gelatinisation, water intake of the animals and water binding capacity of the feed, fluidity and mixing of stomach content and feed viscosity. Furthermore, the used ingredients could also play a role to promote these clumps. Digestibility of feeds was not decreased in the balance trial. Therefore, the negative effects on performance seemed only be related to a longer ingesta retention time associated with a persistent satiety and lower feed intake. However, restrictive feeding of the balance trial could have prevented development of clumps.

Table 7. Stomach associated parameters and relative organ weights (LSMeans).

Variable	Coarsely ground feed				Finely ground feed				PSE*	*p*-Value		
	M▿	P‡	Ex□	ExP•	M	P	Ex	ExP		Grinding	Hydro-thermal treatment	Grinding x Hydro-thermal treatment
Stomach weight [g/kg BW]	5.95	5.89	6.12	6.13	5.67	5.65	5.77	6.00	0.17	0.055	0.333	0.942
Stomach score†	0.42^{e}	3.08^{a}	1.50^{cd}	1.91^{bcd}	2.83^{ab}	2.33^{abc}	1.25^{de}	1.58^{bcd}	0.24	0.113	< 0.001	< 0.001
Stomach content												
pH	3.82	3.45	4.20	4.33	3.89	3.82	4.45	4.25	0.32	0.494	0.083	0.900
Dry matter [%]	21.3	15.0	20.6	18.7	16.	19.2	23.0	20.2	2.15	0.653	0.174	0.141
Liver [g/kg BW]	16.8^{b}	18.1^{ab}	19.2^{a}	18.5^{ab}	18.5^{ab}	17.6^{ab}	19.2^{a}	18.0^{ab}	0.41	0.599	0.002	0.028
Kidney [g/kg BW]	3.14^{b}	3.08^{b}	3.60^{a}	3.39^{ab}	3.23^{ab}	3.13^{b}	3.44^{ab}	3.39^{ab}	0.09	0.932	< 0.001	0.476
Spleen [g/kg BW]	1.58	1.65	1.70	1.54	1.78	1.65	1.66	1.65	0.08	0.207	0.676	0.406

Notes: ▿M, Meal; ‡P, Pelleted; □Ex, Expanded; •ExP, Expanded and re-pelleted; *PSE, Pooled standard error; ‡Score: 0 = intact epithelium, score 4 = severe lesions and scarring; ^{abcd} LSMeans of different feeding groups not sharing the same superscripts are significantly different ($p < 0.05$)

4.3. Organ weights

Although no differences were found in BW, significantly higher liver and kidney weights were determined in pigs fed expanded feed compared with those which received coarsely ground meal. Several authors ascribed higher weights of liver and kidneys after feeding diets with higher CP contents (Anugwa et al. 1989; Kerr et al. 1995). The authors hypothesised that organ hypertrophy is caused by functional adaptation which is necessary to compensate surplus of CP or higher removal of nitrogenous compounds.

In the present study, there was a trend of higher stomach weights when coarsely ground feed was offered. Pelleted feed without expanding and finely ground feed without hydro-thermal treatment showed the lowest weights of this organ. This was in agreement with earlier studies, in which fine grinding and pelleting was associated with lower stomach weights (Eisemann and Argenzio 1999b; Hedemann et al. 2005). This could possibly be related to the decreased dry matter content in animals of these feeding groups. There was no significant impact of hydro-thermal treatment on stomach weight. However, expanded feed with and without pelleting induced heavier stomachs despite secondary grinding during these more intensive processing methods. This effect could be related to the development of clumps in stomach which reached proportions of approximately 1.6 kg. Consequently, a thicker tunica muscularis of the stomach could have developed for better mixing of stomach contents. Hedemann et al. (2005) suggested that higher stomach weights are associated with higher stomach motility.

4.4. Stomach health

Lowest stomach scores were obtained by feeding coarsely ground feed without hydro- thermal treatment. Highest stomach scores indicating severe lesions of the pars nonglandularis were obtained in animals fed pelleted feed without expanding and finely ground feed without hydro-thermal treatment compared with coarsely ground mash feed. Although differences were not significant, animals of these feeding groups also had the lowest proportions of dry matter content in the stomach and the lowest pH value. Eisemann and Argenzio (1999b) and Grosse Liesner et al. (2009) also reported lower gastric dry matter content when pigs received pelleted feed. An increased mixing of more fluid stomach content in animals fed finely ground or pelleted feed could be the reason for this effect (Fladlandsmo and Slagsvold 1971). In contrast, coarsely ground feed allows a development of a pH gradient (Maxwell et al. 1972; Mösseler et al. 2010). Mixing of the stomach content is inhibited and harmful substances of pyloric region can hardly get in contact with the nearly unprotected *Pars nonglandularis*. It is assumed that high dry matter content and a high ability for water binding and a mushy consistency are important to inhibit separation of sediment and fluid and therefore to protect against stomach lesions. Interestingly, animals of Group CgM achieved a similar pH level compared to pigs fed pelleted feed. However, the stomachs of these animals showed a higher dry matter content. These results indicate that pH-value is not the main precursor of stomach lesions and dry matter content plays a key role in protection from stomach lesions. However, it has to be emphasised that the pH-values were measured in mixed stomach content. The subordinate relevance of the pH-value of stomach content was also described by Mösseler et al. (2010). Mikkelsen et al. (2004) also reported that the consistence of stomach content plays a key role in protection of stomach lesions. In general, several authors suggested that high fluidity stomach content induced stomach lesions (Maxwell et al. 1967; Nuwer et al. 1967; Baustad and Nafstad 1969; Fladlandsmo and Slagsvold 1971). A further interesting fact was that animals that received expanded feeds or expanded and re-pelleted showed a moderate stomach score despite possible secondary grinding or pelleted form. In the present study pigs fed expanded feed had a numerically higher pH-value of stomach content and higher dry matter content compared to other groups except for Group CgM. This could be the explanation for lower stomach score when pigs received expanded or expanded and re-pelleted feed compared with feed which was only pelleted. Another reason could be that the clumps which were developed in stomachs mimic a gradient which was described in earlier studies after feeding coarsely ground feed without hydro-thermal treatment (Maxwell et al. 1972; Mösseler et al. 2010). Disadvantages of these clumps on stomach health could be a slower acidification of stomach content which could promote colonisation of pathogenic microbes. In agreement with previous studies by Kirchgessner et al. (1985) and Dirkzwager et al. (1998) performance of pigs seemed not to be influenced by severe lesions.

5. Conclusions

Fine grinding and hydro-thermal treatments improved digestibility of crude nutrients in pigs. Finely ground pelleted feed which was not expanded resulted in high ADG and decreased FGR. However, pelleting promoted the development of stomach lesions. Despite improvements in the digestibility of crude nutrients, pigs fed expanded feed are not able to accomplish performances similar to the other feeding groups because of decreased feed intake caused by possibly induced persistent satiety, which was promoted by clumping of stomach content caused by starch gelatinisation. With regard to higher processing costs, expansion of whole feed is not to be favoured in fattening of pigs. Coarsely ground feed also had beneficial effects on weight gain and feed intake and further protected gastric pars nonglandularis against lesions. However, coarsely ground feed resulted in higher FGR, which maybe an artefact caused by possible higher feed wastage.

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Disclosure statement

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5 Paper II

Effects of diets differing in protein source and technical treatment on digestibility, performance and visceral and biochemical parameters of fattening pigs

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Abstract

The aim of the experiment on 100 cross-bred barrows was to compare commercial diets for fattening pigs based on either soya bean meal (SBM) imported from non-European countries with diets based on a mixture of locally produced rape seed meal distillers' dried grains with solubles and soya beans as main protein sources. In addition, these both types of diets were processed by two different technical feed treatments, i.e. coarse grinding without hydrothermal treatment or fine grinding and pelleting. With only few exceptions, nutrients of the diet without SBM were more digestible ($p < 0.05$) resulting in a higher metabolizable energy (ME) content. Fine grinding and pelleting increased also the ME content and the nutrient digestibility with the exception of crude fibre. Higher feed intake of animals that fed diets without SBM ($p < 0.01$) resulted in higher average daily gain ($p < 0.01$). However feeding this diet, the higher digestibility was not reflected in a decreased feed-to-gain ratio (FGR), but fine grinding and pelleting reduced FGR ($p < 0.001$). A higher pH value and a lower DM content of caecal chymus were detected in animals that received coarsely ground feed ($p < 0.05$). Animals that fed finely ground and pelleted feed had higher slaughter and relative liver weights and higher blood cholesterol concentrations ($p = 0.040$). The urea concentrations of blood were lower ($p = 0.019$) after feeding diets without SBM. In conclusion, SBM imported from non-European countries can be replaced by alternative local protein sources without compromising digestibility or performances of animals. Although fine grinding and thermal treatment particularly seemed to be advantageous for digestibility and performance, the possible risk of development of stomach lesions should be considered.

Keywords: Blood physiology; digestibility; fattening performance; particle size; pelleting; pigs; protein sources; soyabean oilmeal

1. Introduction

Soya bean meal (SBM) is the most important protein source in broiler and pig nutrition relative to the requirements of the animals, especially with regard to the amino acid pattern. However, public acceptance of SBM in central Europe has decreased in the last few years, caused by the discussion on the usage of genetically modified plants and environmental problems in producing countries. Therefore, alternative local protein sources are at the front of research efforts. Available alternative protein feeds in Germany are, among others, by-products of the biofuel industry, i.e. rapeseed meal and distillers' dried grains with solubles (DDGS). Difficulties in feeding such protein sources are mainly caused by anti-nutritional factors or a lower protein quality. Furthermore, anti-nutritional factors are often associated with lower protein quality, a lower digestibility and reduced acceptance by the animals (Bell 1984; Lindermayer et al.2011).

In literature, different processing methods are discussed to enhance the digestibility of nutrients (O'Doherty et al. 2001; Oryschak and Zijlstra 2002; Park et al. 2003). Consequences of higher digestibility are a higher availability of nutrients, which can reduce the feed-to-gain ratio (FGR) of animals. In addition to these effects, there are also indications that organ weights are affected, including liver and kidney in pigs (Liermann et al. 2015). However, a lower digestibility seems to have also beneficial effects, especially with regard to gut-health. Canibe et al. (2005) described that a lower digestibility of coarsely ground feed causes more starch to arrive the hindgut, which seemed to have beneficial effect on the microbiota. Furthermore, a thermal treatment of feedstuffs can reduce protein quality by the development of Maillard reaction products (Parsons et al. 1992; Anderson-Hafermann et al. 1993) and requires a high energy input (Traylor et al. 1999). With regard to reduce costs and to diminish the impact on environment and climate, it would be necessary to minimise the energy input for feed processing and to adapt it to the special requirements.

Therefore, the aim of this study was to investigate the replacement potential of imported SBM by more local protein sources (rapeseed meal, DDGS, soya beans from Central Europe) and possible improvements of the feeding value of alternative protein sources by different common technical feed treatments. In contrast to earlier studies which investigated the replacement of SBM by one or only a few other local protein sources, the approach of the present study was to reduce the imported SBM proportion in complex diets reflecting the more practical feeding situation. In addition, the study laid a special focus on the effects of particle size distribution and hydrothermal treatment as well as health aspects.

2. Material and methods

2.1. Experimental diets and design

The four feeding groups differed on the one hand in used protein sources and on the other hand in technical treatment (Table 1). To meet the requirements of growing and finishing pigs (GfE 2008), the SBM imported from non-European countries and used for Diet 1 was replaced in Diet 2 by rapeseed meal, toasted soya beans (originating from Central Europe) and DDGS as far as possible from a nutritional and economical position (Table 2). The two different technical treatments included coarsely ground meal or finely ground and pelleted feed. Coarse feed was ground with a roller mill (0.5 mm gap width). In contrast, finely ground feed was processed with a hammer mill with a 3-mm screen. Pellets were pressed at 74°C and a steam supply of 3% using a 3 mm pelleting die. The fattening period was divided into three phases. For the determination of nutrient digestibility based on a marker method using HCl-insoluble ash (GfE 2005), 1% silicon oxide (Diamol; SiO2 75%) was added to all diets.

Table 1. Experimental design.

	Diet 1		Diet 2	
	With soya bean meal		Replacement of soya bean meal	
	Group 1 coarsely ground meal (n=25)	Group 2 finely ground and pelleted (n=25)	Group 3 coarsely ground meal (n=25)	Group 4 finely ground and pelleted (n=25)
Number of n for specific estimations				
Nutrient digestibility	11-12	11-12	11-12	
Fattening performance	25	25	25	25
Slaughtering traits	25	25	25	25
Organ weights	25	24-25	25	25
Prancreas weight	13	13	13	13
Caecum parameters	13	13	13	11-13
Biochemical parameters	13	13	13	13

Table 2. Ingredients of experimental diets in the starter, grower and finisher phases.

	Starter phase (< 70 kg BW)		Grower phase (70-100 kg BW)		Finisher phase (< 100 kg BW)	
	Diet 1	Diet 2	Diet 1	Diet 2	Diet 1	Diet 2
Ingredients [g/kg diet]						
Soybean meal	206.6	-	184.0	-	112.5	-
Rapeseed meal	-	150.0	-	150.0	-	150.0
Soya beans toasted	-	87.4	-	62.0	-	-
DDGS*	-	60.0	-	60.0	-	60.0
Maize	250.0	331.0	250.0	330.0	190.0	323.0
Barley	100.0	100.0	100.0	100.0	100.0	100.0
Wheat	271.0	217.5	226.6	218.0	288.0	273.6
Wheat bran	33.8	-	100.8	24.0	110.0	34.0
Maize gluten	-	19.0	-	21.0	61.5	1.2
Maize meal	100.0	-	100.0	-	100.0	26.0
Vinasse	10.0	10.0	10.0	10.0	10.0	10.0
Plant oil	2.3	-	5.4	-	6.0	-
Lysine HCl	2.8	4.8	1.1	3.2	2.0	3.2
DL-methionine	0.3	0.1	0.1	-	-	-
Premix 1†	13.2	10.2	12.0	11.8		
Premix 2‡					10.0	9.0
Diamol#	10.0	10.0	10.0	10.0	10.0	10.0

Notes: *DDGS, distillers' dried grains with solubles; †Premix 1 delivers per kg feed: 20 mg copper; 6.9 g calcium; 5.4 g phosphorus; 1.8 g sodium; 10,000 IU vitamin A; 1000 IU vitamin D; 75 mg vitamin E; ‡Premix 2 delivers per kg feed: 20 mg copper; 6.5 g calcium; 5.2 g phosphorus; 1.8 g sodium; 10,000 IU vitamin A; 1000 IU vitamin D; 75 mg vitamin E; #Diamol, SiO2 75%.

2.2. Growth experiment

To examine the impact of the four different feeds, 100 cross-bred barrows ((German Landrace × German Large White) × Piétrain) were fattened for 10 weeks between May and August. Animals had an initial average body weight (BW) of 34.7 ± 3.6 kg and were allotted to feeding groups on the basis of BW. Animals were housed in a mechanically ventilated building. Pigs of each feeding group were individually penned in 25 concrete-floored boxes of 3.1 m2 each without bedding. All boxes were equipped with individual troughs and nipple drinkers. Feed was offered dry and ad libitum according to trough fill-level until immediately before slaughtering. Water was also available ad libitum. Animals were weighed and feed was reweighed weekly during the fattening period. In each fattening phase, faeces of 12 animals of each feeding group were collected three times per week in the morning (Table 1). Before sampling of faeces, a 7-day adaptation phase was given after each feed changeover. Faeces samples of each animal were pooled for each fattening phase and kept frozen.

After reaching a BW of 120 kg (126.6 ± 5.1 kg), pigs were slaughtered according to conventional standards. Three animals per group were slaughtered each day. Pigs were electrically stunned and killed by bleeding. Blood samples from 13 animals were taken during bleeding from the middle of the bloodstream. Weights of emptied stomach, liver without bile bladder, kidneys, spleen and pancreas (pancreas from 13 animals per group) were recorded. Chymus of caecum were also collected from 13 animals per group for analysis of dry matter (DM) content, starch content and pH measurements.

Incisions of cutlet were made from dorsal to ventral, between the 13 and 14 thoracic vertebras in the chilled, left half of carcass for photographical capturing according to standards for calculation of lean meat percentage after the Bonner Equation (ZDS 2007).

2.3. Analytical methods

Dry sieve analyses corresponding to DIN 66165-1:1987-04 (DIN 1987a) and DIN 66165-2:1987-04 (DIN 1987b) were used to investigate particle size distribution of coarsely ground meal diet and finely ground feed before pelleting. Therefore, particles of 72.20-g feed sample were separated by sieves according to DIN ISO 3310/1 (DIN 2001). The remaining fractions of each sieve were reweighed and the ratio to the weight of the initial feed sample was calculated.

Feed and faeces samples were analysed according to methods of the Association of German Agricultural Analysis and Research Centres (VDLUFA). Thus, DM of feed, faeces and chymus of caecum were analysed by methods of Naumann and Bassler (1976). Nitrogen (N) was determined using the combustion method according to Dumas. Methods of VDLUFA 5.1.1, 6.1.1 and 8.1 were used to analyse ether extract (EE), crude fibre (CF) and crude ash, respectively. HCl-insoluble ash was analysed according to the VDLUFA Method 8.2. Furthermore, sugar concentration was determined corresponding to Luff-Schoorl. Concentrations of starch in feed and caecum content were measured polarimetrically. As an indicator of adequate thermal pretreatment of soya beans, urease activity and protein solubility (PS) were examined in coarsely ground Diet 1, coarsely ground Diet 2 and finely ground and pelleted Diet 2 (VDLUFA Methods 20.1 and 20.2). Furthermore, PS in potassium hydroxide (PS-KOH) was determined according to Van Eys (2012). Glucosinolates were determined in rapeseed-containing feeds by HPLC in accordance to the European Union method L170/28 (ISO Norm 1992). A pH-meter, type WTW pH 530, was used for pH measurements of mixed contents in caecum (BLB, Brunswick, Germany).

Blood serum samples were used to analyse parameters of clinical chemistry including albumin, total protein, glucose, cholesterol, aspartate-amino-transferase, γ-glutamyltranferase, bilirubin, triglycerides, urea, β-hydroxybutyrat and non-esterified fatty acids. Briefly, mentioned parameters were measured by an automatic clinical chemistry analyser (Eurolyser CCA180, Erolab, Austria). Two haemolytic samples were excluded from aspartate-amino-transferase analyses.

2.4. Calculations and statistical analysis

Metabolisable energy (ME) of feed was calculated on the basis of digestible nutrients (as analysed) according to the equation of the GfE (2008) as follows:

$$\begin{aligned} \text{ME [MJ/kg]} = {} & 0.0205 \cdot \text{DCP [g]} + 0.0398 \cdot \text{DEE [g]} + 0.0173 \cdot \text{Starch [g]} \\ & + 0.0160 \cdot \text{Sugar [g]}\ 0.0147 \cdot (\text{DOM} - \text{DCP} - \text{DEE} - \text{Starch} - \text{Sugar})\ \text{[g]} \end{aligned}$$

where DCP is the digestible crude protein, DEE is the digestible ether extract and DOM is the digestible organic matter (OM).

Average daily weight gain (ADG) was defined as the quotient of the difference between final BW and initial BW and the length [d] of the corresponding feeding period. Daily feed intake (DFI) was calculated by dividing the feed consumption by the length [d] of feeding period. FGR was calculated based on the ratio between feed intake and weight gain.

Carcass lean meat percentage was calculated according to the Bonner Equation (ZDS 2007):

$$\begin{aligned} \text{Leanmeat percentage [\%]} = {} & 59.704 - 0.147 \cdot \text{Fat surface of cutlet [cm}^2\text{]} \\ & + 0.222 \cdot \text{Meat surface of cutlet [cm}^2\text{]} \\ & - 1.744 \cdot \text{Backfat, loin [cm]} - 1.175 \cdot \text{Back fat, middle [cm]} \\ & - 0.809 \cdot \text{Back fat, withers [cm]} \\ & - 0.378 \cdot \text{Side fat thickness [cm]} \\ & - \text{Fat thickness over surface of back muscle [cm]} \end{aligned}$$

Values of globulin concentrations [g/l] in blood samples were calculated by subtraction of total protein [g/l] by the concentration of albumin [g/l] in blood (Harr 2006).

MIXED procedure of SAS Enterprise Guide 6.1 was used for statistical analyses. The model included fixed effects of diet and technical feed treatment as well as their interaction. Animals were considered as repeated factor in the case of statistical analyses of digestibility of nutrients and ADG, DFI and FGR, respectively. Differences were declared as significantly different at $p \leq 0.05$. The correlation coefficient according to Pearson was estimated using Statistica 12 and declared to be significantly different from zero at $p < 0.05$.

3. Results

3.1. Feed analyses

Diets 1 and 2 were designed to be isoenergetic and isonitrogenous. While in the starter phase, the soya bean protein content was reduced by 57.9% in Diet 2 compared with Diet 1, it was reduced by 66.3% in the grower phase and was completely replaced by other protein sources in the finisher phase. Nutrient contents of experimental diets are shown in Table 3.

After the same technical feed treatment, the particle size distribution in the different fattening phases was similar. There were only minor differences between particle size distributions of the two different diets when comparing the same technical feed treatment (Table 4), except for finely ground feed of the starter phase. When comparing coarsely ground feed and finely ground feed, the main differences existed between particles smaller than 355 μm and particles greater than 1005 μm. Generally, coarsely ground meal of both diets had more particles greater than 1005 μm and finely ground particles had more particles smaller than 355 μm.

