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Benzoic acid as feed additive in pig nutrition: Effects of diet composition on performance, digestion and ecological aspects

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KATHRIN BÜHLER
Dipl. Sc. Nat. ETH

born May 2, 1979
citizen of Winterthur ZH

accepted on the recommendation of

Prof. Dr. C. Wenk, examiner
Dr. B. Bucher, co-examiner
Dr. J. Broz, co-examiner

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Abbreviations

ADF	Acid detergent fibre
AGP	Antimicrobial growth promoter(s)
AP	Total alkaline phosphatase
BMC	Bone mineral content
BMD	Bone mineral density
BW	Body weight
$BW^{0.75}$	Metabolic body weight
CA	Crude ash
CP	Crude protein (N * 6.25)
d(xy)	Apparent digestibility of nutrient xy
DE	Digestible energy
DM	Dry matter
DWG	Daily weight gain
FCR	Feed conversion ratio
GE	Gross energy
MT	Metatarsus
NDF	Neutral detergent fibre
NH_3	Ammonia
OC	Osteocalcin
SCL	Serum crosslaps
SD	Standard deviation
SEM	Maximal standard error of the means
SI	Small intestine
VFA	Volatile fatty acid(s)

Experimental diets:

LPr-	Low protein diet without benzoic acid
LPr+	Low protein diet with 1 % benzoic acid
HPr-	High protein diet without benzoic acid
HPr+	High protein diet with 1 % benzoic acid
CC	Low-P diet without phytase, without benzoic acid
CB	Low-P diet without phytase, with 0.5 % benzoic acid
PhyC	Low-P diet with 750 U/kg phytase, without benzoic acid
PhyB	Low-P diet with 750 U/kg phytase, with 0.5 % benzoic acid
LF-	Low fibre diet without benzoic acid
LF+	Low fibre diet with 0.5 % benzoic acid
HF-	High fibre diet without benzoic acid
HF+	High fibre diet with 0.5 % benzoic acid

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Summary

Modern animal production is trapped between consumer's concerns on animal and human health, environmental aspects and an increasing demand for animal products. To overcome the ban on antimicrobial growth promoters in Europe alternatives are needed to maintain high productivity. A promising approach in pig nutrition is the use of organic acids. Naturally, they occur in many fruits and in the organisms' metabolism and are therefore considered to be harmless. In addition, these acids show strong antimicrobial activity which makes them a valuable alternative for the banned growth promoters. The most potent of the organic acids seems to be benzoic acid. However, the effects of this acid vary with the diets used and the animal's age. Furthermore, it is unknown whether the use of benzoic acid may also help to reduce environmental problems caused by animal production.

The aim of these studies was to examine the effects of benzoic acid added to different diet types. The main question thereby was how benzoic acid influences performance and digestion in growing-finishing pigs. The selection of the diets additionally allowed conclusions on how benzoic acid may affect nutrient excretion and thus the environmental impact of pig production.

To answer these questions three 2 x 2 factorial digestibility studies with growing-finishing pigs were conducted. The diets were, each without and with benzoic acid: two levels of crude protein in study one (nitrogen study), low-P diet without or with phytase supplementation in study two (phytase study) and two levels of fibre in study three (fibre study). The animals were fed restrictively one of the four experimental diets during the grower and the finisher period. Parameters examined were weight gain, feed conversion ratio and nutrient digestibility and, depending on the study, urinary pH, bone metabolism and volatile fatty acid production.

Independent of the protein content in the nitrogen study, animals fed the diets supplemented with benzoic acid showed an improved weight gain (+ 7 %) and better feed conversion ratio (- 3 %) but the differences were not statistically significant. N excretion increased with increasing protein content. However, N balance was similar for all diets but apparent N digestibility in the grower period was higher with benzoic acid in the diet. At both protein levels 1 % of benzoic acid reduced urinary pH by about one pH-unit and decupled the excretion of hippuric acid. There was no interaction of benzoic acid and dietary protein content observed.

Neither benzoic acid nor phytase had any effect on animal performance in the phytase study. Benzoic acid tended to reduce the apparent digestibility of Ca and P but at the same

time it was increased by phytase. Additionally, a negative interaction between benzoic acid and phytase on nutrient digestibility (- 3 %) could be observed. Phytase showed some positive effects on short time blood parameters describing bone metabolism but no long term effects of any of the two additives were noticed. Concerning bone density, bone composition and bone stability benzoic acid in diets with a low P content reduced these parameters but phytase could compensate for the losses. These effects of benzoic acid were more pronounced in the grower pigs than in the finisher pigs.

In the fibre study weight gain in the grower period was significantly higher (14 %) in pigs fed diets with benzoic acid. The addition of fibres reduced the apparent nutrient digestibility whereas it was improved by benzoic acid. However, this effect could only be observed in the grower period. In addition, benzoic acid also increased digestibility of energy and fibres as well as the production of butyric acid in the gut. The total amount of volatile fatty acids in the gut was comparable for all diets. Similar to the nitrogen study, there were no interactions between benzoic acid and dietary fibre content.

In conclusion it can be said that the growth enhancing properties of benzoic acid were not significant but there were signs of increased performance and better nutrient digestion in all three studies. The results of the phytase study revealed that benzoic acid can interact with other additives such as phytase. From the environmental point of view, benzoic acid reduced urinary pH and increased the nutrient utilization of fibrous rich diets. Apart from these effects the addition of benzoic acid could neither reduce nor did it worsen the impacts of pig production on the environment.

To fully understand and exploit the potential of benzoic acid, the interaction with other additives as well as the exact mode of action should be in focus in future studies. Another point of interest is the influence of benzoic acid on total urinary and faecal nutrient excretion to describe the ecological aspects of this acid in more detail.

Zusammenfassung

Die moderne Tierproduktion bewegt sich im Spannungsfeld zwischen den Sorgen der Konsumenten um Gesundheit und Umwelt und dem steigenden Bedarf an tierischen Produkten. Um die hohe Produktivität auch nach dem Verbot der antimikrobiellen Leistungsförderer zu gewährleisten, sind neue Ideen nötig. Ein vielversprechender Ansatz in der Schweinefütterung ist der Einsatz organischer Säuren. Diese kommen in vielen Früchten sowie im Metabolismus verschiedenster Organismen vor und werden deshalb als natürlich und unbedenklich eingestuft. Zudem können sie starke antimikrobielle Eigenschaften zeigen. Dies macht sie zu einer möglichen Alternative für die verbotenen antimikrobiellen Leistungsförderer. Die Säure mit dem grössten Wirkungspotential scheint Benzoesäure zu sein. Allerdings hängt die Wirkung dieser Säure stark von der Zusammensetzung des Futters sowie dem Alter der Tiere ab. Zudem ist unklar, ob der Zusatz von Benzoesäure negative Umwelteinflüsse der Tierproduktion verringern kann.

Ziel dieser Studien war, die Wirkung von Benzoesäure in unterschiedlich zusammengesetzten Rationen zu untersuchen. Das Hauptaugenmerk lag dabei auf der Leistung und der Nährstoffverdaulichkeit bei wachsenden Schweinen. Die verwendeten Futtervarianten wurden so gewählt, dass zusätzlich Rückschlüsse gezogen werden konnten, ob Benzoesäure die Nährstoffausscheidung und damit die Umweltbelastung durch die Schweineproduktion beeinflussen kann.

Um diese Fragen zu beantworten, wurden drei 2 x 2 faktorielle Verdauungsversuche mit wachsenden Schweinen durchgeführt. Die Futterzusammensetzung, jeweils ohne und mit dem Zusatz von Benzoesäure, war: zwei Rohproteinstufen in Versuch 1 (Stickstoffversuch), reduzierter P-Gehalt ohne und mit dem Zusatz von Phytase in Versuch 2 (Phytaseversuch) und zwei Fasergehalte in Versuch 3 (Faserversuch). Die Tiere wurden sowohl in der Jager- als auch in der Ausmastperiode restriktiv mit einem der jeweils vier Versuchsfutter gefüttert. Untersucht wurde die Mastleistung, die Nährstoffverdaulichkeit und, abhängig vom Versuch, Harn-pH, Knochenmetabolismus und die Produktion flüchtiger Fettsäuren.

Unabhängig vom Proteingehalt im Stickstoffversuch zeigten die mit Benzoesäure gefütterten Tiere einen besseren Zuwachs (7 %) und eine verbesserte Futterverwertung (- 3 %) als jene, die keine Benzoesäure erhielten. Die Unterschiede waren jedoch nicht signifikant. Mit zunehmendem Proteingehalt stieg auch die N-Ausscheidung, doch die N-Bilanz war in allen Varianten vergleichbar. Andererseits war in der Jagerperiode die scheinbare N-Verdaulichkeit durch Benzoesäure erhöht. Bei beiden Proteinstufen senkte 1 % Benzoesäure

den Harn-pH im Mittel um eine pH-Einheit und verzehnfachte die Ausscheidung von Hippursäure. Es konnte keine Interaktion von Benzoesäure und Proteingehalt beobachtet werden.

Im Phytaseversuch hatten weder Benzoesäure noch Phytase einen Einfluss auf die Mastleistung. Die Verdaulichkeit von Ca und P wurde durch Benzoesäure leicht gesenkt und durch Phytase gesteigert. Zusätzlich bestand eine negative Interaktion zwischen der Säure und Phytase (- 3 %). Bei den Blutparametern des Knochenmetabolismus konnten kurzzeitige positive Effekte der Phytasezugabe beobachtet werden. Langzeiteffekte, die durch Benzoesäure oder Phytase hervorgerufen wurden, konnten jedoch nicht gefunden werden. Bezüglich Knochendichte, -zusammensetzung und -stabilität zeigte Benzoesäure in Futter mit tiefem P-Gehalt eine reduzierende Wirkung, die aber durch den Zusatz von Phytase kompensiert werden konnte. Dies war besonders ausgeprägt in der Jagerperiode.

Im Faserversuch steigerte die Benzoesäure den Zuwachs in der Jagerperiode signifikant (+ 14 %). Der Zusatz der Säure verbesserte zudem die scheinbare Nährstoffverdaulichkeit, während der erhöhte Fasergehalt zu einer Verschlechterung führte. Dieser Effekt konnte jedoch nur in der Jagerperiode beobachtet werden. Innerhalb des Verdauungstraktes erhöhte Benzoesäure die Verdaulichkeit der Energie und der Fasern und führte zu einer gesteigerten Produktion von Buttersäure. Der Gesamtgehalt der gebildeten flüchtigen Fettsäuren war für alle Futter dieses Versuchs vergleichbar. Ähnlich wie im Stickstoffversuch wurden keine Interaktionen zwischen Benzoesäure und Fasergehalt gefunden.

Die Leistungsverbesserung durch den Zusatz von Benzoesäure konnte nicht abschliessend nachgewiesen werden. Es bestand jedoch in allen drei Versuchen eine allgemeine Tendenz zu verbesserter Leistung und Nährstoffverdaulichkeit. Andererseits zeigten die Resultate auch, dass Benzoesäure mit anderen Additiven, wie Phytase, interagieren kann. Neben der Reduktion des Harn-pH und der verbesserten Verwertung faserreichen Futters konnten keine Effekte zur Verminderung der Umweltbelastung durch den Einsatz von Benzoesäure in der Schweineproduktion gefunden werden.

Um das ganze Potential der Benzoesäure verstehen und nutzen zu können, sind weitere Studien zur Interaktion mit anderen Additiven wie auch zur genauen Wirkungsweise nötig. Ebenfalls von Interesse ist der Einfluss der Benzoesäure auf die Gesamtausscheidung von Nährstoffen, damit die ökologischen Aspekte dieser Säure genauer beschrieben werden können.

General Introduction

Animal products play an important role in human nutrition. However, the intensification of animal production has led to increasing concerns related to health issues and the environment. The former is mainly connected to diseases and the use of antibiotics in animal production whereas the latter has to be looked at from two angles: first the pollution of the environment through excessive nutrient deposition, second the competition between man and animal for food. There is a balancing act between satisfying an increasing demand for animal products and reducing the impact of animal production on man and nature at the same time. One approach to overcome this challenge consists in various dietary measures, such as making use of new additives and their effects in different diets. Since, as stated by Aarnink and Verstegen (2007), "nutrition is a key factor in reducing environmental problems".

Nutrient composition

Nitrogen

In feed and in the body nitrogen (N) is mainly present as amino acids and thus as proteins making it a crucial element for normal body function. Nevertheless, pigs are only capable of utilizing about 30 % of the N intake (Dourmad et al. 1999a; Dourmad et al. 1999b; van der Peet-Schwering et al. 1999a). The remaining N is excreted via faeces and urine in the ratio of about 1:3 (Jongbloed and Lenis 1992; Ferket et al. 2002) (Figure 1.1).

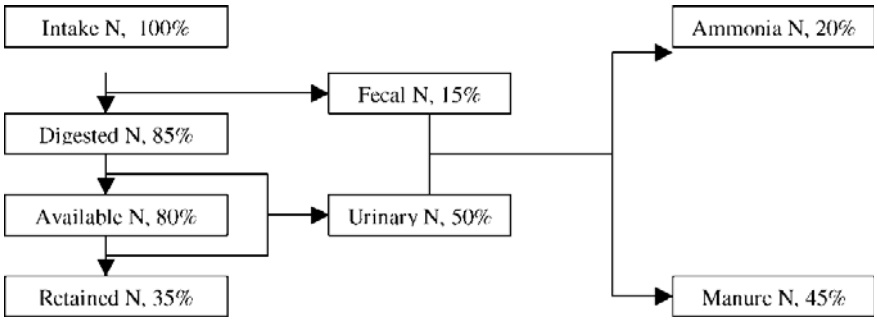

Figure 1.1. Nitrogen flow in poultry and swine (Ferket et al. 2002)

The exact amount of retained N can vary considerably (van der Peet-Schwering et al. 1999a) and depends on the protein digestibility of the dietary components (Gatel and Grosjean 1992) as well as on the requirement of the animals, which depends on sex, age, genotype and physiological status (Jongbloed and Lenis 1992; Dourmad and Jondreville 2007). In case of excess N in the diet or if the composition of amino acids does not meet the animals' requirements, N excretion is increased (Jongbloed and Lenis 1992). Such a situation can occur

when safety margins and economic pressure for cheap ingredients lead to an inadequate N and protein supply (Gatel and Grosjean 1992; Aarnink and Verstegen 2007). The excessive excretion of N has led to severe environmental problems in regions with intense animal production.

Whereas N in slurry evaporates mainly as ammonia (NH_3) during storage, N applied to the fields is lost by nitrate leakage or as NH_3 and nitrous oxide. Leakage can pollute surface and groundwater, causing eutrophic water bodies. Ammonia and other gaseous N components, on the other hand, can cause acid rain and undesired N deposition in normally N deficient ecosystems such as bogs and rough pasture. The excessive deposition of N also causes soils to become more acidic resulting in less resistance of plants to droughts and higher susceptibility of trees to storm damages (Van der Eerden 1998). On top of that, NH_3 is an odorous nuisance and even toxic for humans and animals.

In order to prevent N pollution, several countries established legislation and defined upper limits or reduction goals for nitrates and gaseous N emissions. The most important ones in Europe are the Gothenburg Protocol (also ratified by the USA and Canada) 'to abate acidification, eutrophication and ground-level ozone' of 1999, the IPPC directive on pollution prevention and control (96/61 EC, now renamed to 2008/1/EC), the nitrate directive (91/676/EC) to protect water and the NEC directive (2001/81/EC) defining ceiling levels for atmospheric pollutants. Within these legislations several countries such as Denmark and the Netherlands have set up their own measures to reduce the environmental impact of agriculture (Jongbloed et al. 1999; Schroder and Neeteson 2008). Legislations in Switzerland on the other hand are less strict (Gassner 2006), but each Canton can set up its own, more restrictive, legislation.

To fulfil these restrictions it is necessary to reduce nitrogen excretion and NH_3 emission. So far, there are three well known and intensely described dietary measures that are additive (Jongbloed 2007). Within these measures it is important that the nutrient requirements of the animal are met. This supports optimal performance and good health and also helps to reduce environmental pollution:

- The concept of the ideal protein, a reduction of total dietary protein and phase feeding improve N utilisation and help to better meet the animal's N requirements thus reducing excessive N excretion (Fuller et al. 1989; Gatel and Grosjean 1992; Canh et al. 1998b; Philippe et al. 2006; Dourmad and Jondreville 2007). However, the protein should be of high availability to ensure optimal use of this nutrient.

- A high fibre content in the diet decreases the urine-N/faeces-N ratio as more microbial protein is built and excreted via faeces. In this form N is more resistant to degradation than when it is excreted as urea. As a consequence of the slower degradation, NH_3 emissions from slurry are reduced and N availability for plants is improved (Canh et al. 1998c; Hansen et al. 2006).
- Acidification of urine, faeces or slurry or all three of them reduces the activity of urease. This enzyme is present in faeces and degrades the urea from the urine to NH_3 when the two come into contact. On the other hand, the ammonia/ammonium equilibrium ($NH_3 + H_3O^+ \leftrightarrow NH_4^+ + H_2O$) is shifted to ammonia at a low pH. Thus, both mechanisms are capable of reducing NH_3 emission especially from stables and from stored slurry (van Kempen 2001; Hansen et al. 2007).

Phosphorus

Between 75 to 85 % of the phosphorus (P) in the body is stored in bones (Poulsen 2000; Liesegang et al. 2002) but it is also present in teeth, cell membranes, cellular energy carriers such as ATP and it plays a key role in a variety of different cellular mechanisms. Thus sufficient P supply is essential for good skeletal health and normal body functions. However, P digestibility in feedstuffs varies considerably: it ranges from 65 – 90 % for inorganic P and P from animal origin to 10 – 50 % for P in plants (Gebert 1998). The low P digestibility in plants is mainly due to the fact that in these organisms 50 – 85 % of the P is stored as phytate (Eeckhout and De Paepe 1994), the salt of phytic acid (Figure 1.2). In this form, P is almost unavailable for monogastric animals (Eeckhout and De Paepe 1994; Pallauf and Rimbach 1997).

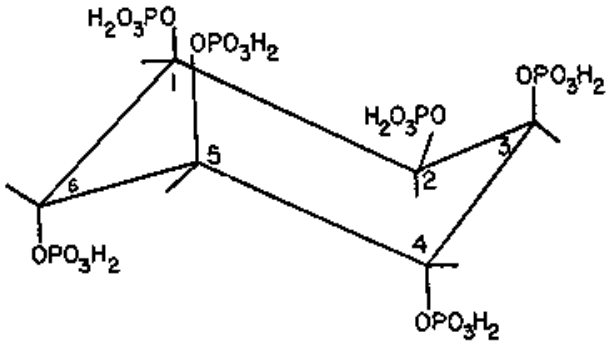

Figure 1.2. Structure of phytic acid (FAO)

The main alternative source is inorganic P as meat and bone meals for animal nutrition are prohibited (Rodehutscord et al. 2002). However, this additional P, which should prevent a P deficiency in the diet, also increases P excretion. The effects of massive P excretion on the environment are similar to those observed with N: excessive P leaks into the water and causes an eutrophication of lakes and rivers. For this reason, legislations set up for nitrogen in most cases also include limits for P. Therefore, measures are needed to obtain sufficient P supply and to reduce P losses.

A very efficient way of reducing P excretion is the reduction of the overall P content in the diet and the improvement of P availability in breaking down the phytate molecule with the enzyme phytase. This enzyme hydrolyses phytate, either starting at position 3 (3-phytases, E.C. 3.1.3.8) or at position 6 (6-phytases, E.C. 3.1.3.26) and is synthesized by plants and microorganisms (Pallauf and Rimbach 1997) but not by animals and humans. It has been reported repeatedly (Pallauf and Rimbach 1997; Jongbloed et al. 2000; Lei and Stahl 2000; Gentile et al. 2003; Jendza et al. 2005; Thacker et al. 2006) that the addition of phytase to the diet reduces the need for additional P and the excretion of P.

The disadvantages of native phytase, however, are low heat stability and a narrow pH-range for optimal activity. These aspects limit its use. Techniques of genetic modifying and 'bio-engineering' allowed the improvement of phytases in terms of heat stability, efficiency and proteolytic stability. This has led to a variety of commercially available and thus widely used phytases and the research – also for new phytases – is still going on (Greiner et al. 1993; Igbasan et al. 2000; Simon and Igbasan 2002; Augspurger et al. 2003; Vats and Banerjee 2004; Greiner and Farouk 2007).

Today, the use of phytases in non-ruminant nutrition is well established. This is not only due to environmental issues and the need to achieve an even P balance but also to the fact that the natural reservoirs of inorganic P are almost depleted. This scarceness causes a massive rise in prize and an urgent need for economic usage of this mineral. Furthermore, phytase can improve performance and nutrient digestibility (Mroz et al. 1994; Gebert et al. 1999; Johnston et al. 2004; Jendza et al. 2005) which makes the use of this enzyme even more interesting.

Dietary fibre

Dietary fibre, also referred to as fibres, is defined as "the sum of plant polysaccharides and lignin that are not hydrolysed by endogenous enzymes of the mammalian digestive system" (Wenk 2001). Based on this definition, fibres form a very heterogeneous cluster (Figure 1.3).

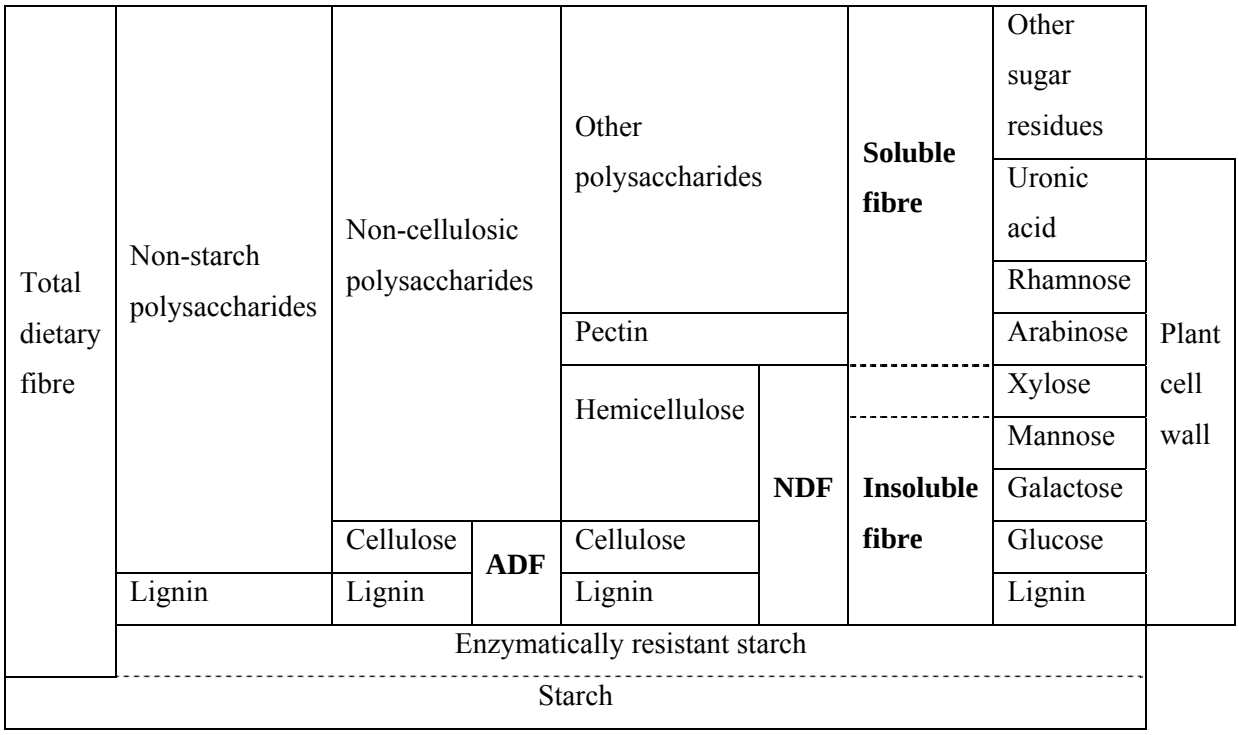

Figure 1.3. Analytical fractionations used in relation to dietary fibre analysis. Broken lines indicate boundaries that are not absolute. (Adopted and modified from Asp et al. (1992)).

There are two main approaches to classify the different groups of fibres:

- Analytical approach: crude fibre (CF) consists of cellulose, lignin and some hemicelluloses. Neutral detergent fibre (NDF) includes hemicellulose, cellulose and lignin whereas cellulose and lignin are termed as acid detergent fibre (ADF). Non starch polysaccharides (NSP) consists of cellulose, pectin, β-glucans, pentosans and xylans (Bach Knudsen 2001; Souffrant 2001). Unfortunately, this grouping is an ideal assumption which cannot be followed in the corresponding analytical methods. The analysis of CF has two major flaws: first, it is the oldest of the analytical methods and is based on chemical and not enzymatical extraction (Mertens 2003). Secondly, a considerable amount of lignin is dissolved and thus removed during CF analysis resulting in a reduced CF content which in addition mainly consists of cellulose (Asp et al. 1992; Lipiec et al. 1994; Mertens 2003). Similarly, during NDF analysis some NSP may be lost and a part of the starch and the protein may not be removed. In the case of ADF, some hemicelluloses may remain in the residues after ADF analysis thus pretending a higher amount of ADF (Bach Knudsen 2001). For the determination of the dietary fibre content, NDF or ADF but not CF analysis should be considered (Asp et al. 1992; Mertens 2003).

- Physiological approach: some hemicelluloses and pectin can bind water in high quantities and are therefore called soluble fibre. In contrast to that certain hemicelluloses, cellulose and lignin belong to the insoluble fibre (Pisarikova et al. 2007).

As there is no such thing as 'the' fibre, the differences in structure, physical and physico-chemical properties define the effects of fibres after ingestion in detail. According to Canibe and Knudsen (2001) the most important factors are thereby: solubility, viscosity, hydration properties, particle size, fermentability and binding capacity for organic compounds. It seems that the type of fibre even plays a more important role than the amount (Stanogias and Pearce 1985a; Wenk 2001). On the animal's side the capacity to use fibre rich diets correlates with age and live weight (Fernandez and Jorgensen 1986; Noblet and Shi 1993; Le Goff et al. 2002b).

Despite this variety, fibres have some common effects on the behaviour and the digestive physiology of pigs. Whittaker et al. (1999) and Ramonet et al. (2000) reported less aggression and less stereotypic behaviour in sows fed high fibre diets. Also the well-being of fattening pigs is improved with such diets (Wenk 2001; Gerrits et al. 2003). High fibre diets reduce the rate of gastric emptying and thus increases satiety. This is especially important in pregnant sows to prevent them from becoming too heavy and adipose and thus from having problems at farrowing (Ramonet et al. 2000). Fibres have also been reported to increase transit time (Dierick et al. 1989; Varel and Yen 1997). As they are not degraded in the small intestine, fibres increase the amount of material reaching the large intestine, causing higher gut content and thus reduced transit time and more faeces (Bach Knudsen 2001). Other effects observed with high fibre diets are less stomach ulcers (Baustad and Nafstad 1969; Amory et al. 2006; Bolhuis et al. 2007), increased digestive secretion (Dierick et al. 1989; Wenk 2001; Souffrant 2001) and morphological changes of the gut lumen (Jin et al. 1994; Hedemann et al. 2006; Serena et al. 2008).

Nevertheless, there are also some undesired effects that have to be considered when feeding fibre rich diets. Most of the adverse effects of such diets are due to the fact that fibres are not digested by enzymes but fermented by microorganisms. Enzymatic digestion takes place in the small intestine where almost all nutrients can be easily absorbed by the gut lumen. Microbial fermentation on the other hand is located in the caecum and colon. There the absorption of energy and nutrients, especially of proteins, is limited (Wenk 2001) and more nutrients (e.g. microbial protein) are excreted with the faeces. Additionally, fibres may reduce nutrient absorption in the small intestine because of increased transit time or higher viscosity

(Dierick et al. 1989; Bach Knudsen 2001; Souffrant 2001). This leads to a dilution of energy and nutrients (Mosenthin et al. 1999), which can have detrimental effects on animal performance (Drewry 1981).