PS in water was between 10% and 20% in all analysed feeds (Table 3). The values of PSKOH of feeds of the starter phase were higher than 72%. PS-KOH was also higher than 72% in the coarsely ground Diet 1 of the grower and finisher phases. However, PS-KOH values of Diet 2 in meal and in pelleted form were lower than 72% in the grower and finisher phases. Urease activity of all analysed feeds was lower than 0.5 mg N g−1 min−1. Only traces of glucosinolates were detected in feeds which contained rapeseed meal (data not shown).

In the starter and the finisher phases, the digestibility of all nutrients was higher for Diet 2 than for Diet 1. The only exceptions were the DEE in the starter phase and the DCP in the finisher phase, which were unaffected by different diet composition (Table 5). In the grower phase, the digestibility of EE was higher in Diet 2. However, in this fattening phase, higher proportions of DCP and DOM were measured for Diet 1. ME and digestibility of CF were not significantly affected by diet in grower phase. Pooled means of digestibility of all nutrients of Diet 2 were higher compared with nutrient digestibility of Diet 1 except for CP in the grower phase (data not shown).

Fine grinding combined with pelleting increased the digestibility of all nutrients significantly. The only exception was the digestibility of CF in starter and finisher feed, which was not significantly affected by technical feed treatment.

3.2. Performance

Fattening performance of pigs is represented in Table 6. Significant influences of diet on ADG were found in the starter phase (p = 0.004). Animals that fed Diet 2, which included no SBM, reached highest ADG in this phase. Further, these pigs tended to consume more feed compared with animals that fed Diet 1 (p = 0.080) and tended to need less feed for gain (p = 0.065). Additionally, there was a trend towards reducing FGR by feeding finely ground and pelleted feed. In the grower phase, there were no effects of diet on fattening performance of the animals. However, technical feed treatment significantly affected DFI and FGR. In addition to lower DFI, the FGR of pigs that fed finely ground, pelleted feed decreased compared with pigs that fed coarsely ground feed. There was only a trend to influence ADG by technical feed treatment but a significant interaction between diet and technical feed treatment was demonstrated in the grower phase (p = 0.005). In the finisher phase, there was no significant influence of technical feed treatment on the fattening performance of animals. Pigs that fed finely ground and pelleted feed achieved numerically higher ADG and needed less feed for gain. On the other hand, animals which were fed Diet 2 consumed significantly more feed per day and reached a lower FGR. Diet 2 also increased ADG in this phase.

Considering the effects of diet and technical feed treatment of the total fattening period, significant influences of diet on ADG and DFI and a significant effect of technical feed treatment on FGR were detected. Feed treatment tended also to affect ADG and DFI. Generally, animals fed Diet 2 consumed more and achieved higher ADG than animals of the remaining feeding groups. Pigs receiving finely ground and pelleted feed tended to consume less feed than animals that fed coarsely ground feed. There was also a trend to higher ADG in pigs receiving finely ground and pelleted feed compared with animals that fed coarsely ground meal. Additionally, fine grinding and pelleting significantly decreased FGR compared with coarse grinding without hydrothermal treatment. There was no significant interaction between diet and technical feed treatment when considering fattening performance of the entire fattening period.

Table 3. Contents of metabolisable energy (ME) and analysed nutrients in the experimental diets (on dry matter basis).

	Starter phase (< 70 kg BW)				Grower phase (70 – 100 kg BW)				Finisher phase (> 100 kg BW)			
	Diet 1[#]		Diet 2[§]		Diet 1		Diet 2		Diet 1		Diet 2	
	Finely ground, pelleted	Coarsely ground	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted
ME* [MJ/kg]	15	13.7	14.1	15.5	13.6	14.8	13.3	15.2	14.1	15.1	14.5	15.4
Dry matter [%]	88.3	89.1	88.7	88.5	88.8	88.2	88.7	88.3	88.9	89.1	89.2	88.9
Crude protein [g/kg]	190	197	215.	196	186	182	177	184	178	159	172	159
Ether extract [g/kg]	51	55	41	37	35	39	39	49	45	41	42	42
Crude fibre [g/kg]	46	57	42	38	40	40	47	51	46	39	55	48
Crude ash [g/kg]	66	69	67	55	79	76	72	67	70	54	65	56
Starch [g/kg]	448	420	434	526	495	487	505	489	464	540	482	528
PS[†] [%]	-	16.8	14.5	15.2	11.4	-	12.2	14.4	16.8	-	19	17.4
PS-KOH[‡] [%]	-	76.1	83.6	96.8	81.8	-	68.8	70.9	76.4	-	62.2	68.6
Urease activity [mg N g^{-1} min^{-1}]	-	0.02	< 0.01	< 0.01	0.10	-	< 0.01	< 0.01	< 0.01	-	< 0.01	< 0.01

Notes: *ME, metabolisable energy calculated on the basis of digestible nutrients (as analysed) according to GfE (2008); [#]Diet 1, with soya bean meal (SBM); [§]Diet 2, replacement of SBM; [†]PS, protein solubility in water; [‡]PS-KOH, protein solubility in potassium hydroxide.

Table 4. Particle size distribution of experimental diets.*

	Starter phase (< 70 kg BW)				Grower phase (70 – 100 kg BW)				Finisher phase (> 100 kg BW)			
	Diet 1 [#]		Diet 2[§]		Diet 1		Diet 2		Diet 1		Diet 2	
Particle size distribution [%]	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted
< 125	10.1	13.0	12.7	20.9	10.9	16.1	9.3	18.6	12.9	22.7	12.5	24.1
125-355 µm	16.0	14.1	15.5	24.0	15.2	21.2	14.6	26.5	19.9	21.0	16.4	23.2
360-1000 µm	37.4	38.5	32.3	38.1	37.7	42.1	42.1	39.9	39.1	39.9	33.6	37.9
1005-2000 µm	34.5	30.8	37.2	16.8	34.6	20.4	29.6	14.8	27.1	16.3	35.6	14.6
> 2000 µm	2.0	3.6	2.3	0.3	1.6	0.2	5.1	0.3	1.1	0.1	1.9	0.2

Notes: *Particle size distribution for pelleted diets was estimated before pelleting; [#]Diet 1, with soya bean meal (SBM); [§]Diet 2, replacement of SBM.

Table 5. Contents of metabolisable energy (ME) and digestibility of nutrients in experimental diets (LSMeans).

	Diet 1 (with soya bean meal)		Diet 2 (replacement of soya bean meal)		PSE[†]	*p* -Value		
	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted		Diet	Technical feed treatment	Diet x Technical feed treatment
Starter phase (< 70 kg)	*n* = 12	*n* = 11[‡]	*n* = 12	*n* = 12				
ME* [MJ/kg]	13.7^{c}	15.0^{b}	14.1^{c}	15.5^{a}	0.11	< 0.001	< 0.001	0.607
Dry matter [%]	92.6^{c}	93.8^{b}	93.8^{bc}	95.7^{a}	0.31	< 0.001	< 0.001	0.203
Crude protein [%]	73.3^{c}	79.5^{b}	77.7^{b}	83.9^{a}	0.90	< 0.001	< 0.001	0.967
Ether extract [%]	65.0^{b}	82.5^{a}	65.7^{b}	79.4^{a}	1.01	0.232	< 0.001	0.064
Crude fibre [%]	46.7^{ab}	44.0^{b}	50.1^{ab}	52.4^{a}	2.15	0.008	0.930	0.234
NfE[§] [%]	85.6^{b}	91.1^{a}	87.3^{b}	93.0^{a}	0.65	0.008	<0.001	0.873
Organic matter [%]	79.4^{d}	86.0^{b}	82.4^{c}	89.0^{a}	0.69	< 0.001	< 0.001	0.976
Grower phase (70-100 kg)	*n* = 12	*n* = 12	*n* = 12	*n* = 12				
ME [MJ/kg]	13.6^{c}	14.8^{b}	13.3^{c}	15.2^{a}	0.07	0.564	< 0.001	< 0.001
Dry matter [%]	93.8^{b}	94.9^{a}	92.8^{c}	95.0^{a}	0.17	0.006	< 0.001	0.001
Crude protein [%]	74.2^{b}	82.4^{a}	67.8^{c}	81.4^{a}	0.86	< 0.001	< 0.001	0.002
Ether extract [%]	48.5^{c}	78.1^{b}	49.1^{c}	83.2^{a}	1.07	0.009	< 0.001	0.034
Crude fibre [%]	32.3^{b}	47.9^{a}	30.7^{b}	53.5^{a}	2.18	0.363	< 0.001	0.095
NfE [%]	88.5^{b}	91.8^{a}	87.3^{c}	92.0^{a}	0.30	0.094	<0.001	0.019
Organic matter [%]	81.7^{b}	87.5^{a}	79.1^{c}	87.3^{a}	0.46	0.005	< 0.001	0.010
Finisher phase (> 100 kg)	*n* = 11[‡]	*n* = 11[‡]	*n* = 11[‡]	*n* = 12				
ME [MJ/kg]	14.1^{d}	15.1^{b}	14.5^{c}	15.4^{a}	0.07	< 0.001	< 0.001	0.339
Dry matter [%]	94.1^{c}	94.9^{b}	95.1^{ab}	95.4^{a}	0.13	< 0.001	< 0.001	0.091
Crude protein [%]	78.8^{b}	81.9^{a}	78.7^{b}	83.0^{a}	0.77	0.499	< 0.001	0.466
Ether extract [%]	63.0^{c}	80.1^{b}	64.6^{c}	83.6^{a}	0.50	< 0.001	< 0.001	0.068
Crude fibre [%]	37.9^{b}	42.5^{b}	56.6^{a}	56.7^{a}	1.81	< 0.001	0.208	0.226
NfE [%]	87.8^{c}	90.8^{b}	90.9^{b}	92.9^{a}	0.29	< 0.001	< 0.001	0.087
Organic matter [%]	82.4^{c}	86.8^{b}	85.5^{b}	89.0^{a}	0.42	< 0.001	< 0.001	0.276

Notes: *ME, metabolisable energy calculated on base of digestible nutrients (as analysed) according to the formula of the GfE (2008); [†]PSE, pooled standard error; [§]NFE, nitrogen-free extractives; [‡]One pig was omitted due to insufficient amounts of faeces; $^{a–d}$Means not sharing the same superscripts are significantly different ($p < 0.05$) .

Table 6. Fattening performance of experimental groups (LSMeans; n = 25).

	Feeding group					*p*-Value		
	Diet 1 (with soya bean meal)		Diet 2 (replacement of soya bean meal)					
	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted	PSE[*]	Diet	Technical feed treatment	Diet x Technical feed treatment
Starter phase (< 70 kg BW)								
DFI[†] [g]	2014	2040	2152	2067	46.81	0.080	0.530	0.242
ADG[‡] [g]	1010^{b}	1057^{ab}	1101^{a}	1090^{a}	21.17	0.004	0.389	0.174
FGR[$] [kg/kg]	2.05	1.98	1.98	1.93	0.03	0.065	0.072	0.794
Grower phase (70-100 kg BW)								
DFI [g]	3334^{a}	3055^{b}	3370^{a}	3208^{ab}	60.16	0.121	<0.001	0.334
ADG [g]	1076^{ab}	1054^{b}	1049^{b}	1133^{a}	19.71	0.165	0.091	0.005
FGR [kg/kg]	3.16^{ab}	2.98^{bc}	3.32^{a}	2.89^{c}	0.06	0.565	<0.001	0.063
Finisher phase (> 100 kg BW)								
DFI [g]	3554^{b}	3599^{b}	3960^{a}	4014^{a}	88.32	<0.001	0.580	0.958
ADG [g]	1121	1180	1191	1228	31.60	0.065	0.133	0.722
FGR [kg/kg]	3.26	3.14	3.48	3.36	0.09	0.021	0.200	0.993
Total fattening period								
DFI [g]	2850^{ab}	2758^{b}	3001^{a}	2913^{ab}	49.85	0.003	0.074	0.964
ADG [g]	1058^{b}	1081^{ab}	1098^{ab}	1135^{a}	15.72	0.004	0.064	0.642
FGR[kg/kg]	2.74^{ab}	2.61^{b}	2.81^{a}	2.60^{b}	0.04	0.376	<0.001	0.251

Notes: *PSE, pooled standard error; [†]DFI, daily feed intake; [‡]ADG, average daily gain; [$]FGR, feed-to-gain ratio. ^{abc}Means not sharing the same superscripts are significant different between feeding groups ($p < 0.05$).

3.3. Slaughter traits and organ weights

Pigs receiving Diet 2 were significant heavier at slaughter than pigs of Diet 1. Furthermore, pigs which were fed finely ground and pelleted feed had higher slaughter weights compared with pigs of the other feeding groups (Table 7).

Technical feed treatment significantly affected slaughtering traits of tested animals, except for the meat surface of cutlet ($p < 0.05$). Carcass yield and fat surface of cutlet was larger in animals which received finely ground and pelleted feed. These animals also had a lower carcass lean meat percentage. Highest values of this parameter were achieved by feeding coarsely ground feed. The fat surface of cutlet was also influenced by diet. Higher values were observed in animals that fed Diet 2 compared with Diet 1 with the same technical feed treatment.

Table 7. Slaughtering traits (n = 25), organ weights (n = 25), pancreas weight (n = 13) and parameters of caecum content (n = 13) (LSMeans).

Parameter	Feeding group				PSE*	*p*-Value		
	Diet 1 (with soya bean meal)		Diet 2 (replacement of soya bean meal)					
	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted		Diet	Technical feed treatment	Diet x Technical feed treatment
Body weight (BW) [kg]	123.0^{c}	126.5^{b}	126.6^{b}	130.1^{a}	0.89	< 0.001	< 0.001	0.991
Carcass yield [%]	82.1^{b}	82.9^{ab}	82.4^{ab}	83.2^{a}	0.25	0.273	0.003	0.865
Meat surface [cm^2]	62.6	63.3	65.0	64.3	0.12	0.129	0.980	0.542
Fat surface [cm^2]	25.5^{b}	27.2^{ab}	27.6^{ab}	29.8^{a}	0.99	0.019	0.049	0.811
Carcass lean meat Percentage [%]	56.1^{a}	55.4^{ab}	56.0^{a}	53.9^{b}	0.54	0.122	0.010	0.189
Organ weights [g/kg BW]								
Stomach	4.7^{a}	4.3^{b}	4.8^{a}	4.0^{b}	0.09	0.318	< 0.001	0.112
Liver	14.6^{b}	15.7^{a}	15.8^{a}	16.4^{a}	0.28	0.001	0.004	0.378
Kidney	2.9	2.9	3.1	3.6	0.39	0.228	0.449	0.522
Spleen	1.3	$1.4^{\dagger}$	1.4	1.4	0.06	0.432	0.503	0.459
Pancreas‡	1.3	1.4	1.3	1.3	0.04	0.479	0.888	0.216
Caecum content								
pH	6.02^{ab}	5.95^{b}	6.10^{a}	5.89^{b}	0.05	0.908	0.011	0.162
DM$^{\$}$	13.9^{b}	17.7^{a}	14.0^{b}	16.7^{a}	0.41	0.291	< 0.001	0.165
Starch [g/kg DM]	67.5	62.8	67.0	$61.5^{\P}$	3.25	0.779	0.134	0.911

Notes: *PSE, pooled standard error; $^{\dagger}n = 24$; $^{\ddagger}n = 13$; $DM, dry matter; $^{\P}n = 11$. ^{abc}Means not sharing the same superscripts are significant different between feeding groups ($p < 0.05$).

Stomach weights were significantly influenced by technical feed treatment (p < 0.001). Coarsely ground feed significantly increased stomach weights compared with finely ground and pelleted feed.

There was no effect of diet and technical feed treatment on organ weights of kidney, spleen and pancreas related to BW (Table 7). A significant impact of diet and technical feed treatment was observed on liver weights. Feeding of the diet without SBM (Diet 2) resulted in higher liver weights compared to pigs that fed Diet 1. Further, finely ground and pelleted feed increased liver weights compared to coarsely ground feed with same ingredients. Ingredients of Diet 1 combined with coarse grinding caused significantly lower liver weights than fine grinding and pelleting. There were no obvious pathological alterations in named visceral organs.

3.4. Caecum content

No alterations in collected parameters of caecum content were detected when feeding the different diets (Table 7). However, technical feed treatment had significant effects on pH and DM percentage of caecum content. Feeding of finely ground and pelleted feed significantly decreased pH values of caecum content compared with coarsely ground feed ($p = 0.011$). Furthermore, DM content in chymus of caecum region of pigs that fed finely ground and pelleted feed was significantly higher compared with the other feeding groups ($p < 0.001$). Numerically higher starch concentrations related to DM were detected in the caecum content of pigs that fed coarsely ground feed.

3.5. Clinical chemistry

Glutamate-dehydrogenase activity in all blood samples was below the limit of detection. There was no effect of diet and technical feed treatment on the parameters of clinical chemistry except for the concentration of cholesterol and urea in blood serum (Table 8). While cholesterol was significantly influenced by technical feed treatment, urea was significantly affected by diet. Finely ground and pelleted feed resulted in significantly higher blood cholesterol concentration compared to the coarsely ground feed. Urea concentrations were higher in the blood of animals which consumed the diet with SBM (Diet 1). Technical treatment tended also to affect the concentration of total protein in blood and activity of γ-glutamyltransferase. Total protein and activity of γ-glutamyltransferase were higher in animals that fed finely ground and pelleted feed compared with animals that fed coarsely ground feed.

4. Discussion

One focus of the present study was to replace SBM in diets of fattening pigs. Therefore, a diet which included SBM was compared with a diet containing soya beans, rapeseed meal and DDGS. Subsequently, the soya bean protein was reduced by considerable parts in starter and grower phases and completely in finisher phase. Furthermore, the role of technical feed treatment to improve feed quality of diets with and without SBM was tested. Thus, the two diets were either coarsely ground or finely ground and additionally pelleted.

4.1. Feed analysis

Initially it was ensured that used pretreated ingredients, especially SBM, soya beans and rapeseed meal of diets, were adequately pretreated before creation of the diets to exclude impacts of, for example, anti-nutritive substances. Urease activity was used as an indirect indicator for the activity of trypsin inhibitors of soya beans. All of the analysed feeds showed urease activities lower than the VDLUFA reference level of 0.5 mg N g−1min−1 (Naumann and Bassler 1976). Furthermore, only traces of glucosinolates were detected. Therefore, an inadequate thermal treatment of feed ingredients, especially soya beans and rapeseed meal, and an impact of trypsin inhibitors on animal performance or further collected parameters such as weights of visceral organs or clinical chemistry can be excluded.

Besides the inactivation of anti-nutritive substances, an overheating of feedstuffs can also result in thermal damage of amino acids or development of amino acid–carbohydrate complexes which are poorly digestible (Parsons et al. 1992; Anderson-Hafermann et al. 1993; Qin et al. 1996; Siljander-Rasi et al. 1996). An indicator of overheating is the PS. PS of all analysed feeds was within the optimal range between 10% and 35% according to VDLUFA for SBM (Naumann and Bassler 1976). Bellof and Steiner (2009) described PS between 10% and 20% as an indication of overheating of soya beans. In general, a PS value lower than 15% has to be assessed as the critical range (Menke and Huss 1987). Trials of Bellof and Steiner (2009) with laying hens indicated reduced performances when the fed soya bean cake had PS values lower than 20%, while broilers and piglets receiving same feed ingredients show no reduced performances. Especially in feeds of the grower phase, PS values lower than 15% were measured in the present study. PS-KOH is another indicator of overheating of SBM (Araba and Dale 1990; Parsons et al. 1991). Lowest PS-KOH values were measured in Diet 2 in the grower and finisher phases. Parsons et al. (1991) reported decreased feed efficiencies in chickens when PS-KOH was lower than 59% and in pigs when PS-KOH was lower than 66%.

Table 8. Parameters of clinical chemistry (LSMeans, n = 13).

Variable	Feeding group				PSE[*]	*p*-Value		
	Diet 1		Diet 2					
	Coarsely ground	Finely ground, pelleted	Coarsely ground	Finely ground, pelleted		Diet	Technical feed treatment	Diet x Technical feed treatment
Albumin [g/l]	37.8	37.9	37.2	38.6	0.58	0.900	0.194	0.240
Globulin [g/l]	19.7	20.1	19.5	23.1	1.24	0.275	0.118	0.196
Ratio albumin/globulin	2.7	2.0	2.0	1.7	0.38	0.211	0.218	0.521
Total protein [g/l]	57.5	57.9	56.6	61.7	1.42	0.316	0.060	0.109
Glucose [mg/dl]	88.0	93.5	84.1	93.7	4.94	0.714	0.133	0.671
Cholesterol [mg/dl]	95.0	97.8	92.1	101.4	2.86	0.906	0.040	0.252
AST[†] [IU/l]	19.3	25.7[‡]	23.9	23.8	4.12	0.746	0.447	0.431
γ- glutamyltransferase [IU/l]	20.16	22.77	19.6	25.9	2.25	0.573	0.054	0.417
Bilirubin [mg/dl]	0.1	0.1	0.1	0.2	0.01	0.540	0.365	0.287
Triglycerides [mg/dl]	16.7	17.2	15.0	21.5	2.61	0.629	0.189	0.263
Urea [mg/dl]	30.4	31.2	24.8	27.5	1.90	0.019	0.364	0.606
β- hydroxybutyrat [mmol/l]	0.18	0.16	0.17	0.20	0.03	0.405	0.828	0.383
NEFA[§] [mmol/l]	0.20	0.22	0.21	0.22	0.01	0.573	0.373	0.641

Notes: *PSE, pooled standard error; [†]AST, aspartate-amino-transferase; [‡]$n = 12$ (haemolytic samples were excluded); [§]NEFA, non-esterified fatty acids.

Araba and Dale (1990) assumed that nutritive value for chickens could be impaired when PS KOH are less than 70% and values lower than 65% indicating overheating. Van Eys (2012) assessed a minimum of 72% PS-KOH. In contrast, Anderson- Hafermann et al. (1993) described the values of 35% as a critical range when analysing canola meal. In the present study, the PS-KOH values were higher than 72% in all tested feeds of starter phase and in coarsely ground meal of Diet 1 in grower and finisher phases. However, in the grower and finisher phases for Diet 2, the measured PS-KOH levels were lower than 72%.

4.2. Digestibility and fattening performance

In general, the pooled means of nutrient digestibility of Diet 2 were higher compared with Diet 1 excepting the digestibility of EE in the starter feed and excepting the digestibility of DM, CP, OM and NFE in the grower phase. Interestingly, PS values lower than 15.0% and PS-KOH values lower than 72.0% were only measured in feeds of Diet 2 of grower phase at the same time. Especially the DCP of the coarsely ground Diet 2 was significantly lower than all other feeds in this feeding phase. This could be due to the fact that this diet type contained protein sources which were thermally treated before being used in the present experiment with the consequence of a lower PS. The reduction in protein digestibility of this feed was also associated with a decreased ADG despite high DFI in the grower phase, while ADG of Diet 2 was higher compared with Diet 1 in all other fattening phases. FGR also increased when fed the coarsely ground Diet 1 in grower phase.

Considering the whole fattening phase, animals of Diet 2 showed higher ADG than animals of Diet 1. This could be explained by higher feed intake which possibly depends on used components. However, in the whole fattening phase, Diet 2 resulted in higher FGR compared to Diet 1 despite the slightly higher digestibility. In contrast, animals that fed finely ground and pelleted feed consumed less feed and had higher ADG than animals of coarsely ground meal, which subsequently resulted in lower FGR. This effect is explainable by higher digestible nutrients of finely ground and pelleted feed compared to coarsely ground meal, except for CF of feeds in the starter and finisher phases. An increase of nutrient digestibility by fine grinding and pelleting is probably based on an increase of particle surface. Furthermore, pelleting induce gelatinisation of starch. Both effects promote a higher availability of nutrients to digestive enzymes (Jensen and Becker 1965; O'Doherty et al. 2001; Döll et al. 2007). Furthermore, fine grinding and pelleting resulted in higher damage of cell structure which increased utilisation of nutrients (Medel et al. 2004; Döll et al. 2007). However, possible lower feed wastage of pelleted feed compared with coarsely ground meal could also be a reason for decreased FGR which was earlier observed in studies of O’Doherty et al. (2001) and Medel et al. (2004). However, the possible adverse effects on stomach, such as erosions and ulcera, have to be considered (Liermann et al. 2015), when using pelleted and or finely ground feed.

4.3. Analysis of caecum content

In agreement with studies by Canibe et al. (2005), a lower digestibility of coarsely ground feed caused numerically higher starch content in caecum of slaughtered animals compared to finely ground and pelleted feed. Some authors described positive effects of higher starch content in caecum and colon on gram-positive bacteria counts, especially on lactobacilli (Canibe et al. 2005; Papenbrock et al. 2005; Kamphues et al. 2007; Visscher et al. 2009). Furthermore, the promotion of lactobacilli and generation of lactic acids results in suppression of the concentration of enterobacteria in the digestive tract which have positive effects on the gut health (Canibe et al. 2005). The pH value of caecum content was significantly higher in pigs that fed coarsely ground meal compared with finely ground and pelleted feed. Visscher et al. (2009) described opposite effects when comparing coarsely ground crumbled feed with finely ground crumbled feed. Mikkelsen et al. (2004) and Papenbrock et al. (2005) found no significant differences of pH in caecum when comparing coarsely ground feed with finely ground and pelleted feed. In the present study, there was a significant negative correlation between starch content related to DM and pH of caecum content ($r = -0.318$; $p = 0.020$). DM content in caecum was lower in animals receiving coarsely ground feed compared with animals that fed finely ground pelleted feed. Canibe et al. (2005) did not find differences between DM content in caecum after feeding coarsely ground feed and finely ground pelleted feed; however, measurements of DM content of proximal colon and mid colon resulted in comparable DM content in caecum as measured in the present study.

4.4. Slaughter traits and stomach weights

The larger fat surface of cutlet and decreased carcass lean meat percentage of animals that fed finely ground pelleted feed is likely related to the faster gaining of animals which also resulted in higher BW at slaughter. Park et al. (2003) reported higher backfat and lower carcass lean meat percentage in pigs receiving pelleted feed compared to pigs that fed mash feed and attributed these effects to higher energy level caused by the higher digestibility of nutrients. Stomach weights of animals that fed finely ground and pelleted feed were significantly lower than stomach weights of the other feeding groups. Earlier studies also described lower stomach weights when finely ground pelleted feed was offered (Eisemann and Argenzio 1999; Hedemann et al. 2005). This could be related to a more fluid stomach content which was observed in animals that fed finely ground pelleted feed in various studies (Fladlandsmo and Slagsvold 1971; Canibe et al. 2005). It was assumed that the higher stomach weights after feeding coarsely ground diets could be associated with higher motility of the stomach.

4.5. Liver weights and clinical chemistry

Higher liver weights in finely ground and pelleted feed compared to coarsely ground meal could be explained by functional adaptation caused by higher availability of nutrients (Kerr et al. 1995; Liermann et al. 2015). This could also be the explanation for the higher liver weights of pigs that fed Diet 2 compared to liver weights of pigs receiving Diet 1. With regard to analysed parameters of clinical chemistry, there was no indication of pathological alterations of liver caused by feeding. It was assumed that higher total protein concentration and cholesterol concentrations of blood in animals that fed finely ground and pelleted feed could be an indication of higher liver metabolism depending on higher availability of nutrients and resulting higher synthesis rate of the liver. However, correlations between liver weights and blood cholesterol were not significant, nor were correlations between other measured parameters of clinical chemistry and liver weights. Correlations between γ-glutamyltransferase and cholesterol were not significant; however, studies in cows of Dänicke et al. (2014) could support the aspect that the activity of γ-glutamyltransferase is closely related to cholesterol concentrations. If such relationships exist also for pigs, it needs to be examined further.

Animals receiving Diet 1 had higher blood urea concentrations compared with animals that fed Diet 2. Feed intake can be excluded as the main impact factor on this parameter, because animals that fed Diet 2 consumed more feed in the finisher phase. Furthermore, CP contents and DCP of the different diets were similar in this phase. Therefore, the effect could be associated with the choice of the different protein sources and the amino acid pattern. Besides CP content of diets and CP intake, the protein quality plays a key role by affecting serum urea content (Eggum 1970; Bassily et al. 1982; Cho et al. 2007). Eggum (1970) and Bassily et al. (1982) reported decreased blood urea content when diets with high protein quality were fed. They described the determination of blood urea as a rapid method to evaluate protein quality. Therefore, a higher protein quality of Diet 2 could be assumed.

5. Conclusion

Results of the present study indicated that it is possible to replace imported SBM with local protein sources without declining nutrient digestibility or performances of fattening pigs. However, successful fattening depends strongly on adequate feed treatment and processing to avoid the impact of anti-nutritional factors and overheating. Finely grinding and pelleting was useful to enhance the nutrient digestibility and reduced FGR by avoiding feed wastage by the animals. However, the possible adverse effects on stomach, such as erosions and ulcera, should be kept in mind when optimising technical feed treatments.

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Disclosure statement

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6 Paper III

Effects of two commercial diets and technical feed treatment on stomach lesions and immune system of fattening pigs

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Summary

The impact of technical feed treatment and diet on stomach lesions and traits of the local and systemic immune system were investigated in fattening pigs. Feeding groups differed in technical feed treatment (standard ground meal vs. finely ground and pelleted feed) and diet (soya bean meal vs. rapeseed meal/DDGS/soya beans). Pigs were fattened approximately 10 weeks by *ad libitum* feeding and slaughtered subsequently. Gastric alterations were assessed by a macroscopic scoring system [macroscopic stomach score (MSC) 0 = normal to 4 = severe lesions]. For immunological investigations, lymphocytes from blood and jejunal tissues were isolated. T-cell phenotyping was carried out by staining intestinal lymphocytes with monoclonal antibodies for CD4 and CD8 and flow cytometric measurements. MSC was higher in animals fed finely ground and pelleted feed compared with their counterparts. Significant interactions between diet and feed treatment considering the MSC were observed ($p = 0.027$). There was no effect of diet or technical feed treatment on T cells of blood, *Lymphonodi gastrici* or lamina propria (LP) and intraepithelial cells. However, technical feed treatment significantly affected subsets of CD4+, CD8+, CD8low, CD4/CD8 double-positive T cells, the mean fluorescence intensity of CD4+ T cells and the ratio of CD8low/CD8high T cells in Peyer's patches (PP). All named parameters were reduced in PP of animals fed finely ground and pelleted feed compared with animals fed standard ground meal. Furthermore, significant differences between T cells of lymph nodes and LP were observed between animals with middle MSC (MSC = 1–2.5) and animals with high MSC (MSC = 3–4). Significant alterations in T cells of PP were observed between animals of low (MSC = 0–0.5) and high MSC. The observed effects provide the evidence that the impact of technical feed treatment is not limited on the stomach lesions. Possible stimuli and consequences of the immune system should be studied in more detail.

Keywords: pigs, pelleting, stomach, intestinal immune system, $CD4^+$ cells, $CD8^+$ cells

Introduction

A high number of studies deal with the effect of technical feed treatment on stomach lesions and stomach milieu of pigs. Therefore, it is well known that particle size plays a key role in development of gastric lesions. Furthermore, hydrothermal treatment such as pelleting and expanding can promote lesions in the stomach (Friendship, 2003; Liermann et al., 2015). In contrast, the knowledge about possible effects of technical treatment on intestinal health is far away from being researched in depth. However, the intestine plays a key role in regulation of the nutrient absorption and protection against potential pathogens. Furthermore, there are clear evidences that technical feed treatment also has an influence on this part of the digestive system, for example, by influencing the morphological structure or the secretion of mucins (Brunsgaard, 1998; Hedemann et al., 2005). Studies of Liu et al. (2006) with broiler chickens even indicated that particle size affects immune cells of the intestine. They demonstrated that standard ground feed compared with finely ground feed significantly reduced the number of mast cells in the intestine of broilers and contributed to develop mast cell cluster in the apical region of the villi. While technical feed treatment per se seems to influence the immune system, it is likely that technical feed treatment induces a higher release rate or a development of new epitopes or allergens from the feed which can also modulate the immune response (Betscher et al., 2010).

A further aspect which can affect the intestinal health is the formulation of the diet. Soya bean meal is the most important protein source in fattening pig nutrition, and the effects on performances have been studied in detail. However, some studies of Barratt et al. (1978) and Stokes et al. (1987) showed that soya bean protein provoke morphological alterations in the villi and the lamina propria in calves and piglets. Furthermore, some nutrients or low concentrations of nutrients as well as its physical and chemical aspects of diet have direct or indirect regulatory effects on the immune response of the intestine (Klasing, 1998). Therefore, even in balanced diets it seemed to be reasonable to research the influence of diet on the digestive tract and especially on the immune response of the intestine in fattening pigs. The aim of the present trial was to study possible alterations in T-cell subsets as a part of the adaptive immune system of fattening pigs fed feeds differing in technical feed treatment and diet. Therefore, technically treated feeds that are associated with protective effects against stomach lesions were compared to technically treated feeds that are known to provoke stomach lesions. Furthermore, two diets differing mainly in protein sources (with soya bean meal vs. rapeseed meal/DDGS/soya beans) were used to study the impact of composition of common diet on the immune system of fattening pigs.

Material and methods

Experimental diets and design

One hundred cross-bred barrows [(German Landrace x German Large White) x Piétrain] were fattened for 10 weeks at the research station of the Friedrich-Loeffler- Institut in Brunswick, Germany. The experiment was part of a fattening trial which was published by Liermann et al. (2016).

The initial body weight (BW) of used animals was 34.6 ± 3.6 kg. According to regulation EU-RL 2010/ 63 (2010), the experimental pigs were individually housed in concrete-floored boxes (3.1 m^2 per pen) without bedding which were integrated in a mechanically ventilated building. All boxes were equipped with individual nipple drinkers. Feed and water were available *ad libitum* until immediately before slaughtering.

Pigs were divided into four feeding groups (25 animals per group) according to BW. The two basal experimental diets differed mainly in protein sources (soya bean meal vs. rapeseed meal/soya beans/DDGS) (Table 1). They were formulated according to common pig feed and the requirements of growing and finishing pigs (GfE, 2008), respectively, and were designed isoenergetically and isonitrogenously. Furthermore, two different technical feed treatments were compared (standard ground meal vs. finely ground and pelleted feed). While standard feeds were ground by roller mill, finely ground feeds were processed by hammer mill. The particle size distribution of standard feed was milled corresponding to commercial pig feed. In contrast, finely ground feed before pelleting had a higher degree of fineness than commercial pig feed (Table 2). Pellets (3 mm diameter) were produced by processing temperature of 74 °C and under steam conditions of 3%.

Table 1 Composition of experimental diets

	Starter phase (< 70 kg BW)		Grower phase (70 – 100 kg BW)		Finisher phase (> 100 kg BW)	
	Diet 1	Diet 2	Diet 1	Diet 2	Diet 1	Diet 2
Ingredients [g/kg diet]						
Soya bean meal	206.6	-	184.0	-	112.5	-
Rapeseed meal	-	150.0	-	150.0	-	150.0
Soya beans toasted	-	87.4	-	62.0	-	-
Maize	250.0	331.0	250.0	330.0	190.0	323.0
Barley	100.0	100.0	100.0	100.0	100.0	100.0
Wheat	271.0	217.5	226.6	218.0	288.0	273.6
DDGS*	-	60.0	-	60.0	-	60.0
Wheat bran	33.8	-	100.8	24.0	110.0	34.0
Maize gluten meal	-	19.0	-	21.0	61.5	1.2
Maize meal	100.0	-	100.0	-	100.0	26.0
Vinasse	10.0	10.0	10.0	10.0	10.0	10.0
Feed oil	2.3	-	5.4	-	6.0	-
Lysine HCL	2.8	4.8	1.1	3.2	2.0	3.2
DL-methionine	0.3	0.1	0.1	-	-	-
Diamol	10.0	10.0	10.0	10.0	10.0	10.0
Premix	13.2†	10.2†	12.0†	11.8†	10.0‡	9.0‡
Analysed feed components on dry matter basis						
ME§ [MJ/kg]						
Standard ground	13.7	14.1	13.6	13.3	14.1	14.5
Finely ground and pelleted	15.0	15.1	14.8	15.2	15.4	15.4
Crude protein [g/kg]						
Standard ground	197	215	186	177	178	172
Finely ground and pelleted	190	196	182	184	159	159

*DDGS, Distillers dried grain with soluble; †Premix delivers per kg feed: 20 mg copper; 6.9 g calcium; 5.4 g phosphorus; 1.8 g sodium; 10000 IU vitamin A; 1000 IU vitamin D; 75 mg vitamin E; ‡ Premix delivers per kg feed: 20 mg copper; 6.5 g calcium; 5.2 g phosphorus; 1.8 g sodium; 10000 IU vitamin A; 1000 IU vitamin D; 75 mg vitamin E; § ME, Metabolisable energy calculated on base of digestible nutrients (as analysed) according to the formula of the GfE (2008)

Table 2 Particle size distribution

Diet	Feed treatment	Particle size distribution [%] < 125 µm	125 –355 µm	360 –1000 µm	1005–2000 µm	> 2000 µm	D50† [mm]
Diets starter phase (<70 kg)							
Diet 1	Standard ground	10.13	16.00	37.38	34.50	2.00	0.86
	Finely ground, pelleted*	12.98	14.12	38.55	30.79	3.56	0.43
Diet 2	Standard ground	12.69	15.52	32.25	37.25	2.29	1.04
	Finely ground, pelleted*	20.86	23.96	38.09	16.82	0.27	0.71
Diets grower phase (70-100 kg)							
Diet 1	Standard ground	10.91	15.24	37.71	34.56	1.58	0.92
	Finely ground, pelleted*	16.15	21.16	42.09	20.38	0.22	0.41
Diet 2	Standard ground	9.34	14.61	28.82	29.63	5.14	0.73
	Finely ground, pelleted*	18.56	26.54	39.87	14.77	0.26	0.53
Diets finisher phase (> 100 kg)							
Diet 1	Standard ground	12.87	19.90	39.09	27.06	1.07	0.70
	Finely ground, pelleted*	22.66	21.01	39.87	16.33	0.13	0.39
Diet 2	Standard ground	12.48	16.37	33.56	35.64	1.94	0.57
	Finely ground, pelleted*	24.09	23.17	37.90	14.61	0.23	0.43

*Particle size distribution before compacting analysed by dry sieve analysis according DIN 66165-1:1987-04 and DIN 66165-2:1987-04; degree of fineness could be higher after pelleting; † D50, cumulative particle size distribution at 50 %

Sampling procedures

Animals were slaughtered in the slaughterhouse of the institute according to European regulations (EG 853/ 2004, 2004; EG 854/2004, 2004) at an average age of 5 months and after reaching a BW of 120 kg (126.5 ± 5.1 kg). Every day, three of every group were slaughtered, 12 animals all together. The chronical order of groups differed from day to day. Pigs were electrically stunned, killed by bleeding and scalded. Blood samples were collected during bleeding from 13 animals per feeding group in commercial tubes containing EDTA. Stomachs of all animals were dissected and cut longitudinally along *Curvatura major* for collection of mixed stomach content. As an indicator of stomach health, *Pars nonglandularis* were assessed by a macroscopic scoring system according to Liermann et al. (2015) (macroscopic stomach score – MSC; score 0 – intact epithelium, smooth and glistening white surface; score 1 – mild changes, partially bile staining and hyperkeratosis; score 2 – moderate degree of hyperkeratosis and bile staining over entire surface; score 3 – high-degree hyperkeratosis; and score 4 – severe lesions and scarring). Scoring of each stomach was carried out by the same person and blind to the treatment.

Further examined tissues were dissected from 13 pigs per feeding group corresponding to blood samples. *Lymphonodi gastrici* were collected from the region of *Curvatura gastrica minor*. The whole small intestine was dissected and separated from the mesenterium. Afterwards, the small intestine was placed in 1-m loops to locate the middle of this organ. Seventeen centimetres of Jejunal tissue was dissected 5 cm proximal from the located middle of the entire small intestine for isolation of epithelial cells. Proximal starting from the mentioned tissue, a further jejunal tissue (20 cm) was dissected for cell isolations of lamina propria. Furthermore, at least three Peyer's patches were separated from the jejunum. All tissue samples were stored in CUSTODIOL® (Dr. FRANZ KÖHLER Chemie GmbH, Bensheim, Germany) at 4 °C until further processing.

Analytical Methods

Stomach content

Dry matter of stomach chymus was analysed by methods corresponding to the Association of German Agricultural Analysis and Research Centres (VDLUFA) of Naumann and Bassler (1976). A pH meter, type WTW pH 530, was used (BLB, Brunswick, Germany) for pH measurements of mixed contents in stomach.

Haematology

Whole blood was analysed for red blood cell counts by using the haematology analyser Celltac-α (MEK 6450, Nihon Kohden Corporation, Tokyo, Japan) according to Stelter et al. (2013). Differentiation of white blood cells was performed by counting of at least 200 cells of blood smears according to morphological characteristics which were stained by Pappenheim solution.

Isolation of cells from lymph nodes

Cells from *Lymphonodi gastrici* were isolated by modified methods corresponding to Todd et al. (1999) and Solano-Aguilar et al. (2000). Briefly, surrounding tissues and serosa were removed from lymph nodes. Lymph nodes were fragmented by scalpel within cold phosphate-buffered saline (PBS). Then, the tissue-cell-suspension was extruded by cell sieves with a mash diameter of 50 μm.

Isolation of intraepithelial lymphocytes (IEL)

Isolation of intraepithelial cells was performed according to modified methods of Kearsey and Stadnyk (1996); Todd et al. (1999) as well as Davis et al. (2004). For obtaining of intraepithelial cells, 17-cm jejunal tissue without Peyer's patches was dissected and gently rinsed with PBS. The intestine was filled with PBS and the ends were tied up by sewing material. After 90 min of resting time, PBS was removed. The intestine was gently flushed by 10 ml of HEPES-buffered, Hanks balanced salt solution (HBSS without Ca^{2+} and Mg^{2+}; Biochrom GmbH, Berlin, Germany) containing 10 m_M dithiothreitol (DTT; Sigma-Aldrich® Chemie GmbH, Munich, Germany) and 10% foetal bovine serum (Biochrom GmbH, Berlin, Germany) five times in a row. The total of the five flushes was collected in a 50-ml tube and subsequently centrifuged for 5 min at 350 x *g* at room temperature. The supernatant was discarded by pipetting, and the resulting pellet was resuspended in 25 ml of 30% Percoll solution (diluted by HBSS; Sigma-Aldrich®, Chemie GmbH). The solution was centrifuged for 15 min at 350 x *g* at room temperature. The supernatant was removed once again and the pellet was resuspended in 5 ml of Roswell Park Memorial Institute medium (RPMI-1640, Biochrom GmbH, Berlin, Germany). Subsequently, cell suspension was sieved by CellTrics® (Partec GmbH, Görlitz, Germany) with a mash diameter of 50 µm. Viability of IELs assessed by the trypan blue exclusion method was >91%. Cell suspension was stored on ice until flow cytometric measurements.