During fibre fermentation volatile fatty acids (VFA) are produced. VFA are short (C2 – C6) fatty acids which are volatile at room temperature, the most important being in descending order: acetic acid, propionic acid and butyric acid. The concentrations of these VFA in the hindgut are on average 70 % : 20 % : 10 % (Friend et al. 1963; Argenzio and Southworth 1975; Bergman 1990; Kirchgessner and Müller 1991). Unlike other nutrients VFA can easily be absorbed in the large intestine (Jorgensen et al. 1997). Similar to common fatty acids, VFA can be metabolized by the body and thus contribute to the pig's energy requirements. Depending on the study, VFA can provide between 10 % and 30 % of the energy needed (Dierick et al. 1989; Vervaeke et al. 1989; Bergman 1990; Yen et al. 1991; Kirchgessner and Müller 1991). The wide range can partly be explained by the relation of fibre content to VFA production (Stanogias and Pearce 1985b; Bach Knudsen and Jensen 1991; Anguita et al. 2006). Apart from being an energy source, VFA also have other important metabolic functions. Muscles, the kidneys and the heart use primarily acetic acid as energy source. It also takes part in the synthesis of long chain fatty acids (Bergman 1990; Blottiere et al. 1999). Propionic acid is completely metabolized in the liver and can act as precursor for glucose synthesis (Bergman 1990; Blottiere et al. 1999). Most of the butyric acid is taken up and metabolized by the gut epithelium. There, it serves as an important energy source for colonocytes. Furthermore, butyric acid plays a part in cell maturation, cell differentiation and apoptosis (Freire et al. 2000; Bach Knudsen et al. 2003).

In contrast to N and P the content of dietary fibre in pig nutrition has been steadily increased in the last years. One reason is that fibre rich feed ingredients are often cheaper than usual ones (Le Goff et al. 2002a). Secondly, the use of fibre rich products can reduce the competition with food (cereals) for human nutrition (Varel and Yen 1997).

Organic acids as feed additives

When it was clear in the late 1990s that a total ban on antimicrobial growth promoters (AGP) is only a matter of time, organic acids came into focus as a possible 'natural' alternative. The total ban on AGP in Switzerland in 1999 (LwG, Art. 160, Abs. 8) and the EU in 2006 (1831/2003 EC) was the consequence of a series of studies on the resistance inducing properties of subclinical dosages of antibiotics used in animal nutrition. The discussion on the

safety of AGP began as early as 1951, when Starr and Reynolds (1951) reported observations of bacterial resistance to AGP. Their observation was confirmed in the Swann report (1969), which even suggested a restriction in the use of AGP, especially of substances also used in humans. Ongoing research on development and occurrence of antibiotic resistances (Bates et al. 1993; Barton 2000; DANMAP 2004; Ghosh and Lapara 2007; Alexander et al. 2008) as well as increasing concerns of consumers finally led to the ban on AGP.

Organic acids are weak acids with at least one carboxylic group (-COOH) and a carbon chain having not more than 10 C–atoms, normally C1 to C7. This distinguishes them from the fatty acids with longer carbon chains (Theron and Lues 2007). In general organic acids, which were added to the diets, are absorbed quite rapidly and without previous degradation in the stomach or no later than in the small intestine. After absorption, some organic acids can serve as an additional energy source for the animal. The two extremes in providing digestible energy are benzoic acid (0 MJ/kg) and propionic acid (20.6 MJ/kg). Organic acids occur in or are produced by plants, animals and bacteria and are an important compound in the metabolism of all organisms. Because of their natural origin and because organic acids show strong antimicrobial and antifungal properties (Brul and Coote 1999; Ricke 2003) they are considered to be harmless in adequate doses and seem to be a promising alternative to AGP.

There are several organic acids used in animal nutrition for performance enhancement with different origins and characteristics. Many of them have been used for a long time as preserving agents in feed and food (Ricke 2003). They are either supplemented as free acids or as salts, the latter having the advantage of being less corrosive, less odorous and easier to handle than the free acid. Another, rather new form used in animal nutrition is coated acids. The coating allows a stepwise release of the acid so that it can pass through the small intestine and also act directly in the large intestine (Piva et al. 1997; Piva et al. 2002). Factors influencing the decision of which acid to use in which form and which dosage in animal nutrition not only depends on the efficacy but also on the costs which can vary considerably among the different commercially available organic acids.

Benzoic acid

Benzoic acid is the simplest of the aromatic carboxylic acids and forms colourless to white crystals. It occurs in different resins, in fruits and berries, especially from the genus *Vaccinium*, but also in milk and milk products and animal tissue and gland secretions (CICA 2000).

This acid has been used for food preservation in human nutrition (E-number: E210) for a long time, but it was only introduced to pig nutrition in 2003, now listed in the group of "other zootechnical additives" (1138/2007/EC). In pig nutrition, benzoic acid has been shown to increase daily weight gain and to improve feed/gain ratio (van der Peet-Schwering et al. 1999b; Maribo et al. 2000; Dierick et al. 2004; Guggenbuhl et al. 2007b) in piglets and growing-finishing pigs. In *in vitro* studies benzoic acid was very potent in reducing overall caecal microflora (Biagi and Piva 2005) and also in imposing adverse effects on coliforms as well as on lactic acid bacteria (Knarreborg et al. 2002). Results from *in vivo* studies are less consistent. Some are in accordance to the *in vitro* results (Maribo et al. 2000), others show counts of lactic acid bacteria which can increase or decrease depending on the amount of benzoic acid in the diet (Kluge et al. 2006). Finally, some even describe increasing numbers of *E. coli* (Dierick et al. 2004; Torrallardona et al. 2007). Part of this variability can be explained by the fact that the effects depend on the examined section of the gastrointestinal tract. The effects can even be oppositional depending on the gastrointestinal segments examined.

Beside its antimicrobial effects, benzoic acid can also reduce urinary pH (van der Peet-Schwering et al. 1999b; Kluge et al. 2006; Plitzner et al. 2006). After absorption from the small intestine, the acid is transported to the liver where it conjugates with the amino acid glycine to hippuric acid. In this form benzoic acid is then excreted to 90 – 100 % within 24 h via the urine (Bridges et al. 1970). This reduction of urinary pH can markedly reduce ammonia emission (Mroz et al. 2000; Hansen et al. 2007), which is of great importance in areas with intense pig production.

Citric acid

Citric acid is a rather complex acid with three carboxylic groups. At room temperature it is present as white and odourless crystals with a sour taste. Citric acid is mainly present in vegetables, berries and fruits, especially in citrus fruits. As one of the first intermediate in the citric acid cycle, citric acid and its salts can be found in almost all organisms.

Due to its importance in the metabolism, many microorganisms are adapted to this acid. For this reason citric acid is not such an efficient antimicrobial agent as other acids (Partanen and Mroz 1999). Despite this, the addition of citric acid showed positive effects on performance of piglets (Giesting and Easter 1985; Radcliffe et al. 1998; Tsiloyiannis et al. 2001a) and chickens (Boling et al. 2000; Rafacz-Livingston et al. 2005; Liem et al. 2008). To

what amount these effects are based on antimicrobial activity and how much they are caused by the digestible energy content of 10.2 MJ/kg or by boosting the citric acid cycle is unclear.

Apart from its growth enhancing properties citric acid can also improve the utilization of phytate P in chickens (Boling et al. 2000; Liem et al. 2008; Ebrahimnezhad et al. 2008). This has also been described for pigs but there the effect is less pronounced (Boling et al. 2000).

Formic acid

Formic acid, a colourless liquid with a pungent odour, is the simplest of the organic acids. It can be found in plants and insects where it mainly serves as defending agent. On the other hand, formate is an integral part of the metabolism, especially in the transfer of 1-C intermediates and in the synthesis of purines (Partanen and Mroz 1999).

In animal nutrition formic acid is mainly used as formate, as the salt is less corrosive and less toxic than the free acid. There is an abundance of studies in piglets and growing-finishing pigs describing growth enhancing and antimicrobial effects of formic acid and its salts (Bolduan et al. 1988a; Eidelsburger et al. 1992c; Overland et al. 2000; Partanen et al. 2001; Ettle et al. 2004; Eidelsburger et al. 2005; Eisemann and van Heugten 2007). When adding formic acid to the diet in excess disturbances in the acid-base balance of the animals and symptoms of intoxication may occur (Eckel et al. 1992). This in turn can reduce performance.

Fumaric acid

Fumaric acid is present in a white, crystalline form and has a fruit like taste. Naturally it is found in plants, fungi and lichens. Similar to citric acid it is also an intermediate of the citric acid cycle. Additionally, fumaric acid is produced by the urea cycle and by the degradation of the amino acids phenylalanine and tyrosine (Partanen and Mroz 1999).

The growth promoting effects of fumaric acid were first described by Kirchgessner and Roth in the late 1970s (1978). Since then, many more studies were conducted, mainly with piglets but some with broilers, too. In poultry, daily weight gain and feed conversion ratio was increased when about 2.5 – 3 % of fumaric acid were added to the diets (Rafacz-Livingston et al. 2005; Liem et al. 2008) but not at lower inclusion levels (Pirgozliev et al. 2008). In swine, especially piglets, fumaric acid is added to the feed in the amount of 0.5 – 2 %, rarely higher. In general, fumaric acid increased daily weight gain and improved feed conversion ratio at least numerically (Edmonds et al. 1985; Thacker et al. 1992; Risley et al. 1993; Krause et al. 1994; Tsiloyiannis et al. 2001a) but there are also studies showing no (Bosi et al. 1999) or

even negative effects (Henry et al. 1985; Mroz et al. 2000). Fumaric acid can also increase the concentration of VFA in caecum and colon (Sutton et al. 1991; Roth et al. 1992b) and tends to reduce lactobacilli and E. coli (Sutton et al. 1991; Gedek et al. 1992b).

Lactic acid

Lactic acid is a colourless liquid mainly produced by lactic acid bacteria such as *Lactobacillus, Bifidobacterium* and others through fermentation of carbohydrates. In animals, lactate is produced in muscles from pyruvate, in the brain and in erythrocytes.

In weaning pigs lactic acid plays an important role in lowering gastric pH. This supports beneficial bacteria and suppresses *E. coli* (Thomlinson and Lawrence 1981) and can prevent postweaning diarrhoea (Tsiloyiannis et al. 2001a). Various studies showed that performance is increased when lactic acid is added to diets for piglets (Tsiloyiannis et al. 2001a; Tsiloyiannis et al. 2001b; Kil et al. 2006). At the same time diarrhoea and mortality can be massively reduced (Tsiloyiannis et al. 2001a; Tsiloyiannis et al. 2001b). Positive effects on performance and nutrient digestibility were also described for older animals (Kemme et al. 1999a; Kemme et al. 1999b; Jongbloed et al. 2000).

Propionic acid

Propionic acid is a colourless oily liquid with rancid odour. It is a degradation product of certain fatty acids and amino acids and it is produced in the hindgut by bacterial fermentation. When added as feed additive it has been found to improve weight gain in growing pigs (Thacker et al. 1992) and to reduce it in weaned piglets (Giesting and Easter 1985). However, the only significant change in performance and survival rate was described by Tsiloyiannis et al. (2001a), adding 1 % propionic acid to the diet of weaned pigs.

Sorbic acid

At room temperature sorbic acid forms colourless crystals. It naturally occurs in the berries of rowan (*Sorbus aucuparia)*. In the body sorbic acid is absorbed and metabolised as a fatty acid (β-oxidation) and can be used as additional energy source (Stopforth et al. 2005). Sorbic acid can be metabolised under certain circumstances by the microorganisms (Stopforth et al. 2005), a fact that has to be considered when using this acid as an antimicrobial agens. The effects of sorbic acid in animal nutrition were described for the first time in 1995 by Kirchgessner et al. (1995). At the dosages of 1.2 %, 1.8 % and 2.4 %, sorbic acid increased

overall performance of pigs up to 26 kg. However, in a choice study with 1.2 % acid, pigs selected preferably the acid free diet (Ettle et al. 2004). In growing-finishing pigs 0.85 % sorbic acid was found to reduce levels of indole and skatole as well as the numbers of bacteria in the gut (Overland et al. 2007; Overland et al. 2008). In contrast to the results in pig studies, sorbic acid in diets for broilers does not seem to improve performance (Pirgozliev et al. 2008).

Other acids and acid blends

Acetic acid is a colourless acid with a distinct taste and odour. It is produced when bacteria such as *Acetobacter* oxidise ethanol to acetic acid. In a study by Valencia and Chavez (2002), 1 % acetic acid slightly improved daily weight gain and feed conversion ratio in weaned piglets.

Butyric acid is also a colourless liquid with a rancid odour. Its origin is in bacterial fermentation under anaerobic condition. There are studies indicating that butyric acid may improve performance, nutrient digestibility and VFA production in pigs of different ages (Mroz et al. 2000; Biagi et al. 2007).

Gluconic acid, a colourless to brown liquid, is formed by the oxidation of glucose. It is originally found in plants and fruits but also in wine and honey. As additive in broiler diets different salts of gluconic acid are used. Depending on the form, different effects were observed but there seems to be an overall tendency of improved performance and higher tibia ash (Rafacz-Livingston et al. 2005). On the other hand Biagi et al. (2006) used the free acid in a pig study and reported also growth promoting effects of gluconic acid.

Malic acid forms colourless crystals. This acid is present in green apples, quince, grapes, gooseberries and similar fruits and berries. Furthermore, it is another intermediate of the citric acid cycle and it takes part in the fixation of C4 in plants. When fed to pigs it improves animal performance and can prevent diarrhoea (Tsiloyiannis et al. 2001a). As additive in diets for broilers malic acid reduces body weight measured at day 16 but slightly improves feed conversion ratio, bone ash and mineral digestibility (Liem et al. 2008). However, in pig and poultry the effects depend on the buffering capacity of the diet (Krause et al. 1994).

Beside single acid addition, also blends of different acids are used. These blends can consist of different organic acids or of mixtures of organic, inorganic and fatty acids. Acids often used in mixtures are formic, lactic and propionic acid. In most cases, two acids are mixed but there are also studies with blends of four and more components (Omogbenigun et al. 2003; Namkung et al. 2004). The combination of different acids makes it possible to target

more microbial species than with one acid alone. Nevertheless, blending of organic acids also poses the risk that the dosage of each acid becomes too low to act effectively. Another possible advantage can be that blends are less prone to variations in diet or management conditions. However, studies to support this assumption are very scarce as are studies using the same blend. Bosi et al. (1999) found differences in the effects on performance in two studies with the same blend. According to their variation in composition the effects of organic acids blend are very variable. A formic acid-sorbate blend improved weight gain and reduced the feed conversion ratio (Partanen et al. 2002a), whereas the 50:50 mixture of propionic and formic acid with an oligosaccharide source resulted in a slightly reduced weight gain but clearly improved feed conversion ratio (Owens et al. 2008).

Mode of action

Independent of the organic acid used, the exact mode of action is not yet clear. According to the current knowledge the potential of organic acids to change from the undissociated (RCOOH) to the dissociated ($RCOO^- + H^+$) form and vice versa seems to be a crucial element. An important point in the equilibrium between the two forms is the pK_a, defined as the "pH value at which there exists equal proportions of molecular acid and charged anions" (Lambert and Stratford 1999). This makes organic acids the more potent antimicrobials the lower the pH is (Lambert and Stratford 1999). The undissociated acid, which dominates in an acidic environment, is lipophilic and can cross the bacterial or fungal cell membrane. Once inside the microbial cell the acid dissociates as the cellular pH is close to neutral (Figure 1.4). The increase of H^+ leads to a decrease in pH forcing the cell to actively remove the protons from the cytoplasma. This is an ATP dependent and thus energy consuming mechanism making the bacterial or fungal cell less competitive or even killing it (Brul and Coote 1999; Ricke 2003; Kim et al. 2005; Theron and Lues 2007). Furthermore, the decrease in cytoplasmic pH hampers enzymatic reactions and nutrient transport systems (Cherrington et al. 1991).

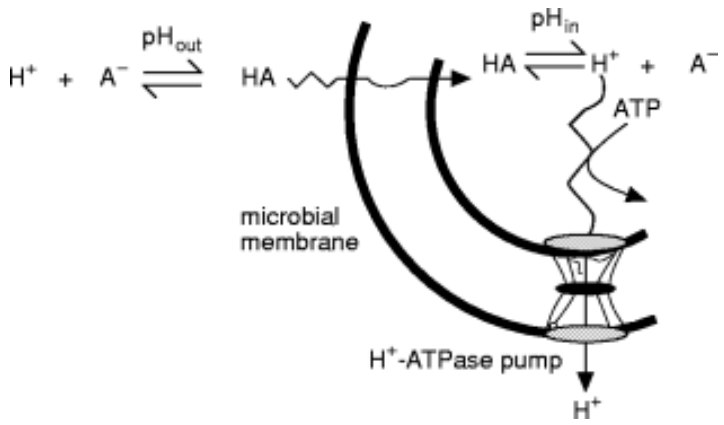

Figure 1.4. Predicted cytoplasmic weak-acid/anion equilibrium. Only uncharged weak-acid molecules (HA) can diffuse freely across the plasma membrane. Charged anions (A^-) and protons (H^+) are retained within the cell; cytoplasmic protons are expelled by the membrane-bound H^+-ATPase (Lambert and Stratford 1999)

On the other hand the accumulation of polar anions is toxic for the cell and causes an osmotic pressure (Russell 1992; Roe et al. 1998). Other possible modes of action include membrane disruption and inhibition of bacterial metabolism as it was reviewed by Brul and Coote (1999). Under these conditions and in combination with reduced pH in the gastrointestinal tract, beneficial microorganisms such as lactic acid bacteria are favoured over potential pathogens such as *E. coli* and *Salmonella* spp. (Mathew et al. 1991; Overland et al. 2000; Knarreborg et al. 2002). Indirect effects of organic acids include reduction of gastric emptying rate, increased intestinal enzyme secretion and activity, additional energy source, improved protein digestion and increased mineral availability (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Partanen 2001; Kim et al. 2005).

So far there has been no report on bacterial resistance to organic acids but acid induced protection and adaptation systems do exist (Lin et al. 1996; Guilfoyle and Hirshfield 1996; Lambert et al. 1997; Lambert and Stratford 1999; Brul and Coote 1999; Piper et al. 2001; Richard and Foster 2003; Ricke 2003). It has been shown several times that organic acids act the better, the younger the animal is (Gabert and Sauer 1994; Roth and Kirchgessner 1998). This age dependent effect was explained by the fact that the immature intestinal microflora is more susceptible to acid induced changes than the mature microbial community found in older animals (Jensen 1998; Partanen and Mroz 1999). How much a possible adaptation of the microorganisms to organic acids is involved in this observation is unclear.

Other additives than organic acids also proposed to be an alternative for AGP are prebiotics (oligosaccharides), probiotics (mainly *Lactobacilli)*, herbs and essential oils (Wenk 2000). Comparing the alternatives it seems that organic acids are the most promising substances – beside strict management and hygiene measurements (Adjiri-Awere and Van Lunen 2005) – to compensate for the ban on AGP (Jensen 1998; Metzler et al. 2005). However, results depend on diet type, diet composition, dietary buffering capacity, age and health of the animal as well as on the length of the feeding period (Ravindran and Kornegay 1993; Gabert and Sauer 1994).

Aim of the study

With the ban on antibiotic growth promoters new additives needed to be established. Of the alternatives known so far organic acids seem to come very close to the effects observed with AGP. However, the effects of organic acids vary widely depending on the acid used, the dosage and the dietary composition. Compared to other organic acids used in animal nutrition, benzoic acid has unique properties such as its aromatic structure and its metabolic pathway. These properties make it especially difficult to predict how the acid acts in different diets for swine.

For this reason a series of studies with benzoic acid added to different diets was conducted. The experimental approach and diets were:

- 1 % benzoic acid and two levels of crude protein
- 0.5 % benzoic acid in diets with a low total P-content and phytase *(Peniophora lycii)*
- 0.5 % benzoic acid and two levels of fibre

The aim was to describe the effects of benzoic acid on animal performance and digestion in growing-finishing pigs more profoundly. In addition, the selection of the experimental diets allowed conclusions on whether benzoic acid can positively affect ecological aspects of pig production.

The influence of benzoic acid and dietary protein level on performance, nitrogen metabolism and urinary pH in growing-finishing pigs

based on K. Bühler, C. Wenk, J. Broz and S. Gebert
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Abstract

An experiment was conducted to examine the effects of benzoic acid and two dietary protein levels on pig performance, nitrogen (N) balance and urinary pH. 24 crossbred barrows (26 kg to 106 kg bodyweight) received one of four diets: low protein without and with 1 % benzoic acid (LPr- and LPr+) and high protein without and with 1 % benzoic acid (HPr- and HPr+) added. The animals were restrictively fed a grower and a finisher diet and were kept in metabolic cages in week 3, 6, 9, and 12 of the experiment. The addition of benzoic acid did not statistically improve ($p > 0.05$) weight gain and feed conversion ratio. N intake and digested N were only influenced by dietary protein level ($p < 0.01$) but N balance was similar ($p > 0.05$) in all four diets. Dietary benzoic acid improved ($p < 0.01$) N digestibility in the grower period but not in the finisher period. The addition of benzoic acid reduced urinary pH by about one pH-unit ($p < 0.01$) in both feeding periods independent of the protein level of the diet ($p > 0.05$) and highly increased ($p < 0.01$) the concentration of urinary hippuric acid. The results of this study indicate positive influences of dietary benzoic acid on pigs especially in the low protein diet and in the grower period.

Introduction

The widespread use of antibiotics as feed additives either as prophylactic medication or as growth promoter has been related to increased antibiotic resistance of pathogens found in animals and humans (Wegener et al. 1998; DANMAP 2004). For this reason antibiotic growth promoters have been forbidden in animal nutrition in Switzerland since 1999 and in the European Union since 2006. A possible alternative for antibiotics as growth promoters are organic acids. According to Canibe et al. (2001a) organic acids can positively influence the microflora in the gastrointestinal tract, thus improving nutritional uptake and health of the pigs. Organic acids naturally occur in different organisms and are also produced by the metabolism. Therefore, they are not considered to be harmful in adequate doses (Partanen and Mroz 1999).

Compared to other organic acids, benzoic acid seems to fulfil the tasks of replacing antibiotics as growth promoters very well (Den Brok et al. 1999; Canibe et al. 2001a). Another advantage of benzoic acid is its potential to reduce ammonia (NH_3) emissions from excrements. This is of particular importance as the negative effects of NH_3 on the environment can be reduced. Furthermore, NH_3 is an olfactory nuisance and it is known to cause respiratory problems in humans and animals (Ferket et al. 2002). Most of the NH_3 in

pig waste is a degradation product from urea produced by the bacterial enzyme urease found in faeces (van Kempen 2001). In contrast to other organic acids, benzoic acid is metabolised in the liver to hippuric acid which is excreted by the urinary pathway (Bridges et al. 1970). The concentration of hippuric acid in the urine lowers urinary pH and thus the activity of urease which depends on pH. Benzoic acid is found in fruits and berries and is used as a preservative in human nutrition. Since May 2003 benzoic acid has been provisionally registered in the European Union as additive in pig nutrition (EC 877/2003).

To gain more information on the influence of benzoic acid in pig nutrition two diets with different protein levels were used in this experiment. The aim was to determine the effect of dietary benzoic acid on performance and nitrogen (N) metabolism in growing-finishing pigs. Secondary, it should be examined whether the reduction of urinary pH and the excretion of hippuric acid are influenced by dietary protein level.

Materials and methods

Animals and experimental design

A total of 24 barrows (16 Duroc x (Landrace x Large White) and 8 Large White x Large White) in six series were used for this experiment. The animals originated from the experimental stations 'UFA Bühl' and 'ETH Chamau'. The average initial and final bodyweights (BW) were 26.0 ± 1.2 kg and 105.6 ± 2.8 kg (mean ± SD), respectively. For the experiment the animals of each group were randomly allotted to one of four dietary treatments: low protein diet without benzoic acid (LPr-), low protein diet with 1 % of benzoic acid (LPr+), high protein diet without benzoic acid (HPr-) and high protein diet with 1 % of benzoic acid added (HPr+). Benzoic acid was provided as VevoVitall® by DSM Nutritional Products Ltd., Basel.

The animals were kept in individual pens and were fed once a day according to a BW-based feeding scale (190 g diet * $BW^{0.569}$) with water available *ad libitum*. Feed was offered as pellets. During the first 6 weeks of the experiment the animals were fed with a grower diet, afterwards with a finisher diet. Body weight was recorded weekly.

In weeks 3, 6, 9, and 12 of the experiment the animals were kept in metabolic cages for four days each. This allowed two sampling periods each for feed, faeces and urine during the grower and the finisher period.

Feed samples were taken once in a sampling period and pooled for analysis. Faeces of each animal were collected every 24 h and pooled for individual and sampling period. Urine

was collected in containers containing 25 ml of 10 % thymole-isopropanole solution as preserving additive. An aliquot of 1 % of total daily urine volume was pooled to a 96 h sample and stored at -20°C. Twice in a sampling period, all urine voided from 9 am to 1 pm was collected in a cooled container without preserving additive for measuring urinary pH.

The experimental procedures described were approved by the official veterinary authority of the canton of Zurich (authorization number ZH 153/2004).

Diets

The diets were based on wheat, barley, triticale, peas and soybean expellers (Table 1.1). Depending on the protein level, wheat was partially replaced by potato protein and amino acid supplementation was adjusted. For the experimental diets 1 % of benzoic acid was added to the control diets. In the diets LPr- and LPr+ the calculated crude protein (CP) content was 165 g/kg for the grower diet and 134 g/kg for the finisher diet. The calculated CP content in the diets HPr- and HPr+ was 189 g/kg and 156 g/kg, respectively. The digestible energy (DE) content of all diets was calculated as 13 MJ/kg. The Lys/DE ratio was calculated as 0.74 g/MJ for grower diets LPr- and LPr+ and 0.55 g/MJ for the respective finisher diets. In the diets HPr- and HPr+ the ratio was 0.94 g/MJ for the grower and 0.71 g/MJ for the finisher diets.

Table 1.1. Composition of the experimental diets (g/kg).

	Grower period		**Finisher period**	
Protein level	**Low**	**High**	**Low**	**High**
Barley	200		200	
Triticale	300		300	
Wheat	90	55	220	185
Peas	200		160	
Potato protein	15	50	0	35
Soybean expellers	120		50	
Molasses	20		20	
Limestone	10		10	
NaCl	5		5	
Dicalcium phosphate	12		8	
L-lysine-HCl	0.7	1.9	1	1.3
DL-methionine	0.7	1.5	0	0.2
L-threonine	0.2	0.8	0.3	0.4
Celite 545[1)]	23.40	20.80	23.45	22.85
Vitamin/mineral premix[2)]	3		2.25	

[1)] Given is the content of Celite 545 in diets without benzoic acid. For diets with benzoic acid, Celite 545 was reduced by 10 g/kg. [2)] Supplied per kg of grower (finisher) diet: 8000 (6000) IU vitamin A, 1000 (750) IU vitamin D_3, 36 (27) mg vitamin E, 1110 (830) µg vitamin B_1, 3.0 (2.2) mg vitamin B_2, 2.2 (1.7) mg vitamin B_6, 18.0 (13.5) µg vitamin B_{12}, 1.3 (1.0) mg vitamin K_3, 11 (8) mg Ca-pantothenate, 22 (16) mg niacine, 570 (430) µg folic acid, 69 (52) µg biotine, 105 (79) mg choline, 105 (79) mg Fe, 37 (27) mg Mn, 9.0 (6.8) mg Cu, 540 (405) µg I, 180 (135) µg Se and 60 (45) mg Zn.

Analytical methods

The feed samples were ground in a centrifugal mill (Retsch ZM 1, Arlesheim, Switzerland) using a 0.75 mm sieve. The feed content of dry matter (DM), crude ash (CA), crude fibre, crude fat and CP (6.25 * N) were determined by the standard procedure of VDLUFA (Naumann and Bassler 1997). Gross energy (GE) was analysed calorimetrically (Calorimeter C7000, IKA-Werke, GmbH & Co., KG, Staufen, Germany). Digestibility was estimated by means of the indicator method, using the nutritionally inert substance Celite 545 (acid-washed diatomaceous earth) as indicator in the diets (Table 1.1).