Isolation of cells from Lamina propria (LP)

The isolation procedure of cells from *Lamina propria* was adapted from the method of Davis et al. (2004).

For isolation, 20 cm of jejunal tissue without Peyer's patches was used. Firstly, surrounding mesenterial tissue was cut and the intestine was incubated in PBS for 30 min on ice. The intestine was cut into small pieces of approximately 2 cm. Pieces were transferred into Erlenmeyer flask containing 100 ml of HBSS. A magnetic stir bare was added and samples were gently stirred for 20 min. After sedimentation of pieces, the supernatant was rejected. The wash procedure was repeated. Two incubation procedures followed with 25 ml of RPMI-1640 containing 180 U of collagenase type V (Sigma-Aldrich® Chemie GmbH) at moderate stirring for 20 min. The supernatant was transferred after each step into a 50-ml tube which was stored on ice. Then, 25 ml of RPMI-1640 was added which contained 260 U of collagenase. Samples were stirred for 30 min at a low speed. The supernatant was also transferred into the 50-ml tubes. Collected supernatants were centrifuged at 191 x *g* for 10 min at room temperature. The resulting pellet was washed three times by centrifugation with 30 ml of RPMI-1640. Subsequently, the pellet was resuspended in 5 ml of RPMI-1640 and sieved by CellTrics® with a mash diameter of 50 µm. Viability of isolated cells assessed by the trypan blue exclusion method was on average 81%. Cell suspension was stored on ice until flow cytometric measurements.

Isolation of cells from Peyer´s patches (PP)

Cells of Peyer´s patches were isolated by using a modified method according to Solano-Aguilar et al. (2000).

Peyer's patches were rinsed by PBS, fragmented into pieces of approximately 2 cm and transferred into a 50-ml tube containing 40 ml of HBSS-DTT (HBSS without Ca^{2+} and Mg^{2+}, 2 m_M DTT, 0.01 $_M$ Hepes). Tubes were stored in a water bath (37 °C) for 20 min. After incubation, samples were shaken for 15 s. After sedimentation of tissue pieces, the supernatant were discarded. The pieces of PP were resuspended in 45 ml of HBSS/EDTA/HEPES (HBSS without Ca^{2+} and Mg^{2+}, 1 m_M EDTA, Sigma-Aldrich® Chemie GmbH, Munich, Germany, 1 m_M HEPES) and incubated in water bath (37 °C) for 20 min. Samples were vortexed once again and the supernatant was discarded. Then, the tissues were fragmented into smaller pieces by scalpel and transferred into a 50-ml tube containing 50 ml of medium. Samples were centrifuged at 600 x *g* for 10 min. Supernatant containing remained tissue pieces was gently rejected. The pellet was resuspended in 13 ml of a 40% Percoll gradient. A total of 6.5 ml of a 75% Percoll gradient which was filled in a 15-ml tube was covered by 6.5 ml of cell suspension. The tubes were centrifuged at 1000 x *g* for 30 min without break. Subsequently, the lymphoid cells were pipetted from the interphase between the two Percoll gradients. Cells were washed twice with 5 ml of RPMI-1640 by centrifugation for 10 min at 300 x *g* at room temperature. The resulting pellet was resuspended in 2 ml of RPMI-1640 and sieved by a CellTrics® with a mash diameter of 50 µm. Viability of isolated cells assessed by the trypan blue exclusion method was on average 91%. Cell suspension was stored on ice until flow cytometric measurements.

Flow cytometric measurements

Flow cytometric analyses were performed as previously described by Stelter et al. (2013). Briefly, isolated leucocytes and blood cells were stained with monoclonal antibodies for $CD4^+$

(mouse anti-pig CD4a: FITC; AbD Serotec, Oxford, United Kingdom) and CD8$^+$ (mouse anti-pig wCD8a: PRE; AbD Serotec, Oxford, United Kingdom). For corresponding isotype controls, cells were treated with mouse IgG2a negative control: RPE and mouse IgG2b negative control: FITC (AbD Serotec, Oxford, UK). Samples were incubated for 30 min at room temperature in the dark and washed by centrifugation in HBSS. Red blood cells of whole blood samples were lysed in a lysis buffer (BD FACS^{TM} Lysing Solution, BD Biosciences, San Jose, USA) before centrifugation. After pipetting in a 96- well microplate, samples were measured by FACS Canto II (BD Biosciences). An acquisition gate was set for the leucocyte populations in accordance with its side scatter and forward scatter properties. At least 10000 cells were counted. Non-specific signals of FITC and PE were evaluated and compensated by the BD FACSDiva^{TM} Software (BD Biosiences).

Calculation and statistics

The ratio of CD4$^+$ and CD8$^+$ T cells was calculated by dividing measured proportions of total CD4$^+$ T cells by proportions of CD8$^+$ T cells. CD8^{low} and CD8^{high} were defined according to the mean fluorescence intensity of CD8$^+$ cells. Dividing of CD8^{low} T cells and CD8^{high} T cells resulted in the ratio of CD8^{low} and CD8^{high} T cells.

For statistical analyses, MIXED procedure of SAS Enterprise Guide 4.3 was used. The variance analysis of parameters of stomach and stomach content, red and white blood cell counts and T cells included fixed effects of diet and technical feed treatment as well as their interaction. Further variance analyses were used to examine the impact of the different localisations on T-cell subsets. In a second step, animals were divided according to the MSC classes on the basis of the degree of alterations in the *Pars nonglandularis* in low MSC = 0–0.5, middle MSC = 1–2.5 and high MSC 3–4. Differences in T-cell subsets and mean fluorescence intensity of T cells between animals with different MSC classes were also proved by the variance analysis. Differences were characterised as significant when $p \leq 0.05$. The correlation coefficient according to Pearson was determined using STATISTICA 12 and assessed to be significantly different from zero at $p \leq 0.05$.

Results

Parameters of stomach health and stomach content

Collected data of stomach and stomach content are summarised in Table 3. Effects of technical feed treatment on stomach score (MSC) were highly significant. Standard ground feed resulted in stomach score of 0.60 (Diet 1) and 0.68 (Diet 2). In contrast, animals of both feeding groups with finely ground and pelleted feed showed higher average MSC. Further, MSC of animals fed finely ground and pelleted Diet 1 were significantly higher than MSC of animals fed finely ground and pelleted Diet 2. A significant interaction was observed between diet and technical feed treatment considering the MSC ($p = 0.027$). In addition, pH values of stomach content of animals fed finely ground and pelleted feed were significant lower than in chymus of pigs fed standard ground feed with standard diet (Diet 1) ($p = 0.025$). There was no significant effect of technical feed treatment on DM content in stomach chymus, although standard ground Diet 1 resulted in higher DM contents than other feeds. Only the diet tended to affect the stomach score ($p = 0.088$).

Table 3 Parameters of stomach score and stomach content

Variable	Feeding group				PSE*	*p*-Value		
	Diet 1		Diet 2					
	Standard ground	Finely ground, Pelleted	Standard ground	Finely ground, pelleted		Diet	Technical feed treatment	Diet x Technical feed treatment
Stomach score	0.60^{c}	3.08^{a}	0.68^{bc}	2.48^{b}	0.15	0.088	< 0.001	0.027
pH mixed stomach content	4.76^{a}	4.17^{b}	4.29^{ab}	4.07^{b}	0.18	0.120	0.025	0.314
DM mixed stomach content [%]	13.43	10.88	12.15	12.39	1.32	0.931	0.381	0.293

PSE, pooled standard error; DM, dry matter; different subscripts characterise significant differences between feeding groups ($p < 0.05$).

Haematology

Neither diet nor technical feed treatment significantly affected parameters of red blood cells or white blood cell counts (Table 4). However, there was a significant interaction between diet and technical feed treatment in case of red blood cell ($p = 0.014$) and lymphocyte counts ($p = 0.032$).

Table 4 Parameters of red and white blood cell count (LSMeans; n = 13)

Variable	Feeding group				PSE	*p*-Value		
	Diet 1		Diet 2					
	Standard ground	Finely ground, Pelleted*	Standard ground	Finely ground, pelleted		Diet	Technical feed treatment	Diet x Technical feed treatment
Red blood cells								
RBC [10^6/µl]	6.72	7.28	7.25	6.95	0.17	0.544	0.435	0.014
HGB [g/dl]	14.10	14.81	14.84	14.40	0.38	0.674	0.723	0.140
HCT [%]	38.51	40.77	40.68	39.89	1.07	0.553	0.499	0.162
MCV [fl]	57.28	56.02	56.16	57.34	0.65	0.872	0.953	0.066
MCH [pg]	20.99	20.36	20.47	20.70	0.23	0.701	0.395	0.071
MCHC [g/dl]	36.67	36.36	36.48	36.07	0.22	0.287	0.104	0.826
Normoblasts [%]	0.00	0.00	0.00	0.03	0.01	0.237	0.237	0.237
White blood cells								
WBC [$\cdot 10^3$/µl]	15.10	16.11	16.41	16.92	1.27	0.408	0.553	0.843
Lymphocytes [%]	74.12	70.86	67.81	73.06	1.95	0.608	0.294	0.032
Segmented neutrophils [%]	18.48	24.81	19.25	19.23	2.02	0.237	0.123	0.120
Banded neutrophils [%]	0.15	0.27	0.70	0.15	0.27	0.441	0.423	0.223
Monocytes [%]	4.83	4.50	6.00	3.75	0.87	0.808	0.143	0.272
Eosinophils [%]	1.71	2.29	1.81	2.27	0.37	0.163	0.912	0.880
Basophils [%]	0.71	0.91	0.81	1.52	0.32	0.162	0.277	0.428

*Haemolytic samples were excluded, n = 12; PSE, pooled standard error; RBC, red blood cells; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; Mean corpuscular haemoglobin; ‡‡ MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells

Flow cytometric measurements

Immune cell distribution depending on different localisation

The differences between T-cell subsets are summarised in Table 5. Lowest proportions of $CD4^+$ T cells were measured in the epithelium and highest proportions in lymph nodes considering all feeding groups regardless of the possible impact of diet or technical feed treatment. Subsets of $CD4^+$ T cells of all localisations differed significantly from each other except for proportions of $CD4^+$ T cells of lamina propria (LP) and Peyer's patches (PP). Proportions of $CD8^+$ T cells were highest in the epithelium. PP had the lowest proportions of $CD8^+$ T cells when comparing the different localisations. Proportions of $CD8^+$ T cells of blood and LP were similar. Furthermore, proportions of $CD8^+$ T cells of lymph nodes also equalled proportions of LP. Differences between subsets of $CD8^+$ T cells to other localisations were significant. Proportions of $CD8^{low}$ T cells were lowest in LP and highest in lymph nodes when comparing the different localisations. All localisations differed significantly from each other in proportions of $CD8^{low}$ T cells. In blood and PP, the lowest proportions of $CD8^{high}$ T cells were measured. Highest proportions of these phenotypes were measured in the epithelium. Blood had equal proportions of $CD8^{high}$ T cells of lymph nodes and PP. Proportions of $CD8^{high}$ T cells of lymph nodes and PP were also similar. Proportions of $CD8^{high}$ T cells of other localisations differed significantly from each other. Proportions of CD4/CD8 double- positive T cells were lowest in LP and highest in blood and lymph nodes. Subsets of CD4/CD8 double positive T cells were similar in blood and lymph nodes. Similar proportions of these cell types were also measured in epithelium and LP. Proportions of $CD4^+$ T cells were lower than proportions of $CD8^+$ T cells in case of blood, intraepithelial lymphocytes (IEL) and LP. However, the range between these cells was highest in the epithelium which is also reflected in the very low ratio of $CD4^+$ and $CD8^+$ T cells. In contrast, PP had higher proportions of $CD4^+$ than of $CD8^+$ T cells. In lymph nodes, proportions of these cell types were nearly similar. While $CD8^{low}$ T-cell proportions were higher than that of $CD8^{high}$ T cells in blood and lymph nodes, an opposite relation was observed in localisations of the intestine.

Table 5 T-cell subsets depending on localisation (LSMeans)

T-cell phenotype	Blood *n* = 47[*]	Lymph node *n* = 47[*]	Epithelium *n*= 52	Lamina propria *n* = 52	Peyer´s patches *n* = 52	PSE[*]	p- Value
CD4^{+} total [%]	19.6^{c}	40.6^{a}	8.7^{d}	30.7^{b}	27.6^{b}	1.5	< 0.001
CD8^{+} total [%]	33.2^{c}	39.3^{b}	79.8^{a}	34.8^{bc}	23.5^{d}	1.5	<0.001
CD8^{low} [%]	19.8^{b}	24.0^{a}	13.3^{c}	5.5^{d}	10.2^{e}	0.8	<0.001
CD8^{high} [%]	13.4^{c}	15.3^{c}	66.6^{a}	29.3^{b}	13.3^{c}	1.3	< 0.001
Double stained[‡] [%]	9.8^{a}	9.8^{a}	4.1^{c}	3.6^{c}	7.3^{b}	0.6	< 0.001

PSE, pooled standard error; [*]Five animals have to be excluded because of technical difficulties; [†]double stained cells of CD4^{+} and CD8^{+}; LSMeans within rows without a common subscript differ significantly ($p < 0.05$).

Immune traits depending on feeding groups

Diet and technical feed treatment had no effects on analysed T cells of blood and Lymphonodi gastrici as well as on IELs or cells of LP ($p > 0.05$) (data not shown). However, proportions of total CD4^{+}, total CD8^{+}, CD8^{low} and CD4/CD8 double-positive stained T cells were significantly influenced by technical feed treatment in case of PP ($p < 0.05$) (Table 6). Proportions of all named cell types were less frequent in animals fed finely ground pelleted feed. Additionally, the density of expressed CD4^{+} molecules on T-cell surface, described by the mean fluorescence intensity (MFI), and the ratio of CD8^{low} and CD8^{high} T cells were also decreased in animals of finely ground and pelleted feed compared with pigs which consumed standard ground meal.

Table 6 Effect of diet and feed treatment on T-cells of Peyer´s patches (*n* = 13)

Variable	Feeding group				PSE[*]	p-Value		
	Diet 1		Diet 2					
	Standard ground	Finely ground, Pelleted	Standard ground	Finely ground, pelleted		Diet	Technical feed treatment	Diet x Technical feed treatment
Peyer´s patches								
CD4^{+} total [%]	33.6^{a}	23.6^{ab}	30.7^{ab}	22.6^{b}	2.8	0.492	0.002	0.743
CD4^{+} total MFI[†]	754^{a}	539^{ab}	659^{ab}	527^{b}	60	0.377	0.006	0.489
CD8^{+} total [%]	27.3	21.0	26.4	19.4	2.8	0.671	0.022	0.909
CD8^{+} total MFI[†]	2013	1666	1976	1572	269	0.809	0.169	0.916
CD8^{low} [%]	12.8^{a}	8.6^{bc}	12.2^{ab}	7.3^{c}	1.1	0.387	< 0.001	0.726
CD8^{high} [%]	14.5	12.3	14.2	12.2	2.3	0.919	0.355	0.978
CD8^{low}/CD8^{high} ratio	1.0	0.9	1.1	0.7	0.1	0.523	0.026	0.349
CD4^{+}/CD8^{+} ratio	1.3	1.3	1.2	1.2	0.1	0.329	0.848	0.729
Double stained[*] [%]	8.3^{ab}	6.4^{ab}	9.9^{a}	4.8^{b}	1.0	0.964	0.001	0.115

PSE, pooled standard error; MFI, mean fluorescence intensity; [*]Double stained cells of CD4^{+} and CD8^{+}; LSMeans within rows without a common subscript marks significant differences between feeding groups ($p < 0.05$).

Immune traits depending on MSC classes

Significant differences were also observed when dividing animals on the basis of their macroscopic stomach changes using the MSC classes low (MSC = 0–0.5), middle (MSC = 1–2.5), high (MSC = 3–4) (Tables 7 and 8). The low MSC class included 11 animals fed the standard ground Diet 1, one animal fed the finely ground and pelleted Diet 2, nine animals fed the standard ground Diet 2 and one animal fed the finely ground and pelleted Diet 2. Two animals fed the standard ground diet 1, four animals fed the finely ground and pelleted Diet 2, four animals fed standard ground Diet 2 and seven animals fed the finely ground and pelleted Diet 2 were allotted to the middle MSC class. The high MSC class contained eight animals fed the finely ground and pelleted Diet 1 and five animals fed the finely ground and pelleted Diet 2.

Animals with high MSC had significantly lower proportions of $CD4^+$ T cells and a lower ratio of $CD4^+$ and $CD8^+$ T cells in lymph nodes than animals with middle MSC. Furthermore, CD4/CD8 double-positive T cells of lymph nodes were significantly reduced in animals with high MSC compared with animals with low MSC. In contrast, proportions of $CD4^+$ T cells and the ratio of $CD4^+$ and $CD8^+$ T cells of LP were significantly increased in animals with high MSC compared with animals with middle MSC. There were also significant alterations in proportions of $CD8^{low}$ T cells according to the MSC classes. Highest proportions of these cell types were measured in animals with high MSC in this localisation. $CD4^+$ T cells tended to decrease in animals with high MSC compared with animals which had a low MSC. Additionally, proportions of $CD8^{low}$ T cells and CD4/CD8 double-positive T cells of PP were significantly reduced in animals with high MSC compared with animals which had a low MSC. The ratio of $CD8^{low}$ and $CD8^{high}$ T cells was significantly lower in animals with middle MSC compared with animals with low MSC in case of PP.

Table 7 T cells of blood and *Lymphonidi gastrici* depending on MSC classes

Variable	MSC				p-Value
	Low (0 – 0.5) $n = 19^{\dagger}$	Middle (1 – 2.5) $n = 16^{\ddagger}$	High (3 – 4) $n = 12^{\dagger}$		
Blood					
CD4$^+$ total [%]	19.4	20.8	17.4	1.0	0.087
CD4$^+$ total MFI‡	840	835	732	78	0.589
CD8$^+$ total [%]	34.7	33.5	30.0	1.7	0.171
CD8$^+$ total MFI‡	4784	4747	4409	316	0.693
CD8^{low} [%]	21.0	20.0	17.4	1.3	0.148
CD8^{high} [%]	13.7	13.5	12.6	1.0	0.722
CD8^{low}/CD8^{high} ratio	1.7	1.6	1.4	0.2	0.514
CD4$^+$/CD8$^+$ ratio	0.6	0.6	0.6	0.0	0.359
Double stained* [%]	10.9	9.8	8.1	0.8	0.090
Lymphonodi gastrici					
CD4$^+$ total [%]	41.3^{ab}	42.3^{a}	36.3^{b}	1.4	0.019
CD4$^+$ total MFI‡	1554	1584	1319	86	0.099
CD8$^+$ total [%]	39.0	37.5	42.0	1.6	0.193
CD8$^+$ total MFI‡	3729	3360	3786	275	0.507
CD8^{low} [%]	22.7	23.9	26.0	2.0	0.525
CD8^{high} [%]	16.2	13.6	16.0	2.0	0.554
CD8^{low}/CD8^{high} ratio	4.3	5.5	2.4	2.3	0.702
CD4$^+$/CD8$^+$ ratio	1.1^{ab}	1.1^{a}	0.9^{b}	0.1	0.022
Double stained* [%]	11.1^{a}	9.6^{ab}	8.3^{b}	0.7	0.034

MSC, macroscopic stomach score; PSE, Pooled standard error; MFI, Mean fluorescence intensity; *Double stained cells of CD4$^+$ and CD8$^+$; †Three animals and ‡two animals have to be excluded because of technical difficulties. Different subscripts characterise significant differences between MSC – classes ($p < 0.05$).

Table 8 T cells isolated from intestine depending on MSC classes

Variable	MSC			PSE	p-Value
	Low (0 – 0.5) $n = 22$	Middle (1 – 2.5) $n = 17$	High (3 – 4) $n = 13$		
Intraepithelial cells					
CD4$^+$ total [%]	10.3	6.3	9.1	2.5	0.490
CD4$^+$ total MFI	417	213	275	95	0.262
CD8$^+$ total [%]	80.9	80.8	76.8	3.1	0.613
CD8$^+$ total MFI	9496	9628	9313	665	0.953
CD8^{low} [%]	12.8	14.4	12.6	1.6	0.686
CD8^{high} [%]	68.1	66.3	64.2	3.1	0.694
CD8^{low}/CD8^{high} ratio	0.2	0.2	0.2	0.0	0.536
CD4$^+$/CD8$^+$ ratio	0.1	0.1	0.1	0.2	0.503
Double stained* [%]	5.5	2.2	4.5	1.4	0.246
Cells of Lamina propria					
CD4$^+$ total [%]	31.8^{ab}	21.8^{b}	40.6^{a}	3.7	0.007
CD4$^+$ total MFI	821	640	925	80	0.067
CD8$^+$ total [%]	36.9	33.1	33.3	2.9	0.560
CD8$^+$ total MFI	4308	4080	3702	420	0.614
CD8^{low} [%]	6.1	4.0	6.4	0.7	0.037
CD8^{high} [%]	30.9	29.1	26.9	2.7	0.598
CD8^{low}/CD8^{high} ratio	0.2	0.2	0.2	0.0	0.069
CD4$^+$/CD8$^+$ ratio	1.0^{ab}	0.7^{b}	1.5^{a}	0.0	0.024
Double stained* [%]	3.6	2.4	5.0	1.0	0.264
Peyer´s patches					
CD4$^+$ total [%]	31.8	25.5	23.3	2.5	0.042
CD4$^+$ total MFI	693	581	547	54	0.136
CD8$^+$ total [%]	26.0	22.5	20.7	2.5	0.314
CD8$^+$ total MFI	1879	1797	1698	237	0.872
CD8^{low} [%]	12.4^{a}	9.2^{ab}	7.8^{b}	1.0	0.006
CD8^{high} [%]	13.6	13.3	12.9	2.0	0.970
CD8^{low}/CD8^{high} ratio	1.1^{a}	0.8^{b}	0.8^{ab}	0.1	0.026
CD4$^+$/CD8$^+$ ratio	1.3	1.2	1.3	0.1	0.271
Double stained* [%]	9.2^{a}	6.4^{ab}	5.3^{b}	0.9	0.010

MSC, macroscopic stomach score; PSE, Pooled standard error; MFI, Mean fluorescence intensity; *Double stained cells of CD4$^+$ and CD8$^+$; different subscripts mark significant differences between MSC – classes ($p < 0.05$).

Correlations between parameters of stomach and T-cells

Significantly negative correlations between MSC and the proportions of CD8$^+$ T cells of blood as well as MSC and proportions of CD4$^+$, CD8^{low} and CD4/CD8 double-positive T cells of PP were observed (Table 9). Additionally, the MSC was negatively correlated with the ratio of CD8^{low} and CD8^{high} subsets of PP. The pH of stomach content was correlated with the MFI of CD8$^+$ T cells and the ratio of CD8^{low} and CD8^{high} T cells of PP. Furthermore, positive correlations were observed between DM of stomach content and proportions of CD8$^+$ T cells of lymph nodes, proportions of CD4$^+$ of LP and proportions of CD4$^+$ of PP as well as between DM and the ratio of CD4$^+$ and CD8$^+$ T cells and proportions of CD4/CD8 double-positive T cells of LP. The MFI of CD4$^+$ T cells of LP was negatively correlated with the DM of stomach content.

Table 9 Significant correlations between parameters of stomach score or stomach content and T cells

Variable	Correlation coefficient (*r*)	p- Value
MSC to …		
CD8$^+$ total [%] of blood	- 0.288	0.050
CD4$^+$ total [%] Peyer´s patches	-0.357	0.001
CD8^{low} [%]of Peyer´s patches	-0.447	0.001
CD8^{low}/CD8^{high} ratio Peyer´s patches	-0.274	0.049
Double stained* of Peyer´s patches	-0.388	0.004
pH of stomach content to …		
CD8$^+$ total MFI‡ of Peyer´s patches	0.284	0.041
CD8^{low}/CD8^{high} ratio of Peyer´s patches	-0.348	0.012
DM of stomach content to …		
CD8$^+$ total [%] of lymph nodes	0.309	0.035
CD4$^+$ total [%] of lamina propria	0.394	0.004
CD4$^+$ total MFI of lamina propria	-0.304	0.029
CD4$^+$/CD8$^+$ ratio of lamina propria	0.361	0.008
Double stained* of lamina propria	0.281	0.044
CD4$^+$ total [%] of Peyer´s patches	0.310	0.025

MSC, macroscopic stomach score; PSE, pooled standard error; MFI, mean fluorescence intensity; ^{*d}ouble stained cells of CD4$^+$ and CD8$^+$

Discussion

The present study focused on the effects of technical feed treatment and diet on stomach lesions and stomach content as well as on the local and systemic immune system of fattening pigs. Further, the relationships between the stomach lesions and the immunological response of the intestine were studied.

No animal received medical treatment during fattening period or had to be excluded prematurely from the trial. Except for stomach lesions, no obvious pathological alterations were noticed during fattening period or at slaughtering, which was also indicated by the parameters of the red and white blood cell counts.

Stomach score and stomach content

The stomach score (MSC) and the pH value of the mixed stomach content were mainly influenced by the technical feed treatment. Animals fed standard ground meal had a lower MSC indicating less stomach lesions in *Pars nonglandularis* compared with finely ground and pelleted feed. This aspect agrees with several previous studies which compared coarsely ground feed or feed with similar particle size distribution of the standard feed with finely ground pelleted feed (Eisemann and Argenzio, 1999; Mikkelsen et al., 2004; Grosse-Liesner et al., 2009; Liermann et al., 2015). The named authors suggested that the more fluid stomach content and higher mixing of stomach content after feeding finely ground and pelleted feed provoke the contact between harmful substances of pyloric region and the nearly unprotected *Pars nonglandularis* which induces lesions in stomach of pigs. In contrast, coarsely ground meal promotes the physiologic stratification of stomach content which separates these regions from each other (Mösseler et al., 2012). In the present study, a lower pH value was measured in mixed stomach content of animals fed finely ground and pelleted feed compared with their counterparts. However, there were no significant correlations between pH values of stomach content and MSC. Mösseler et al. (2012) described a pH gradient after feeding coarsely ground meal while finely ground and pelleted feed resulted in nearly homogenous pH values. Therefore, pH value in mixed stomach content as measured in the present experiment does not necessarily reflect the situation at the *pars nonglandularis* which induced these stomach lesions. Differences between DM of mixed stomach contents of feeds were not significant. It is possible that the degree of fineness of the standard ground feed was insufficient to provoke a markedly higher DM content in stomach. Although there was no significant influence of technical feed treatment on DM, a significantly negative correlation was found between DM of stomach content and MSC ($r = -0.294$; $p = 0.003$). Therefore, it can be assumed that significantly higher MSC of animals fed finely ground and pelleted feed of Diet 1 compared with feed of Diet 2 with same technical feed treatment was possibly induced by numerically lower DM of stomach content. The importance of DM percentage and of consistency of stomach content to protect the *Pars nonglandularis* was also suggested by Mikkelsen et al. (2004) and Liermann et al. (2015).

Immune cell distribution depending on different localisation

Isolation of T cells from different localisations of the intestine, lymph nodes and blood allowed a comparison between T-cell subsets of the local and systemic immune system in fattening pigs despite a potential influence of technical feed treatment and diet. High subsets of $CD8^+$ T cells were observed in particularly striking manner in case of isolated intraepithelial lymphocytes (IEL). In contrast, only a few $CD4^+$ T cells were found in this region. The higher number of $CD8^+$ T cells compared with CD4+ T cells was also previously described by Rothkötter et al. (1991), Vega-Lopez et al. (1993) and Stokes et al. (1994). In the present study, remarkable higher $CD8^{high}$ T cells could also be observed within the epithelium. The high number of $CD8^+$ T cells that are able to attack directly pathogen-infected cells and are generally referred as cytotoxic cells (Calder, 2001) along with CD8^{high} T cells reflects the natural function of the epithelium as the first defence between the harsh conditions of the lumen and the tissues of the intestine. Besides CD8^{+} T cells, a high number of CD4^{+} T cells were observed in *Lymphonodii gastrici*, LP and PP which function as helper/inducer cells (Miceli and Parnes, 1993) by expressing cytokines and are connected for a specific memory function. The balanced proportions of CD4^{+} and CD8^{+} emphasise the regulatory function of these tissues. Similar results were demonstrated by Zuckermann and Gaskins (1996). The higher subsets of CD4/CD8 double-positive T cells in blood and lymph nodes compared with other localisations agree with the results of Zuckermann and Gaskins (1996) although these authors found no significant differences between lymph nodes and PP. Furthermore, T cells of this phenotype were significantly higher in PP compared with the epithelium and the LP. CD4/CD8 double-positive T cells were suggested to be comprised of memory cells because of the higher abundance, especially in older pigs. Therefore, it is likely that they have a key function in protective immunity and in immune regulation (Zuckermann, 1999).

Immune traits depending on feeding groups

In previous studies, it was demonstrated that soya bean protein provokes morphological alterations and damage of the villi and LP in calves and piglets (Barratt et al., 1978; Stokes et al., 1987). It is also known that different nutrients as well as its physical and chemical aspects have direct or indirect regulatory effects on the immune response of the intestine (Klasing, 1998). Therefore, a possible impact of diet was hypothesised and motivated the choice to consider two diets which differ especially in protein source within the present trial. Already Sun et al. (2008) demonstrated that feeding glycinin, which is regarded as an important feed antigen of soya beans, induced an increase of CD4^{+} T cells and CD8^{+} T cells in the blood of piglets in a dose-dependent manner. In the present study, there was an effect of diet neither on the T cells isolated from blood nor on T cells of different localisations of the jejunal intestine or lymph nodes. The lack of different diets to affect the immune system could possibly be explained by deactivation of antinutritional substances by hydrothermal pre-treatment of feed stuffs such as soya beans, soya bean meal or rapeseed meal, which was proved by decreased urease activity or glucosinolates (Liermann et al., 2016). The age of the pigs could be another reason for the absence of significant influences of diet on T cells. Barratt et al. (1979) demonstrated an age-dependent immune modulation to soya bean proteins in calves while younger calves showed a higher immune response after feeding a soya diet than older calves. Although the effect on T cells was not considered in studies of Barratt et al. (1979), it is possible that T-cell subsets react also on different diets in an age-dependent manner. Despite the lack of T-cell alteration in response to diet, an immune response caused by different diets cannot be excluded because only T-cell subsets and the mean fluorescence intensity (MFI) of them were examined in the present study. Therefore, in following studies, further parameters such as B-cell subsets should be considered in the examinations. Additionally, immunoglobulin A and immunoglobulin G or cytokines such as interleukin-4 and interleukin- 6 could be considered, which were affected by soya bean proteins in studies of Li et al. (1990) and Sun et al. (2008) in pigs. While no effect of diet on T cells were observed, there was an influence of technical feed treatment on T-cell subsets of PP. Interestingly, subsets of $CD4^+$ and $CD8^+$ T cells were higher in jejunal PP of animals fed standard ground meal compared with animals fed finely ground and pelleted feed. In studies with mice, Alvarez et al. (1998) and Perdigon et al. (1999) observed an increase of $CD4^+$ and $CD8^+$ T cells in LP by oral administration of *Lactocacillus casei* and *Lactobacillus plantarum*. However, $CD4^+$ T-cell subsets or CD8+ T-cell subsets of PP were not determined in these studies. There is also a natural occurrence of several lactic acid bacteria in the intestine such as *Lactobacillus plantarum* (Leser et al., 2002). Canibe et al. (2005) found significantly higher density of lactic acid bacteria in stomach and distal small intestine of pigs fed standard ground meal compared with animals fed finely ground and pelleted feed. These authors demonstrated further that standard ground feed resulted in significantly higher concentrations of lactic acid in stomach and slightly higher concentrations of lactic acid in proximal and mid-small intestine compared with finely ground and pelleted feed. Microbial status or lactic acid concentrations of intestinal chymus were not determined in the present study; however, it could be possible that microbial alterations within the gastrointestinal tract could induce the observed immune modulation of PP after feeding different technically treated feeds. The higher MFI of $CD4^+$ T cells observed in PP of animals fed standard ground meal compared with finely ground and pelleted feed may indicate a higher density of expressed $CD4^+$ molecules on the T-cell surface. An influence of microbial status could be also assumed in case of MFI; however, this parameter was not described in the prior studies. The same applies to changes of CD4/CD8 double-positive T cells. The significant effect of technical feed treatment on $CD8^{low}$ T cells could be associated with higher CD4/CD8 double- positive cells. A strongly significant positive correlation ($r = 0.799$; $p < 0.001$) was observed between $CD8^{low}$ and CD4/CD8 double-positive T cells of PP. However, $CD8^{high}$ T cells were not correlated with CD4/CD8 double-positive T cells ($r = 0.180$; $p = 0.202$). Zuckermann (1999) reported also that double-positive CD4/CD8 T cells mainly exhibit a $CD8^+$ low phenotype.

Immune traits depending on MSC classes

Interestingly, significant alterations in T cells were observed after dividing animals into groups according to the MSC classes of low (MSC = 0–0.5), middle (MSC = 1–2.5) and high stomach score (MSC = 3–4) on the basis of the physiologic relevance of the observed stomach lesions. Except for CD4/CD8 double- positive T cells isolated from lymph nodes, significant differences between T-cell subsets of lymph nodes and LP were observed between animals with middle MSC and animals with high MSC. However, the T cells of lymph nodes and LP were affected in an opposite manner. Significant alterations in T-cell subsets of PP were mainly observed between animals of low and high MSC expect for the ratio between $CD8^{low}$ and $CD8^{high}$ of T cells isolated from PP. The immune modulation of animals from different MSC classes could be a clear evidence for relations between the development of stomach lesions and alterations in T-cell subsets in lymph nodes, LP as well as PP or MFI in CD4+ of LP; especially with regard to examination of tissues close to the stomach, it could be assumed that these alterations were associated with the stomach milieu. Interestingly, a correlation existed between the pH value and the DM of stomach content and almost all examined T-cell parameters of LP and PP which altered significantly between MSC classes.

Although further significant differences were observed between animals with different MSC classes, no further correlations to the examined parameters of stomach content were found. This indicated that further parameters which can affect the stomach lesions can also influence the T cells of different tissues of the intestine or lymph nodes. Potential factors in stomach contents which differed in accordance with alterations in stomach lesions in previous studies were the concentrations of organic acids or the microbial composition (Mikkelsen et al., 2004). Furthermore, it is likely that several pathogens can pass through the stomach lesions into the blood and induce systemic effects which could explain the immune modulation in the *Lymphonodi gastrici*.

Conclusion

With regard to animal welfare and health aspects, according to the present results, standard ground meal is to be preferred to finely ground and pelleted feed. The present study clearly shows that technical feed treatment had effects besides influences on the stomach lesions. Different technically treated feeds can also affect the T-cell population of Peyer's patches within the small intestine. Further studies are needed to clarify the reasons and mechanism of observed immune modulations. Furthermore, the relationships between alterations in MSC and intestinal immune modulation should be studied in more detail. This could be useful for deeper understanding of local and systemic immunological mechanisms and development and function of T cells in pigs.

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7 General discussion

The aim of the present examinations was to balance the benefits and disadvantages of different technical feed treatments in pig fattening. For this reason coarsely ground and finely ground mixed feed were tested against each other. While the degree of fineness of “coarsely ground feed” used in the present studies corresponded to the standard degree of fineness of common German pig feed, the degree of fineness of used “finely ground feed” was higher compared to common German pig feed. Additionally, the effects of the hydro-thermal treatments pelleting, expanding and expanding and re-pelleting of a complete mixed feed were compared to feed without hydro-thermal treatment. Subsequently, the combination of the mentioned processing methods allowed the direct comparison of the impact of feeds differing in feed processing degree in pig fattening. In the following discussion the classification of the various feed processing degrees as shown in Table 4 is based on the one hand on the extent of grinding and on the other hand on the level of heat and pressure supply used during processing. While coarsely ground meal feed was classified as the feed with the lowest processing degree, finely ground expanded and re-pelleted feed was defined as the feed with the highest processing degree.

Table 4 Classification of processing degree of various technically treated feeds

Technical treated feed	Processing degree
Coarsely ground meal (without hydrothermal treatment)	1
Finely ground meal (without hydrothermal treatment)	2
Coarsely ground, pelleted feed	3
Coarsely ground, expanded feed	4
Coarsely ground, expanded and re-pelleted feed	5
Finely ground, pelleted feed	6
Finely ground, expanded feed	7
Finely ground, expanded and re-pelleted feed	8

Besides the effects of these technical feed treatments on nutrient digestibility and the performance of fattening pigs a special focus was put on the impact on health aspects of these animals. Thereby, stomach lesions and the stomach chyme were focused. Furthermore, morphological alterations of the small intestine and the intestinal immune system were considered.

7.1 Influence of different technical feed treatments on the digestibility of feed and performance of fattening pigs

Heterogenic results were described in previous studies concerning the impact of grinding degree and hydro-thermal treatment on the performance of fattening pigs. Furthermore, the different experimental designs and different used feed components made a direct comparison of the common technical feed treatments difficult. The results of the present examinations concerning digestibility and performance of pigs are summarised in Table 5.

Table 5 Effects of higher processing degrees of feeds on digestibility and performance of fattening pigs compared to coarsely ground meal (according to results of Paper I and II)

Variable	Finely ground meal	Coarsely ground			Finely ground		
		Pelleted	Expanded	Expanded + pelleted	Pelleted	Expanded	Expanded + pelleted
Starch disintegration	n.e.	↑	↑↑	↑↑↑	↑	↑↑	↑↑↑
Metabolisable energy	(↑)	n.e.	↓	(↑)	↑↑	↑	↑↑↑
Digestibility							
Crude protein	(↑)	(↑)	(↓)	(↑)	↑	↑	↑
Ether extract	↑ starter phase	↑	↑ starter phase (↓) grower phase	↑	↑↑	↑↑	↑↑↑
Crude fibre	n.e.	n.e.	(↓)	(↑)	↑	(↑)	↑
NFE	n.e.	n.e.	n.e.	(↑) starter phase	↑ Exp. II	↑	(↑)
Organic matter	(↑)	n.e.	(↓)	(↑)	↑	(↑)	(↑)
Fattening performance							
Daily feed intake	(↓)	(↓)	(↓)	(↓)	(↓)	↓	(↓)
Average daily gain	n.e.	n.e.	↓	n.e.	(↑)	(↓)	n.e.
Feed to gain ratio	(↓)	↓	n.e.	(↓)	↓	(↓)	(↓)
Slaughtering Performance							
Carcass yield	n.e.						
Carcass lean meat	↓ Exp. II finely ground and pelleted feed						

Notes: n.e., no effect; (↑) / (↓), numerically higher / lower; ↑, ↑↑, ↑↑↑, significantly, markedly enhanced; ↓, significantly lower; Exp. II, only in the second experiment (Paper II); NFE, N-free extractives

In accordance with studies of Wondra et al. (1995a) and Wondra et al. (1995b) a reduction of feed particle size increased the digestibility of nutrients which in turn reduced the FGR in the current study (**Paper I**). The increase of digestibility is mainly caused by the increase of particle surface and the resulting facilitated accessibility of nutrients for digestive enzymes. However, the differences between finely ground meal feed and coarsely ground meal feed were less pronounced compared to finely ground feed which was additionally hydro-thermally treated. An effect of particle size reduction on the ADG and DFI or on the slaughtering performance was not determined. However, comparing the development of ADG throughout the entire fattening phase it is noticeable that pigs fed a coarsely ground feed (Figure 2) show more constant ADG compared to animals fed finely ground feed (Figure 3). Fluctuations of ADG after feeding finely ground feed with and without hydro-thermal treatment were particularly determined in the grower phase (45 – 72 kg BW) and after feed changeover (after day 21 and after day 42 of the trial). Alterations of sensory characteristics of the feed due to fine grinding might be the reason of this effect. However, similar fluctuations could be not observed when comparing the temporal development of the DFI.

Hydro-thermal feed treatment also significantly influenced the digestibility of CP, ether extract or the organic matter ($p < 0.05$) which in turn affected the content of metabolisable energy (**Paper I**). In general a higher processing degree resulted in a higher digestibility of the mentioned nutrients. As mentioned before finely grinding before pelleting, expanding or expanding and re-pelleting intensified this impact. However, a significant increase of the digestibility of nutrients was mostly only achieved by an extremely high extent of the processing degree. Finely ground expanded feed and finely ground, expanded and re-pelleted feed showed the highest digestibility of the mentioned nutrients. On the one hand an increase of the digestibility of nutrients is based on the rupture of cell walls (Nahm, 2002). On the other hand, a secondary grinding by hydro-thermal treatment which was also reported by Goelema et al. (1999), Große-Liesner et al. (2009) and Wolf et al. (2010) increases the particle surface of the feed. This increase in turn promotes a higher accessibility of nutrients for digestive enzymes. Also, starch modification due to hydro-thermal treatment is associated with a higher digestibility because of a facilitated accessibility for digestive enzymes (Medel et al., 2004). In several studies a higher gelatinisation of starch were determined by hydro-thermal feed treatment (Goelema et al., 1999; Johnston et al., 1999; Rowe et al., 1999, Lundblad et al. 2010). Furthermore, in the current examinations higher starch disintegration was measured in feed with hydro-thermal treatment which is strongly related to the gelatinisation of starch. The modification of starch by different technical processing methods was visualised by scanning electron microscopy (SEM) (Figure 4A-F) in broiler feed according to methods of Bochnia et al. (2015a) and Bochnia et al. (2015b).