Faeces were lyophilised and allowed to reach air dry mass. For analysis they were ground in a centrifugal mill (Retsch ZM 1, Arlesheim, Switzerland) using a 0.5 mm sieve. Faeces were analysed for DM, CA, CP and GE according to the methods used for the feed samples.

Urine was analysed for N, pH and hippuric acid. The amount of hippuric acid in the urine was determined according to the method of Tomokuni and Ogata (1972). Urinary pH of each pig was measured with a 691 pH Meter (Metrohm, Switzerland).

Statistical analysis

The mixed model procedure of SAS with Bonferroni-adjustment for multiple comparisons (SAS System for Windows, SAS Institute Inc., Cary (NC), USA; Version 8.2) was used for statistical analysis. Differences were considered to be significant if $p < 0.05$. The significance of the effects of protein level (Pr), dietary benzoic acid (B) and protein level x dietary benzoic acid interaction (Pr x B) was obtained from the mixed model.

Results and discussion

Diets

The experimental diets did not differ in the calculated and analysed nutrient content. The analysed amount of benzoic acid was in accordance with that expected from the formulation (Table 1.2). In the diets CP was slightly higher than calculated, but the differences for low and high protein level and for both dietary periods were as intended. In vivo DE calculated from gross energy digestibility was similar in all diets. The regression (Agroscope Liebefeld-Posieux 2004) resulted in a lower DE than found in vivo but it was still slightly higher than calculated.

Table 1.2. Analysed nutrient content of the experimental diets per kg diet.

	Grower period				Finisher period			
	LPr-[1)]	**LPr+**	**HPr-**	**HPr+**	**LPr-**	**LPr+**	**HPr-**	**HPr+**
Benzoic acid (g/kg)[2)]	0	10.2	0.3	10.2	0	10	0.1	10.2
CP (g/kg)	177.1 ± 1.3		202.0 ± 0.6		144.7 ± 0.7		166.3 ± 0.6	
DM (g/kg)	899.1 ± 0.4				899.3 ± 2.5			
Ash (g/kg)	69.7 ± 6.3				62.6 ± 3.8			
DE (MJ/kg)[3)]	14.2 ± 0.4				14.1 ± 0.3			
DE (MJ/kg)[4)]	13.6 ± 0.2				13.6 ± 0.2			
Crude fibre (g/kg)	38.2 ± 1.8				36.2 ± 1.2			
Crude fat (g/kg)	28.5 ± 0.5				20.8 ± 0.3			

[1)] LPr: low protein diet; HPr: high protein diet; -: without benzoic acid; +: with 1 % of benzoic acid. [2)] Analysed by ADICEA Laboratory, F 35460 St Etienne en Cogles, France (on behalf of DSM Nutritional Products). [3)] determined in vivo. [4)] Calculated according to table values (Agroscope Liebefeld-Posieux 2004).

Body weight and pig performance

The animals were similar ($p > 0.05$) in initial and final BW. Dietary protein level as well as dietary benzoic acid had an influence on fattening duration, but comparative analysis did not result in significant differences ($p > 0.05$) among the four diets (Table 1.3). Animals fed diets with benzoic acid reached slaughter weight (105 kg) 99 ± 7 d after the start of the experiment whereas the corresponding number was 103 ± 8 d without dietary benzoic acid (data not shown). Animals fed diets LPr+ and HPr+ showed increased daily weight gain and better feed conversion ratio in both periods but the improvements were statistically not significant ($p > 0.05$).

Table 1.3. Daily weight gain (DWG), feed intake and feed conversion ratio (FCR).

	Diet[1)]					*p*-values[2)]		
	LPr-	**LPr+**	**HPr-**	**HPr+**	**SEM**	**Pr**	**B**	**Pr x B**
Grower period								
DWG (g/d)	662	754	703	746	50	0.64	0.06	0.47
Feed intake (kg/d)	1.5	1.6	1.5	1.5	0.1	0.80	0.06	0.20
FCR (kg/kg)	2.21	2.08	2.12	2.06	0.11	0.50	0.27	0.64
Finisher period								
DWG (g/d)	807	838	839	874	40	0.17	0.17	0.94
Feed intake (kg/d)	2.2	2.3	2.2	2.3	0.03	0.26	0.05	0.27
FCR (kg/kg)	2.72	2.70	2.68	2.61	0.09	0.32	0.38	0.58

1) LPr: low protein diet; HPr: high protein diet; - without benzoic acid; +: with 1 % of benzoic acid.

2) Effects of protein level (Pr), benzoic acid (B) and their interaction (Pr x B). SEM: maximal standard error of the means.

The results of this experiment are similar to van der Peet-Schwering et al (1999b) who found significantly improved weight gain and feed conversion ratio in growing-finishing pigs when 1 % of benzoic acid was added to the diet.

In this study, the effect of dietary benzoic acid was more pronounced in the grower period and when added to the low protein diet. The age depending efficacy of organic acids was also observed in other studies (Gabert and Sauer 1994; Roth and Kirchgessner 1998). This has been related to the immaturity of digestive organs and microflora in piglets (Hampson 1986; Jensen et al. 1997). In grower pigs, the gastrointestinal tract is still susceptible to changes triggered by organic acids. In contrast to that it has reached its final stage in the finisher pig and can efficiently regulate the intestinal conditions.

The animals were not examined in detail after slaughter but they all seemed healthy and no apparent changes in the urinary or digestive tract were observed. The addition of 1 % of benzoic acid thus did not have any obvious negative impact on the welfare of the animals.

N metabolism

In both feeding periods N intake, digested N and urinary N were influenced by the protein level of the diet but N balance was similar in all diets (Table 1.4). In diet LPr- N retention in

the grower period was 1.65 g/kg BW $^{0.75}$/d. This was lower than in the other diets during the grower period but differences were statistically not significant ($p > 0.05$) among the diets.

The addition of benzoic acid significantly increased N digestibility (d(N)) in the grower period by almost 4 % ($p < 0.01$). In grower diets with dietary benzoic acid d(N) was 0.87 ± 0.03 compared to 0.84 ± 0.04 in the other grower diets. Comparison among the four diets, however, only resulted in a significant difference ($p < 0.05$) between the diets LPr- and HPr+. In the finisher period, d(N) was statistically not influenced by benzoic acid and it was similar in all diets.

Table 1.4. N metabolism (g /kg BW$^{0.75}$/d) and N digestibility (d(N)).

	Diet[1)]					***p*-values[2)]**		
	LPr-	**LPr+**	**HPr-**	**HPr+**	**SEM**	**Pr**	**B**	**Pr x B**
Grower period								
N intake	2.63^{a}	2.74^{a}	3.12^{b}	3.03^{b}	0.09	< 0.01	0.83	0.08
N digested	2.46^{a}	2.58^{a}	2.94^{b}	2.89^{b}	0.08	< 0.01	0.44	0.11
Urinary N	0.81^{ab}	0.78^{b}	1.11^{a}	1.07^{ab}	0.09	< 0.01	0.69	0.98
N balance	1.65	1.80	1.83	1.82	0.09	0.22	0.37	0.32
d(N)	0.83^{a}	0.86^{ab}	0.84^{ab}	0.88^{b}	0.01	0.08	< 0.01	0.61
Finisher period								
N intake	1.99^{a}	1.98^{a}	2.28^{b}	2.24^{b}	0.03	< 0.01	0.24	0.51
N digested	1.89^{a}	1.88^{a}	2.17^{b}	2.15^{b}	0.03	< 0.01	0.43	0.61
Urinary N	0.71^{b}	0.70^{b}	0.97^{a}	1.00^{a}	0.05	< 0.01	0.94	0.73
N balance	1.18	1.19	1.20	1.15	0.05	0.94	0.71	0.58
d(N)	0.87	0.87	0.88	0.89	0.01	0.16	0.30	0.75

1) LPr: low protein diet; HPr: high protein diet; - without benzoic acid; +: with 1 % of benzoic acid.
2) Effects of protein level (Pr), benzoic acid (B) and their interaction (Pr x B). Different superscripts in a row indicate significant differences ($p < 0.05$) among diets. SEM: maximal standard error of the means.

The strong dependence of N secretion and especially of urinary N of dietary protein level supports the results found by Canh et al. (1998b).

It is known that dietary benzoic acid affects the gastrointestinal microflora (Maribo et al. 2000), resulting in a reduced excretion of microbial nitrogen. Together with the increase of urinary N this may be the reason for the effect of benzoic acid on d(N). However, further

studies are necessary to determine the changes in microbial nitrogen excretion and a possible increase of N utilisation by the pig.

Urinary pH and urinary hippuric acid

Dietary benzoic acid significantly ($p < 0.05$) lowered urinary pH from 7.8 ± 0.5 to 6.9 ± 0.5 in the grower and from 7.8 ± 0.4 to 6.8 ± 0.7 in the finisher diet but it was neither influenced by the protein level of the diets nor by the Pr x B interaction (Table 1.5).

Although urinary pH was generally higher than reported by van der Peet-Schwering et al. (1999b), the results of this study support their findings. It is possible that the collecting period of four hours in this experiment rose urinary pH due to bacterial activity in the urine despite cooling. On the other hand the results showed that benzoic acid keeps urinary pH lowered over several hours. In this context it is interesting to note that the reduction of urinary pH is independent of the dietary protein level. This indicates that dietary benzoic acid may be used in different food types without losing its acidifying capacities.

Table 1.5. Urinary pH and excretion of hippuric acid.

	Diet[1)]					***p*-values[2)]**		
	LPr-	**LPr+**	**HPr-**	**HPr+**	**SEM**	**Pr**	**B**	**Pr x B**
Grower period								
Urinary pH	7.93[a]	7.09[b]	7.77[a]	6.76[b]	0.1	0.13	< 0.01	0.65
Hippuric acid (mg/ml)	0.82[a]	7.9[b]	0.86[a]	8.7[b]	1.1	0.72	< 0.01	0.76
Hippuric acid (mg/g feed)[3)]	1.03[a]	9.85[b]	1.42[a]	13.22[c]	0.8	0.01	< 0.01	0.04
Finisher period								
Urinary pH	7.81[a]	6.95[b]	7.77[a]	6.71[b]	0.2	0.55	< 0.01	0.66
Hippuric acid (mg/ml)	0.97[a]	11.2[b]	1.27[a]	13.9[b]	1.6	0.20	< 0.01	0.29
Hippuric acid (mg/g feed)	1.15[a]	10.16[b]	1.34[a]	13.87[c]	0.9	< 0.01	< 0.01	< 0.01

1) LPr: low protein diet; HPr: high protein diet; - without benzoic acid; +: with 1 % of benzoic acid. **2)** Effects of protein level (Pr), benzoic acid (B) and their interaction (Pr x B). **3)** Calculated as total hippuric acid/feed intake. Different superscripts in a row indicate significant differences ($p < 0.05$) among diets. SEM: maximal standard error of the means.

The concentration of hippuric acid in the urine of animals receiving diets LPr+ and HPr+ was almost ten times higher ($p < 0.01$) than in the other diets. Urinary hippuric acid

concentration was in the grower (finisher) period 0.84 ± 0.3 mg/ml (1.12 ± 0.5 mg/ml) for diets without benzoic acid and 8.3 ± 2.9 mg/ml (12.7 ± 4.1 mg/ml) for diets with benzoic acid. A similar increase was found in the amount of hippuric acid excreted per gram feed (Table 1.5). In addition, this parameter was significantly affected by Pr and Pr x B ($p < 0.01$).

Hippuric acid is the metabolic product of benzoic acid and glycine. For this reason, an increase in the concentration of hippuric acid was expected but the extent was remarkable. It is known that pigs excrete up to 86 % of ingested benzoic acid as hippuric acid (Bridges et al. 1970). In this study the excretion rate in diet LPr+ was similar to this but it was much higher in diet HPr+, indicating a dietary dependence of hippuric acid excretion. The potato protein added to the high protein diet may be the reason for this increase. Also animals fed diets LPr- and HPr- excreted a certain amount of hippuric acid. This supports the findings by Nehring et al. (1965) that hippuric acid is a normal by-product of the pig's metabolism.

Conclusion

The results found in this study hint towards positive effects of dietary benzoic acid on pig performance and N metabolism, especially in the grower period and when benzoic acid was added to the low protein diet. However, it is not known how much of the changes in N metabolism result in an additional nutritional value for the pigs. As the number of animals used in this study was very low, experiments with more animals of different age and sex are necessary to gain certainty on the results of this study.

This study also showed that dietary benzoic acid is potent in reducing urinary pH without harming the animals. A reduced urinary pH is very efficient in reducing ammonia emission and thus in minimising environmental problems in pig production. In this experiment the effect of benzoic acid on NH_3 emission was not examined. Further studies should therefore also concentrate on the question how diets of different compositions may influence the potential of benzoic acid in reducing NH_3 emission.

Effect of benzoic acid and phytase in low-phosphorus diets on performance and nutrient digestibility of growing-finishing pigs

based on K. Bühler, B. Bucher, C. Wenk and J. Broz

submitted to Archives of Animal Nutrition

Abstract

Two identical 2 x 2 factorial digestibility studies (experiment A and B) with 16 growing (25 – 66 kg BW) and 32 growing-finishing (26 – 108 kg BW) crossbred gilts, respectively, were conducted. The effects of 0.5 % benzoic acid and 750 U/kg phytase *(Peniophora lycii)* on performance and apparent nutrient digestibility were examined. During the experiments, the animals were fed restrictively one of four experimental diets: control diet without any supplementation (CC), control diet with benzoic acid (CB), phytase diet without benzoic acid (PhyC) and phytase diet with benzoic acid supplementation (PhyB). Total P content of diets was reduced to 4 g/kg diet. Overall performance of the animals was not affected by the additives. In the grower period of experiment B, digestibility of crude protein and energy was negatively affected ($p < 0.01$) by the interaction of benzoic acid and phytase. In the grower period of experiment A benzoic acid reduced ($p < 0.05$) and phytase improved ($p < 0.01$) P digestibility whereas in the finisher period of experiment B both additives increased P digestibility. Phytase also increased Ca digestibility ($p < 0.01$) during the finishing period but a negative combined effect of the two additives was observed ($p < 0.01$). The findings of this study indicate that the combination of benzoic acid and phytase in low-P diets can negatively affect nutrient and mineral availability without influencing growth performance. So far, the mechanisms behind this interaction remain unclear.

Introduction

Up to 70% of phosphorus (P) found in plants is bound as phytate (Eeckhout and De Paepe 1994) and thus as such nearly unavailable to the pigs. To meet the pig's requirements, additional inorganic P is usually added to the diet. This surplus has led to severe environmental problems due to excessive P excretion (Jongbloed 2007). It has been shown repeatedly (Simons et al. 1990; Pallauf and Rimbach 1997; Thacker et al. 2006) that the addition of microbial phytase to the diet increases P availability and reduces P excretion. Along with better P utilisation, phytase can improve performance (Gebert et al. 1999; Jendza et al. 2005) and increase digestibility of crude protein (Mroz et al. 1994) and energy (Johnston et al. 2004) as well as the availability of minerals such as calcium (Ca) (Gebert et al. 1999; Jongbloed et al. 2000; Veum and Ellersieck 2008).

As the activity of phytase is pH dependent (Vats and Banerjee 2004), its effects should be enhanced when the gastrointestinal pH is lowered. Feed additives known to have this capacity are organic acids (Eidelsburger et al. 1992a). The combined use of organic acids and phytase

has been tested in several studies with piglets and young pigs but the results are inconsistent. Some studies described positive synergistic effects of these two additives (Jongbloed et al. 1996; Jongbloed et al. 2000; Valencia and Chavez 2002) whereas others did not (Radcliffe et al. 1998). To our knowledge no studies related to this topic were performed so far with growing-finishing pigs or with benzoic acid. Benzoic acid is listed as an acidity regulator (EC 757/2007) and beside its capacity to reduce urinary pH (Mroz et al. 2000), it seems to be the least degradable of the organic acids used in pig nutrition (Maribo et al. 2000; Canibe et al. 2001a).

Therefore the present study was conducted to evaluate the effects of benzoic acid and phytase in low-P diets on fattening performance and apparent nutrient and mineral digestibility in growing-finishing pigs.

Material and methods

Animals and housing

The experiments took place simultaneously at two sites with a total of 48 crossbred gilts (Large white x (Landrace x Large white)) obtained from 'UFA Bühl', Hendschikon (CH). The 16 animals of experiment A were kept at ETH Zurich under ideal housing conditions. Initial and final body weight (BW) of these animals was 25.2 ± 2.0 kg and 66.0 ± 2.7 kg (mean ± SD), respectively. Experiment B was conducted with 32 animals at the research station 'Chamau' of ETH Zurich under practical conditions. The pigs of this experiment had an initial BW of 26.1 ± 1.1 kg and their final BW was 108.3 ± 2.9 kg. The animals were housed in single pens with wood shavings (experiment A) or straw (experiment B) as bedding. The experimental procedures were approved by the official veterinary authorities of the canton of Zurich (CH) (ZH 181/2007 for experiment A) and Zug (CH) (ZG 44/06 for experiment B).

One animal (diet CC) of experiment B had to be slaughtered at a BW of 86.50 kg due to severe walking problems (paralysis) of unknown origin. For this reason, digestibility but not performance data of the finisher period of this animal were included into statistical analysis.

Diets and feeding

Diets and feeding were the same in both experiments. The amount of feed required for both experiments were prepared in one batch and then distributed to the two sites.

Animals were fed with one of four experimental diets: control diet without phytase and without benzoic acid (CC), control diet with 0.5 % benzoic acid (CB), phytase diet without

benzoic acid and with 750 U/kg phytase (PhyC) and phytase diet with 0.5 % benzoic acid and 750 U/kg phytase (PhyB). Feed was offered as pellets at a daily amount of 190 g diet * $BW^{0.569}$. Water was available *ad libitum.* After 6 weeks (BW: 57.4 ± 2.6 kg), diets of the animals in experiment B were changed from grower to finisher diets. Animals in experiment A only received grower diets.

The diets were based on cereals, peas and soybean expellers (Table 2.1). For the grower period crude protein (CP) content of the diets was calculated as 181 g/kg and as 149 g/kg for the finisher period, respectively. Calculated digestible energy (DE) was 13.3 MJ/kg for all diets with a Lys/DE ratio of 0.84 g/MJ for the grower period and 0.62 g/MJ for the finisher period. P content was set at 4.0 g/kg in the grower and 3.5 g/kg in the finisher period, respectively which is below the recommendation of ALP (Agroscope Liebefeld-Posieux 2004). Calculated digestible P in the grower period was 1.4 g/kg for the control diets and 2.6 g/kg for the phytase diets. The corresponding values in the finisher period were 1.2 g/kg and 2.4 g/kg, respectively. Calcium (Ca) to P ratio was set at 1.3 for grower and 1.5 for finisher diets. Celite 545 (acid insoluble ash) was added as digestibility marker to the diets. To inactivate native phytase diets were expanded before adding the commercial phytase. Benzoic acid and phytase were provided as VevoVitall® and Ronozyme® P, respectively, by DSM Nutritional Products Ltd., Basel (CH).

Table 2.1. Composition of the experimental diets (g/kg).

	Grower period	**Finisher period**
Barley	300	300
Wheat	300	500
Peas	200	80
Soybean expellers	110	15
Potato protein	12.5	25
Molasses	20	25
Fat	5	4
Limestone	10.6	12
NaCl	3.4	3.5
Monocalcium phosphate	0.5	0.7
L-lysine-HCl	2.4	2.6
DL-methionine	1.4	0.1
L-threonine	0.9	0.6
Celite 545[1)]	28.3	26.5
Vitamin/mineral premix[2)]	5	5

[1)] Content of Celite 545 in diets without phytase and without benzoic acid. For the other diets, Celite 545 was reduced according to supplementation. [2)] Supplied per kg of diet: 8000 IU vitamin A, 1000 IU vitamin D_3, 50 mg vitamin E, 1.5 mg vitamin B_1, 3.5 mg vitamin B_2, 2 mg vitamin B_6, 15 µg vitamin B_{12}, 430 µg vitamin K_3, 10 mg Ca-pantothenate, 20 mg niacine, 600 µg folic acid, 239.5 mg choline, 175 mg Fe, 30 mg Mn, 9.8 mg Cu, 750 µg I, 250 µg Se and 83.5 mg Zn.

Collection and analysis of samples

In a three week rhythm faeces were collected during four consecutive days and pooled for each animal and for each sampling period. This scheme resulted in two sampling periods in experiment A and four in experiment B. In the latter, there were two sampling periods in the grower and two in the finisher period. Samples of feed were taken at each site once during each sampling period.

Faeces were lyophilised and then allowed to reach air dry mass. For analysis they were ground to a size of 0.5 mm in a centrifugal mill (Retsch ZM 1, Arlesheim, Switzerland). Feed samples were ground to 0.75 mm, using the same mill. Samples of feed and faeces were analysed for dry matter, crude ash, crude fat (only feed samples) and CP (6.25 * N) by the standard procedures adopted in our laboratory (Naumann and Bassler 1997). Neutral

detergent fibre (NDF) and acid detergent fibre (ADF) in feed samples were analysed according to the method of Robertson and Van Soest (1981). Gross energy (GE) was analysed using a bomb calorimeter (Calorimeter C7000, IKA-Werke, GmbH & Co., KG, Staufen, Germany). For the analysis of P and Ca, feed and faeces were ashed for 13 h (feed) and 24 h (faeces) at 550°C. The content of both minerals was determined by colorimetry with an autoanalyser (COBAS MIRA®, Roche-Autoanalyser, Basel, Switzerland). The commercial kits used were CALC 20 (Axon Lab AG, Baden, Switzerland) for Ca and ABX Pentra (Horiba ABX, Montpellier, France) for P. Apparent digestibility values of nutrients were estimated by means of the indicator method (acid insoluble ash), using the nutritionally inert substance Celite 545 (Table 2.1), an acid-washed diatomaceous earth, as indicator

Analysis of benzoic acid with reversed phase liquid chromatography and UV-detection followed the DSM Research Analytical Method DSM-RES 3-E. Activity of phytase was measured at the laboratories of Biopract GmbH, Berlin (D) with their standard procedure.

Statistical analysis

For both experiments the mixed model procedure of SAS for multiple comparisons (SAS System for Windows. SAS Institute Inc., Cary (NC), USA); Version 8.2) was used for statistical analysis. Phytase (Phy), benzoic acid (B) and the interaction of the two additives (Phy x B) were the defined effects. Similarity of results in the grower period between the same diets in the two experiments was determined with a two sample t-test. The significance level for all statistical analyses was defined as $p < 0.05$ (Bonferroni-adjustment). Unless otherwise stated, results are presented as means ± SD.

Results and discussion

Diets

Analysed CP and P contents of feed samples (in DM) were similar to the calculated values (as-fed basis) (Table 2.2). DE_{exp} was in all diets slightly higher than expected. This was also true for the Ca/P ratio which was markedly higher in the samples of experiment B. To inactivate native phytase diets were expanded before adding the phytase. However, phytase activity in control diets was between 150 and 200 U/kg. This activity was clearly lower than in the phytase supplemented diets (Table 2.2). As intended, no benzoic acid could be found in diets CC and PhyC whereas the content of benzoic acid in diets CB and PhyB was close to 0.5 % with the exception of grower diet PhyB in experiment B (Table 2.2).

Despite being processed at the same time, two feed samples of experiment B showed noticeable differences to the expected amount of additives. Feed sample PhyB of the first sampling period revealed a benzoic acid and phytase content as low as 0.2 g/kg and 253 U/kg, respectively. For this reason only sampling period two of experiment B was used for statistical analysis of digestibility parameters in the grower period. The other conspicuous feed sample was diet CC of the fourth sampling period with a phytase activity of 498 U/kg. However, this activity was still markedly lower than in the phytase diets. The reason of these discrepancies is unclear as all samples of experiment A as well as samples of the second and third sampling periods showed normal levels of benzoic acid and phytase activity.

Table 2.2. Analysed nutrient composition of experimental diets fed in experiments A and B.

	Experiment A				Experiment B							
	Grower period[1)]				Grower period				Finisher period			
	CC[2)]	CB	PhyC	PhyB	CC	CB	PhyC	PhyB[2)]	CC	CB	PhyC	PhyB
Benzoic acid (g/kg)	0 ± 0	5.2 ± 0	0 ± 0	6.1 ± 0	0 ± 0	5.9 ± 0.1	0 ± 0	2.8 ± 3.6	0 ± 0	5.5 ± 0.6	0 ± 0	5.8 ± 0.2
Phytase (U/kg)	179 ± 6	155 ± 2	1248 ± 110	992 ± 156	206 ± 35	156 ± 3	930 ± 30	669 ± 588	335 ± 231	147 ± 13	834 ± 104	603 ± 45
DM (g/kg)	883.6 ± 2.6				886.5 ± 4.2				882.6 ± 3.4			
Crude Ash (g/kg DM)	67.8 ± 8.5				66.5 ± 5.6				65.6 ± 5.6			
CP (g/kg DM)	213.0 ± 7.4				211.2 ± 5.0				181.1 ± 12.2			
Crude Fat (g/kg DM)	25.7 ± 2.5				25.1 ± 2.6				18.7 ± 1.8			
NDF (g/kg DM)	145.4 ± 6.3				136.3 ± 8.7				138.1 ± 10.7			
ADF (g/kg DM)	59.5 ± 3.9				57.5 ± 2.8				51.3 ± 5.6			
P (g/kg DM)	4.6 ± 0.2				4.5 ± 0.2				4.1 ± 0.3			
Ca (g/kg DM)	6.6 ± 0.6				7.0 ± 0.6				7.4 ± 0.6			
Ca/P	1.4 ± 0.1				1.6 ± 0.2				1.8 ± 0.1			
DE_{exp} (MJ/kg)[3)]	13.5 ± 0.3				13.6 ± 0.3				13.4 ± 0.1			

[1)] No finisher period in experiment A. [2)] C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. [3)] Calculated as $(GE_{intake} - GE_{excretion})/ Feed_{intake}$.

Pig performance

Neither benzoic acid nor phytase influenced ($p > 0.05$) daily weight gain (DWG) of the animals (Table 2.3). Average DWG in experiment A was 769 ± 31 g/d with a maximal DWG of 809 g/d in diet CB (data not shown). In experiment B DWG in the grower period was 746 ± 24 g/d and in the finisher period 789 ± 22 g/d. The highest DWG in the grower period was 782 g/d in diet CB compared to the mean of 734 g/d in the other diets (Table 2.3). In the finisher period animals fed diet PhyB had a DWG of 817 g/d, being the highest of all diets. Compared to diet PhyC, these animals gained 51 g/d more but the difference was not significant ($p > 0.05$).

Feed conversion ratio (FCR) was similar in all diets and independent ($p > 0.05$) of the addition of benzoic acid or phytase (Table 2.3). Animals receiving grower diets needed 2.06 ± 0.04 kg feed per kg weight gain in experiment B. In the finisher period this value rose to 3.18 ± 0.09 kg feed per kg weight gain. The FCR of animals fed diet PhyB was in the finisher period almost 7 % better than that of the animals fed diet PhyC but the difference was not significant. Those animals with the highest DWG also showed the best FCR. This was also true for experiment A. FCR of these animals was on average 2.08 ± 0.08 (data not shown).

There was no difference of DWG or FCR in the grower period between experiment A and experiment B ($p < 0.05$).