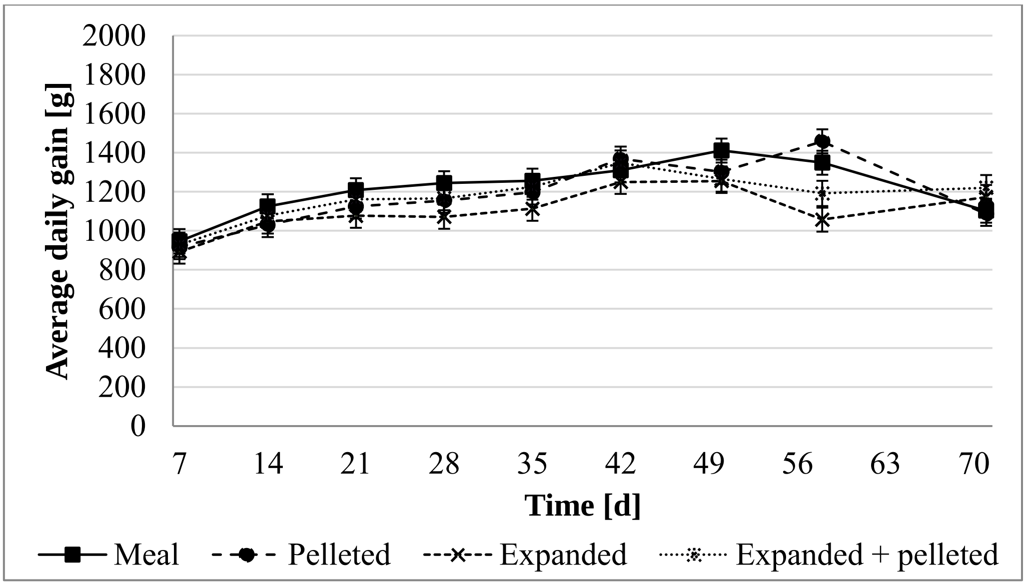

Figure 2 Average daily gain after feeding coarsely ground feed with and without hydro-thermal treatments (LSMeans ± SE)

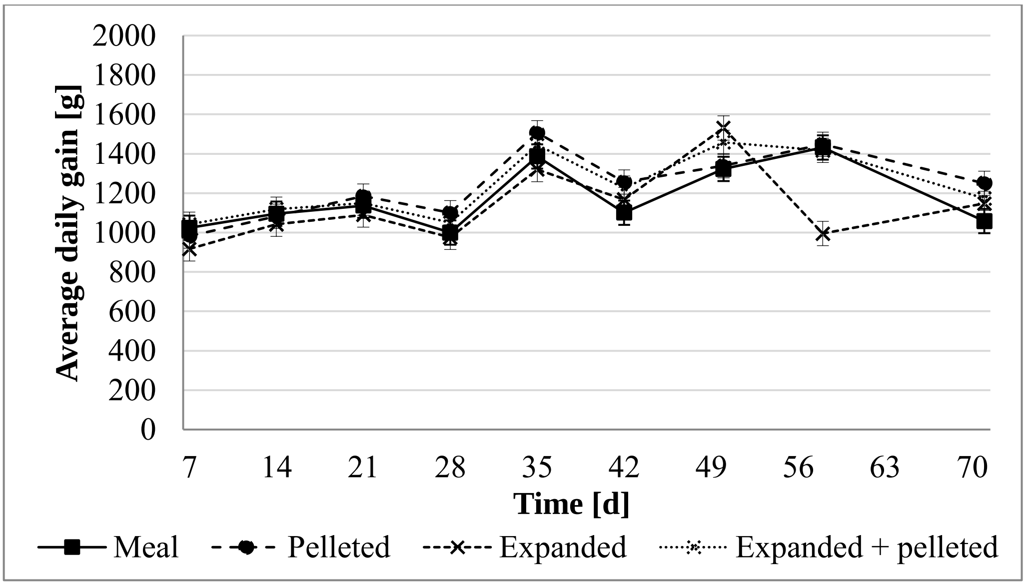

Figure 3 Average daily gain after feeding finely ground feed with and without hydro-thermal treatments (LSMeans ± SE)

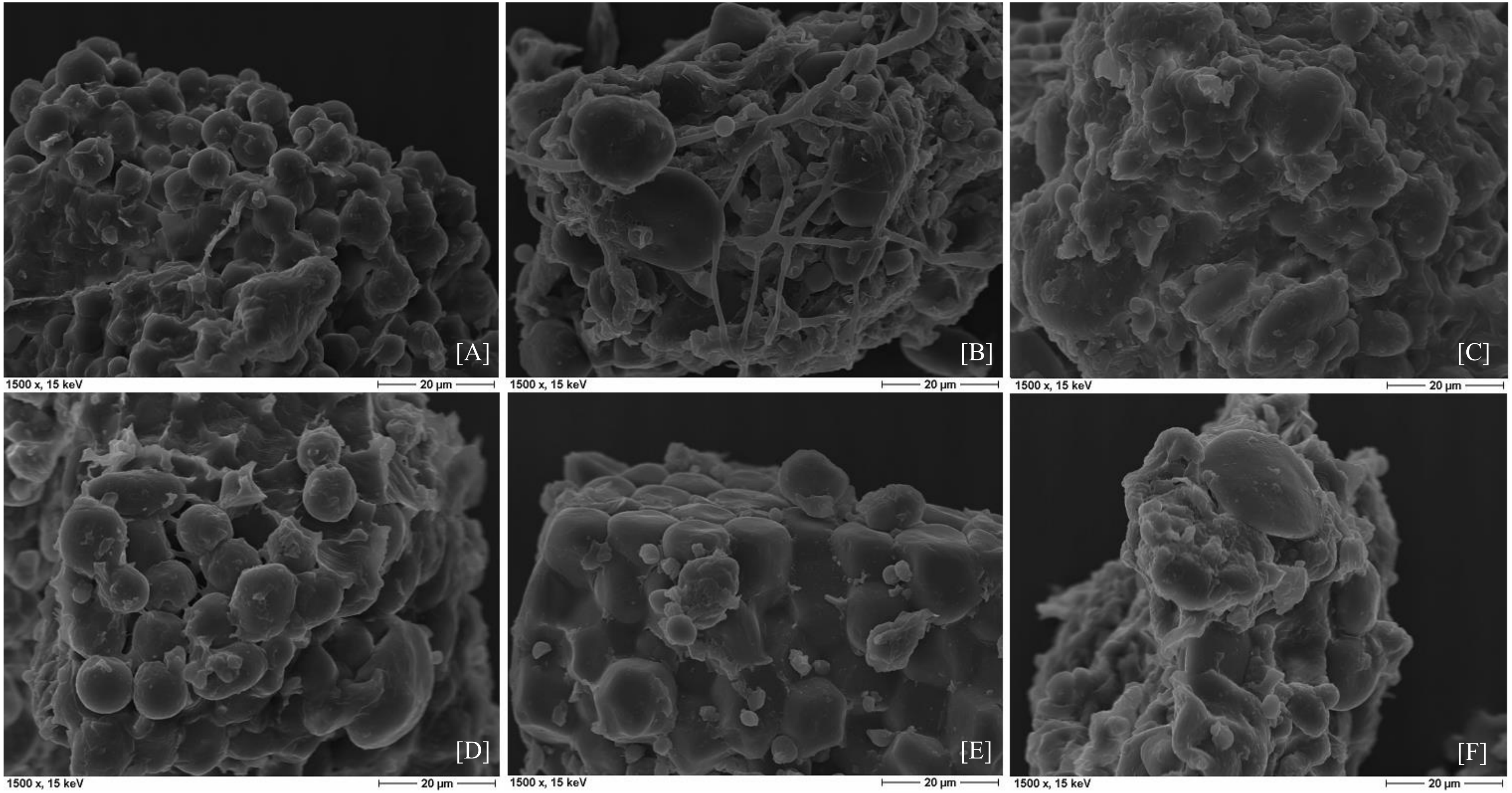

Figure 4 SEM picture from starch granules of broiler feed (x 1500) ([A] coarsely ground meal; [B] coarsely ground and pelleted; [C] coarsely ground, expanded and re-pelleted; [D] finely ground meal; [E] finely ground and pelleted; [F] finely ground, expanded and re-pelleted) (created with the friendly assistance of the Martin Luther University Halle-Wittenberg, Prof Dr Annette Zeyner and Dr Mandy Bochnia)

While the starch granules of coarsely ground meal and finely ground meal have a spherical shape and are reliably separated, the starch granules of hydro-thermal treated feed seemed to lose their shape and swell and melt. Especially the swelling and melting of the granules may offer a higher target surface for digestive enzymes. Thereby, the most marked effects of hydro-thermal feed were demonstrated in expanded and re-pelleted broiler feed. However, it has to be considered that only the complete feed was analysed by SEM. Therefore, an assignment of starch origin is not possible. The size of starch granules varies between types of plant tissue and range from 1 µm to more than 100 µm (French, 1973; Buleon et al., 1998). Furthermore, some cereals such as oat, wheat and barley possess large and small granules. While large starch granules of wheat average 30 µm the size of small granules amount 2 - 3 µm. The granule size of different maize varieties ranges from 5 µm to 30 µm (Buleon et al., 1998). Therefore, it could not be excluded that the differences between the sizes of assessed starch granules may result from different ingredients. The size of the granules measured by SEM varied between 2.6 µm and 23.3 µm.

The lower starch disintegration and the resulting lower digestibility of starch furthermore explains the higher amount of starch entering the caecum of animals fed coarsely ground feed compared to finely ground and pelleted feed (**Paper II**).

While the digestibility of nutrients of coarsely ground feed and finely ground and pelleted feed were similar in the first experiment (**Paper I**) finely grinding and pelleting significantly improved the digestibility of nutrients compared to coarsely ground meal in the second experiment (**Paper II**). It has to be considered, that the digestibility in the first experiment was examined by a quantitative sampling method and during the second experiment with a marker based method. Because of the faecal recovery of markers lower than 100 % (Kavanagh et al., 2001) and the calculations according to the GfE (2005) the marker based method can overestimate the digestibility of crude nutrients. However, in this case the overestimation of the digestibility would apply for all feeds of the second experiment. Therefore an influence on the differences between coarsely ground feed and finely ground and pelleted feed seems unlikely. Moreover, no differences of energy and nutrient digestibility were observed comparing the quantitative sampling method and the marker based method in studies of Kavanagh et al. (2001) and McCarthy et al. (1977). Because of the similar processing conditions between both trials these results indicate that the increase of digestibility of nutrients by hydro-thermal treatment is strongly related to the used feed components. On the one hand chemical composition of feedstuffs as well as their structure and gelatinisation properties of contained starch can alter shear forces which in turn affect conditions within the pellet press (Goelema et al., 1999). On the other hand gelatinisation temperature of different feedstuffs varies (Thomas et al., 1998). These aspects directly affect the extent of the increase in digestibility and explain why an applying of the present results is not possible to all diets.

While in the second experiment (**Paper II**) the lower FGR of finely ground and pelleted feed could be explained by the higher digestibility of nutrients the minor effects of pelleting on the digestibility of nutrients of the first experiment (**Paper I**) made it difficult to explain the lower FGR by this aspect. Another reason which was often described in previous studies is the lower feed wastage by the compaction of the feed (O'Doherty et al., 2001; Medel et al., 2004). In contrast spillage and rummage of animals result in higher feed wastage when feeding coarsely ground feed. It has to be emphasized that the feed of the present examination were offered dry. Liquid feeding may reduce the feed wastage.

In both experiments hydro-thermal treatment had no beneficial effect on DFI or ADG compared to coarsely ground meal considering the entire fattening phase (**Paper I** and **II**). Moreover, expanded feed markedly reduced DFI and ADG of animals compared to coarsely ground feed (**Paper I**). Also Johnston et al. (1999), O'Doherty et al. (2001) and Millet et al. (2012) described decreased DFI after offering expanded feed which in some cases also decreased ADG. A higher energy level or changes in the palatibility of the feed were assumed as the main reasons for these adverse effects of expanded feed (Johnston et al., 1999; Millet et al., 2012). However, in the current investigations the clumping of stomach content is regarded to play a key role in the disadvantages of expanded feed.

It is assumed that particularly the physico-chemical changes of the feed during hydrothermal treatment are the main reasons for the formation of ingesta clumps in stomach of animales fed these feeds. These suggestion is supported by the fact that these alterations were not detected in pigs fed meal feed. Especially the modification of starch such as the higher starch disintegration and gelatinisation is suggested to provoke the formation of clumps. Lundblad et al. (2011) suggested a relationship between starch gelatinisation and the feed extract viscosity. Analysis of the feed used during the finisher phase of the first experiment by viscosimeter (DV-ii+ Viscometer, Brookfield Engineering Laboratories Vertriebs GmbH, Lorch) (**Paper I**) revealed a slightly higher viscosity of pelleted feed extract compared to meal feed extract (Figure 5). A more considerable increase of feed extract viscosity was measured in expanded feed with and without re-pelleting compared to meal feed. These results are in accordance to the findings of Lundblad et al. (2011).

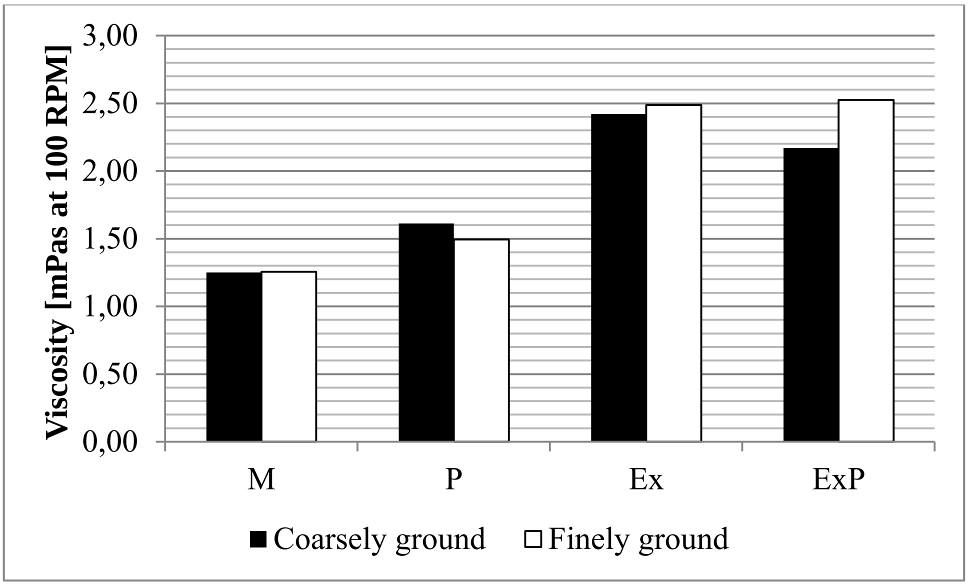

Figure 5 Influences of hydro-thermal feed treatment on feed extract viscosity of finisher feed of the first experiment (Paper I) (Means)

Notes: M, meal feed, without hydro-thermal treatment; P, pelleted feed; Ex, expanded feed; ExP, expanded and re-pelleted feed

Changes in feed extract viscosity could in turn affect the emptying of the stomach (Furuya et al., 1978; Ehrlein and Pröve, 1982; Dänicke et al., 1999). Studies of Pröve and Ehrlein (1982) indicated that the viscosity of meal has a significant impact on the indentations of the gastric waves which were markedly deeper in dogs fed low viscosity liquid meal compared to dogs fed medium and high viscous meal. These authors suggested that a higher viscosity could provoke a higher flow resistance in stomach lumen which in turn produce a high wall tension. The high wall tension could inhibit intrinsic or extrinsic reflexes by stimulation of a tension receptor and affect peristaltic waves. The forwarding of the content could be inhibited in turn. Therefore, it is assumed that expanded feed and further the formation of stomach clumps have an adverse influence on the passage rate of the feed and could particularly inhibit the stomach emptying. This could result in a persistent satiety which in turn decreases the DFI.

The delaying of stomach emptying caused by higher feed extract viscosity could also have positive effects. In studies of Furuya et al. (1978) feeding of sodium polyacrylate which is associated to increase the viscosity of water based compounds resulted in a higher protein digestibility in growing pigs. The authors suggested that the delaying of stomach emptying caused by an increased viscosity of chyme could provoke this beneficial effect. It is conceivable that the efficiency of the starting protein digestibility could be promoted by a higher retention time in the stomach. However, with regard to the physiological function of the stomach also disadvantages of ingesta-clumps on nutrient digestibility are conceivable because the presence of clumps could also inhibit the acidification of the stomach content. Actually, the stomach content of animals fed expanded feed showed a slightly higher pH-value than pigs of the other feeding groups (**Paper I**). Especially the secretion of pepsinogen and the activation of pepsin are strongly linked to the down regulation of the pH value within the stomach content by the secretion of hydrochloric acid (Scharrer and Wolffram, 2005). Furthermore, the products of the starting protein digestibility within the stomach such as peptides are also the stimuli of the gastrin secretion. This peptide hormone in turn is a stimulus of the hydrochloric acid secretion (Low, 1990). Therefore, the breakdown of the peptide bonds of the dietary protein could be inhibited which could have subsequently an adverse effect on the digestibility of crude protein. The important role of the stomach and malfunctions of this organ in protein digestibility was pointed out in studies of Cunningham (1967) with gastrectomised pigs which showed a reduction of protein digestibility of 14.0 % compared to control pigs.

In the present study the formation of clumps seemed to have no adverse effects on the digestibility of crude nutrients. However, in contrast to the fattening trial in which the development of clumps was detected the animals of the balance trial were fed restrictively and examinations of the stomach content were not possible (**Paper I**). The feeding method could be a relevant factor in formation of clumps. Therefore, further studies are needed to examine the impact of the feeding technique and of the level of feed intake on the formation of clumps. Also, the influence of expanding on the digestibility of crude nutrients in *ad libitum*-fed pigs should be explored.

Considering the feeding systems in animal farms the development of clumps could also be a problem with regard to mash or liquid feeding. Circa 44 % of the animal farms use mash feed and 33 % liquid feed in Germany (Hoy, 2008). Only 23 % offer dry feed to the animals. With regard to the viscosity of feed and a possible higher risk of development of feed clumps by water addition the cleanliness and cleaning of the feeding systems could be difficult and cleanliness insufficient. Furthermore, this aspect could have adverse effects on the flow properties of the feed which could provoke blockages. Moreover, expanded feed has a lower bulk density (Heidenreich, 1994) which has a negative impact on storage and transport quality.

Summarised, expanding of a complete mixed feed is not advisable in pig fattening. However, in studies of Johnston et al. (1999) it was shown that expanding of individual feedstuff might have beneficial effects on the performance of nursey pigs compared to the complete expanded feed. Therefore, this approach could be a solution to exploit the benefits of expander treatment on feed. This would also result in a reduction of the electrical energy input and technical wear and tear which in turn decrease the processing costs of the feed (Graf von Reichenbach, 2016, personal communication of 07 September 2016, unreferenced).

7.2 Influence of different technical feed treatments on the gastrointestinal tract of fattening pigs

7.2.1 Influences on stomach associated parameters

The current examinations have shown a protective impact of coarsely ground meal feed against stomach lesions (**Paper I; Paper III**). In contrast, fine grinding of feed and pelleting promoted stomach lesions in fattening pigs (Table 6) which is in accordance to several previous studies (Mahan et al., 1966; Chamberlain et al., 1967; Reimann et al., 1968; Picket et al., 1969; Maxwell et al. 1970; Flatlandsmo and Slagsvold, 1971; Wondra et al. 1995a; Eisemann and Argenzio, 1999; Mikkelsen et al., 2004).

Table 6 Effects of higher processing degrees of feed on stomach associated parameters compared to coarsely ground meal (according to results of Paper I and II)

Variable	Finely ground meal	Coarsely ground			Finely ground		
		Pelleted	Expanded	Expanded + pelleted	Pelleted	Expanded	Expanded + pelleted
Stomach weight			↓ Exp. II finely ground and pelleted feed				
Stomach score	↑↑	↑↑↑	↑	↑	↑↑	(↑)	↑
Dry matter content	(↓)	(↓)	n.e.	(↓)	(↓)	(↑)	n.e.
pH stomach content	n.e.	(↓)	(↑)	(↑)	↓ Exp. II	(↑)	(↑)

Notes: n.e., no effect; (↑) / (↓), numerically higher, lower; ↑, ↑↑, ↑↑↑, significantly enhanced; ↓, significantly decreased; Exp. II only in experiment II (**Paper III**)

A coarsely grinding of the feed before pelleting had no beneficial effects on stomach lesions. It is possible that the coarsely ground feed increased secondary grinding during pelleting compared to finely ground feed because of a higher shearing and shattering effect within the pellet press. Furthermore, a lower pellet durability and hardness by coarsely ground material could provoke a higher abrasion which in turn increases the proportion of fine particles. This was also proven in the first experiment by the standard method of the American Society of Agriculture Engineers (ASAE, 1970). The pellets of coarsely ground meal had a higher abrasion than pellets from finely ground meal in all fattening phases (Table 7). The difference between the abrasion of pellets from coarsely ground feed and the abrasion of pellets from finely ground feed was lower when expander treatment was used before pelleting. No difference was observed in pellet-abrasion of coarsely ground, expanded and re-pelleted feed compared to its counterpart.

Table 7 Abrasion of pellets related to different grinding extent before hydro-thermal treatment (Pellets used in the first experiment; Paper I)

Fattening phase	Abrasion [%]		Difference coarsely ground and finely ground pelleted feed [%]	Abrasion [%]		Difference coarsely ground and finely ground pelleted +expanded feed [%]
	Coarsely ground Pelleted	Finely ground Pelleted		Coarsely ground Expanded + pelleted	Finely ground Expanded + pelleted	
Starter	2.3	1.5	34.8	2.1	1.5	28.6
Grower	2.3	1.8	21.7	1.7	1.5	11.8
Finisher	1.6	1.4	12.5	1.6	1.6	0.0

Considering the results of the second study (**Paper III**) the degree of pathological alterations of the *Pars nonglandularis* seems also to be related to the components used in the basal diet.