Table 2.3. Daily weight gain (DWG) and feed conversion ratio (FCR) in experiment B

	Diets[1)]					***p*-values[2)]**		
	CC	**CB**	**PhyC**	**PhyB**	**SEM**	**Phy**	**B**	**Phy x B**
Grower Period								
DWG (g/d)	738	782	733	732	20	0.14	0.24	0.22
FCR (kg/kg)	2.07	2.00	2.08	2.07	0.06	0.39	0.40	0.52
Finisher Period								
DWG (g/d)	778	793	766	817	30	0.77	0.12	0.39
FCR (kg/kg)	3.19	3.19	3.28	3.05	0.10	0.75	0.11	0.12

1) C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. **2)** Effects of phytase (Phy), benzoic acid (B) and their interaction (Phy x B). SEM: maximal standard error of the means. n = 8

The performance data of this study neither showed significant performance enhancing effects of benzoic acid nor of phytase (Table 2.3) which stands in contrast to previous

research. It is known that the influence of organic acids is more pronounced in younger animals mainly due to the immaturity of their digestive system (Partanen and Mroz 1999). Similarly, the addition of phytase to low P diets has been described to improve pig performance in several studies (Harper et al. 1997; Jendza et al. 2005; Thacker et al. 2006). Explanations for these effects are higher feed intake (Beers and Jongbloed 1992; Jongbloed et al. 2000) and the increased digestibility of minerals (Gebert et al. 1999; Jendza et al. 2005). On the other side, Radcliffe et al. (1998) could not find any effect of phytase on animal performance. Reasons for the lack of performance enhancement by organic acid and phytase addition found in the present study are manifold. As the animals in this study were fed restrictively there was no effect of feed intake on performance. This explanation is contradicted by Valencia and Chavez (2002) who found improved DWG without increased feed intake in piglets fed low P diets with phytase and acetic acid. Furthermore, the low number of animals used in addition with the quite high average DWG could have masked any effect of the additives.

Apparent nutrient digestibility

In the grower period of experiment B digestibility of crude protein (d(CP)) and energy (d(E)) was negatively influenced ($p < 0.01$) by the interaction of benzoic acid and phytase (Table 2.4). In experiment A this effect could only be observed for d(E) ($p = 0.05$). In both experiments the reduction of digestibility in diet PhyB was about 5 % compared to the highest value (Table 2.4) causing this dietary treatment to differ significantly from the others ($p < 0.05$). There were no effects of the additives on d(CP) and d(E) in the finisher period ($p > 0.05$).

In experiment A benzoic acid decreased ($p < 0.05$) and phytase increased ($p < 0.01$) apparent P digestibility (d(P)) but there was no influence on Ca digestibility (d(Ca)) (Table 2.4). In the grower period of experiment B no effect of benzoic acid or phytase on d(P) could be observed ($p > 0.05$) but d(Ca) increased with the addition of phytase from 0.50 ± 0.01 to 0.55 ± 0.04. There was no interaction of benzoic acid and phytase on mineral digestibility in the grower period in any of the experiments. In the finisher period of experiment B digestibility of P was influenced by both, benzoic acid and phytase ($p < 0.05$) but there was no interaction. The lowest d(P) with 0.30 was found in diet CC, differing clearly from the other diets (Table 2.4). On the other hand d(Ca) was improved ($p < 0.01$) when phytase was added to the diet. At the same time a negative interaction of benzoic acid and phytase markedly ($p < 0.01$) reduced d(Ca) in diet PhyB (Table 2.4). Digestibility of Ca in diet PhyB

was 0.54 and differed significantly ($p < 0.05$) from diet PhyC with a coefficient of 0.62 but it was similar to the C-diets.

All digestibility coefficients in the grower period were comparable between the two experiments ($p > 0.05$).

Table 2.4. Apparent total tract digestibility of crude protein (d(CP)), energy (d(E)), phosphorus (d(P)) and calcium (d(Ca)) in experiments A and B.

	Diets[1)]					*p*-values[2)]		
	CC	**CB**	**PhyC**	**PhyB**	**SEM**	**Phy**	**B**	**Phy x B**
Experiment A (n = 4)								
d(CP)	0.82	0.83	0.83	0.80	0.01	0.35	0.25	0.16
d(E)	0.849^{ab}	0.847^{ab}	0.852^{a}	0.814^{b}	0.01	0.11	0.04	0.05
d(P)	0.32^{ab}	0.27^{a}	0.41^{b}	0.34^{ab}	0.03	< 0.01	0.02	0.62
d(Ca)	0.45	0.44	0.47	0.43	0.03	0.94	0.39	0.68
Experiment B (n = 8)								
Grower Period								
d(CP)	0.80^{ab}	0.83^{a}	0.82^{a}	0.78^{b}	0.02	0.16	0.37	< 0.01
d(E)	0.84^{a}	0.85^{a}	0.85^{a}	0.81^{b}	0.01	0.05	0.06	< 0.01
d(P)	0.25	0.32	0.30	0.28	0.05	0.83	0.46	0.27
d(Ca)	0.50	0.49	0.57	0.52	0.03	0.04	0.24	0.46
Finisher Period								
d(CP)	0.85	0.86	0.85	0.85	0.06	0.99	0.38	0.23
d(E)	0.87	0.87	0.87	0.87	0.03	0.55	0.22	0.53
d(P)	0.30^{a}	0.37^{b}	0.37^{b}	0.38^{b}	0.02	< 0.01	0.02	0.06
d(Ca)	0.50^{a}	0.53^{a}	0.62^{b}	0.54^{a}	0.02	< 0.01	0.23	< 0.01

[1)] C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. [2)] Effects of phytase (Phy), benzoic acid (B) and their interaction (Phy x B). Different superscripts in a row indicate significant differences ($p < 0.05$). SEM: maximal standard error of the means. No finisher period in experiment A.

Data presented here indicate that an interaction of benzoic acid and phytase negatively affects d(CP) and d(E) of animals up to 57 kg BW. Concerning mineral digestibility, benzoic acid tended to decrease Ca and P digestibility while phytase increased them. Further benzoic acid reduced d(Ca) in the finisher period when it was added to the Phy-diet (Table 2.4). The fact that statistical effects differed between experiments despite similar results is most presumably because of the optimal conditions in experiment A and the higher number of animals used in experiment B.

It has been reported that organic acids can enhance apparent nutrient digestibility or nutrient retention (Jongbloed et al. 2000; Kluge et al. 2006; Partanen et al. 2007) but results vary, depending on the diet and the type of organic acids used (Partanen and Mroz 1999). The proposed mode of action is a reduction of the gastrointestinal microflora making more nutrients available to the pig. Similar to performance enhancement by organic acid the effects on digestibility are the more prominent the younger the animals are (Gabert and Sauer 1994). This age dependency can explain why in this study almost no effects of benzoic acid on d(CP) and d(E) in finishing pigs were observed. In the case of the influence of organic acids on mineral digestibility the findings of previous studies are more controversial. There are some studies indicating that organic acids may improve digestibility of P and Ca by 14 % (Kirchgessner and Roth 1980; Mroz et al. 2000). Others found no effect (Radecki et al. 1988; Radcliffe et al. 1998) or an increased elimination via urine (Hess and Gutzwiller 2008). An increase of apparent mineral digestibility caused by the addition of benzoic acid to low-P diets can therefore indicate that more minerals were excreted in the urine and less in the faeces.

The activity of phytase not only releases P from phytate but also other nutrients and minerals chelated by this substance thus increasing digestibility (Pallauf and Rimbach 1997; Gebert et al. 1999; Johnston et al. 2004). Even though some effects of phytase on mineral digestibility were found in the present experiment, the increase is not as clear and consistent as expected. Beside pH the Ca/P ratio influences phytase activity (Lei and Stahl 2000). The Ca/P ratio in our study was higher than 1.2:1 which is considered to be the optimum for phytase activity (Lei and Stahl 2000). Although being calculated to be 1.3:1, the analyzed Ca/P ratio of the experimental diets was much higher (Table 2.2), a fact that could well have reduced any phytase effects. Furthermore, it is possible that the amount of available P in this experiment was above the animal's requirements, a fact that could have been caused by two mechanisms: one is that the higher P availability applied to all diets. In this case, the additional P released by phytase was not absorbed but only excreted by the pig and thus no

improvement in d(P) could be observed. The other mechanism is that the remaining activity of native phytase found in the C-diets could have covered the phytase effect in the Phy-diets.

Considering the combined use of organic acids and phytase, Jongbloed et al. (2000) found that phytase and lactic acid or formic acid solely as well as synergistically increased P and Mg digestibility. Similar results were found by Valencia and Chavez (2002) and Kemme et al. (1999b) using acetic acid and lactic acid, respectively. On the other hand Li et al (1998) and Radcliffe et al. (1998) did not find an increase in phytase efficiency when citric acid was added to the diet. However, no study has so far showed negative interactions when organic acids and phytase were used in combination in low-P diets. In the aforementioned studies an *Aspergillus niger* phytase was used whereas the phytase tested in this study was derived from *Peniophora lycii*. The physical, chemical and biological properties of latter differ substantially from the *A. niger* phytase (Simon and Igbasan 2002; Ullah and Sethumadhavan 2003; Vats and Banerjee 2004) but effects have been described to be similar (Payne et al. 2005; Guggenbuhl et al. 2007a). The most striking differences are that the *A. niger* phytase starts to hydrolyse phytate at position 3 and has two activity optima at pH 2.5 and 5.5, respectively, compared to the *P. lycii* phytase starting at position 6 and having a single activity optimum at pH 4 – 4.5. One reason of the observed interaction could have been that benzoic acid lowered the pH in the stomach below the pH optimum of the phytase used. A study by Kluge et al. (2006) stands against this explanation as they did not find such an acidification of digesta in piglets. On the other hand it has been shown that high doses of citric acid may decrease phytase activity in the stomach but without affecting its efficiency (Radcliffe et al. 1998). Perhaps benzoic acid did affect phytase activity as well as phytase efficiency in the stomach.

Another reason could be the aromatic nature of benzoic acid. It is known that phenylglyoxal can inhibit the active site of *A. ficuum* phytaseA (Ullah and Sethumadhavan 1998). Despite also being encoded in the phyA gene the geometry of the active site of *P. lycii* phytase is different (Ullah and Sethumadhavan 2003). Thus the reaction of *P. lycii* phytase on phenylglyoxal needs not to be the same as in *Aspergillum* phytases and in diet PhyB no reduction of phytase activity was found. However, the fact that the interaction did not occur in all digestibility parameters and fattening periods examined is in opposition to the inhibitor hypothesis.

Interestingly, the reduced digestibility did not influence performance. The reason for this finding is unclear but it may be related to the rather low number of animals used in this study. Therefore it is crucial that more studies with more animals are conducted about this topic to gain certainty on the results presented here.

Conclusion

The results of this study show that benzoic acid may reduce while phytase can improve mineral digestibility in pigs fed low-P diets. On the other hand there are indications that the combined use of benzoic acid and phytase in low-P diets may be detrimental to nutrient availability due to a negative interaction between the two additives. However, this interaction does not influence pig performance. The cause and the kind of the observed interaction remain unclear. Another question is whether these findings are only observed in the combined use of benzoic acid and a *P. lycii* phytase or whether this is a general reaction of benzoic acid and phytases. Further experiments are important to answer the question whether some restrictions are necessary when using low-P diets supplemented with benzoic acid and phytase.

Influence of benzoic acid and phytase in low-phosphorus diets on bone parameters in growing-finishing pigs

based on K. Bühler, A. Liesegang, B. Bucher, C. Wenk and J. Broz

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Abstract

In two simultaneous studies (experiment A and B) the effects of benzoic acid and phytase in low-phosphorus (P) diets on bone metabolism, bone composition and bone stability were examined. Experiment A was conducted with 16 crossbred gilts in the weight range of 25 to 66 kg body weight (BW) whereas in experiment B 32 crossbred gilts (25 – 108 kg BW) were used. All animals were restrictively fed one of four diets with a total P content of 4 g/kg: unsupplemented control diet (CC), control diet with 0.5 % benzoic acid (CB), phytase diet with 750 U/kg phytase but without benzoic acid (PhyC) and phytase diet with 750 U/kg phytase and 0.5 % benzoic acid (PhyB). Blood samples were taken at the beginning of the experiment and before slaughter as well as throughout the experiment to measure bone metabolism. Bone composition and bone stability were examined using the metatarsi and tibiae of the animals after slaughter. No long-term effect of diets on bone metabolism could be detected but phytase ($p < 0.05$) increased P and serum crosslaps concentrations and SCL/OC ratio in some of the sampling times examined. Benzoic acid negatively affected ($p < 0.05$) Ca content in bones and distal bone density, especially in the younger animals. Phytase increased ash, P and Ca content in bone as well as bone density and breaking strength ($p < 0.05$), independent of the animal's age. The results of this study indicate that for a healthy skeleton benzoic acid in low-P diets should not be used without the addition of phytase.

Introduction

Excessive excretion of phosphorus (P) in intensive animal production has led to severe environmental problems (Mallin and Cahoon 2003; Jongbloed 2007). As a consequence legislations on nutrient management in agriculture were adopted to control the use of inorganic P in farm animal diets (Jongbloed et al. 1999; Deunert et al. 2007). However, for monogastric animals it is almost impossible to achieve the required amount of available P in diets of plant origin because there P is bound to phytate (Eeckhout and De Paepe 1994).

Low P-availability can be a cause of skeletal problems (Nicodemo et al. 1998; Gutzwiller et al. 2007b) as 75 % of P in the body is stored in bones (Poulsen 2000). When microbial phytases are added to low-P diets, P availability and thus bone stability are increased (Radcliffe et al. 1998; Liesegang et al. 2005; Veum and Ellersieck 2008).

The use of organic acids in pig diets is of increasing importance mainly due to the ban on antimicrobial performance enhancers in the EU in 2006. Despite the fact that some organic acids such as citric and fumaric acid are able to increase the availability of phytate P (Boling

et al. 2000; Liem et al. 2008), they are thought to reduce bone stability when added to diets with reduced P content. So far this effect has been observed with fumaric acid (Liesegang et al. 2002) and benzoic acid (Gutzwiller et al. 2007a), being more pronounced in the latter. Benzoic acid as feed additive has been authorized since May 2003 (EC 877/2003) and is now listed in the group of zootechnical additives (EC 1138/2007). Through its metabolic end product hippuric acid, benzoic acid has the capacity to markedly reduce urinary pH (Mroz et al. 2000). This acidification can cause an increased renal mineral excretion and thus reduce bone mineralization (Kraut et al. 1986). So far it has not been tested whether these adverse effects of benzoic acid can be avoided with the addition of phytase to low-P diets but results in the study of Radcliffe et al. (1998) indicate such a protective effect.

In this study the response of bone metabolism, bone composition and bone stability to low-P diets supplemented with benzoic acid and phytase was examined. To gain knowledge on age dependent effects the experiment was conducted with growing and finishing pigs.

Material and methods

Animals and housing

The experiments were carried out simultaneously at two sites with a total of 48 crossbred gilts (Large white x (Landrace x Large white)). The 16 animals of experiment A were kept at ETH Zurich. Initial and final body weight (BW) of these animals was 25.2 ± 2.0 kg (mean ± SD) and 66.0 ± 2.7 kg, respectively. Experiment B was conducted with 32 animals at the research station 'Chamau' of ETH Zurich. The pigs of this experiment had an initial BW of 26.1 ± 1.1 kg and a final BW of 108.3 ± 2.9 kg.

All animals originated from the experimental station 'UFA Bühl', Hendschikon (CH). They were kept in single pens with wood shavings (experiment A) or straw (experiment B) as bedding. The experimental procedures were approved by the official veterinary authority of the canton of Zurich (CH) (ZH 181/2007 for experiment A) and Zug (CH) (ZG 44/06 for experiment B).

One animal of experiment B (diet CC) had to be slaughtered at a BW of 86.50 kg due to severe walking problems (paralysis) of unknown origin. As the problems were not related to diet the data of bone analysis were included into statistical calculation.

Diets and feeding

Diets and feeding were the same in both experiments. The diets for the experiments were prepared in one batch and then distributed to the two sites.

Animals were fed with one of four experimental diets: control diet without any supplementation (CC), control diet with 0.5 % benzoic acid (CB), phytase diet without benzoic acid and with 750 U/kg phytase (PhyC) and phytase diet with 0.5 % benzoic acid and 750 U/kg phytase (PhyB). Feed was offered as pellets at a daily amount of 190 g diet * $BW^{0.569}$. Water was available *ad libitum.* After 6 weeks (BW: 57.4 ± 2.6 kg), the diet in experiment B was changed from grower to finisher diet. Animals in experiment A only received grower diet.

The diets were based on cereals, peas and soybean expellers (Table 3.1). For the grower period crude protein (CP) content of the diets was calculated as 181 g/kg (all values as-fed) and as 149 g/kg for the finisher period, respectively. Digestible energy (DE) was calculated for all diets as 13.3 MJ/kg with a Lys/DE ratio of 0.84 g/MJ for the grower diets and 0.62 g/MJ for the finisher diets. Total P content was set at 4.0 g/kg in the grower and 3.5 g/kg in the finisher period which is below the recommendation of ALP (Agroscope Liebefeld-Posieux 2004). Calculated digestible P in the grower period was 1.43 g/kg for the control diets and 2.63 g/kg for the phytase diets. The corresponding values in the finisher period were 1.2 g/kg and 2.4 g/kg, respectively. Recommended values of ALP are 5.3 g/kg (2.67 g/kg) and 4.3 g/kg (2.16 g/kg) for total P (digestible P), respectively, in the two fattening periods.

Calcium (Ca) to P ratio was set at 1.3 for grower and 1.5 for finisher diets. Celite 545 (acid insoluble ash) was added as digestibility marker to the diets as the same experiment also included digestibility measurements (see Benzoic acid and phytase I). To inactivate native phytase diets were expanded before adding the commercial phytase. Benzoic acid and phytase were provided as VevoVitall® and Ronozyme® P, respectively by DSM Nutritional Products Ltd., Basel (CH).

Table 3.1. Composition of the experimental diets (g/kg).

	Grower period	**Finisher period**
Barley	300	300
Wheat	300	500
Peas	200	80
Soybean expellers	110	15
Potato protein	12.5	25
Molasses	20	25
Fat	5	4
Limestone	10.6	12
NaCl	3.4	3.5
Monocalcium phosphate	0.5	0.7
L-lysine-HCl	2.4	2.6
DL-methionine	1.4	0.1
L-threonine	0.9	0.6
Celite 545[1)]	28.3	26.5
Vitamin/mineral premix[2)]	5	5

[1)] Content of Celite 545 in diets without phytase and without benzoic acid. For the other diets, Celite 545 was reduced according to supplementation. Phytase addition was 1.2 g per kg diet. [2)] Supplied per kg of diet: 8000 IU vitamin A, 1000 IU vitamin D_3, 50 mg vitamin E, 1.5 mg vitamin B_1, 3.5 mg vitamin B_2, 2 mg vitamin B_6, 15 µg vitamin B_{12}, 430 µg vitamin K_3, 10 mg Ca-pantothenate, 20 mg niacine, 600 µg folic acid, 239.5 mg choline, 175 mg Fe, 30 mg Mn, 9.8 mg Cu, 750 µg I, 250 µg Se and 83.5 mg Zn.

Analysis of diets (see section: Collection and analysis of samples) showed that the composition was close to the calculation. Ca content was slightly higher than intended rising the Ca/P ratio to 1.51 ± 0.13 in the grower period and to 1.82 ± 0.07 in the finisher period. For unknown reasons analysis of one feed sample (PhyB) taken three weeks after the start of experiment B showed very low levels of benzoic acid and phytase. All other feed samples taken during the experiment were within the expected values. Therefore no influence on blood and bone parameters was expected and all data of animals fed diet PhyB in experiment B were used for statistical analysis.

Collection and analysis of samples

Feed samples were taken at both sites once every third week. The samples were ground to 0.75 mm in a centrifugal mill (Retsch ZM 1, Arlesheim, Switzerland) and analysed for dry matter, crude ash, crude fat CP (6.25 * N), neutral detergent fibre (NDF) and acid detergent fibre (ADF) by the standard procedures adopted in our laboratory (Robertson and Van Soest 1981; Naumann and Bassler 1997). Gross energy (GE) was analysed using a bomb calorimeter (Calorimeter C7000, IKA-Werke, GmbH & Co., KG, Staufen, Germany). For the analysis of P and Ca the feed samples were ashed for 13 h at 550°C. The content of both minerals was determined by calorimetry with an autoanalyser (COBAS MIRA®, Roche-Autoanalyser, Basel, Switzerland). The commercial kits used were CALC 20 (Axon Lab AG, Baden, Switzerland) for Ca and ABX Pentra (Horiba ABX, Montpellier, France) for P.

Analysis of benzoic acid was done with reversed phase liquid chromatography and UV-detection and followed the DSM Research Analytical Method DSM-RES 3-E. Activity of phytase was measured at the laboratories of Biopract GmbH, Berlin (D) with their standard procedure.

Blood samples were taken from the jugular vein at arrival of the animals and before slaughter. Additional samples were taken at week 3 (SP 1) and 6 (SP 2) in experiment A and in experimental week 7 (end of grower period) in experiment B. For technical reasons blood samples of the animals in experiment A were taken in the morning before feeding and of the animals in experiment B in the evening 10 hours after the last meal. Blood serum was analysed for Ca, P, total alkaline phosphatase (AP) and for the bone markers serum crosslaps (SCL, bone resorption marker) and osteocalcin (OC, bone formation marker). The mineral content in the serum was determined with the same method and the same kits as used for the feed samples. One-step ELISA (Osteometer, Biotech, Copenhagen, Denmark) was used to analyse SCL concentrations. Concentration of OC in serum was measured with radioimmunoassay using a commercial kit (Nichols Diagnostics, San Juan Capistrano, California).

After slaughter the left tibiae and metatarsi (MT) were collected. Bones were manually cleaned from attached tissue and frozen until further analysis. For determination of P and Ca the MT were dried (105°C, 24 h), ashed (550°C, 24 h) and then analysed the same way as the diet samples.

The tibiae were analysed at 50 % tibia length (midshaft) and at 10 % tibia length (distal metaphyse) for bone mineral density (BMD) and bone mineral content (BMC) with a peripheral quantitative computer tomography (pQCT, Stratec XCT 2000 bone scanner, Stratec

Medizinaltechnik GmbH, Pforzheim, Germany). Calculation of cortical BMD and BMC was done with automatic computation (cortical mode 2; threshold for cortical bone >640 mg/cm^3), values for trabecular BMD and BMC were: peel mode 2 and >710 mg/cm^3 as threshold for trabecular bone). Breaking strength – the maximal force applied to the bone before its failure – of MT II and III (before ashing) and tibia (after pQCT) was measured with three point force application (Stabl Mircro Systems TA-HD Texture Analyser, maximal force 2.5 kN, for MT and Zwick Z010 testXpertV10.11, maximal force 10 kN, for tibiae).

Statistical analysis

For the statistical analysis of both experiments the mixed model procedure of SAS for multiple comparisons (SAS System for Windows. SAS Institute Inc., Cary (NC), USA); Version 8.2) was used. Phytase (Phy), benzoic acid (B) and their interaction (Phy x B) were the defined effects. To determine whether changes in blood parameters in the experiment were affected by diet, the mixed model was run with the defined effects diet (D), sampling period (SP) and the interaction D x SP. If the Bonferroni-adjustment resulted in $p < 0.05$, differences were considered to be significant. Unless otherwise stated, data are shown as mean ± SD.

Results

Blood parameters

Phytase increased P concentration in serum in SP 1 of experiment A ($p = 0.01$) whereas Ca concentration remained constant ($p > 0.05$) (Figure 2.1A, B). With the addition of phytase, P increased from 2.72 ± 0.09 mmol/l to 3.03 ± 0.07 mmol/l but there were no statistical differences among diets (Figure 2.1B). In all other samples and in experiment B (data not shown) phytase had no effect ($p > 0.05$) on mineral concentration in the blood.

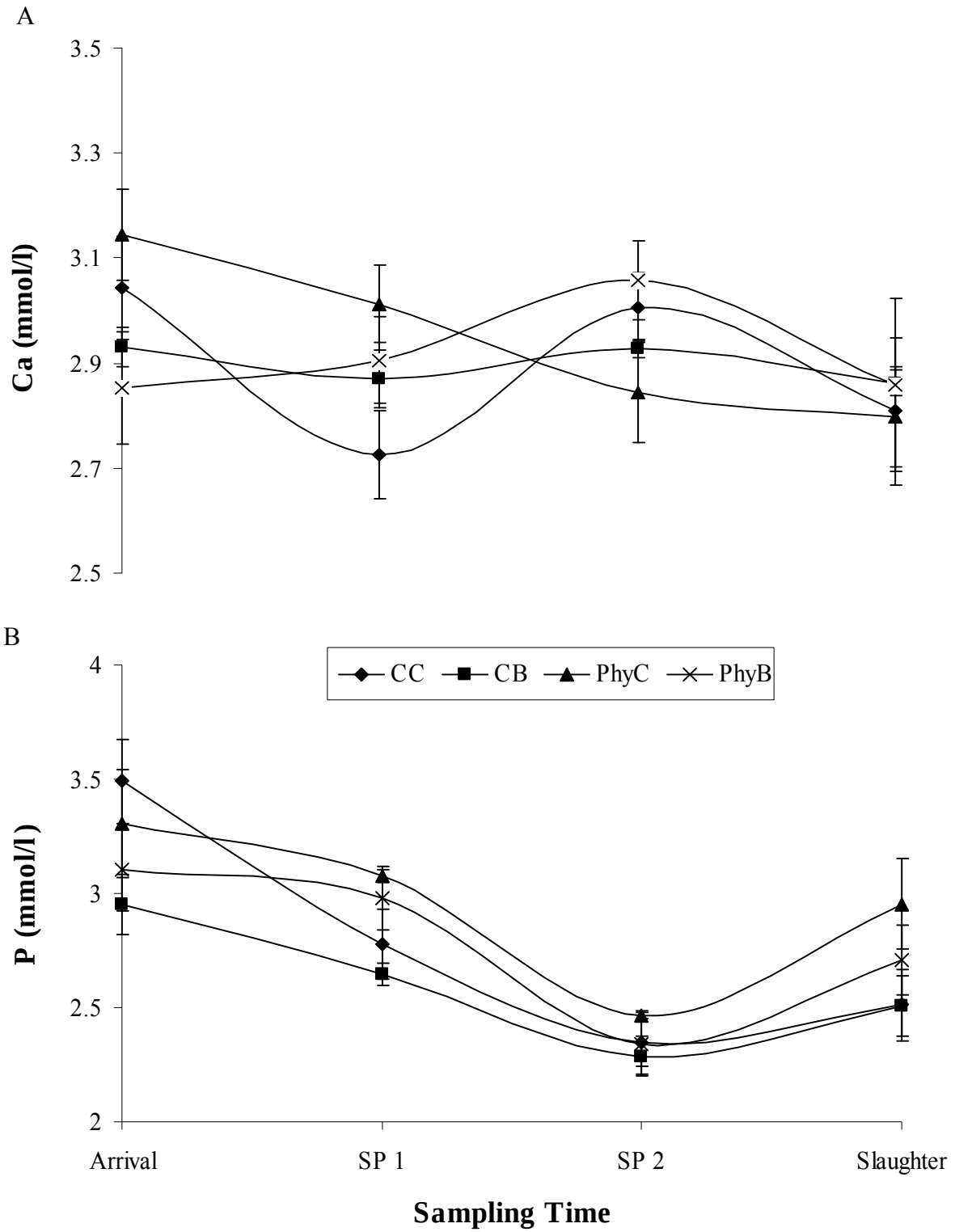

Figure 2.1. Mean (± SE) serum calcium (A) and phosphor (B) concentrations (mmol/l) of the four groups in experiment A. CC: no additives; CB: 0.5 % benzoic acid; PhyC: 750 U/kg diet phytase, no benzoic acid; PhyB: 0.5 % benzoic acid and 750 U/kg diet phytase. n = 4.

In SP 1 unspecific alkaline phosphatase (AP) showed a negative reaction ($p = 0.02$) to the interaction of benzoic acid x phytase (Figure 2.2A). However, this effect disappeared in the other samples and could not be observed at all in experiment B (data not shown). Activity of AP in SP 1 of experiment A was the lowest in diet PhyB with 171 U/l, followed by diets CC, CB and PhyC with 183 U/l, 220 U/l and 226 U/l, respectively but it did not differ from the other diets ($p > 0.05$).

The concentration of serum crosslaps (SCL) was increased ($p \leq 0.04$) when phytase was added to the diet. This effect could be measured in SP 2 and at the end of experiment A (Figure 2.2B) as well as at the end of the grower period in experiment B (data not shown). On average, SCL in animals fed with the phytase diets was 16.6 % higher than in the control groups. Except for diets CB and PhyC ($p < 0.05$) at the end of the grower period in experiment B (0.29 ng/ml versus 0.37 ng/ml, data not shown) there were no statistical differences among diets ($p > 0.05$). The concentration of osteocalcin (OC) was similar in all diets at all sampling times and in experiment A (Figure 2.2 C) and B (data not shown) ($p > 0.05$).

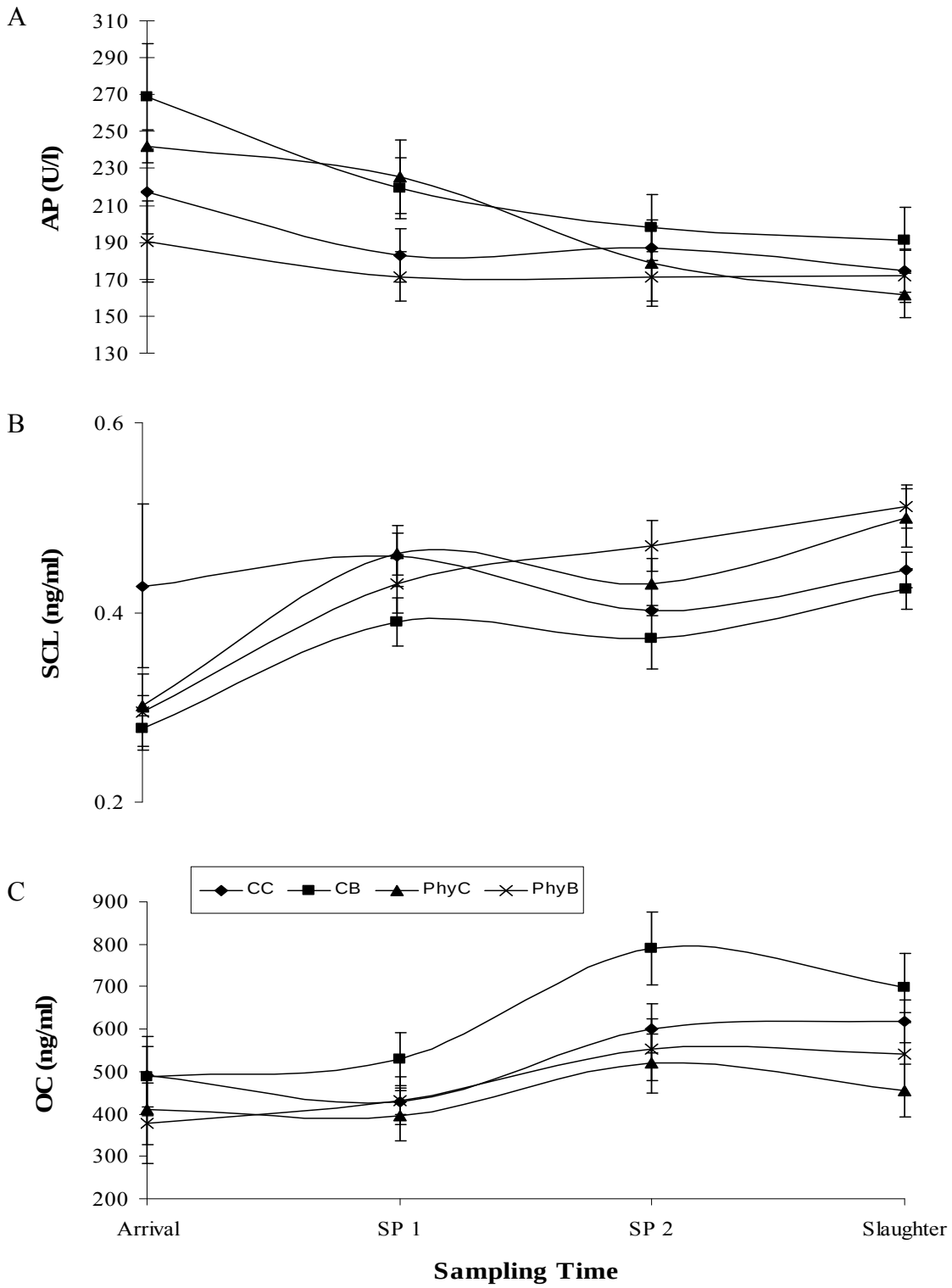

Figure 2.2. Mean (± SE) serum alkaline phospatase (AP) activities (U/l, A) and serum crosslaps (SCL, B) and osteocalcin (OC, C) concentrations (ng/ml) of the four groups in experiment A. CC: no additives; CB: 0.5 % benzoic acid; PhyC: 750 U/kg diet phytase, no benzoic acid; PhyB: 0.5 % benzoic acid and 750 U/kg diet phytase. n = 4.

Phytase increased ($p \leq 0.01$) SCL/OC ratio in SP 2 and before slaughter in experiment A (data not shown). No changes of the SCL/OC ratio were observed in experiment B. The SCL/OC ratio in Phy-diets of experiment A was elevated by 52 % (SP2) and 61 % (before slaughter), respectively, compared to the control diets (data not shown) but there were no statistical differences among diets ($p > 0.05$).

The addition of benzoic acid did not change any of the blood parameters nor the SCL/OC ratio examined at any time point ($p > 0.05$). Neither in experiment A nor in experiment B diet related long-term changes of mineral concentrations, AP or bone markers in serum including SCL/OC ratio could be observed ($p > 0.05$).

Bone composition

The addition of phytase significantly increased ash and mineral content of bones ($p \leq 0.02$) in experiment A and experiment B. Benzoic acid significantly influenced Ca content in experiment A ($p = 0.01$) and numerically reduced the other parameters of bone composition (Table 3.2). There were no interactions of benzoic acid and phytase observed ($p > 0.05$).

In experiment A phytase increased ash of MT from 28.9 ± 1.4 % to 32.9 ± 0.8 %. For P and Ca the increase was from 0.47 ± 0.01 % to 0.54 ± 0.02 % and from 1.2 ± 0.1 % to 1.4 ± 0.1 %, respectively. In experiment B the increase in ash, P- and Ca-content in the Phy-diets compared to the control diets was 19, 0.3 and 0.75 g/kg DM, respectively (Table 3.2). In both experiments Ca in bones was reduced by 6 % when benzoic acid was added to the diet. In general, bone composition of diet CB was significantly different from those of diet PhyC ($p < 0.05$). In experiment A the former also differed from diet PhyB ($p < 0.05$). Independent of the age of the animals ash, P and Ca content of the MT increased in the order CB < CC < PhyB < PhyC (Table 3.2).

Table 3.2. Ash (g/kg DM) and mineral content (g/kg DM) in metatarsal bones at 66 kg (experiment A) and at 108 kg (experiment B) BW.

	Diets[1)]					*p*-values[2)]		
	CC	**CB**	**PhyC**	**PhyB**	**SEM**	**Phy**	**B**	**Phy x B**
Experiment A (n = 4)								
Ash	298.9^{ac}	278.7^{a}	334.2^{b}	322.9^{bc}	10.9	< 0.01	0.06	0.58
P	48.2	46.1	55.3	52.3	3.2	< 0.01	0.25	0.85
Ca	123.0^{ac}	115.8^{a}	139.8^{b}	130.6^{bc}	4.0	< 0.01	0.01	0.73
Experiment B (n = 8)								
Ash	358.6^{ab}	343.7^{a}	374.7^{b}	365.6^{ab}	6.8	< 0.01	0.06	0.65
P	55.6	54.7	58.2	58.1	1.5	0.02	0.65	0.75
Ca	149.0^{ab}	143.9^{a}	154.9^{b}	152.9^{ab}	3.3	< 0.01	0.18	0.56

[1)] C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. [2)] Effects of phytase (Phy), benzoic acid (B) and their interaction (Phy x B). Different superscripts in a row indicate significant differences ($p < 0.05$). SEM: maximal standard error of the means.

Bone stability

Total and cortical bone mineral density (BMD) and bone mineral content (BMC) of the midshaft were markedly improved with phytase in the diet ($p \leq 0.03$) in both experiments. This was not the case for the cortical BMD in tibia obtained from finishing pigs in experiment B where no effect of this additive could be found (Table 3.3). The increase of BMD and BMC triggered by phytase in experiment A ranged from 4.7 % (cortical BMD) up to 21.9 % (cortical BMC). The bones of the finishing pigs showed a similarly distinctive improvement between 7 % for total BMD and 15 % for cortical BMD.

Apart from the cortical BMC in growing pigs of experiment A ($p = 0.03$) benzoic acid did not influence bone stability of the midshaft. Compared to diets without phytase cortical BMC was reduced by 16 mg/cm^3 in control diets and 6 mg/cm^3 in the phytase diets. In general, BMD and BMC of animals fed diet CB were markedly lower ($p < 0.05$) than the values obtained for diet PhyC (Table 3.3).

Table 3.3. Bone mineral density (BMD, mg/cm^3) and bone mineral content (BMC, mg/cm) in the midshaft of the tibia at 66 kg (experiment A) and at 108 kg (experiment B) BW.

	Diets[1)]					***p*-values[2)]**		
	CC	**CB**	**PhyC**	**PhyB**	**SEM**	**Phy**	**B**	**Phy x B**
Experiment A (n = 4)								
BMD_{tot}	659^{ab}	592^{a}	761^{b}	717^{ab}	35	< 0.01	0.08	0.70
BMC_{tot}	164^{ab}	153^{a}	185^{b}	181^{b}	6	< 0.01	0.08	0.38
BMD_{cor}	1088^{ab}	1061^{a}	1124^{b}	1123^{ab}	19	< 0.01	0.33	0.38
BMC_{cor}	136^{ab}	120^{a}	159^{c}	153^{bc}	7	< 0.01	0.03	0.32
Experiment B (n = 8)								
BMD_{tot}	670	641	695	705	21	0.03	0.60	0.32
BMC_{tot}	234^{a}	253^{ab}	272^{a}	265^{b}	11	< 0.01	0.43	0.08
BMD_{cor}	1093	1088	1108	1105	12	0.11	0.66	0.91
BMC_{cor}	193^{a}	210^{ab}	235^{b}	227^{b}	9	< 0.01	0.49	0.05

1) C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. **2)** Effects of phytase (Phy), benzoic acid (B) and their interaction (Phy x B). Tot: total, cor: cortical. Different superscripts in a row indicate significant differences ($p < 0.05$). SEM: maximal standard error of the means.

In animals of experiment A phytase increased total as well as trabecular BMD and BMC of the distal metaphyse ($p \leq 0.02$). In experiment B this effect could only be observed in total BMD and BMC ($p \leq 0.01$) but not in trabecular bone tissue (Table 3.4). In experiment A total BMD and BMC as well as trabecular BMD were negatively affected by 0.5 % benzoic acid ($p \leq 0.03$), resulting in significant differences between diets CB and PhyC. Total BMD of growing animals fed phytase diets was about 15 % higher than that of the control. Regarding BMC the increase was even 20 % for total BMC and 30 % for trabecular BMC (Table 3.4). On the other hand benzoic acid decreased total BMD and total BMC on average by 9 % and trabecular BMD by 12 %. In experiment B total BMD (total BMC) was reduced from 408 ± 8 mg/cm^3 (464 ± 13 mg/cm) to 382 ± 5 mg/cm^3 (410 ± 8 mg/cm) in C-diets compared to the Phy-diets.

Table 3.4. Bone mineral density (BMD, mg/cm^3) and bone mineral content (BMC, mg/cm) in the distal metaphyse of the tibia at 66 kg (experiment A) and at 108 kg (experiment B) BW.

	Diets[1)]					***p*-values[2)]**		
	CC	**CB**	**PhyC**	**PhyB**	**SEM**	**Phy**	**B**	**Phy x B**
Experiment A (n = 4)								
BMD_{tot}	301^{ab}	264^{a}	343^{b}	318^{b}	18	< 0.01	0.03	0.66
BMC_{tot}	281^{ab}	265^{a}	344^{c}	312^{bc}	11	< 0.01	0.03	0.40
BMD_{trab}	317^{ab}	277^{a}	363^{b}	320^{ab}	18	< 0.01	0.01	0.82
BMC_{trab}	133	125	165	171	29	0.02	0.95	0.67
Experiment B (n = 8)								
BMD_{tot}	378	385	413	402	12	0.01	0.87	0.40
BMC_{tot}	404^{a}	416^{ab}	473^{c}	454^{b}	11	< 0.01	0.75	0.13
BMD_{trab}	354	354	373	363	10	0.11	0.56	0.56
BMC_{trab}	299	295	310	300	14	0.49	0.55	0.78

1) C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. **2)** Effects of phytase (Phy), benzoic acid (B) and their interaction (Phy x B). Tot: total, trab: trabecular. Different superscripts in a row indicate significant differences ($p < 0.05$). SEM: maximal standard error of the means.

In both experiments phytase improved breaking strength of the tibia and the metatarsal bones ($p < 0.01$). Benzoic acid showed no statistical influence on bone stability but tibia and MT of animals in experiment A fed diets CB or PhyB were weaker than those fed diet CC or PhyC (Table 3.5). In experiment B breaking strength of tibia in diet CB was higher than that of diet CC and in general bones of animals fed diets with benzoic acid were slightly weaker ($p > 0.05$). In experiment A, the tibia of animals fed the control diet broke at a force of 1.66 ± 0.44 kN which was significantly ($p < 0.01$) less than the 2.04 ± 0.07 kN measured when phytase was added to the diets. The corresponding values for experiment B were 3.09 ± 0.06 kN and 3.75 ± 0.19 kN, respectively (Table 3.5) with an intermediate value of 3.61 kN for diet PhyB. Breaking strength of MT in diet CB was with 0.46 kN significantly lower ($p < 0.05$) than that of the Phy-diets with 0.67 ± 0.03 kN. At a BW of 108 kg, a force of 0.97 kN was needed to break MT of diet CB which was 37 % less ($p < 0.05$) than in diet PhyC (Table 3.5).

Table 3.5. Breaking strength (kN) of tibia and metatarsal bones II + III at 66 kg (experiment A) and at 108 kg (experiment B) BW.

	Diets[1)]					*p*-values[2)]		
	CC	**CB**	**PhyC**	**PhyB**	**SEM**	**Phy**	**B**	**Phy x B**
Experiment A (n = 4)								
Tibia	1.68[a]	1.63[a]	2.09[b]	1.99[b]	0.11	< 0.01	0.34	0.72
Metatarsi	0.54[ab]	0.46[a]	0.69[b]	0.65[b]	0.06	< 0.01	0.18	0.71
Experiment B (n = 8)								
Tibia	3.05[a]	3.13[a]	3.88[b]	3.61[ab]	0.16	< 0.01	0.54	0.24
Metatarsi	1.06[ab]	0.97[a]	1.33[b]	1.19[ab]	0.10	< 0.01	0.18	0.75

[1)] C: without phytase, Phy: with 750 U/kg phytase, C: without benzoic acid, B: with 0.5 % benzoic acid. [2)] Effects of phytase (Phy), benzoic acid (B) and their interaction (Phy x B). Different superscripts in a row indicate significant differences ($p < 0.05$). SEM: maximal standard error of the means.

Discussion

In this study it was shown that almost all parameters examined in the bones were improved by the addition of phytase, independent of the animal's age. In contrast to that benzoic acid in low-P diets can have an adverse effect on bone composition and bone stability, mainly in younger animals.

Changes observed in the bones being caused by benzoic acid were not mirrored by the parameters examined in the blood as they were, if at all, only affected by phytase. It is known that serum Ca an P are of limited value to describe bone mineralization (Koch and Mahan 1986). Activity of alkaline phosphatase on the other hand has been reported to correlate with bone stability in growing pigs between 16 and 31 kg BW (Boyd et al. 1983) but not in pigs of the weight range 65 to 95 kg (Koch and Mahan 1986). It is thus possible that the negative interaction benzoic acid x phytase in SP 1 in experiment A reflected a momentary decline in bone stability. With a BW of 34.33 ± 2.07 kg the pigs at this time were only slightly above the weight range examined by Boyd et al. (1983). It has to be taken into consideration that total AP originates from several tissues (Christenson 1997) and only bone specific AP strongly correlates to bone metabolism (Epstein 1988). The changes in AP concentration found in this

study can thus also hint to changes in the metabolism of other tissues and not only in the bone. However, how this interaction triggered the distinct reduction of AP is unclear. A possibility is that the additives increased P availability and thus decreases AP activity as it is described by Boyd et al. (1983). However, in a digestibility study conducted with the same animals and diets as this study, we could not find any interaction in apparent P digestibility; in fact, it was even decreased by the addition of benzoic acid (see: Benzoic acid and phytase I).

In contrast to what could have been expected, SCL concentration was increased in animals fed the Phy-diets. Generally, this bone marker is reduced when bone stability is increased (Briot and Roux 2005; Vasikaran 2008) and in this study phytase clearly increased breaking strength, BMD and BMC. A possible explanation is that the elevated concentration of SCL was not a sign of decreased bone stability but of accelerated bone turnover. In fact, the increased SCL/OC ratio in SP2 and before slaughter found in experiment A is a sign for such an intensified turnover.

It has been claimed that OC is the better predictor of bone stability than AP (Carter et al. 1996) as not bone specific AP was examined. However, Nicodemo et al. (1998) could not find a correlation between OC and bone mineralization, which supports the results presented here. Additionally, no effect of any of the additives on OC was found in our study. This is in accordance to the study of Liesegang et al. (2002). Their explanation was that the low dietary P content and fumaric acid influenced osteoblasts producing bone specific alkaline phosphatase more than those producing OC. Whether this is the case and whether this is a specific reaction to organic acids in general needs further investigation.

When comparing concentrations of the bone markers SCL and OC with the actual bone structures, it has to be taken into consideration that these parameters are static short-term pictures of a highly dynamic system. For example, the concentrations of SCL and OC in several species including humans follow a circadian rhythm (Hansson et al. 1974; Lepage et al. 1991; Hassager et al. 1992; Muhlbauer and Fleisch 1995; Liesegang et al. 1999; Liesegang et al. 2003). It is possible that changes in bone turnover were masked by this rhythm. However, it is unclear whether this time dependency can also be found in growing-finishing pigs but it is known from piglets that their OC concentration is the highest after midnight and the lowest around noon (Guo et al. 2000). Furthermore, it is possible that the time intervals for blood samplings chosen in this experiment were too long and thus changes in bone turnover were missed. This would explain why benzoic acid did not show any effect on bone markers in serum but nevertheless affected bone structure in experiment A.

As it has been described in several studies for pigs and poultry (Harper et al. 1997; Liesegang et al. 2005; Payne et al. 2005; Sacakli et al. 2006; Veum and Ellersieck 2008), this experiment showed that bone composition and bone stability was positively influenced when phytase was added to low-P diets. These effects could be observed in animals with BW 66 kg as well as in those with BW 108 kg.

Concerning the type of organic acid it has been described for citric acid that it can slightly increase metatarsal ash in young pigs (Boling et al. 2000). However, compared to pigs, the effects observed are stronger in broilers (Boling et al. 2000) and are also described when malic or fumaric acid are used as additives (Liem et al. 2008). In this study benzoic acid decreased Ca, BMD and BMC especially in the younger animals and in the distal bone. Benzoic acid is the only organic acid in pig nutrition which reduces urinary pH through its metabolic end product hippuric acid. This acidification is the cause that more Ca is excreted with the urine to re-establish the acid-base balance of the body as it was shown in rats (Kraut et al. 1986). In this study this effect could be well observed in the reduced Ca content in MT of the younger animals. This contradicts the findings of Budde and Crenshaw (2003) who reported that the additional Ca was not of skeletal origin.

So far the negative effects of benzoic acid on bone parameters have only been described by Gutzwiller et al. (2007a) and Hess and Gutzwiller (2008) feeding nitrogen and P reduced diets (NPr-diets). In the former study, the effect of benzoic acid on bones in younger animals was more pronounced than in the study described here. This may be based on the fact that the rather high amount of crude protein in the diets used in the present experiment could act as buffer for the effects of the acid which was not possible in the NPr-diets. Findings describing strong effects of organic acids on bones in low buffering diets (Kornegay et al. 1994; Biagi et al. 2003) support this theory. It seems that in diets with a high buffer capacity, the P content in the diet is the first limiting factor of bone stability before the changes induced by benzoic acid. Beside statistical effects (n = 4 in experiment A, n = 8 in experiment B), this could explain the age dependent effects of the results on bone composition and bone stability. It is possible that in the finisher period the animals needed less P per kg feed than provided and that thus the effects of benzoic acid on bone parameters disappeared. The limiting effect of P is also supported by the results of a similar study where 1 % benzoic acid added to normal and high protein diets with sufficient total P had no effect on bone parameters in finishing pigs (Bühler et al. 2007).

In the present study the impact of benzoic acid on bone metabolism did not lead to impaired health of the pigs despite being very pronounced in the growth zone of the distal

bone. However, the pigs used in this study were fed a normal diet until the start of the experiment. Thus, further research is needed with animals receiving the experimental diets described here from weaning on as well as with mature sows and boars, so that long-term effects of such diets on skeletal health can be determined.

Conclusion

This study could not detect any long-term effects of benzoic acid or phytase on mineral, AP and bone marker concentrations in serum but positive part time effects of phytase on some of these parameters could be observed. On the other hand, the bones themselves showed signs of reduced stability when benzoic acid was added to low-P diets compared with the control groups. Nevertheless, these adverse effects could be almost completely compensated for with the addition of phytase. The results also showed that the negative effect of benzoic acid on bone stability disappears with increasing age whereas the positive effect of phytase persists. Results of this study indicate the necessity of using phytase in low-P diets supplemented with benzoic acid to prevent bone problems.

Influence of benzoic acid in high fibre diets on nutrient digestibility and VFA production in growing-finishing pigs

based on K. Bühler, B. Bucher, C. Wenk and J. Broz
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Abstract

In a feeding study with 32 crossbred gilts (13 – 108 kg body weight) the effect of 5 g/kg benzoic acid in low (150 g/kg DM NDF) and high (202 g/kg DM NDF) fibre diets on performance and apparent nutrient digestibility was examined. The animals were restrictively fed one of four grower and finisher diets: low fibre diet without and with benzoic acid (LF- and LF+) and a high fibre one without and with benzoic acid (HF- and HF+). At 56 kg body weight four animals per diet were slaughtered to obtain data on volatile fatty acid (VFA) production in the gut. In the grower period digestibility of nitrogen, energy and neutral detergent fibre (NDF) was positively influenced by benzoic acid ($p < 0.01$) and reduced by fibre addition ($p < 0.01$). The concentration of butyric acid in caecum and colon was increased by benzoic acid ($p < 0.05$). Dietary fibre content did not influence VFA concentrations. It is concluded that the addition of benzoic acid helped animals to better exploit high fibre diets.

Introduction

High fibre diets have been used for a long time for sows to prevent problems at farrowing (Ramonet et al. 2000) and such diets are now of increasing interest in pig fattening as well (Noblet and Le Goff 2001)**.** One reason is that high fibre diets are often cheaper than conventional ones (Le Goff et al. 2002a). Another is the beneficial effect of such diets on the pig's health and well-being (Wenk 2001).

When fermenting dietary fibre, the gastrointestinal microorganisms produce volatile fatty acids (VFA) which can be absorbed by the pigs (Jorgensen et al. 1997) and serve as an additional energy source for the animal (Argenzio and Southworth 1975; Kirchgessner and Müller 1991). In contrast to VFA, the absorption of nutrients in the large intestine is limited (Wenk 2001). This reduces digestibility of nutrients (Mosenthin et al. 1999; Noblet and Le Goff 2001) and makes efficient pig fattening difficult (Drewry 1981). The importance of microorganisms in fibre digestion should make it possible to modulate this process by changing the gastrointestinal microflora. One possibility is the use of organic acids, such as benzoic acid. In the European Union, benzoic acid at dietary levels of 0.5 to 1.0 % has been authorized since May 2003 as a feed additive for growing-finishing pigs. It is currently included in the functional group of other zootechnical additives with the claim "urinary pH decrease" (EU regulation No. 1138/2007/EC). This decrease of urinary pH is caused by hippuric acid, the metabolic end-product of benzoic acid (Bridges et al. 1970).

So far, there is no study in which the effect of benzoic acid was studied in high fibre diets. However, there are indications that organic acids are able to improve the nutritive value of high fibre diets. Two studies of Partanen et al. (2001; 2002a) showed that the addition of formic acid to such diets increased apparent ileal and faecal digestibility of nutrients and changed the VFA pattern in the gut but did not influence performance.

The aim of this study was to determine whether these effects can be reproduced when straw meal and soybean hulls as fibre sources and benzoic acid are added to the diet. The focus was on nutrient and fibre digestibility of the diet as well as the influence of benzoic acid and fibre content on VFA production in growing-finishing pigs.

Material and methods

Animals and housing

A total of 32 gilts (Large White x (Landrace x Large White)) with an initial bodyweight (BW) of 13.4 ± 0.9 kg (mean ± SD) were used in this experiment. Final BW was 56.3 ± 4.4 kg and 108.4 ± 3.9 kg, respectively. The animals originated from the experimental station 'UFA Bühl', Hendschikon (CH). One animal developed an umbilical hernia and had to be replaced after the second sampling period. Full-length wheat straw was provided as bedding. An additional experiment showed (Bühler et al. 2009) that there was no compensatory intake of straw in animals fed the low fibre diet. For this reason straw intake was considered to be equal in all diets. The experimental procedure was approved by the official veterinary authority of the canton of Zug (CH) (authorization number ZG 44/06).

Diets and feeding

The animals were randomly distributed in individual pens and received one of four experimental diets: low fibre diet without benzoic acid (LF-), low fibre diet with 0.5 % benzoic acid (LF+), high fibre diet without benzoic acid (HF-) and high fibre diet with 0.5 % benzoic acid added (HF+). Benzoic acid was provided as VevoVitall® by DSM Nutritional Products Ltd., Basel, Switzerland.

The LF diets were based on cereals, peas and soybean expellers (Table 4.1). For the experimental diets 0.5 % benzoic acid was added to the control diet. In diets HF 5 % soybean hulls and 5 % untreated wheat straw meal were added at the expense of LF.

Table 4.1. Composition of the experimental diets (g/kg).

	Grower period	**Finisher period**
Barley	200	200
Triticale	300	300
Wheat	73	203
Peas	200	160
Soybean expellers	120	50
Potato protein	33	18
Molasses	20	20
Limestone	10	10
NaCl	5	5
Dicalcium phosphate	12	8
L-lysine-HCl	1.3	1.2
DL-methionine	1.1	0.1
L-threonine	0.5	0.35
Celite 545[1)]	22.1	23.15
Vitamin/mineral premix[2)]	3	2.25
Fibre source[3)]:		
Soybean hulls	50	50
Wheat straw meal	50	50

[1)] Content of Celite 545 in diets without benzoic acid. For diets with benzoic acid, Celite 545 was reduced by 5 g/kg. [2)] Supplied per kg of grower (finisher) diet: 8000 (6000) IU vitamin A, 1000 (750) IU vitamin D_3, 36 (27) mg vitamin E, 1110 (830) µg vitamin B_1, 3.0 (2.2) mg vitamin B_2, 2.2 (1.7) mg vitamin B_6, 18.0 (13.5) µg vitamin B_{12}, 1.3 (1.0) mg vitamin K_3, 11 (8) mg Ca-pantothenate, 22 (16) mg niacine, 570 (430) µg folic acid, 69 (52) µg biotine, 105 (79) mg choline, 105 (79) mg Fe, 37 (27) mg Mn, 9.0 (6.8) mg Cu, 540 (405) µg I, 180 (135) µg Se and 60 (45) mg Zn. [3)] Only added to the high fibre diets

Crude protein (CP) content of the diets LF was calculated as 177 g/kg in the grower and as 145 g/kg in the finisher period. The digestible energy (DE) content was calculated for all diets as 13 MJ/kg with a Lys/DE ratio of 0.84 g/MJ for the grower diets and 0.63 g/MJ for the finisher diets.

Feed was offered as pellets in two equal rations per day. To obtain the same intake of benzoic acid and energy for all pigs, animals fed on diets HF received daily 200 g * $BW^{0.569}$ of diet whereas animals fed on diets LF received 180 g diet * $BW^{0.569}$. At a body weight of

54.6 ± 6.4 kg diet was changed from grower to finisher diet for all animals. Water was available *ad libitum*.