It is suggested that the development of stomach lesions is mainly caused by the modifications of stomach content by feeding different technically treated feeds. Correlations between the macroscopic stomach score (MSC) and the pH-value of the stomach content were significant ($r = - 0.2873$; $p = 0.005$) according to the results of the first experiment (**Paper I**). However, in the second experiment no significant correlations between these parameters could be detected (**Paper III**). Therefore, it is assumed that the pH-value of the mixed stomach content is not the main inducer of stomach lesions. However, according to the present examinations the dry matter content of the stomach seems to play a key role to prevent stomach lesions. This suggestion is confirmed by the negative correlation between the DM content and the stomach score examined in the first experiment ($r = - 0.3467$; $p < 0.001$). A similar relation was also proven in the second experiment ($r = - 0.294$; $p = 0.003$) (**Paper III**). It is conceivable that specific DM content is required for the formation of an ingesta gradient between the nearly unprotected *Pars nonglandularis* and the pyloric region which was described in studies of Maxwell et al. (1970), Regina et al. (1999) and Mößeler et al. (2010). The higher fluidity of stomach content after feeding finely ground feed or pelleted feed which was described in previous studies of Reimann et al. (1968), Maxwell et al. (1970), Flatlandsmo and Slagsvold (1971) and Healy et al. (1994) seems to avoid the development of the described ingesta gradient. Rather the inhibition of the physiologic stratification results in a higher mixing of stomach content. Therefore, the more acidic stomach content and possible harmful substances such as chloride of the pyloric region may contact the *Pars nonglandularis*.

The importance of DM content has been pointed out even more by studies of Friendship et al. (2000) which showed a higher incidence of stomach lesions in fasting pigs compared to pigs fed *ad libitum*. Therefore, a constant stomach fill seems to protect against stomach lesions. Another solution could be to decelerate the stomach emptying. This assumption will be supported by the findings of Kokue et al. (1974) which reported a lower risk of gastric lesions in rats after administration of the viscosity increasing additive sodium polyacrylate. This could be in turn the explanation for the moderate MSC of animals fed expanded feed which showed also a higher extract viscosity and reduced the stomach emptying as mentioned before. In contrast finely ground feed or pelleted feed which increased the incidence of stomach lesions also increases the passage rate through the alimentary tract (Maxwell et al., 1970; Seerley et al., 1962). Furthermore, the detected clumps in animals fed expanded feed could mime an ingesta gradient between the *Pars nonglandularis* and the pyloric region.

However, as described in the chapter 7.1 the presence of clumps could have an inhibiting effect on the acidification of the stomach content. The measurements of pH values inside the developed clumps show a neutral pH value (data not shown). Furthermore, the pH-values of the mixed stomach content of animals fed expanded feed (with and without re-pelleting) were numerically higher compared to animals from the other feeding groups. However, as reported in studies of Williams Smith and Jones (1963) the down regulation of the gastric pH-value is particularly important to prevent the bacterial overgrowth, including pathogenic microorganisms in the small intestine which could be ingested with the feed. Therefore, a higher colonisation of pathogenic microorganism could be promoted in animals fed feeds which promote the formation of stomach content clumps.

In the present examinations and in studies of Dirkzwager et al. (1998) the performance of animals was not influenced by stomach lesions. Moreover, there were no animal losses during the present fattening trials.

It has to be emphasized that animals were housed individual with visual contact to other animals. Therefore, stressors such as agonistic interactions were excluded in the present experiments. However, it is conceivable that stomach lesions could be more severe under conventional housing conditions which could result in animal losses. Especially in studies of Pickett et al. (1969) the promoting impact of a high stocking density on stomach lesions were reported. Furthermore, in studies of Ramis et al. (2005) the incidence of stomach lesions increased under standard housing conditions (conventional barns with partially slatted floors) compared to enriched conditions (large open-front sawdust bedded barns). Subsequently, the problem of stomach lesions with regard to animal welfare could also arise to an economic problem with regard to possible animal losses under conventional conditions especially considering the average MSC (> 2.3) of groups fed finely ground meal feed or pelleted feed without expander treatment (**Paper I and III**).

7.2.2 Influences on intestine associated parameters

A great number of studies deals with the effect of different technically treated feeds on the stomach content or stomach lesions. Only a few studies focused on the impact of technical feed treatment on the small intestine, even though it has a key function in the defence of pathogens and the absorption of essential nutrients. In some studies, also effects of technical feed treatment on physico-chemical changes such as the DM content, fluidity or the pH-value of the gut chyme were examined (Betscher, 2010; Canibe et al., 2005). Therefore, morphological changes or changes of the local immune system due to alterations of chyme are conceivable. Thus, the present examinations also aimed at studying the impact of different technically treated feeds on the small intestine (**Paper III**).

As a part of a master thesis morphological alterations of the small intestine were examined (Strutzke, unpublished data, unreferenced). Formalin-fixed (4%) and paraffin-embedded tissue samples of the jejunum which were taken 3 cm proximal to the located middle from the small intestine of animals (13 animals per group) used in the second experiment (**Paper III**) were stained with hematoxylin and eosin. None of the animals showed gross-macroscopical lesion at this localisation. For measurements of the thickness of the jejunal epithelium and the *Tunica muscularis* as well as the crypt depth a microscope (Leica DMi

6000 B; Leica Microsystems CMS GmbH, Wetzlar, Germany) and the software program ImageJ were used. Two fields of view per histological section (4 sections per animal) were considered for measurements of epithelial thickness and the thickness of the *Tunica muscularis*. The depth of approximately 32 crypts per pig was measured.

No influences of diet or technical feed treatment were shown on the thickness of the epithelium or on the *Tunica muscularis* of the jejunum (data not shown). There was also no effect of diet on crypt depth ($p > 0.05$). However, the technical feed treatment significantly influenced the crypt depth of fattening pigs ($p < 0.001$). Interestingly, feeding of finely ground and pelleted feed resulted in higher crypt depth compared to coarsely ground feed (Figure 6). In contrast, Hedemann et al. (2005) described deeper crypts in animals fed coarsely ground meal feed compared to finely ground and pelleted feed. In studies of Brunsgaard et al. (1998) increased crypt depths were found in animals fed a diet including coarsely ground grain compared to animals fed a diet containing finely ground grain. It is noticeable that both mentioned studies only considered a feeding period of four weeks. In the present examinations pigs were fed for 10 weeks (**Paper II**). Therefore, the longer feeding period could be a reason for the observed opposite effects.

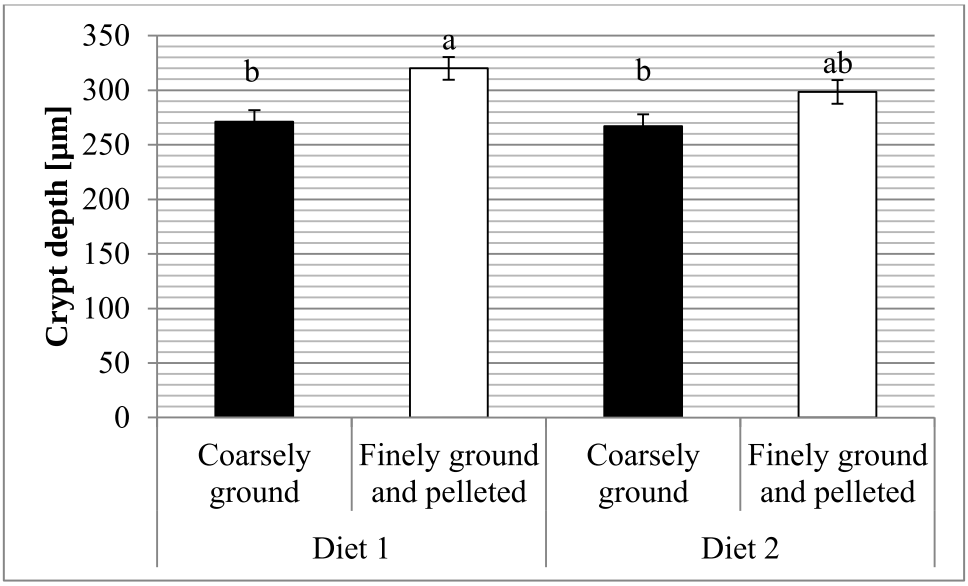

Figure 6 Crypt depth related to different technical feed treatments used in the second experiment (Paper III) (LSMeans ± SE; *n* = 13)
Notes: Diet 1, standard diet with soybean meal; Diet 2, diet with rapeseed meal, DDGS, soybeans; different subscripts mark significant differences between feeding groups (p < 0.05).

In studies of Dauncey et al. (1983) greater crypt depth was measured in animals that consumed a higher amount of feed and had a higher energy intake.

In accordance to findings of Dauncey et al. (1983) the crypt depth of animals used in the present study was positively correlated to the content of the metabolisable energy ($r = 0.492$; $p = 0.009$) of the finisher feed used in the second experiment. Further, positive correlations were found between the crypt depth and the digestibility of the organic matter ($r = 0.450$; $p = 0.018$), CP ($r = 0.602$; $p < 0.001$) and ether extract ($r = 0.442$; $p = 0.021$) of the finisher feed used in the second experiment (**Paper III**). A negative correlation was detected between the crypt depth and the crude ash digestibility ($r = -0.432$; $p = 0.024$). In contrast to studies of Dauncey et al. (1983) the crypt depth was also negatively correlated to the feed intake of the entire fattening period. There was also a trend towards a negative correlation between the crypt depth and the feed intake of the finisher period ($r = -0.238$; $p = 0.086$).

Summarised it is suggested that finely ground and pelleted feed enhanced the nutrient accessibility which in turn influences morphological alterations of the small intestine. Deeper crypts increase the intestinal surface which enables the absorption of the higher nutrient offer.

To visualise possible relationships between morphological traits of stomach and the small intestine, parameters of chyme, nutrient digestibility and animal performance as mentioned a Principal Component Analysis (PCA) including 19 variables was conducted (Figure 7A). The filtered first two principle components (PC 1 and PC 2) extracted approximately 49.3 % of their total variance. A scree plot was used to visualise consecutive principal components and their eigenvalue. The scree plot showed a pronounced break after extraction of the first three components which signalised the transition point between the main important components and components with no significant impact on the total explained variance. The mean eigenvalue of 1.0 of all components corresponded to a total of 6 extracted components. They explained cumulatively 79.2 % of the total variance between the 19 variables.

The additional projected variable, *Technical feed treatment shows a significant correlation to both PC1 and PC2 which is indicated by its localisation close to the outer circle. However, while a negative correlation seems to exist between technical feed treatment and PC 1 the technical feed treatment is positive correlated to PC 2. Moreover, this variable clusters with the MSC, the jejunal crypt depth and the digestibility of CP, the digestibility of ether extract, the digestibility of organic matter and ME. This cluster indicates a relationship between these variables which was signalised above. In contrast the additional variable *Diet correlated negatively to PC 2 and clustered with the digestibility of crude fibre and crude ash and performance parameters. Therefore, these parameters are rather related to the composition of feed. A further cluster is formed by DM of stomach content and the stomach weight as well as the starch content of caecum content. All these traits show an opposite trend to the MSC which indicated that the lower MSC the higher extent of the mentioned three parameters. Also the opposite trend of crypt depth compared to the DFI and the DFI of finisher phase as well as to crude ash digestibility as indicated by mentioned correlations is highlighted by the performed PCA. While the jejunal crypt depth is highly positive correlated to PC 2 the DFI of the finisher phase and the DFI of the entire fattening period and the digestibility of crude ash are highly negatively correlated to this PC.

Projection of the cases which demonstrated the individual pigs based on the 19 variables clearly show a separation between pigs fed coarsely ground meal and pigs which consumed finely ground, pelleted feed (Figure 7B).

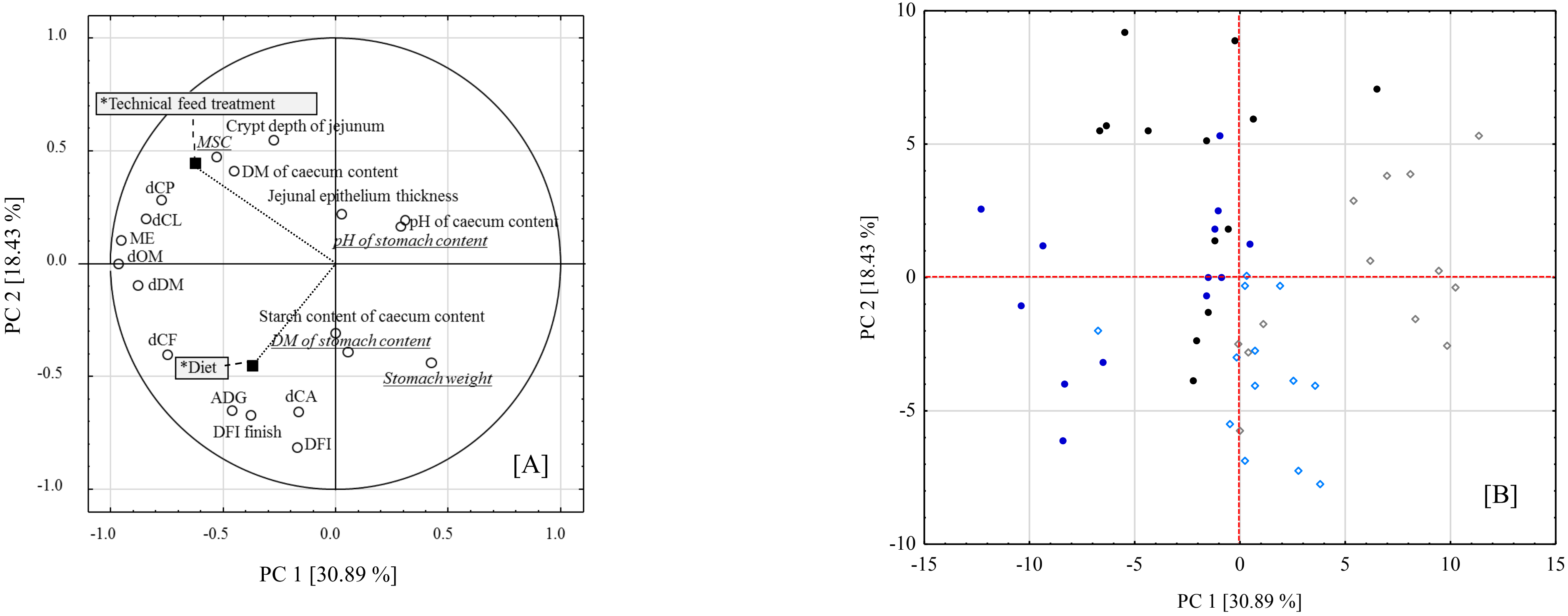

Figure 7 Visualisation of relationships between morphological alterations of stomach and small intestine, parameters of chyme, nutrient digestibility and animal performance of the second experiment (Paper II; III) by Principal Component Analysis

Notes: PC, principle components; **Variables for the analysis:** MSC, macroscopic stomach score; stomach weight; crypt depth of jejunum; jejunal epithelium thickness; dry matter (DM) of stomach content; pH of stomach content; DM of caecum content, pH of caecum content; starch content of caecum content; ME, metabolisable energy of feed; calculated on base of digestible crude nutrients (as analysed) according to the formula of the GfE (2008); dCP, digestibility of crude protein; dCL, digestibility of ether extract; dOM, digestibility of organic matter; dDM; digestibility of dry matter; dCF, digestibility of crude fibre; dCA, digestibility of crude ash; ADG, average daily gain of the entire fattening period; DFI, daily feed intake of the entire fattening period; DFI finish, daily feed intake in finisher phase; ***Additional variables**: Diet, diet composition; Technical feed treatment, grinding extent/hydro-thermal feed treatment; [A] Projection of variables, [B] Projection of cases (pigs): ◊ coarsely ground meal, Diet 1 (standard diet with soybean meal); ◊ coarsely ground meal, Diet 2 (rapeseed meal/DDGS/soybeans); ● finely ground and pelleted feed, Diet 1; ● finely ground and pelleted feed, Diet 2

Besides the mentioned effects of technical feed treatment on morphological alterations of the small intestine, in the present examinations also changes in the intestinal immune cell populations of fattening pigs after feeding different technically treated feeds were investigated (**Paper III**). Only the differences of T-cell subsets and mean fluorescence intensity (MFI) of $CD4^+$ T-cells of leucocytes isolated from peyer's patches (PP) were significant between animals fed coarsely ground meal feed and animals fed finely ground and pelleted feed. It is suggested, that alterations of the physico-chemical characteristics of the chyme could have induced this immune modulation. Furthermore, numerically higher starch concentrations related to DM were detected in the caecum content of animals fed coarsely ground feed compared to finely ground and pelleted feed (**Paper II**). Papenbrock et al. (2005) suggested that higher starch amount entering the caecum promotes the colonisation with gram positive bacteria and especially of lactobacilli. Alvarez et al. (1998) and Perdigon et al. (1999) found similar intestinal immune modulations after oral administration of lactobacilli in studies of mice. Therefore, it also seems to be justified to assume that microbial changes within the intestine caused the observed immune-modulation of pigs fed different technically treated feeds. However, a significant correlation between the starch content of caecum and the T-cell subsets or the MFI of $CD4^+$ T-cells was not observed.

The initially hypothesised impact of the diet formulation on the immune system was not confirmed.

Interestingly, animals with low (MSC = 0-0.5), middle (MSC = 1-2.5) and high (3-4) stomach score showed different modulations of immune-cells isolated from lamina propria and PP. Thereby significant correlations between T-cell subsets of the PP and the MSC were observed (**Paper III**). Significant correlations between the immune cells of the small intestine and the pH of stomach content as well as the DM content were detected (**Paper III**). Therefore, it is suggested that changing physico-chemical alterations of stomach content may have resulted in changes of the intestinal chyme which in turn affected the immune cells of pigs with different MSC-classes. Also alterations of the gastric and intestinal microbiota could have contributed to these effects. In contrast, correlations between the pH value and the DM content of the caecum described in **Paper II** and the immune cells of fattening pigs were not significant ($p > 0.05$). Further studies are needed to identify the possible stimuli of the observed immune modulations.

There were no correlations between alterations of the jejunal morphology and the intestinal immune cell subsets of the animals except for a significant correlation between the crypt depth and the $CD8^+$ T-cell subsets of the PP ($r = -0.315$; $p = 0.031$).

Additionally, significant differences of T-cell subsets of *Lymphonodii gastrici* were observed between animals with different MSC-classes which could be related to a systemic effect induced by pathogens that had passed through the stomach lesions.

There were no correlations between performance parameters and immune cell populations ($p > 0.05$).

Relationships between stomach traits and immunological traits were also visualised by PCA (Figure 8A). A total of 24 variables were included in this statistical analysis. The first two PC explained approximately 35.1 % of the total variance of all components. No obvious transition point between the main important components and the less important components of the total explained variance was extracted by the scree plot. The analysis revealed a total of eight PC which had an eigenvalue of more than 1.0. They explained cumulatively approximately 76.7 % of the total variance of used variables.

Clusters were mainly formed between immunological traits of the different localisation. Interestingly, while the traits of the local immune system form a cluster the immunological traits of blood seem to be markedly delimited. Only $CD4^+$ T-cell subsets of *Lymphonodii gastrici* are included of the cluster of blood immunological traits. This visualised relationship support the hypothesis that differences of T-cell subsets of lymph nodes could be related to systemic effects induced by through stomach lesions penetrating pathogens. Furthermore, these visualisation may show the independent regulation of the intestinal immune system and the peripheral immune system of pigs.

The lack of an influence of diet composition of immune traits as mentioned (**Paper III**) is also demonstrated by its localisation close to the cross of Figure 8A. Interestingly, traits of PP and MSC as well as the additional variable *Technical feed treatment correlated with PC 1; however, in an opposite direction. This seems to visualise a possible relationship between those variables. This could indicate that the higher MSC the lower extent of immunological traits of PP. In reality this hypothesis is supported by the results of the second experiment (Paper III). Subsets of $CD4^+$ T-cells, $CD8^+$ T-cells and $CD8^{low}$ T-cells as well as subsets of CD4/CD8 double positive T-cells were lower in animals with high MSC compared to animals with low MSC.

Also in this PCA the projection of the cases demonstrating the individual pigs based on the evaluated variables clearly revealed a separation between animals fed coarsely ground meal and animals fed finely ground, pelleted feed (Figure 8B).

Subsequently, the present results prove the hypothesis that technical feed treatment influences morphological traits of the small intestine. Moreover, an immune-modulating effect of differently technical treated feeds on T-cells of PP was indicated. Specific mechanism could not reveal with conducted experiments; however, possible relationships to alterations in chyme are conceivable. A direct evidence of a systemic immune-modulation as a consequence of these effects could not be demonstrated.