Chemical analysis of the diet showed that benzoic acid content in the grower period was about 5 % lower than expected whereas DE was higher as calculated but similar in all diets. The difference in neutral detergent fibre (NDF) and acid detergent fibre (ADF) between the low and high fibre diet was about 50 g/kg DM (Table 4.2).

Table 4.2. Analysed nutrient content of the experimental diet.

	Grower period				**Finisher period**			
	LF-[1)]	**LF+**	**HF-**	**HF+**	**LF-**	**LF+**	**HF-**	**HF+**
Benzoic acid (g/kg)[2)]	0	4.8 ± 0.1	0	4.7 ± 0.6	0	5.0 ± 0.0	0	5.0 ± 0.0
NDF (g/kg DM)	154.4 ± 11.9		204.8 ± 8.2		144.0 ± 0.7		202.0 ± 6.7	
ADF (g/kg DM)	59.7 ± 2.6		103.2 ± 3.9		51.4 ± 1.3		97.9 ± 0.2	
DM (g/kg)	878.3 ± 3.7		879.3 ± 1.2		897.1 ± 1.6		900.8 ± 0.9	
Ash (g/kg DM)	76.7 ± 3.7		77.7 ± 2.1		75.5 ± 2.1		78.1 ± 1.5	
CP (g/kg DM)	202.4 ± 1.2		194.1 ± 0.1		161.1 ± 0.03		156.1 ± 0.3	
Crude fat (g/kg DM)	26.3 ± 0.2		27.6 ± 0.4		21.1 ± 0.5		20.4 ± 0.9	
DE_{exp} (MJ/kg)	13.4 ± 0.1		13.3 ± 0.1		13.8 ± 0.1		13.7 ± 0.1	

[1)] LF: low fibre diet; HF: high fibre diet; - without benzoic acid; +: with 0.5 % benzoic acid. Values are given as mean ± SD. DE_{exp} was calculated as $(GE_{intake} - GE_{excreted})/Feed_{intake}$. [2)] Analysed by ADICEA Laboratory, F 35460 St Etienne en Cogles, France (on behalf of DSM Nutritional Products).

Collection of samples

Faeces were collected every third week during four consecutive days resulting in three sampling periods for the grower and two sampling periods for the finisher period. Only fresh faeces were collected and attached straw was carefully removed. Faeces of each animal were pooled for each sampling period. Feed samples were taken once in a sampling period and pooled for analysis.

At the end of the grower period four animals per diet were randomly selected and slaughtered 3 to 4 hours after the last morning feeding. Immediately after killing, the gastrointestinal tract (GIT) was removed and separated into stomach and nine intestinal segments: three equal parts of the small intestine (SI 1-3), caecum (C), four equal parts of the

large intestine and the terminal colon (CO 1-5). The content of these segments was extracted, immediately frozen in liquid nitrogen and stored at -20°C.

Analytical methods

Faeces and digesta were lyophilised and allowed to reach air dry mass. For analysis they were ground in a centrifugal mill (Retsch ZM 1, Arlesheim, Switzerland) using a 0.5 mm sieve. Feed samples were ground to the size of 0.75 mm. Samples were analysed for dry matter, crude ash, crude fat (only feed samples) and CP (6.25 * N) by the standard procedure of VDLUFA (Naumann and Bassler 1997). Analysis of neutral detergent fibre (NDF) and acid detergent fibre (ADF) followed the method of Robertson and Van Soest (1981). Gross energy (GE) was analysed calorimetrically (Calorimeter C7000, IKA-Werke, GmbH & Co., KG, Staufen, Germany). Apparent digestibility of nutrients was estimated by means of the indicator method (acid insoluble ash), using acid-washed diatomaceous earth Celite 545 (Table 4.1) as indicator.

The content of the three VFA acetic acid, propionic acid and butyric acid in caecum and colon was determined in fresh digesta samples with HPLC following the sample preparation described in Doane et al. (1998). HPLC conditions were: HPX-87H column (Bio-Rad Laboratories, Hercules (CA), USA), temperature: 60°C, UV detection at 210 nm. HPLC usage followed the method of Ehrlich et al. (1981).

Statistical analyses

For statistical analysis results of nutrient digestibility in digesta were grouped to small intestine (SI 1-3), caecum (C) and colon (CO 1-5). To determine whether results of VFA analysis differed between caecum and colon a two-sample t-test was used. The mixed model procedure of SAS for multiple comparisons (SAS System for Windows, SAS Institute Inc., Cary (NC), USA; Version 8.2) was used for statistical analysis. Effects were fibre level (F), benzoic acid (B) and fibre level x benzoic acid interaction (F x B). Differences were considered to be significant in all statistical analyses if $p < 0.05$, which was obtained with Bonferroni-adjustment. Unless otherwise stated n = 8 in the grower and n = 4 in the finisher period.

Results and discussion

Pig performance

Benzoic acid significantly increased daily weight gain (DWG) of the animals in the grower period ($p < 0.01$), whereas in the finisher period DWG was higher in the HF diets ($p < 0.01$, data not shown). Average DWG in the grower period increased from 568 ± 15 g/d to 646 ± 24 g/d with benzoic acid in the diet. In the finisher period average DWG of animals fed HF diets was 840 ± 42 g/d compared to 759 ± 17 g/d in the LF-diets. In the grower but not in the finisher period animals on diet LF had an improved FCR ($p < 0.01$, data not shown) with an FCR of 1.89 being the lowest in diet LF-. Benzoic acid had no effect on FCR.

Because of the low number of animals these results have to be looked at with some reservation. In addition, the higher DWG found in HF is most probably not a dietary effect but an artefact caused by the two different feeding regimes (180 g and 200 g diet * $BW^{0.569}$, respectively) used in this experiment. However, it is unclear why the different intake of energy and protein did not change DWG in the grower period. This indicates that the described experimental design covered some mechanisms how benzoic acid and fibre content may influence pig performance.

Apparent nutrient and fibre digestibility

Dietary benzoic acid increased ($p < 0.01$) and fibre addition significantly decreased ($p < 0.01$) apparent total tract digestibility of CP (d(CP)) and energy (d(E)) in the grower period (Table 4.3). Diets with benzoic acid had a numerical increase in d(CP) and d(E) of 0.02 to 0.03 compared to diets without benzoic acid. At the same time apparent digestibility in HF diets was significantly lower ($p < 0.05$) than in LF diets. High fibre content also significantly reduced d(CP) and d(E) in the finisher period ($p < 0.05$) whereas the influence of benzoic acid disappeared ($p > 0.05$).

Benzoic acid increased apparent digestibility of NDF (d(NDF)) ($p < 0.01$) in younger animals (Table 4.3) whereas the high fibre content reduced d(NDF) ($p = 0.01$) in these animals. Later in the fattening period both effects disappeared and d(NDF) of all diets was similar. Apparent digestibility of ADF (d(ADF)) in the finisher period was higher ($p = 0.01$) in the HF diets than in the LF diets (Table 4.3).

Table 4.3. Apparent total tract digestibility of crude protein (d(CP)), energy (d(E)) and neutral and acid detergent fibre (d(NDF), d(ADF)).

	Diet[1)]					*p*-values[2)]		
	LF-	**LF+**	**HF-**	**HF+**	**SEM**	**F**	**B**	**F x B**
Grower period								
d(CP)	0.79^{ac}	0.82^{a}	0.75^{b}	0.77^{bc}	0.01	< 0.01	< 0.01	0.69
d(E)	0.83^{a}	0.84^{a}	0.78^{b}	0.79^{b}	0.01	< 0.01	< 0.01	0.44
d(NDF)	0.55^{ab}	0.61^{a}	0.50^{b}	0.53^{ab}	0.02	< 0.01	0.01	0.26
d(ADF)	0.39	0.42	0.43	0.41	0.03	0.56	0.98	0.36
Finisher period								
d(CP)	0.83	0.85	0.79	0.81	0.02	0.02	0.35	0.88
d(E)	0.84	0.86	0.82	0.82	0.01	< 0.01	0.28	0.44
d(NDF)	0.47	0.54	0.54	0.55	0.04	0.35	0.33	0.51
d(ADF)	0.21	0.33	0.45	0.46	0.07	0.01	0.33	0.35

[1)] LF: low fibre diet; HF: high fibre diet; - without benzoic acid; +: with 0.5 % benzoic acid. [2)] Effects of fibre content (F), benzoic acid (B) and their interaction (F x B). Different superscripts in a row indicate significant differences ($p < 0.05$) among diets. SEM: maximal standard error of the means. n = 8 for the grower and n = 4 for the finisher period.

At 56 kg BW apparent colon digestibility of nitrogen and energy was reduced ($p < 0.01$) with higher fibre content in the diet (Table 4.4). Simultaneously, d(E) in the colon was significantly increased ($p < 0.05$) in diets with 0.5 % benzoic acid. There was no influence of fibre or acid on d(CP) and d(E) in the other parts of the gut. In general, animals fed diet LF+ showed the highest and those fed diet HF- the lowest apparent nutrient digestibility. However, statistical difference among these two diets ($p < 0.05$) could only be found in the colon where d(CP) was 7 % and d(E) 9 % higher for diet LF+ compared to diet HF- (Table 4.4).

Benzoic acid significantly improved d(NDF) ($p < 0.05$) in the small intestine and the colon (Table 4.4). Additionally, a reducing effect ($p = 0.01$) of the fibre content on d(NDF) could be found in the small intestine. In this part of the gut d(NDF) in diet LF+ was doubled ($p < 0.05$) compared to diet LF- whereas in the HF diets this parameter rose with the addition of benzoic acid from 0.13 to 0.23 (Table 4.4). High fibre content reduced d(NDF) from 0.28 ± 0.15 to 0.18 ± 0.07. In the colon d(NDF) of animals fed diet LF- was significantly lower ($p < 0.05$) than that of animals fed diet LF+, which was the highest of all with a value of 0.58.

Digestibility of NDF in animals fed HF diets was about 0.43 and did not differ from the other diets (Table 4.4).

The amount of caecal content in some animals was too small to conduct NDF analyses. This resulted in n = 3 for caecal d(NDF) and no statistical analyses was performed for this parameter. Values of d(NDF) in the caecum (data not shown) ranged from 0.17 (diet LF-) to 0.30 (diets HF- and HF+) and 0.43 (diet LF+).

Table 4.4. Apparent digestibility of nitrogen (d(CP)), energy (d(E)) and neutral detergent fibre (d(NDF)) in different parts of the gastrointestinal tract at 56 kg BW.

	Diet[1)]					***p*-values**[2)]		
	LF-	**LF+**	**HF-**	**HF+**	**SEM**	**F**	**B**	**F x B**
Small Intestine								
d(CP)	0.53	0.63	0.53	0.53	0.09	0.54	0.50	0.52
d(E)	0.49	0.58	0.45	0.49	0.07	0.30	0.30	0.65
d(NDF)	0.17^{a}	0.38^{b}	0.13^{a}	0.23^{ab}	0.05	0.01	< 0.01	0.18
Caecum								
d(CP)	0.79	0.77	0.80	0.76	0.05	0.88	0.27	0.69
d(E)	0.75	0.78	0.74	0.72	0.03	0.11	0.68	0.16
Colon								
d(CP)	0.81^{ab}	0.82^{a}	0.77^{b}	0.78^{ab}	0.01	< 0.01	0.13	0.78
d(E)	0.80^{a}	0.84^{b}	0.77^{a}	0.78^{a}	0.01	< 0.01	0.03	0.09
d(NDF)	0.39^{a}	0.58^{b}	0.42^{ab}	0.44^{ab}	0.04	0.16	0.02	0.07

[1)] LF: low fibre diet; HF: high fibre diet; - without benzoic acid; +: with 0.5 % benzoic acid. [2)] Effects of fibre content (F), benzoic acid (B) and their interaction (F x B). Different superscripts in a row indicate significant differences ($p < 0.05$) among diets. SEM: maximal standard error of the means. n = 4.

It could be shown that, independent of the age of the animals, high dietary fibre content reduced apparent total tract digestibility of nitrogen and energy. On the other hand dietary benzoic acid increased digestibility parameters including d(NDF) in the grower period.

Similar to the performance data the results of the digestibility study hint to age dependent effects of benzoic acid and fibre content. The better nutrient digestibility with increasing age proposed by Noblet and Shi (1993) is confirmed as digestibility values were numerically

higher in the finisher period than in the grower period. Apparent digestibility of NDF and ADF resembled the results for the medium fibre diet (NDF: 200g/kg DM, ADF 60 g/kg DM) used in a study by Partanen et al. (2007).

Results of apparent nutrient digestibility analysed for small intestine, caecum and colon showed that the effects of fibre and benzoic acid were only visible in the colon. This is not true for d(NDF) where an influence of the two additives is already observed in the small intestine. In this case benzoic acid could have directly influenced parameters participating in the degradation of some digestible hemicelluloses in the small intestine. On the other hand the acid could only act indirectly in the colon as it disappears from the gut in the upper part of the small intestine (tested in our laboratory, data not shown). This indirect influence may be based on the fact that changes of substrate and microflora in the small intestine are transported along the gut to the colon. By this, the colon's environment is also modified in a way which enables an improved exploitation of energy and NDF.

Despite the low number of animals used for this study of GIT digestibility, the results are comparable to those in similar studies with formic acid and an organic acid mixture (Partanen et al. 2001; Partanen et al. 2007) but so far there is no conclusive explanation.

Volatile fatty acids

The concentration of butyric but not of acetic acid and propionic acid increased in caecum and colon with benzoic acid in the diet. The fibre content of the diets had no influence on the production of any of the VFA analysed (Table 4.5). The total amount of VFA was independent of benzoic acid and fibre content.

Results showed a significant difference ($p < 0.05$) of butyric acid in caecum and colon between diets without (1.64 % ± 0.09 and 1.65 % ± 0.04) and with (2.32 % ± 0.24 and 2.30 % ± 0.38) dietary benzoic acid. However, there was no statistically significant difference among diets ($p > 0.05$). There was no difference ($p > 0.05$) of VFA concentration in the caecum compared to concentrations in the colon.

Table 4.5. Total volatile fatty acid (VFA) and concentration of acetic, propionic and butyric acid at 56 kg BW.

	Diet[1)]					*p*-values[2)]		
	LF-	**LF+**	**HF-**	**HF+**	**SEM**	**F**	**B**	**F x B**
Caecum								
Total VFA (mmol/l)	53.86	46.11	53.46	50.71	4.95	0.63	0.24	0.57
Acetic acid (%)	78.93	76.69	80.03	76.42	1.87	0.77	0.06	0.63
Propionic acid (%)	19.37	20.82	18.40	21.43	1.78	0.89	0.11	0.55
Butyric acid (%)	1.70	2.49	1.57	2.15	0.31	0.38	0.02	0.70
Colon								
Total VFA (mmol/l)	37.57	37.56	37.68	38.63	3.41	0.82	0.86	0.86
Acetic acid (%)	80.26	78.35	80.02	78.60	1.81	0.998	0.19	0.84
Propionic acid (%)	18.09	19.08	18.37	19.37	1.60	0.80	0.39	0.995
Butyric acid (%)	1.67	2.57	1.62	2.03	0.35	0.30	0.03	0.36

[1)] LF: low fibre diet; HF: high fibre diet; - without benzoic acid; +: with 0.5 % benzoic acid. [2)] Effects of fibre content (F), benzoic acid (B) and their interaction (F x B). SEM: maximal standard error of the means. n = 4.

Data of the proportional content of the three main VFA in the gut of the pig hint to the explanation that soy bean hulls and straw meal in the diet had no influence. At the same time the inclusion of 0.5 % benzoic acid to the diet increased the amount of butyric acid in the gut, mainly at the expense of acetic acid. According to Freire et al. (2000) 20 % soy bean hulls significantly increased total VFA in the caecum of piglets. Similarly, the inclusion of 10 % barley straw slightly increased faecal VFA in barrows of 50 kg BW (Sauer et al. 1991). In our study we only used 5 % straw meal and 5 % soy bean hulls which was probably not enough to alter the VFA pattern in the gut.

Despite the fact that the total amount of VFA (Table 4.5) was markedly lower than reported elsewhere (Kass et al. 1980; Lynch et al. 2007) the relative composition of acetic acid and propionic acid is in accordance to other studies (Friend et al. 1963; Kass et al. 1980). In contrast the percentage of butyric acid is only about a quarter of that reported in the aforementioned studies. The reasons for these two findings are unknown but may be related to an insufficient stabilisation of the digesta during and after sampling as there was no influence of fibre or acid addition noticeable. However, the fact that only butyric acid is reduced contradicts this theory as this is the least volatile of the acids analysed.

The positive influence of benzoic acid on butyric acid concentration and the shift in the proportional composition of total VFA hint at changes in the gastrointestinal microflora which are a proposed mode of action of organic acids (Partanen 2001; Dierick et al. 2004). In a study by Roth et al. (1992a) butyric acid was reduced when formic acid was added, whereas the addition of carbadox or formic acid did not influence this VFA (Partanen et al. 2001). However, it remains unclear why in this study benzoic acid increased the concentration of butyric acid.

VFA in general contribute between 28 % and 50 % of maintenance energy (Yen et al. 1991; Kirchgessner and Müller 1991) and are used in many different tissues and metabolic pathways (Blottiere et al. 1999). Butyric acid is almost completely absorbed by the gut where it serves as an important energy source for gut epithelia (Freire et al. 2000) and helps to maintain normal gut function. The fact that benzoic acid not only modulates the gastrointestinal microflora but also increases the concentration of butyric acid opens new options to explain the positive effects of this acid on health and performance.

Conclusion

In this experiment it could be shown that benzoic acid added to high fibre diets may reduce the negative effects of such diets. Furthermore, benzoic acid is capable of influencing the gastrointestinal microflora in the lower part of the gut and thus improving NDF digestibility. This opens the possibility of a more widespread and more frequent use of fibre rich and cheap feedstuffs. Beside the positive effects of fibre and organic acids the increase of butyric acid in caecum and colon may also help to maintain gut health.

It is unclear whether the difference in fibre content among LF and HF diets was high enough to enable a real influence of fibre. For this reason, further studies with a more pronounced difference in fibre content and diets with only one additional fibre source are needed to confirm the beneficial effect of benzoic acid in high fibre diets for growing and finishing pigs. It should also be examined whether there is an upper limit of fibre content which can still be influenced by organic acids. Research is also needed to discover the mechanisms behind the improvement of digestibility values in fibre rich diets and the increase of butyric acid through organic acids.

Concluding Discussion

Ever since the ban on AGP organic acids have been of increasing importance in animal nutrition. Many years of research on the effects of these additives have shown positive effects on performance and nutrient digestibility. However, the effects vary considerably with the age of the animal, the acid in question and the dietary composition. All organic acids have in common that they are most successful in young animals (weaned piglets). For this reason only a few studies have been performed with growing-finishing pigs. Of similar scarcity are studies on the ecological aspects of organic acids, despite increasing concerns about environmental pollution caused by animal production. With this in mind the studies presented here were conducted to broaden the knowledge on the efficacy and ecological aspect of benzoic acid in different practical diets for growing-finishing pigs.

As a general observation it can be stated that benzoic acid tended to positively enhance performance and digestibility in all diets with emphasis on the grower period. The extent of the influence could be ranked in the order fibre study > nitrogen study > phytase study. However, the fibre study was the only one where these positive effects could be statistically confirmed. Furthermore, the interaction of benzoic acid and phytase occurring in the phytase study reduced the effects of benzoic acid but the addition of phytase was nevertheless necessary for good bone stability. These aspects have to be taken into consideration when the main topics of these studies are discussed more profoundly below.

Performance and digestion

As mentioned above the addition of benzoic acid led to a significant improvement of ADG in the fibre study. Despite this, the overall increase in the three studies of + 5.5 % for ADG and – 3.1 % for FCR is impressive, especially under good experimental conditions. It is to be expected that in practical animal production the effects could even be greater. Despite of all the positive signs, the results of the phytase study showed that the use of benzoic acid in low-P diets supplemented with phytase needs close surveillance. In the digestibility experiment of the phytase study several adverse interactions with benzoic acid occurred. Similarly, the ADG of the phytase diet supplemented with benzoic acid (PhyB) in the grower period of this study was an exception. It was slightly reduced and not increased by the addition of benzoic acid although the performance data were not statistically significant.

The reasons for the performance enhancing effects of benzoic acid are manifold. Higher feed intake or higher palatability as described in other studies (Cole et al. 1968; Bolduan et al. 1988a; Bolduan et al. 1988b) can be excluded in the experimental series described here as

feeding was restricted and not *ad libitum*. An improvement in feed quality caused by the addition of organic acids, which could have reduced the possible presence of moulds, is to be considered here, however.

Another explanation is the close relationship between digestion and performance. Optimal performance is only possible when enough nutrients are absorbed, i.e. the digestion process is not disturbed. This can be achieved with different approaches. The antimicrobial effect of organic acids in general can reduce the microbial population in the gut and thus competition for nutrients (Richards et al. 2005). The fewer organisms are scavenging on the diet for the pig, the more nutrients are available for the pig itself. Despite this host–microorganism competition the gut flora is important for a correct development and a well regulated immune system and gut mucosa (Tlaskalova-Hogenova et al. 2004; Richards et al. 2005). Another effect could be that the addition of benzoic acid favours a beneficial composition of the microorganisms in the intestines. This so called 'Eubiosis' helps to maintain good health, which is of great importance for the animal to reach its genetic potential. It can also help to make better use of otherwise quite unsuitable diets such as those rich in fibres. In the presented studies no microbial examinations were performed. Thus either a reduced microbial population or a more beneficial composition of the microbial community or both could have caused the observed effects on performance and digestibility. Another possibility is that benzoic acid changes the secretion of digestive juices or the turnover of epithelial cells in the gut. Changes in bioavailability of nutrients, especially minerals, are conceivable as wekk. But so far such effects have not been described for benzoic acid.

However, when discussing the effects of dietary composition on digestion it is important to remember that the apparent digestibility also includes endogenous losses. Because these losses appear in the faeces as 'undigested' nutrients, the apparent digestibility is usually lower than the true digestibility. The effects of benzoic acid on apparent digestibility can therefore have two reasons which may again be rooted in the microbial changes:

- the true digestibility of the nutrient has been changed
- there was a change in endogenous losses.

The difference between apparent and true digestibility especially plays a role for proteins and minerals where significant endogenous losses occur. The picture is different for fibres. Animals cannot produce dietary fibres and thus apparent digestibility equals true digestibility. Therefore, it can be said that benzoic acid had a genuine effect on d(NDF) in the fibre study. On the other hand the increase in d(N) in the protein study does not necessarily mean that

more protein was available for the pig. In the context of the influences of benzoic acid on d(P) and d(Ca) the interpretation is even more difficult. First, these parameters were only determined in the phytase study. Because of the inconsistency of the results between experiment A and B, the findings cannot be transferred without limitation to the other studies. Secondly, the reason for the interaction with phytase on d(Ca) remains unknown. This also makes it impossible to single out possible acid related effects. For this reason no final answer on the general effects of benzoic acid on mineral digestibility can be given with the experimental setting applied here.

Animal health

Another factor influencing performance and digestibility is health. The aforementioned effects on the gut flora are assumed to be the main reason that organic acids in general are most efficient in young animals. Especially around weaning the gastrointestinal ecosystem is prone to dramatic changes when the animal is forced to switch from the rather simple milk diet to a solid and complex diet, often without having been prepared for it in advance (van Beers-Schreurs et al. 1998; Geary et al. 1999; Franklin et al. 2002; Castillo et al. 2007). Supporting the 'good' inhabitants of the gut and providing a suitable environment for them, organic acids show their most impressing effects in weaners. At this age the use of organic acids can also markedly reduce the incidence of diarrhoea and mortality of piglets (Maribo et al. 2000; Tsiloyiannis et al. 2001a).

Growing-finishing pigs are more resistant to diarrhoea but transportation, mixing of animals from different origin and the change of the diet can cause an outburst of this disease. In the studies presented here, the health of the animals was not monitored specifically. Of the total of 104 pigs involved in the three studies, 13 animals (12.5 %) had to be treated against diarrhoea. There were no diet related deaths in any of the three studies. However, the distribution of diseases among diets and the three studies was quite unequal. Only one animal (diet LPr+) in the nitrogen study and two (diets CC and PhyB) in the phytase study needed medical treatment, but 9 (2 each fed on diets LF-, LF+ and HF+ and three fed on diet HF-) in the fibre study. This increase may be due to the fact that the animals in the fibre study were younger (around 15 kg BW) when the experiment started than those in the other studies (around 25 kg BW). In this case the question arises whether a continuous addition of benzoic acid from weaning until slaughter shows different effects compared to an addition only in certain stages such as weaning or fattening.

Comparable to good gut health, a good skeleton increases the chances for good performance. The data on bone parameters give a strong indication that phytase is very important in low-P diets supplemented with benzoic acid. Leg weakness and similar bone related diseases can be painful and are therefore of concern for animal welfare. Possible signs are problems in walking and standing. This can be the reason for pressure marks, followed by severe infections, reduced growth because the animal cannot reach the feeder anymore and does not or does only slowly reach slaughter weight.

Apart from the animal wellbeing, better health and thus increased performance is also of significant economic relevance. The most expensive part in pig production are the feed costs. Thus reducing the time as well as the amount of feed needed to gain slaughter weight are efficient ways to reduce production costs. Economic calculations were not part of this experimental setting and the relation between reduced costs through improved performance and feed conversion ratio and the costs of adding benzoic acid to the diet remains open.

Ecological aspects

After the registration of benzoic acid as feed additive for swine and due to its unique capacity to reduce urinary pH, studies on the effects of this acid on NH_3 production and emission were performed. However, there are no studies describing the influence of benzoic acid on nutrient excretion and thus the environmental load of pig production. In the studies presented here the effects of benzoic acid on environmental aspects were only indirectly deduced from the data on urinary pH and nutrient digestibility. As a consequence the results have to be treated as indications that can give some hints but no definite or even quantitative answers.

The main benefit of benzoic acid on the environment is its capacity to clearly reduce urinary pH, which may lead to reduced NH_3 emissions (Canh et al. 1998a; Mroz et al. 2000). Urinary pH was measured neither in the phytase study nor in the fibre study. But as the metabolic pathway of benzoic acid remains the same independent of the diet used, it is highly probable that urinary pH was reduced in the other studies as well. How much the NH_3 emission finally is reduced depends on several factors such as the dosage of dietary benzoic acid. Hansen et al. (2007) found in their study a reduction in the ammonia concentration of 10 % when 1 % of benzoic acid was added and a reduction of 55 % with 3 % in the diet. However, the latter dosage is not practicable for several reasons. On the one hand benzoic acid is only registered for the maximal addition of 1 %. On the other hand the addition of 2 % benzoic acid was described to reduce performance (van der Peet-Schwering et al. 1999b).

Finally, the costs for adding more than 1 % of benzoic acid to pig diets would be too expensive for practical use. Even though the dosages used in the three experiments were lower, it is quite possible that the addition of 0.5 % benzoic acid slightly reduced NH_3 emission. However, even a slight decrease in NH_3 emissions from stables and slurry can markedly reduce the impacts of this noxious gas on the environment and on health.

As mentioned above there were merely hints to improved N digestibility. At least in the nitrogen study no signs for reduced N excretion could be found. In the other studies the focus was on apparent faecal digestibility and not on overall metabolism. Therefore, it remains unclear whether the better N digestibility in the phytase and the fibre study was followed by lower total N excretion.

That a low-P diet and the addition of phytase reduce P excretion is supported by many studies. The results on the effects of benzoic acid on P digestiblity are less clear. As it was shown in the phytase study, benzoic acid reduced P digestibility in the grower period but increased it in the finisher period. The implication of this finding on the total P excretion has yet to be investigated. This notwithstanding, it can be assumed that the influence of benzoic acid on the excretion of P does not neutralise the effects of a reduced total P content in the diet.

Benzoic acid improved the digestion of fibre rich diets, a result that is supported by Partanen et al. (2001; 2007) with the use of formic acid or a blend of organic acids in fibre rich diets. These findings can help to further increase the use of such diets in pig production. Firstly, fibre rich diets reduce the competition with human nutrition as the fibrous ingredients are – unlike, for example, wheat – rarely used for human nutrition. Secondly, fibre rich diets shift the N excretion from urine to faeces. Faecal-N is more inert to chemical decomposition (Jongbloed and Lenis 1992) but at the same time better available for plants. Because of this, fibre rich diets reduce gaseous N losses and slow down N leakage from soil thus supporting an environmentally friendly pig production.

In general it can be said that benzoic acid has a great potential to reduce NH_3 losses at least in stables. On the other hand it is quite difficult to deduce within a given nutrient content from improved nutrient digestibility to reduced nutrient excretion. For example it is possible that the excretion of some nutrients shifted from faeces to urine and vice versa thus changing the measured digestibility but not the total excretion. Nevertheless, the results of these studies allow the conclusion that benzoic acid neither positively nor negatively affected ecological aspects of pig production. From the environmental point of view it can thus be concluded that the addition of benzoic acid only has a marginal effect on the environmental aspects of pig

production. It seems that the established dietary measures such as reduced N and P content, the use of phytase and fibre rich diets remain the most efficient ways to protect the environment from undesired side effects of animal production. However, it is possible that their effects can be supported by certain additives such as benzoic acid.

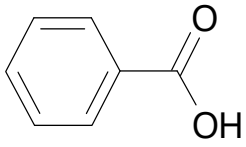
Outlook

The results presented here form a good basis to describe the effects of benzoic acid in the nutrition of fattening pigs. The data could confirm that benzoic acid is beneficial for performance and nutrient digestibility in the weight range of 25 – 108 kg BW in different diets. The data also showed that there is no objection to the further use of benzoic acid in pig nutrition. At the same time several questions remain open. It is essential to explore the exact mode of action as well as the nature of the interaction observed and the impact on the gastrointestinal microflora. To confirm the results described here it is also necessary to conduct additional studies with a higher number of animals. Concerning the ecological aspects of benzoic acid it is necessary to perform further research on its effects on N losses from slurry as well as on NH_3 emission and on the effects on total nutrient excretion. The more is known about the underlying principles of benzoic acid as feed additive, the better this acid can be used as a potent and reliable alternative to antimicrobial growth promoters.

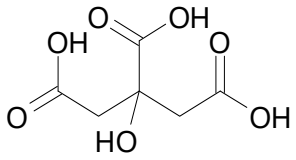
Appendix

Appendix I: Chemical properties of different organic acids

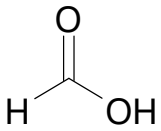
- Benzoic acid: molecular weight: 122.12 g/mol; density: 1.32 g/cm^3; pK_a: 4.21;
  solubility in water: sparingly soluble



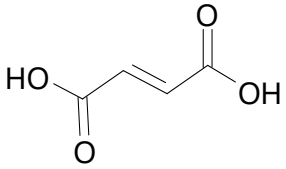
- Citric acid: molecular weight: 192.14 g/mol; density: 1.66 g/cm^3; pK_a: 3.13, 4.76 and 6.4;
  solubility in water: very soluble



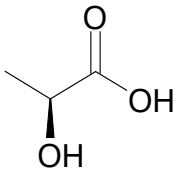
- Formic acid: molecular weight: 46.03 g/mol; density: 1.22 g/cm^3; pK_a: 3.75;
  solubility in water: ∞



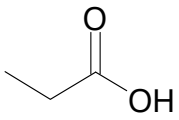
- Fumaric acid: molecular weight: 116.07 g/mol; density: 1.64 g/cm^3; pK_a: 3.02 and 4.38;
  solubility in water: sparingly soluble



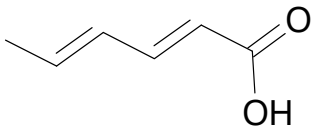
- Lactic acid: molecular weight: 90.08 g/mol; density: 1.21 g/cm^3; pK_a: 3.83;
  solubility in water: very soluble



- Propionic acid: molecular weight: 74.08 g/mol; density: 0.99 g/cm^3; pK_a: 4.88; solubility in water: ∞



- Sorbic acid: molecular weight: 112.14 g/mol; density: 1.20 g/cm^3; pK_a: 4.76; solubility in water: sparingly soluble



Appendix II: Organic acids in animal nutrition – A literature review

The tables below give a review of the research on organic acids in feeds for monogastric animals with focus on performance, nutrient digestibility and selected gastrointestinal parameters. Given is the relative change compared to the control diet(s), an asterisk indicating significant ($p < 0.05$) differences to the control.

- More than one author in a row: several experiments with the same animals and the same experimental setting.
- Two or more dosages: one line per dosage, applies for each parameter.
- 2 x 2 factorial or similar design: numbers in a row indicate the changes to the corresponding control diet. In this case, a short description of the diets is given below the author's name.
- FCR: negative numbers mean a better feed/ weight gain ratio.
- Data show means of the whole experimental period. No distinction between different weaning and fattening stages.
- Unless otherwise stated:
  - the free acid was used
  - bacterial counts were pooled for the whole gastrointestinal tract
  - values for essential and nonessential amino acids were each pooled
- Abbreviations used in Appendix II:

AA	Amino acid
ADF	Acid detergent fibre
ADFI	Average daily feed intake
d(xy)	Apparent digestibility of nutrient xy
dI(xy)	Apparent ileal digestibility of nutrient xy
DWG	Daily weight gain
FCR	Feed conversion ratio
LAB	Lactic acid bacteria
NDF	Neutral detergent fibre
SI	Small intestine
VFA	Volatile fatty acid(s)

- The studies on benzoic acid of authors written **in bold** were supported by DSM, Nutritional Products Ltd., Basel, Switzerland.

Benzoic acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1999	**Van der Peet-Schwering et al. (b)**	Pig	24.3 – 108.9 kg	1 % 2 %	DWG	+ 5* + 0
					ADFI	+ 1* + 0
					FCR	- 3* + 0
					Urinary pH	- 14* - 26*
					Slurry pH	- 5* - 11*
2000	Mroz et al.	Pig	30 – 100 kg	2.4 % Ca-benzoate	DWG	+ 3
					ADFI	+ 0
					dI(dry matter)	+ 1
					dI(organic matter)	+ 1
					dI(ash)	- 12
					dI(protein)	+ 5
					dI(essential AA)	+ 2
					dI(nonessent. AA)	+ 7
					d(dry matter)	+ 1*
					d(organic matter)	+ 1
					d(ash)	+ 15*
					d(energy)	+ 1*
					d(protein)	+ 0
					d(P)	+ 1
					d(Ca)	+ 25*
					d(Mg)	+ 0
					Urinary pH	- 21*
2000	Maribo et al.	Pig	7.5 – 27 kg	2 % for two weeks then 1 %	DWG	+ 16
					ADFI	+ 11
					FCR	- 4
					LAB	- 18*
					Lactobacillus	- 16
					Coliform bacteria	- 10
					Diarrhoea	- 49*
2002	Partanen et al. (b) → weaned at 28 d or 38 d (only overall mean is shown)	Pig	10.5 – 19.2 kg	0.8 % Na-benzoate	Total weight gain	+ 7
					ADFI	+ 8
					FCR	+ 6
					Diarrhoea index	+ 49
2004	Dierick et al.	Pig	8.7 kg, duration: 11 d	1 %	DWG	+ 27
					FCR	- 5
					Bifidobacteria	- 5
					Lactobacilli	- 2
					E. coli	+ 8

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2006	**Kluge et al.**	Pig	7.5 kg – 20.4 kg (performance)	0.5 %	DWG	+ 11
				1 %		+ 14*
					ADFI	+ 8
						+ 10
					FCR	- 2
						- 4
			8.9 kg – 18 kg (balance study)	0.5 %	d(organic matter)	+ 2
				1 %		+ 1
					d(fibre)	+ 22
						+ 9
					d(fat)	+ 3
						+ 1
					d(protein)	+ 2
						+ 1
					Total aerobic bacteria	- 11
						- 18
					Total anaerobic bacteria	- 5
						- 16
					Urinary pH	- 8
						- 14*
2006	**Plitzner et al.**	Pig	32 – 112 kg	0.5 %	DWG	+ 3
				1 %		+ 1
					ADFI	+ 1
						+ 2
					FCR	- 19
						- 17
					Urinary pH	- 4*
						- 9*
2007	**Guggenbuhl et al. (b)**	Pig	7.4 kg, duration: 32 d (performance, GI-parameters)	0.5 %	DWG	+ 13*
					FCR	- 6*
					LAB	- 26
					E. coli	- 92*
			Age of 60 d, Duration: 34 d (dI)	0.5 %	dI(energy)	+ 4*
					dI(nitrogen)	+ 5*
					dI(AA)	+ 3
					dI(essential AA)	+ 3
2007	Hansen et al.	Pig	27 – 100 kg	1 %	NH_3 ppm	- 10
					NH_3-N emission /h/pig	+ 0
					Odour concentr.	- 5
			83 – 100 kg	3 %	NH_3 ppm	- 55*
					NH_3-N emission /h/pig	- 57
					Odour concentr.	+ 13

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2007	**Torrallardona et al.**	Pig	8.9 – 19.6 kg	0.5 %	DWG	+ 16*
					ADFI	+ 10*
					FCR	- 6*
					Lactobacilli	- 3
					E. coli	- 14
					Ileal microbiota similarity	+ 4
					Caecal microbiota similarity	+ 5*
					Urinary hippuric acid concentration	+ 63*
2007	Overland et al.	Pig	28 – 113 kg	0.85 %	Lactic acid bacteria	- 24
2008	Overland et al.				Coliforms	- 21*

Citric acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1984	Falkowski and Aherne	Pig	8.7 kg, duration: 28 d	1 %	DWG	+ 4
				2 %		+ 7
					ADFI	- 1
						- 4
					FCR	- 5*
						- 10*
					d(dry matter)	- 1
						+ 0
					d(protein)	+ 0
						+ 1
1985	Edmonds et al. → All diets in experiment 2 with AGP	Pig	8.7 kg, duration: 21 d	0.75 %	DWG	- 6
					FCR	- 14
			8.6 kg, duration: 21 d	0.75 %	DWG	- 9
				1.5 %		+ 31
					FCR	- 5
						- 13
			9.6 kg, duration: 21 d	1.5 %	DWG	- 8
					FCR	- 5
1985	Giesting and Easter	Pig	7.5 kg, duration: 28 d	2 %	DWG	+ 3
					ADFI	- 4
					FCR	- 7
1985	Henry et al.	Pig	Start with 3.01 kg	3 %	DWG	+ 14*
					ADFI	+ 20
					FCR	- 1
					Free dietary choice	- 54*
1987	Broz and Schulze	Pig	6.9 kg, duration: 28 d	0.5 %	DWG	- 4
				1 %		- 2
				2 %		- 2
					ADFI	- 9
						- 7
						- 6
					FCR	- 5*
						- 5*
						- 5*
					d(organic matter)	+ 1*
						+ 2*
						+ 1*
					d(energy)	+ 1*
						+ 2*
						+ 1
					d(crude protein)	+ 1
						+ 2
						- 1
			7.8 kg, duration: 28 d	0.5 %	DWG	+ 12*
				1 %		+ 3
					ADFI	+ 10

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
						- 1
					FCR	- 3
						- 4
1988	Burnell et al. → in experiment 1 corn-soybean meal (CS) diet and CS with wheat. In experiment 2, 0 and 250 ppm Cu. All diets with AGP.	Pig	7.4 – 16.3 kg	1 %, 2:1 citric acid :Na-citrate	DWG	+ 9, + 4
					ADFI	+ 3, - 1
					FCR	- 6, - 6
			7.4 – 17.6 kg	1 %	DWG	+ 0, + 6
					ADFI	- 2, + 6
					FCR	- 3, - 1
1988	Radecki et al.	Pig	7.1 – 15.3 kg	1.5 %	DWG	- 8
				3 %		- 1
					ADFI	- 5
						- 6
					FCR	+ 3
						- 5
1991	Risley et al. → in experiment 2 with a microbial culture added to the diet	Pig	6.3 kg, duration: 35 d	1.5 %	DWG	+ 4
					ADFI	- 3
					FCR	- 6*
			7.0 kg, duration: 35 d		DWG	- 1
					ADFI	- 3
					FCR	- 4
1993	Höhler and Pallauf → diets with 30 and 70 mg Zn/kg	Pig	9.5 – 18.3 kg	1.5 %	DWG	+ 44, + 8
					ADFI	+ 20, + 4
					FCR	- 6, - 4
					d(P)	+ 14, + 16
					d(Ca)	+ 20, + 13
					d(Mg)	+ 6, + 15
					d(Fe)	+ 61, + 57
					d(Zn)	+ 14, + 48
					d(Cu)	- 9, - 1
1993	Risley et al. → non-challenged and challenged with *E. coli*	Pig	5.5 – 7.8 kg	1.5 %	DWG	- 11, - 6
					ADFI	- 5, - 5
					FCR	- 8, - 1
1994	Höhler and Pallauf → diets with 40 and 60 mg Zn/kg	Pig	11.1 – 24.0 kg	1.5 %	DWG	+ 6, + 2
					FCR	- 1, - 2
					d(P)	+ 9, + 5
					d(Ca)	+ 1, + 10
					d(Mg)	+ 31, + 10
					d(Fe)	+ 12, + 8
					d(Zn)	+ 31, + 5
					d(Cu)	+ 25, + 19

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1994	Krause et al. → diets with or without 1.4 % $NaHCO_3$	Pig	7.0 kg, duration: 28 d	2.5 %	DWG	+ 5, + 3
					ADFI	+ 7, - 4
					FCR	+ 0, - 9
		Broiler	Start with 1 week	2.5 %	DWG	- 3, + 3
					ADFI	- 6, + 1
					FCR	- 3, - 4
1998	Han et al.	Pig	10.7 kg, duration: 42 d	1.5 %	DWG	+ 19
					ADFI	+ 12
					FCR	- 9
1998	Li et al. → phytase diet and phytase + Vit D diet	Pig	Start with 28 d	1.5 %	d(dry matter)	+ 3, + 0
					d(nitrogen)	+ 1, + 0
					d(P)	+ 3, + 3
					d(Ca)	+ 1, + 2
1998	Radcliffe et al	Pig	7.4 kg, duration: 28 d	1.5 %	DWG	+ 7*
				3 %		+ 9*
					FCR	- 5*
						- 10*
					d(dry matter)	+ 0
						+ 0
					d(P)	+ 11
						+ 9
					d(Ca)	+ 8*
						+ 7*
					Stomach pH	- 9*
						- 13*
					Rib shear force	+ 8
						+ 4
					Bone ash	+ 0
						+ 7
			9.6 kg, duration: 28 d	2.0 %	DWG	- 1
					FCR	+ 1
					d(dry matter)	- 1
					d(P)	+ 13
					d(Ca)	- 3
					Stomach pH	- 8*
					Rib shear force	+ 11
					Bone ash	+ 2
2000	Boling et al.	Broiler	8 – 22 d	1 %	DWG	+ 0
				2 %		+ 8*
				4 %		+ 14*
				6 %		+ 22*
					FCR	- 1
						- 1
						- 2
						+ 1
					Tibia ash	+ 4
						+ 14*
						+ 32*
						+ 43*

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
		Pig	Start with 11 kg,	1 %	DWG	+ 8
			22 d experiment	2 %		+ 16*
				3 %		+ 10
					FCR	- 11
						- 15*
						- 18*
					Fibula ash	+ 0
						- 4
						- 5
				6 %	DWG	+ 0
					FCR	- 9*
					Metatarsal ash	+ 7*
2001	Tsiloyiannis et al. (a)	Pig	6.7 – 13.6 kg	1.5 %	DWG	+ 11*
					ADFI	+ 5*
					FCR	- 5*
					Diarrhoea	- 38*
					Mortality rate	- 50*
2001	Tsiloyiannis et al. (b)	Pig	7.2 – 14.6 kg	1.5 %	DWG	+ 13*
					ADFI	+ 6*
					FCR	- 5*
					Mortality rate	- 67*
2004	Snow et al. → in experiment 1 diets with or without Hydroxycolecal-ciferol (HC), in experiments 2 and 3 basal diet and with either phytase, HC or combination of phytase and HC	Broiler	8 – 21 d	4 %	DWG	+ 13*, + 1
					ADFI	+ 9*, + 5
					FCR	- 3, + 4
					Tibia ash	+ 21*, + 10*
			8 – 21 d	3 %	DWG	+ 28*, + 11, + 11*, + 4
					ADFI	+ 13, + 8, + 5, + 5
					FCR	- 12*, - 3, - 5, + 1
					Tibia ash	+ 12*, + 8*, + 6*, + 8*
			8 – 21 d	3 % (less HC)	DWG	+ 25*, + 17*, + 14*, + 6
					ADFI	+ 14*, + 15*, + 10*, + 5
					FCR	- 9*, - 2, - 3, - 4
					Tibia ash	+ 14*, + 14*, + 14*, + 14*,
2005	Rafacz-Livingston et al. → in experiment 1 two nonphytate P levels	Broiler	8 – 22 d	2 %	DWG	+ 5, + 6
					ADFI	+ 5*, - 1
					FCR	+ 1, + 2
					Tibia ash	+ 1, + 0
			8 – 22 d	3 %	DWG	+ 23*
					ADFI	+ 14*
					FCR	- 7*
					Tibia ash	+ 19*

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2008	Liem et al.	Broiler	0 – 16 d	3.23 %	16-d weight	+ 10
					FCR	- 6
					d(P)	+ 34*
					d(Ca)	+ 9
					Bone ash	+ 17*

Formic acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1988	Bolduan et al. (a)	Pig	9 kg, duration: 35 d (21 d for digesta measurements)	0.4 %	DWG	+ 4
				1.3 %		+ 5
				Both as Propandi-ol-formate	ADFI	+ 1
						+ 8
					VFA total (Stomach)	- 40
						- 56
					Stomach pH	- 5*
						+ 0
1988	Bolduan et al. (b)	Pig	9.1 kg, duration: 35 d (21 d for digesta measurements)	0.35 %	DWG	+ 27*
				1.2 %		+ 13
					ADFI	+ 16
						+ 9
					VFA total (Stomach)	- 34
						- 38
					Stomach pH	- 5*
						- 8
1992	Eckel et al.	Pig	6.1 – 22.8 kg	0.6 %	DWG	+ 21*
	Roth et al. (a)			1.2 %		+ 22*
	Gedek et al. (a)			1.8 %		+ 5*
				2.4 %		- 15*
					ADFI	+ 14*
						+ 13*
						+ 4*
						- 12*
					FCR	- 5*
						- 7*
						- 1
						+ 4
					d(dry matter)	+ 1
						+ 1
						+ 1
						+ 1
					d(energy)	+ 1
						+ 0
						+ 1
						+ 2
					d(protein)	+ 2
						+ 2
						+ 3
						+ 4*
					Lactobacilli SI	- 1
						- 11
						- 7
						- 8
					Lactobacilli colon	- 11*
						- 17*
						- 13*

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
						- 13*
					E. coli SI	- 2
						- 3
						+ 24
						+ 6
					E. coli colon	- 11*
						- 18*
						- 18*
						- 15*
					VFA total colon	- 4
						- 3
						- 20*
						- 28*
					Stomach pH	+ 12
						+ 17
						+ 16
						+ 2
					Diarrhoea	- 78
						- 93
						- 93
						- 93
1992	Eidelsburger et al. (a) Eidelsburger et al. (c) Kirchgessner et al.	Pig	6.4 – 24.1 kg	1.8 % Ca-formate	DWG	- 1
					ADFI	- 2
					FCR	- 1
					d(dry matter)	+ 1
					d(energy)	+ 1
					d(protein)	+ 2
					Lactobacilli SI	- 4
					Lactobacilli colon	- 5
					E. coli SI	- 22
					E. coli colon	- 6
					VFA total colon	- 3
					Stomach pH	+ 4
					Small intestine pH	+ 1
			6.4 – 24.1 kg	1.25 %	DWG	+ 7
					ADFI	+ 2
					FCR	- 5*
					d(dry matter)	+ 0
					d(energy)	+ 1
					d(protein)	+ 2
					Lactobacilli SI	- 12
					Lactobacilli colon	- 7
					E. coli SI	- 24
					E. coli colon	- 5
					VFA total colon	+ 5
					Stomach pH	- 15*
					Small intestine pH	+ 0

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1992	Eidelsburger et al. (b) Roth et al. (b) Gedek et al. (b)	Pig	5.8 – 21.9 kg	1.8 % Na-formate	DWG ADFI FCR d(dry matter) d(energy) d(protein) Lactobacilli/Bifido-bacteria E. coli VFA total caecum VFA total colon Stomach pH	+ 3 - 2 - 4* + 1 + 1 + 2 - 7 - 7 - 1 + 12 - 6
1995	Gabert et al. → diets with low and high buffering capacity	Pig	7.8 – 13.8 kg	1 %	dI(dry matter) dI(organic matter) dI(ash) dI(protein) dI(essential AA) dI(non essential AA) d(dry matter) d(organic matter) d(ash) d(energy) d(protein) LAB (Ileum) E. coli (Ileum) total VFA Ileal pH	- 3, - 3 - 3*, - 3* - 13, - 4 - 2, - 5 + 0, - 4 - 1, - 4 - 2, + 2 - 3*, + 2 + 16*, + 12 - 3, + 2 - 2, + 3 - 13, + 3 + 5, + 7 - 12, - 14 + 2, - 1
2000	Jongbloed et al. → diets with and without phytase	Pig	22 – 45 kg	1.6 % and 3.2 % (only overall mean of acid diets were shown)	DWG ADFI FCR d(dry matter) d(organic matter) d(ash) d(P) d(Ca) d(Mg)	+ 10*, + 13* + 3, + 7 - 7, - 6 + 1*, + 2* + 1*, + 1* + 8*, + 12* + 19*, + 24* + 17*, + 11* + 6, + 13
2000	Mroz et al.	Pig	30 – 100 kg	1.38 %	DWG ADFI dI(dry matter) dI(organic matter) dI(ash) dI(protein) dI(essential AA) dI(nonessent. AA) d(dry matter) d(organic matter) d(ash) d(energy) d(protein)	- 10 + 0 + 2 + 2 + 24 + 6 + 5 + 7 + 1 + 1* + 8 + 1 + 2

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
					d(P)	+ 18*
					d(Ca)	+ 11
					d(Mg)	- 13
					Urinary pH	- 4
2000	Overland et al.	Pig	23.1 – 104.5 kg	0.85 % Ca/Na-Formate	DWG	- 1
					ADFI	+ 1
					FCR	+ 1
					Carcass lean	- 2
				0.8 % K-diformate	DWG	+ 3*
					ADFI	+ 2
					FCR	- 1
					Carcass lean	+ 1*
			24.3 – 85.1 kg	0.8 % K-diformate	DWG	+ 12
					ADFI	+ 4
					FCR	- 7
					Odour of meat	+ 2
					Flavour of meat	+ 0
					Tenderness	- 3
			27.1 – 105 kg	0.6 %	DWG	+ 3
				1.2 %		+ 6*
				both as K-diformate	ADFI	+ 2
						+ 3*
					FCR	- 1
						- 3
					Carcass lean	+ 0
						+ 1
2000	Paulicks et al. → in experiment 1 and 2 two diets with different energy levels, in experiment 3 four diets with barley, wheat, corn or mixture of all	Pig	7.3 – 28. 1 kg (single housing)	1.8 % K-diformate	DWG	+ 8, - 1
					ADFI	+ 1, - 3
					FCR	- 6*, - 3
			9.1 – 28.3 kg (group housing)	1.8 % K-diformate	DWG	+ 18*, + 1
					ADFI	+ 8, + 1
					FCR	- 6*, + 1
			8.6 – 30.2 kg	1.8 % K-diformate	DWG	+ 2, + 14*, + 6, + 8
					ADFI	- 4, + 9*, + 6, + 1
					FCR	- 6*, - 4*, + 1, - 7*
2001	Canibe et al. (b)	Pig	28 d, duration: 7d	1.8 % K-diformate	LAB	- 7*
					Coliform bacteria	- 3
			28 d, duration: 29 d	1.8 % K-diformate	DWG	- 2
					ADFI	- 1
					FCR	+ 0
					LAB	- 9*
					Coliform bacteria	- 10

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2001	Partanen et al. → diets with medium and high fibre levels	Pig	39 – 83 kg	0.8 %	dI(organic matter)	+ 0, + 3
					dI(NDF)	+ 17*, + 17*
					dI(protein)	+ 0, + 4
					dI(essential AA)	+ 2, + 3
					dI(nonessent. AA)	+ 1*, + 3*
					dI(P)	+ 6, + 16
					dI(Ca)	+ 5*, + 19*
					d(organic matter)	+ 0, + 2
					d(NDF)	- 1, + 6
					d(protein)	+ 0, + 2
					d(P)	+ 1, + 7
					d(Ca)	+ 0, + 7
2001	Tsiloyiannis et al. (a)	Pig	6.7 – 13.6 kg	1.2 %	DWG	+ 15*
					ADFI	+ 6*
					FCR	- 7*
					Diarrhoea	- 56*
					Mortality rate	- 50*
2002	Partanen et al. (a)	Pig	27 – 107.9 kg	0.8 %	DWG	+ 5
					ADFI	- 4
					FCR	- 5*
					Carcass lean	+ 0
2002	Partanen et al. (b) → weaned at 28 d or 38 d (only overall mean shown)	Pig	10.5 – 19.2 kg	0.8 %	Total weight gain	- 4
					ADFI	+ 10
					FCR	+ 13
					Diarrhoea index	- 63
				0.8 % Ca-formate	Total weight gain	- 30*
					ADFI	- 1
					FCR	+ 58
					Diarrhoea index	+ 102
2004	Ettle et al.	Pig	8.0 – 27.2 kg	2.4 % K-diformate	DWG	+ 5
					ADFI	+ 1
					FCR	- 4
					Free dietary choice	- 5
			8.0 – 27.2 kg	1.2 % K-diformate	Free dietary choice	- 12
			7.3 – 29.6 kg	1.2 %	DWG	+ 9
					ADFI	+ 3
					FCR	- 6*
					Free dietary choice	- 85*
2005	Canibe et al.	Pig	27 – 97 kg 27 – 66 kg (gastrointestinal parameters)	1.8 %	DWG	+ 9
					ADFI	+ 3
					FCR	- 5
					LAB	- 19*
					Enterobacteria	- 21*
					Total VFA	+ 6
2005	Franco et al.	Pig	5.4 kg, 8 d experiment	0.96 %	d(dry matter)	+ 4
					d(protein)	+ 1
					Lactobacilli (Stomach)	- 9

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
					Lactobacilli (SI)	- 8
					Coliforms (Stomach)	+ 20
					Coliforms (SI)	- 8
					Tot VFA (Stomach)	- 44
					Tot VFA (SI)	+ 265
					Stomach pH	- 31
2006	Hernandez et al.	Broiler	1 – 42 d	0.5 %	DWG	+ 1
				1 %		+ 4
					ADFI	- 1
						- 1
					FCR	- 2
						- 4
					dI(dry matter)	+ 0
						+ 5
					dI(protein)	+ 6
						+ 4
					d(dry matter)	- 3
						+ 0
					d(protein)	- 7
						- 2
					Villus length	+ 9*
					(Jejunum)	- 5
					Villus surface area	+ 13
					(Jejunum)	- 20*
					Crypt depth	+ 27*
					(Jejunum)	+ 14*
2006	Kil et al.	Pig	7.4 – 23.1 kg	0.2 %	DWG	- 6
					ADFI	- 5
					FCR	+ 1
			6.2 kg, duration: 25 d	0.2 %	d(dry matter)	+ 0
					d(ash)	- 9
					d(protein)	+ 0
					d(P)	+ 6
					d(Ca)	+ 0
2006	Kluge et al.	Pig	7.5 kg – 20.4 kg	1.2 % K-diformate	DWG	+ 19*
					ADFI	+ 11
					FCR	- 7*
2007	Eisemann and van Heugten → nursery diet with 0 % or with 0.2 % less acid than in the final diet	Pig	21 d – 113 kg	0.6 %	DWG	- 3, + 1
				0.8 %		- 1, + 0
				1 %		+ 2, - 1
				each as formic acid – NH_4-formate	ADFI	- 5, - 3
						- 3, - 5
						- 1, -3
					FCR	- 2, - 4
						- 2, - 5
						- 3, - 2
2007	Overland et al.	Pig	28 – 113 kg	1 %	Lactic acid bacteria	- 21
2008	Overland et al.				Coliforms	- 32*