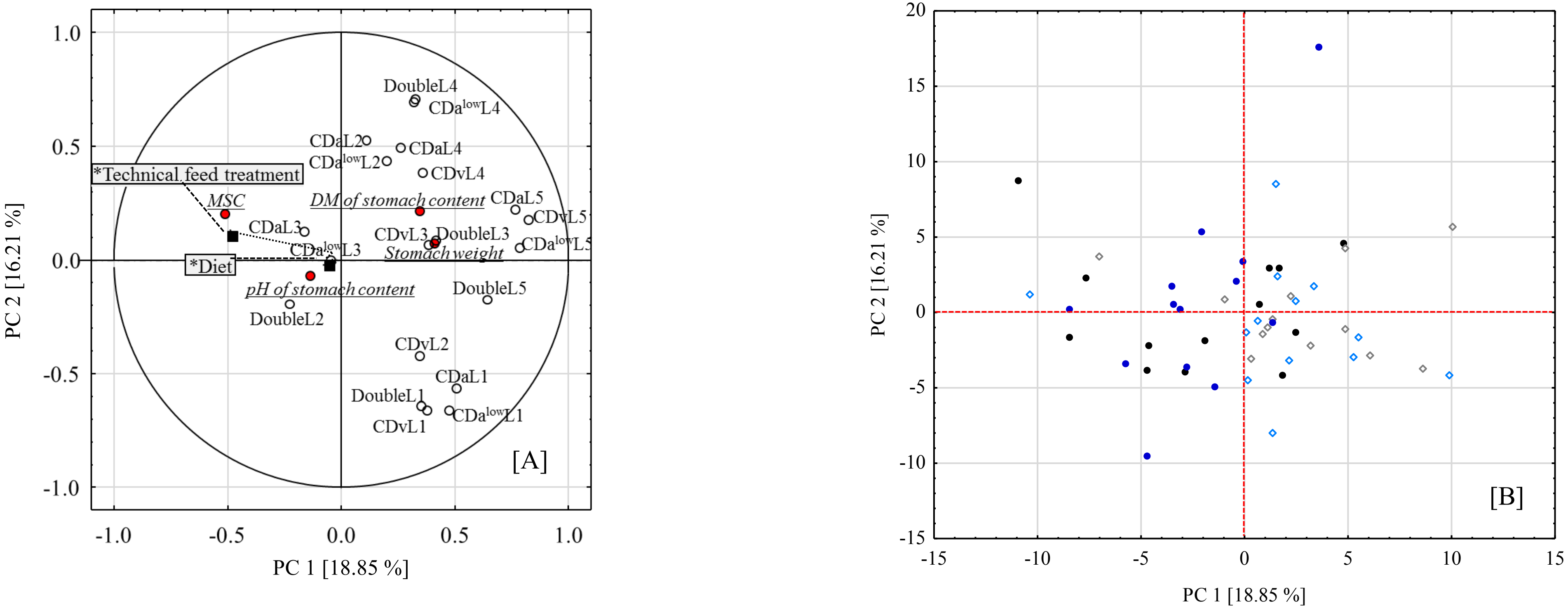

Figure 8 Visualisation of relationships between stomach traits and selected immunological traits of the second experiment (Paper III) by Principle Component Analysis

Notes: PC, principle components; MSC, macroscopic stomach score; stomach weight; dry matter (DM) of stomach content; pH of stomach content; CDvL1, CD4$^+$ T-cell subsets of blood (L1); CDvL2, CD4$^+$ T-cell subsets of *Lymphonodii gastrici* (L2)*;* CDvL3, CD4$^+$ T-cell subsets of jejunal epithelium (L3); CDvL4, CD4$^+$ T-cell subsets of jejunal lamina propria (L4); CDvL5, CD4$^+$ T-cell subsets of peyer's patches (L5); CDaL1, CD8$^+$ T-cell subsets of L1; CDaL2, CD8$^+$ T-cell subsets of L2*;* CDaL3, CD8$^+$ T-cell subsets of L3; CDaL4, CD8$^+$ T-cell subsets of L4; CDaL5, CD8$^+$ T-cell subsets of L5; CDa^{low}L1, CD8^{low} T-cell subsets of L1; CDaL2, CD8^{low} T-cell subsets of L2*;* CDaL3, CD8^{low} T-cell subsets of L3; CDaL4, CD8^{low} T-cell subsets of L4; CDaL5, CD8^{low} T-cell subsets of L5; DoubleL1, CD4/CD8 double positive T-cell subsets of L1; DoubleL2, CD4/CD8 double positive T-cell subsets of L2*;* DoubleL3, CD4/CD8 double positive T-cell subsets of L3; DoubleL4, CD4/CD8 double positive T-cell subsets of L4; DoubleL5, CD4/CD8 double positive T-cell subsets of L5; ***Additional variables**: Diet, Diet composition; Technical feed treatment, Grinding extent/hydro-thermal feed treatment; [A] Projection of variables; ● stomach traits; ○ immunological traits; [B] Projection of cases (pigs): ◊ coarsely ground meal, Diet 1 (standard diet with soybean meal); ◊ coarsely ground meal, Diet 2 (rapeseed meal/DDGS/soybeans); ● finely ground and pelleted feed, Diet 1; ● finely ground and pelleted feed, Diet 2

8 Conclusion

Feed processing influences the physico-chemical characteristics of a feed. The presented and discussed examinations demonstrate the beneficial effects of these changes on the digestibility of nutrients of pig feed. The hypothesis was supported that the higher processing degree of feed used in present examination the higher nutrient digestibility. However, only an extremely high processing degree significantly influences the digestibility compared to coarsely ground meal. Technical feed treatment enables the usage of a wide range of feed components, which could be helpful in diet formulation without soybean meal. The second part of the hypothesis has to be rejected that the higher processing degree the higher animal performance. Coarsely ground feed has equivalent influences on DFI and ADG compared to pelleted feed. However, coarsely ground feed resulted in markedly higher FGR which is strongly related to a higher feed wastage. Therefore, in order to avoid feed wastage the importance of feed compacting processes such as pelleting was verified. The present study also showed that a high extent of feed processing and the resulting changes in feed characteristics can result in adverse effects on stomach chyme. Therefore, expanding of the complete mixed feed cannot be recommended in processing of fattening pig feed.

The hypothesis which stated that the increasing processing degree increased the risk of stomach lesions could not be generally supported. Finely grinding and pelleting enhances the incidence of stomach lesions. Only coarsely ground meal seems to promote the physiological stratification of the stomach content which in turn protects against stomach lesions. In this context, the DM content of the stomach seems to play a key role in protection. Besides the alterations on stomach lesions the physico-chemical changes of the chyme and its microbial colonisation also modulate the intestinal immune system of fattening pigs. The different intestinal immune modulations of animals with different MSC-classes imply the suggestion that promoting factors of stomach lesions are closely linked to the stimuli of the intestinal immune system. An influence of different technically treated feeds on the peripheral immune system could not be proved. An impact of feed composition on the intestinal and peripheral immune system has not been confirmed.

In conclusion, the conflict between high performance and the animal health was reflected by the obtained results. Despite a lack of animal losses within the conducted studies it has to be considered that animal health is a decisive factor for a resource conserving livestock production.

9 Summary

The extending world population, the rising competition for arable land and the enhanced sensitisation of the consumers for animal health are the new challenges of the production of livestock products. Especially the feed and its quality play a key role for a resource conserving production. Many types of technical feed treatment were developed in the past to improve the feed quality and are used on the industrial feed market. The beneficial impact and the disadvantages of technical feed treatment considering the nutritional point of view were often proved in the past. However, different experimental designs and feed ingredients complicated a direct comparison of common technical feed treatments. Therefore, the present examinations aimed to balance the beneficial effects and the disadvantages of technical feed treatment and a higher processing degree with regard to the impacts on the digestibility of feed, the animal performance and health aspects.

In this context two fattening trials were performed. During the first trial a common basal mixed feed was treated by eight technical feed treatments differing in extent of grinding (coarsely grinding (degree of fineness corresponded to common German pig feed) vs. finely grinding (higher degree of fineness than common German pig feed)) and in hydro-thermal treatment (without – meal; pelleted; expanded; expanded and re-pelleted). Additionally, the combination of different grinding extents and hydro-thermal treatments were compared. A total of 96 crossbred barrows which were penned individually and fed *ad libitum* were fattened for ten weeks. Balance trials were performed for digestibility analyses. The two variations of technical feed treatment which during the first experiment showed the most favourable effects on animal performance and health aspects (coarsely ground meal vs. finely ground and pelleted feed) were used to study the relation between differently chosen feed ingredients and the technical feed treatment. Therefore, two diets were compared differing mainly in protein source (soybean meal vs. rape seed meal/distillers′ dried grains with solubles/soy beans). A total of 100 crossbred barrows were used in this trial. Housing conditions were similar to the first trial. A marker method was used for the determination of the feed digestibility during the fattening trial.

The digestibility of nutrients increased when particle size of the feed decreased in the first fattening trial. The higher digestibility resulted in a lower feed to gain ratio; however, the average daily gain and the daily feed intake were not influenced by grinding extent. While animals fed a feed based on coarsely ground feed showed a constant average daily gain, the average daily gain of animals fed a feed based on finely ground feed material fluctuated

markedly. Hydro-thermal feed treatment had also positive effects on the digestibility of nutrients. However, only a high processing degree and high intensity of technical feed treatment resulted in a significant increase of the digestibility compared to coarsely ground meal in the first experiment. Thus, finely ground, expanded and re-pelleted feed resulted in the highest digestibility of nutrients. In contrast, significantly enhanced digestibility of crude nutrients compared to coarsely ground meal was already shown when feeds were finely ground and pelleted during the second trial. Therefore, the choice of feed ingredients for the basal feed plays a key role in the extent of this impact. The hydro-thermal treated feeds had no beneficial effect on the daily feed intake or the average daily gain compared to coarsely ground meal. The feed to gain ratio was significantly reduced by pelleting (without expander treatment) regardless of the extent of grinding compared to coarsely ground meal. Expander treatment of the complete feed without re-pelleting significantly decreased the daily feed intake which in turn resulted in a significantly reduced average daily gain. The main reason of this disadvantage seems to be the formation of ingesta-clumps within the stomach which were detected during slaughtering and which could result in a persistent satiety. The hydro-thermal feed treatment and the resulting modification of starch seems to be a risk factor for the development of ingesta-clumps within the stomach. A significant influence of technical feed treatment on slaughter performance was only detected in the second trial. While the carcass yield of animals fed finely ground and pelleted feed was significantly higher the mean lean meat percentage tended to be lower compared to animals fed coarsely ground meal.

The protective impact of coarsely ground feed against stomach lesions was proved in both fattening trials. In contrast, finely ground meal or pelleted feed increased the risk of stomach lesions. Coarsely grinding before pelleting had no mitigating effect on stomach lesions. In comparison to animals fed solely pelleted feed animals fed expanded feed with and without re-pelleting showed a lower incidence of stomach lesions. However, expanded feed had no beneficial effects on stomach lesions compared to coarsely ground meal. According to the results of the second trial there are indications that the extent of stomach lesions is related to the ingredients of basal diet. Only in the second fattening trial a significant correlation between the pH-value of the stomach content and the extent of stomach lesions was detected. However, the dry matter content of the stomach was significantly negatively correlated to the extent of stomach lesions in both fattening trials.

During the second trial data of the immune system of 13 pigs per feeding group were recorded by T-cell phenotyping of leucocytes isolated from the jejunal epithelium, the lamina

propria and the peyer’s patches besides the impact of technical feed treatment on stomach lesions. A significant influence of different technically treated feeds on the CD4^{+} T-cell subsets (T-helper cells), the CD8^{+} T-cell subsets (cytotoxic cells), the CD8^{low} T-cell subsets and the subsets of CD4 and CD8 double positive T-cells of peyer’s patches was proved. Furthermore, the mean fluorescence intensity of CD4^{+} T-cells and the ratio between CD8^{low} and CD8^{high} T-cells were significantly affected in peyer´s patches by different technically treated feeds. Animals fed finely ground and pelleted feed showed lower values of all these mentioned parameters compared to coarsely ground meal. The T-cell subsets of further considered localisations such as the *Lymhonodii gastrici* or the peripheral immune system from the blood were not influenced by different technically treated feeds. There was no significant effect of different diets on the immune system of the pigs. However, significant differences of the intestinal immune system and the T-cell populations of *Lymphonodii gastrici* were observed in animals with low (0-0.5), middle (1-2.5) and high (3-4) stomach score. At the same time significant correlations between the local immune system and the stomach score or the pH-value as well as the dry matter content of the stomach content were found in some cases.

In summary, pelleted feed has to be assessed as positive with regard to its beneficial effects on feed efficiency and economic efficiency. In relation to animal health and welfare coarsely ground meal is preferable. Expander treatment of the complete feed cannot be recommended due to its promoting effect to develop ingesta-clumps. It follows a conflict of interests between the exploitation of performance potential and animal health and welfare, respectively. With regard to the production of livestock products it has to be pointed out that animal health plays a key role in the resource conserving production of livestock products.

10 Zusammenfassung

Mit dem steigenden Bevölkerungswachstum, der zunehmenden Konkurrenz um landwirtschaftliche Nutzflächen und dem erhöhten Bewusstsein der Verbraucher für das Tierwohl ergeben sich neue Herausforderungen an die Produktion tierischer Lebensmittel. Eine Schlüsselrolle für die ressourcenschonende Erzeugung tierischer Produkte nehmen dabei das Futter und die Futterqualität ein. Eine Vielzahl an technischen Futterbehandlungsverfahren wurde in der Vergangenheit zur Weiterveredelung der Futtermittel und Steigerung der Produktqualität entwickelt und auf dem Mischfuttermittelmarkt eingesetzt. Die Vor- und Nachteile dieser in Bezug auf den tierernährungsspezifischen Nutzen wurden in der Vergangenheit des Öfteren untersucht, wobei jedoch die unterschiedlichen Versuchsdesigns und die Wahl der Futterkomponenten früherer Studien keinen direkten Vergleich der Behandlungsverfahren zulassen. Ziel der vorliegenden Untersuchungen war es daher anhand von auf dem Markt üblichen Futtermischungen, die konventionellen technischen Futterbehandlungsverfahren zu prüfen und die Vor- und Nachteile eines niedrigeren oder höheren Veredelungsgrades des Futters in Bezug auf die Verdaulichkeit der Futtermittel, der Leistung der Tiere aber auch auf gesundheitliche Aspekte gegeneinander abzuwägen.

In diesem Zusammenhang wurden zwei Mastversuche durchgeführt. Im ersten Mastversuch mit 96 männlichen, kastrierten Masthybriden wurde ein konventionelles Mischfuttermittel acht verschiedenen technischen Behandlungsverfahren unterzogen. Dabei wurde zum einen die Art des Vermahlungsgrades (grobes (Feinheitsgrad entsprach dem handelsüblichen deutschen Schweinefuttern) vs. feines Futter (höherer Feinheitsgrad gegenüber handelsüblichem, deutschem Schweinefuttern)) und zum anderen die Art der Druck-Hydrothermischen-Behandlung (ohne – mehlförmiges Futter, pelletiert, expandiert, expandiert und pelletiert) unterschieden. Zusätzlich wurden Kombinationen der unterschiedlichen Vermahlungsgrade und der hydrothermischen Behandlungsmethoden gegenübergestellt. Alle Tiere wurden einzeln aufgestallt und *ad libitum* über zehn Wochen gemästet. In einem Bilanzversuch wurden Verdaulichkeitsuntersuchungen durchgeführt. Die zwei Futtervarianten, die hinsichtlich der Leistung und der gesundheitlichen Aspekte die günstigsten Ergebnisse erbrachten (grobes Schrot und fein vermahlenes und pelletiertes Futter), wurden im zweiten Mastversuch genutzt um deren Auswirkungen an zwei unterschiedlichen Futtermischungen zu testen, die sich vor allem in der Wahl der Proteinträger unterschieden (Standardrezeptur mit Sojaextraktionsschrot vs. Rapsextraktionsschrot/ Bioethanolschlempe/ Sojabohne). Die 100 eingesetzten männlichen, kastrierten Masthybriden wurden korrespondierend zum ersten

Mastversuch unter den gleichen Bedingungen über zehn Wochen gemästet. Die Untersuchung der Verdaulichkeit erfolgte hier mittels unverdaulichem Marker während der Mastperiode.

Die Verdaulichkeit der Rohnährstoffe konnte im ersten Mastversuch durch eine Reduzierung der Partikelgröße im Futter signifikant gesteigert werden. Dies führte wiederum zu einer Verminderung des Futteraufwandes. Die tägliche Lebendmassezunahme und die Futteraufnahme wurden nicht durch den Vermahlungsgrad beeinflusst. Jedoch zeigten die Tiere der Fütterungsgruppen mit grobem Futterausgangmaterial eine deutlich gleichmäßigere Zunahme über die gesamte Mast im Vergleich zu Tieren, die fein vermahlenes Futter mit oder ohne hydrothermische Behandlung erhielten. Auch die hydrothermische Behandlung zeigte positive Auswirkungen auf die Verdaulichkeit der Rohnährstoffe. Feines Vermahlen in Kombination mit Expandieren und Pelletieren des Futters führte dabei zu den höchsten Verdaulichkeiten der Rohnährstoffe. Während jedoch im ersten Mastversuch nur bei einem sehr hohen Veredelungsgrad des Futters eine signifikante Steigerung der Verdaulichkeit gegenüber grobem mehlförmigen Futter zu erreichen war, konnte im zweiten Mastversuch die Verdaulichkeit fast aller Rohnährstoffe durch eine feine Vermahlung und Pelletierung signifikant gegenüber grobem Schrot gesteigert werden. Daher trägt die Zusammensetzung der Futterration maßgeblich zu der Ausprägung des Effektes in Bezug auf die Verdaulichkeit bei. Die hydrothermische Behandlung konnte in beiden Experimenten keine Steigerung der Futteraufnahme oder der täglichen Zunahme bewirken. Der Futteraufwand wurde jedoch ungeachtet des vorherigen Vermahlungsgrades im Vergleich zum grob vermahlenen Schrot durch das Pelletieren signifikant gesenkt. Expandieren des kompletten Futters ohne darauffolgendes Pelletieren führte zu einer signifikanten Senkung der Futteraufnahme, die eine deutliche Reduzierung der Lebendmassezunahme mit sich trug. Als maßgeblicher Einflussfaktor für diesen Effekt wird dabei die Ausbildung von Verklumpungen des Mageninhaltes angenommen, die während der Schlachtung beobachtet wurden und womöglich zu einer persistierenden Sättigung führen. Als Risikofaktor für die Ausbildung dieser Verklumpungen scheinen dabei die hydrothermische Behandlung und die damit verbundene Stärkemodifikation zu fungieren. Ein Effekt der technischen Futtermittelbehandlung auf die Schlachtleistung konnte lediglich im zweiten Mastversuch nachgewiesen werden. Die Ausschlachtung der Tiere, die fein vermahlenes, pelletiertes Futter erhielten, war signifikant höher, der Magerfleischanteil tendenziell niedriger im Vergleich zu Tieren, die grob vermahlenes Schrot erhielten.

In beiden Versuchen wurde die protektive Wirkung grob vermahlenen Schrotes gegenüber Magenläsionen nachgewiesen. Hingegen erweist sich das Verfüttern von feinem

Mischfutter oder pelletierten Futters als Risikofaktor für die Entstehung solcher Läsionen. Eine grobe Vermahlung des Futters vor der Pelletierung konnte keine mildernde Wirkung erzielen. Im Vergleich zu Tieren, die lediglich pelletiertes Futter erhielten, hatten Tiere, die expandiertes Futter mit und ohne Nachpelletierung erhielten, eine geringere Inzidenz für Magenläsionen, konnten jedoch nicht das Niveau der Tiere, die das grobe Schrot erhielten, erreichen. Nach den Ergebnissen des zweiten Mastversuches besteht ebenfalls ein Zusammenhang zwischen der Ausbildung der Magenläsionen und der Futterkomponentenwahl. Signifikante Korrelationen zwischen dem pH-Wert des Mageninhaltes und der Ausbildung von Magenläsionen konnten lediglich im ersten Mastversuch gefunden werden. Jedoch wurden in beiden Mastversuchen signifikante negative Korrelationen zwischen dem Trockenmassegehalt im Magen und dem Auftreten von Magenläsionen nachgewiesen.

Im zweiten Versuch wurden neben den Effekten der behandelten Futtermittel auf die Magengesundheit zusätzlich Daten des intestinalen Immunsystems von 13 Schweinen je Fütterungsgruppe anhand der T-Zell-Phänotypisierung von Leukozyten des jejunalen Epithels, der Lamina propria und der Peyerschen Platten ermittelt. Ein Effekt der technischen Futterbehandlung auf den Anteil der $CD4^+$ T-Zellen (T-helfer Zellen), der $CD8^+$ T-Zellen (zytotoxische Zellen), der $CD8^{low}$ T-Zellen und der Anteile CD4/CD8 doppelt positiv gefärbter T-Zellen sowie der mittleren Fluoreszenzintensität der $CD4^+$ T-Zellen und dem Verhältnis zwischen $CD8^{low}$ und $CD8^{high}$ T-Zellen der Peyerschen Platten wurde nachgewiesen. Dabei wiesen Tiere, die fein vermahlenes, pelletiertes Futter erhielten, geringere Werte der genannten Parameter auf als Tiere, die grobes Schrot erhielten. Die T-Zellpopulationen anderer Lokalisationen sowie die der *Lymphonodii gastrici* oder im Blut wurde nicht durch die Futtermittelbehandlung beeinflusst. Die Wahl der Futterkomponenten hatte keinen Einfluss auf das lokale oder periphere Immunsystem der Tiere. Signifikante Unterschiede wurden in der intestinalen T-Zellpopulation und der T-Zellpopulationen der *Lymphonodii gastrici* zwischen Tieren mit geringem (0-0,5), mittlerem (1-2,5) und hohem Magenscore (3-4) ermittelt. In einigen Fällen konnten signifikante Korrelationen zwischen der Immunmodulation und dem Magenscore sowie dem pH-Wert oder des Trockenmassegehaltes des Mageninhaltes festgestellt werden.

Zusammenfassend sind pelletierte Futtermittel aufgrund der Steigerung der Futtereffizienz und der resultierenden Wirtschaftlichkeit als positiv zu bewerten. In Bezug auf den Aspekt des Tierwohls und gesundheitlicher Aspekte ist grobes mehlförmiges Futter zu bevorzugen. Expandieren der gesamten Futtermischung ist in der Schweinemast nicht empfehlenswert. Es ergibt sich ein Interessenskonflikt zwischen hoher Tierleistung und der Tiergesundheit. In Bezug auf die Produktion tierischer Lebensmittel ist jedoch hervorzuheben, dass die Tiergesundheit in erheblichem Maße zu einer ressourcenschonenden Erzeugung tierischer Lebensmittel beiträgt.

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Eidesstattliche Erklärung / Declaration under Oath

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. *I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.*

Braunschweig, …………………. …………………………..

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