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2008	Li et al.	Pig	7.8kg, duration: 28 d	0.5 % K-diformate	DWG	+ 10
					ADFI	+ 1
					FCR	- 10
					Lactobacilli (Faeces)	+ 0
					E. coli (Faeces)	- 6

Fumaric acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1978	Kirchgessner and Roth → diets slightly differing in protein and fat content	Pig	35 – 50 kg	1.8 %	d(organic matter) d(ash) d(fibre) d(protein)	+ 2*, + 1 - 3, - 7* + 5, - 8 + 1, + 1
1980	Kirchgessner and Roth	Pig	Start with 7 kg	1 % 2 %	d(dry matter) d(ash) d(fibre) d(protein) d(P) d(Ca) d(Mg) 	+ 3* + 2* - 1 + 4* + 11 + 15 + 2 + 3* + 4 + 10* + 1 + 13* + 0 + 3
1984	Falkowski and Aherne	Pig	8.7 kg, duration: 28 d	1 % 2 %	DWG ADFI FCR d(dry matter) d(protein) 	+ 6 + 5 - 1 - 4 - 6* - 7* + 0 + 0 + 1 + 2
1985	Edmonds et al.	Pig	9.6 kg, duration: 21 d	1.5 %	DWG FCR	+ 6 - 4
1985	Giesting and Easter → in experiment 3 two protein levels	Pig	7.5 kg, duration: 28 d	2 %	DWG ADFI FCR	+ 5 - 2 - 7
			8.6 kg, duration: 28 d	1 % 2 % 3 % 4 %	DWG ADFI FCR 	+ 0 - 2 + 13 + 14 - 3 - 11 - 2 + 0 - 4 - 9 - 13 - 13
			10 kg, duration:	1 %	DWG	- 2, + 2

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
			28 d		ADFI	- 8, - 5
					FCR	- 6, - 6
			47.5 – 82.1 kg	1.5 %	DWG	+ 3
				3 %		+ 4
					ADFI	+ 5
						+ 2
					FCR	+ 3,
						+ 0
1985	Henry et al.	Pig	Start with 3.0 kg	1.5 %	DWG	- 10
					ADFI	- 4
					FCR	+ 12
					Free dietary choice	- 52*
1988	Bolduan et al. (a)	Pig	9 kg, duration: 35 d	0.5 %	DWG	+ 11
				1.5 %		+ 12
					ADFI	+ 9
						+ 9
					VFA total (stomach)	- 45
						- 46
					Stomach pH	+ 2
						- 7
1988	Radecki et al.	Pig	7.1 – 13.7 kg	1.5 %	DWG	+ 0
				3 %		- 12
					ADFI	+ 1
						- 7
					FCR	+ 0
						+ 6
			Start with 8.2 kg	1.5 %	d(protein)	+ 0
1991	Giesting and Easter → diets with soybean meal or dried skim milk	Pig	6.2 – 16.1 kg	2 %	DWG	+ 15, + 0
					dI(dry matter)	+ 1, + 0
					dI(nitrogen)	+ 4, + 0
1991	Risley et al. → in experiment 2 with a microbial culture added to the diet	Pig	6.3 kg, duration: 35 d	1.5 %	DWG	+ 2
					ADFI	- 3
					FCR	- 5
			7.0 kg, duration: 35 d	1.5 %	DWG	+ 4
					ADFI	+ 1
					FCR	- 3
1991	Sutton et al. → in experiment 1 control diet with AGP	Pig	8.3 kg, duration: 28 d	0.3 % Na-fumarate	Lactobacillus	- 3
					E. coli	+ 2
					VFA caecum	+ 6
					VFA colon	+ 3
			9.1 kg, duration: 28 d	1 %	Lactobacillus	- 3
					E. coli	+ 2
					VFA caecum	+ 35*
					VFA colon	+ 3

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1992	Eidelsburger et al. (b) Roth et al. (b) Gedek et al. (b)	Pig	5.8 – 21.9 kg	1.8 %	DWG ADFI FCR d(dry matter) d(energy) d(protein) Lactobacilli and Bifidobacteria E. coli VFA total (Caecum) VFA total (Colon) Stomach pH	+ 1 - 2 - 2 + 0 + 0 + 0 - 21* - 15 + 5 - 6 - 15*
1992	Thacker et al.	Pig	8.1 kg, duration: 35 d	2 %	DWG ADFI FCR d(dry matter) d(energy) d(protein)	+ 8 + 1 - 10 - 2 - 2 - 1
1993	Risley et al. → non-challenged and challenged with *E. coli*	Pig	5.5 – 7.8 kg	1.5 %	DWG ADFI FCR	+ 4, + 2 + 6, + 6 + 0, + 3
1994	Krause et al. → with or without 1.4 % $NaHCO_3$	Pig	7.0 kg, duration: 28 d	2.5 %	DWG ADFI FCR	+ 10, + 13* + 3, + 11 - 7, - 2
			65 – 100 kg	2.5 %	DWG ADFI FCR	+ 2, + 0 - 1, + 3 - 3, + 4
		Broiler	Start with 1 week	2.5 %	DWG ADFI FCR	+ 0, - 8 - 1, - 8 - 1, + 0
1999	Blank et al. → in experiment 2 addition of 3 % $NaHCO_3$	Pig	4.7 – 10.4 kg	1 % 2 % 3 %	dI(dry matter)	+ 3 + 4 + 3
					dI(organic matter)	+ 3 + 3 + 2
					dI(energy)	+ 3 + 4* + 2
					dI(protein)	+ 4 + 10* + 6
					dI(essential AA)	+ 3 + 8* + 5
					dI(nonessent. AA)	+ 3 + 9 + 5

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
					d(dry matter)	+ 0
						+ 0
						- 1
					d(organic matter)	+ 0
						+ 0
						+ 0
					d(energy)	+ 0
						+ 0
						- 1
					d(protein)	+ 2
						+ 0
						+ 1
					d(essential AA)	+ 2
						+ 1
						+ 2
					d(nonessent. AA)	+ 1
						+ 1
						+ 1
			5.6 – 11.6 kg	1 %	dI(dry matter)	+ 7
				2 %		+ 7
				3 %		+ 4
					dI(organic matter)	+ 6
						+ 6
						+ 2
					dI(energy)	+ 8
						+ 10
						+ 6
					dI(protein)	+ 5
						+ 7
						+ 8
					dI(essential AA)	+ 3
						+ 6
						+ 6
					dI(nonessent. AA)	+ 5
						+ 10
						+ 6
					d(dry matter)	+ 1
						+ 0
						+ 0
					d(organic matter)	+ 1
						+ 0
						+ 0
					d(energy)	+ 1
						+ 0
						+ 0
					d(protein)	+ 2
						+ 1
						- 1
					d(essential AA)	+ 2

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
						+ 1
						+ 0
					d(nonessent. AA)	+ 2
						+ 2
						+ 0
1999	Bosi et al.	Pig	4.2 – 11.2 kg	0.5 %	DWG	+ 0
					ADFI	- 1
					FCR	- 2
					d(dry matter)	+ 1
					d(protein)	+ 0
			5.0 – 11.5 kg	0.5 %	DWG	- 4
					ADFI	+ 0
					FCR	+ 4
					dI(dry matter)	+ 2
					dI(protein)	+ 5
2000	Mroz et al.	Pig	30 – 100 kg	1.76 %	DWG	- 5
					ADFI	- 1
					dI(dry matter)	+ 2
					dI(organic matter)	+ 2
					dI(ash)	+ 10
					dI(protein)	+ 5
					dI(essential AA)	+ 4
					dI(nonessent. AA)	+ 4
					d(dry matter)	+ 1
					d(organic matter)	+ 1
					d(ash)	+ 11
					d(energy)	+ 1
					d(protein)	- 1
					d(P)	+ 8*
					d(Ca)	+ 25*
					d(Mg)	- 6
					Urinary pH	- 1*
2001	Tsiloyiannis et al. (a)	Pig	6.7 – 13.6 kg	1 %	DWG	+ 13*
					ADFI	+ 6*
					FCR	- 6*
					Diarrhoea	- 47*
					Mortality rate	- 67*
2005	Rafacz-Livingston et al.	Broiler	8 – 22 d	3 %	DWG	+ 8*
					ADFI	+ 5*
					FCR	- 2
					Tibia ash	+ 3
2006	Kil et al.	Pig	7.4 – 23.1 kg	0.2 %	DWG	- 2
					ADFI	+ 1
					FCR	+ 2
			6.2 kg, duration: 25 d	0.2 %	d(dry matter)	+ 1
					d(ash)	- 1
					d(protein)	+ 1
					d(P)	+ 16
					d(Ca)	+ 7

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2008	Liem et al.	Broiler	0 – 16 d	2.9 %	16-d weight	+ 1
					FCR	+ 6
					d(P)	+ 33*
					d(Ca)	+ 33
					Bone ash	+ 5
2008	Pirgozliev et al.	Broiler	14 – 30 d	0.5 %	DWG	- 6
				1 %		- 34*
				1.5 %		+ 0
					ADFI	- 8
						- 23*
						- 6
					FCR	- 5
						+ 14*
						- 7
					Lactic acid bacteria	+ 0
						- 1
						- 8
					Coliforms	- 24
						- 12
						- 15

Lactic acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1999	Kemme et al. (a, b) → interaction with phytase level only for d(P) and phytic acid degradation	Pig	Start with 37 kg	3 %	dI(nitrogen) dI(AA) d(ash) d(P) d(Ca) d(Mg) Degradation of phytic acid (Ileum)	+ 1 + 2 + 9 + 11*, + 26* + 19 + 22 + 99, + 16
2000	Jongbloed et al. → diets with and without phytase	Pig	22 – 45 kg	1.6 % 3.2 % (only overall mean of acid diets were shown)	DWG ADFI FCR d(dry matter) d(organic matter) d(ash) d(P) d(Ca) d(Mg)	+ 10*, + 8* + 5, + 3 - 5, - 6 + 1*, + 1* + 1*, + 0 + 8*, + 8* + 19*, + 10* + 14*, + 6* + 11, - 2*
2001	Tsiloyiannis et al. (a)	Pig	6.7 – 13.6 kg	1.6 %	DWG ADFI FCR Diarrhoea Mortality rate	+ 22* + 9* - 10* - 66* - 67 *
2001	Tsiloyiannis et al. (b)	Pig	7.2 – 14.6 kg	1.6 %	DWG ADFI FCR Mortality rate	+ 14* + 7* - 6* - 60*
2002	Partanen et al. (b) → weaned at 28 d or 38 d (only overall mean shown)	Pig	10.5 – 19.2 kg	0.8 %	Total weight gain ADFI FCR Diarrhoea index	- 11 + 17 + 48 + 10
2005	Pierce et al. → two inulin levels in experiment 2 (not all parameters with interaction)	Pig	7.1 kg, duration: 28 d 8.1 kg, duration: 28 d	1.6 % 1.6 %	DWG ADFI FCR DWG ADFI FCR Lactobacilli/E. coli ratio Total VFA Villus hight Crypt depth Villus/crypt ratio	+ 2 + 4 + 2 - 49, + 72 - 21, - 11 - 32, - 40 - 3, - 8 + 3 + 3, + 5 - 9, + 5 + 8, + 0

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
2006	Kil et al.	Pig	7.4 – 23.1 kg	0.2 %	DWG	+ 6
					ADFI	+ 8
					FCR	+ 2
			6.2 kg, duration:	0.2 %	d(dry matter)	+ 1
			25 d		d(ash)	- 1
					d(protein)	+ 2
					d(P)	+ 15
					d(Ca)	+ 4

Propionic acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1985	Giesting and Easter	Pig	7.5 kg, duration: 28 d	2 %	DWG	- 4
					ADFI	- 11
					FCR	- 7
1988	Bolduan et al. (b)	Pig	9.1 kg, duration: 35 d (21 d for digesta measurements)	0.3 %	DWG	- 11
				1 %		+ 14
					ADFI	- 10
						+ 7
					VFA total (Stomach)	+ 20
						+ 20
					Stomach pH	+ 0
						- 3
1991	Sutton et al.	Pig	8.3 kg, duration: 28 d	0.25 %	Lactobacillus	+ 0
				0.3 % Na-propionate		+ 2
					E. coli	- 3
						- 1
					VFA caecum	+ 1
						- 10
					VFA colon	- 14
						+ 4
1992	Thacker et al.	Pig	25.1 – 98 kg	2.5 %	DWG	+ 4
					ADFI	- 4
					FCR	- 6
					d(dry matter)	+ 4*
					d(energy)	+ 3
					d(protein)	- 5
					Carcass lean	- 1
1992	Mosenthin et al. → diets with and without siliceous earth	Pig	Start with 50 kg	2 %	dI(dry matter)	+ 0, + 9
					dI(organic matter)	+ 0, + 8
					dI(energy)	+ 1, + 8
					dI(protein)	+ 4, + 7
					dI(essential AA)	+ 7, + 7
					dI(nonessent. AA)	+ 7, + 7
					d(dry matter)	+ 2, + 2
					d(organic matter)	+ 2, + 1
					d(ash)	+ 4, + 9
					d(energy)	+ 2, + 2
					d(protein)	+ 3, - 5
					d(essential AA)	+ 4, + 0
					d(nonessent. AA)	+ 2, + 1
2001	Tsiloyiannis et al. (a)	Pig	6.7 – 13.6 kg	1 %	DWG	+ 8*
					ADFI	+ 3*
					FCR	- 5*
					Diarrhoea	- 22*
					Mortality rate	- 50*

Sorbic acid

Year	Author	Species	Age/weight	Dosage	Difference to control (%)	
1995	Kirchgessner et al.	Pig	7.2 – 26.1 kg	1.2 %	DWG	+ 14*
				1.8 %		+ 21*
				2.4 %		+ 27*
					ADFI	+ 9
						+ 15*
						+ 19*
					FCR	- 4*
						- 6*
						- 6*
2004	Ettle et al.	Pig	7.3 – 29.6 kg	1.2 %	DWG	+ 6
					ADFI	+ 1
					FCR	- 4
					Free dietary choice	- 68*
2007	Overland et al.	Pig	28 – 113 kg	0.85 %	DWG	+ 2
2008	Overland et al.				ADFI	- 2
					FCR	- 4
					Lactic acid bacteria	- 27*
					Coliforms	- 17*
					Indole (Colon)	- 9
					Scatole (Colon)	- 27
2008	Pirgozliev et al.	Broiler	14 – 30 d	0.5 %	DWG	- 6
				1 %		- 17
				1.5 %		- 9
					ADFI	- 6
						- 14
						- 8
					FCR	- 2
						+ 2
						+ 1
					Lactic acid bacteria	- 5
						- 8
						- 9
					Coliforms	- 1
						- 28
						- 19

Other organic acids and blends

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
1993	Kirchgessner et al.	Pig	6.6 – 26.6 kg	Tartaric acid	1.2 %	DWG	- 7
					1.8 %		- 6
					2.4 %		- 11
						ADFI	- 10
							- 8
							- 13*
						FCR	- 3
							- 2
							- 3
			6.5 – 25.7 kg	Malic acid	1.2 %	DWG	+ 4
					1.8 %		+ 1
					2.4 %		+ 2
						ADFI	+ 1
							- 2
							- 2
						FCR	- 4*
							- 4*
							- 5*
1994	Krause et al.	Pig	7.0 kg, duration:	Malic acid	2.5 %	DWG	+ 3, - 10
	→ with or without 1.4 % $NaHCO_3$		28 d			ADFI	- 13, - 16*
						FCR	- 20, - 8
		Broiler	Start with 1 week			DWG	- 4, + 3
						ADFI	- 6, + 3
						FCR	- 3, + 0
1999	Bosi et al.	Pig	4.2 – 11.2 kg	Blend of ortho-phosphoric, fumaric, citric and malic acid, triglycerides, free fatty acids	0.5 %	DWG	+ 7
						ADFI	+ 8*
						FCR	+ 1
						d(dry matter)	+ 1
						d(protein)	+ 1*

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
			5.0 – 11.5 kg	Blend of ortho-phosphoric, fumaric, citric and malic acid, triglycerides, free fatty acids	0.5 %	DWG	- 4
						ADFI	+ 6
						FCR	+ 10
						dI(dry matter)	+ 7
						dI(protein)	+ 7
1999	Li et al. → diets with and without enzyme mixture	Pig	10.4 kg, duration: 28 d	Blend of lactic, fumaric, propionic, formic and ortho-phosphoric acid	0.5 %	DWG	+ 5, - 3
						ADFI	+ 1, + 0
						FCR	- 3, + 3
						Lactobacillus	+ 9, + 8
						E. coli	- 9, - 11
						Trypsin activity	- 13, + 9
						Amylase activity	- 34, -34
2000	Mroz et al.	Pig	30 – 100 kg	n-butyric acid	2.67 %	DWG	+ 5
						ADFI	- 2
						dI(dry matter)	+ 2
						dI(organic matter)	+ 1
						dI(ash)	+ 20
						dI(protein)	+ 8*
						dI(essential AA)	+ 5*
						dI(nonessent. AA)	+ 8*
						d(dry matter)	+ 2*
						d(organic matter)	+ 2*
						d(ash)	+ 16*
						d(energy)	+ 2*
						d(protein)	+ 2
						d(P)	+ 10*
						d(Ca)	+ 23*
						d(Mg)	+ 2
						Urinary pH	+ 1*

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
2001	Min et al. → all diets with AGP	Pig	22.3 – 115.4 kg	Organic acid complex of unknown composition	0.5 %	DWG ADFI FCR d(dry matter) d(ash) d(energy) d(protein) d(P) d(Ca)	+ 2 + 0 - 1 + 1 - 5 - 2 + 1 + 4 + 0
2001	Tsiloyiannis et al. (a)	Pig	6.7 – 13.6 kg	Malic acid	1.2 %	DWG ADFI FCR Diarrhoea Mortality rate	+ 10* + 4* - 5* - 38* - 50*
2002	Partanen et al. (b)	Pig	27 – 107.9 kg	Formic acid-sorbate blend	0.8 %	DWG ADFI FCR Carcass lean	+ 9* - 7 - 9* + 0
2002	Valencia and Chavez	Pig	Start with 6.54 kg	Acetic acid	1 %	DWG ADFI FCR d(dry matter) d(protein) d(P) d(Ca) d(Fe)	+ 8 + 5 - 5 + 2* + 4* + 21* + 5 + 50*

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
2003	Omogbenigun et al. → diet with 500 U/kg microbial phytase	Pig	6.4 kg, duration: 28 d	Blend of citric, malic, phosphoric, sorbic, tartaric and lacit acid and aluminate	0.35 %	DWG	+ 3
						ADFI	+ 0
						FCR	+ 1
						dI(protein)	+ 2
						dI(essential AA)	+ 4
						dI(nonessent. AA)	+ 4
						d(dry matter)	+ 0
						d(P)	+ 11
						Stomach pH	+ 0
						Phytate hydrolysis	+ 9
						Mobility score	+ 2
						Bone ash	+ 2
2004	Namkung et al. → FCR values of the first experimental week not included	Pig	4.9 kg, duration: 28 d (14 d for gut morphology)	Blend of acetic, propionic, phosphoric and citric acid	1.1 %	DWG	- 4
						ADFI	- 6
						FCR	- 2
						Lactobacilli	+ 0
						Coliforms	- 5
						Villus height	- 6
						Crypt depth	- 18
				Blend of acetic, propionic, phosphoric and citric acid + lactic acid	1.1 % + 1 %	DWG	+ 2
						ADFI	- 1
						FCR	- 1
						Lactobacilli	- 1
						Coliforms	- 1
						Villus height	+ 33
						Crypt depth	- 10

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
2005	Franco et al.	Pig	5.4 kg, duration: 8 d	Formic and fumaric acid	0.48 % : 0.58 % (1:1 molar mixture)	d(dry matter)	+ 6
						d(protein)	+ 2
						Lactobacilli stomach	- 20
						Lactobacilli SI	- 17
						Coliforms (Stomach)	+ 56
						Coliforms (SI)	- 17
						Total VFA (Stomach)	- 77
						Total VFA (SI)	+ 24
						Stomach pH	+ 64*
				Formic and lactic acid	0.48 % : 0.9% (1:1 molar mixture)	d(dry matter)	+ 6
						d(protein)	+ 3
						Lactobacilli (Stomach)	- 3
						Lactobacilli (SI)	- 10
						Coliforms (Stomach)	+ 9
						Coliforms (SI)	- 33*
						Total VFA (Stomach)	- 38
						Total VFA (SI)	+ 298
						Stomach pH	+ 8
				Formic and lactic acid	0.64% : 0.6 % (2:1 molar mixture)	d(dry matter)	+ 6
						d(protein)	+ 2
						Lactobacilli (Stomach)	- 14
						Lactobacilli (SI)	- 17
						Coliforms (Stomach)	+ 27
						Coliforms (SI)	- 31*
						Total VFA (Stomach)	- 60
						Total VFA (SI)	+ 51
						Stomach pH	+ 4
2005	Rafacz-Livingston et al. → in experiment 2 two nonphytate P levels	Broiler	8 – 22 d	Na-gluconate	1.68 %	DWG	+ 7*
					3.35 %		+ 16*
						ADFI	+ 6
							+ 12*

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
						FCR	- 1
							- 3
						Tibia ash	+ 11*
							+ 19*
				Ca-gluconate	1.65 %	DWG	+ 16*
					3.33 %		+ 7
						ADFI	+ 13*
							+ 8*
						FCR	- 2
							+ 1
						Tibia ash	+ 16*
							+ 14*
				Glucono-δ-lactone	1.5 %	DWG	+ 8*
					3 %		+ 8*
						ADFI	+ 8*
							+ 7
						FCR	+ 0
							- 1
						Tibia ash	+ 7*
							+ 10*
				2-hydroxy-4-methylthio butanoic acid	1.14 %	DWG	+ 2
						ADFI	+ 6
						FCR	+ 0
						Tibia ash	+ 7*
			8 – 22 d	Na- gluconate	2.24 %	DWG	+ 6, - 3
						ADFI	+ 6*, - 3
						FCR	+ 0, + 1
						Tibia ash	+ 7*, + 0
				Ca-gluconate	2.2%	DWG	+ 2, - 4
						ADFI	+ 4, - 2
						FCR	+ 2, + 2

Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
						Tibia ash	+ 3, + 0
			8 – 22 d	Na-gluconate	3.35 %	DWG	+ 18*
						ADFI	+ 12*
						FCR	- 4*
						Tibia ash	+ 17*
				Na-gluconate + citric acid	1.65 % + 1.5 %	DWG	+ 18*
						ADFI	+ 14*
						FCR	- 4*
						Tibia ash	+ 12
2006	Biagi et al.	Pig	7.4 – 25.9 kg	Gluconic acid	3000 ppm	DWG	+ 13*
					6000 ppm		+ 14*
					12000 ppm		+ 5
						ADFI	+ 11
							+ 10
							+ 2
						FCR	- 2
							- 2
							- 2
						Total VFA	+ 60
							+ 53
							+ 40
2006	Sacakli et al. → diets with and without phytase	Quail	3 – 38 d	Lactic and formic acid	0.25 %	DWG	- 2, - 2
						ADFI	- 3, - 2
						FCR	- 1, - 1
						Tibia ash	+ 4*, + 0
2007	Biagi et al.	Pig	6.7 – 27.8 kg	Na-butyrate	1000 ppm	DWG	+ 6
					2000 ppm		+ 3
					4000 ppm		+ 10
						ADFI	+ 4
							+ 1
							+ 8

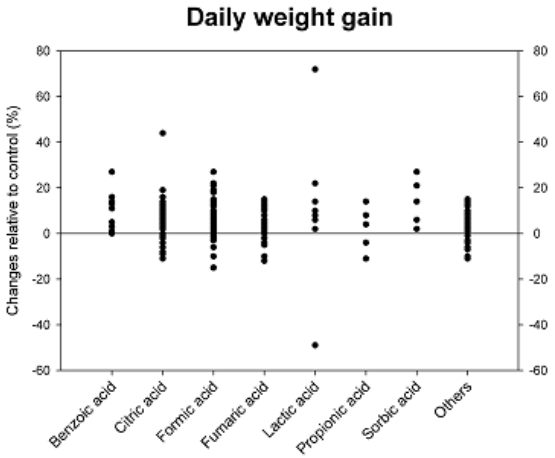
Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
						FCR	- 1
							- 2
							- 2
						Total VFA	+ 3
							- 5
							+ 13
2007	Partanen et al. → three fibre levels	Pig	Start with 34 kg	Blend of formic acid, sorbate and benzoate	0.84 %	dI(dry matter)	+ 0, + 3, + 1
						dI(NDF)	- 20, + 7, + 0
						dI(protein)	+ 2, + 4, + 1
						dI(amino acids)	+ 1, + 3, + 2
						d(dry matter)	+ 1, + 2*, + 0
						d(ash)	+ 9*, + 11*,
							+ 1
						d(NDF)	- 8, + 5, - 1
						d(ADF)	+ 3, + 26,
							+ 11
						d(hemicellulose)	- 8, + 2, - 3
						d(protein)	+ 3, + 3, - 1
						VFA total	+ 16, + 36,
							+ 18
						Digesta pH	+ 2, + 2, + 0
2007	Pastuszewska et al. → diets with three Trp-levels	Pig	16 kg, duration: 24 d	Blend of phosphoric, citric and fumaric acid	0.3 %	DWG	+ 10, + 12,
							- 1
						FCR	- 7, - 9, + 1
						d(protein)	- 3, + 0, + 3
2007	Overland et al.	Pig	28 – 113 kg	Fat coated Ca-butyrate	1.2 %	DWG	+ 7
2008	Overland et al.					ADFI	+ 7
						FCR	- 1
						Lactic acid bacteria	- 10
						Coliforms	- 10
						Indole (Colon)	+ 6

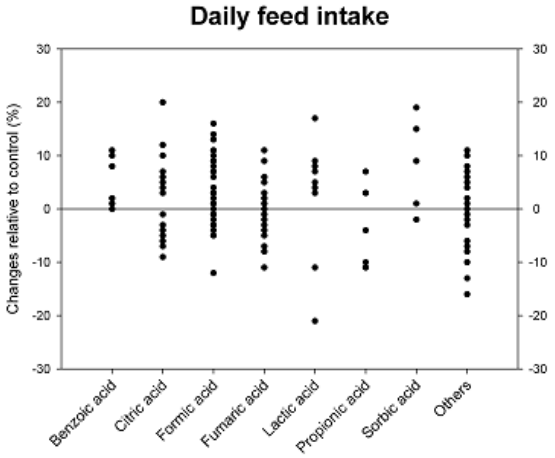
Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
						Scatole (Colon)	+ 7
				Fat and inulin coated Ca-butyrate	1.5 %	DWG	- 3
						ADFI	- 3
						FCR	+ 0
						Lactic acid bacteria	- 16
						Coliforms	- 10
						Indole (Colon)	+ 16
						Scatole (Colon)	- 23
2008	Li et al. → in experiment 2 after 5 d challenged with *E. coli*	Pig	7.8 kg, duration: 28 d	Blend of Ca-salt of 2-hydroxy-4(methylthio)butanoic acid, fumaric and benzoic acid	0.5 %	DWG	+ 15*
						ADFI	+ 1
						FCR	- 13*
						Lactobacilli (Faeces)	+ 1
						E. coli (Faeces)	- 9
			5.9 kg, duration: 14 d	Blend of Ca -salt of 2-hydroxy-4(methylthio)butanoic acid, fumaric and benzoic acid	0.5 % 1 %	DWG	+ 7 + 4
						ADFI	+ 2 - 2
						FCR	- 6 - 6
						Lactobacilli	+ 2 + 2
						E. coli	- 1 - 1
2008	Liem et al.	Broiler	0 – 16 d	Malic acid	2.9 %	16-d weight	- 8
						FCR	+ 10
						Bone ash	+ 4
						d(P)	+ 24*
						d(Ca)	+ 14

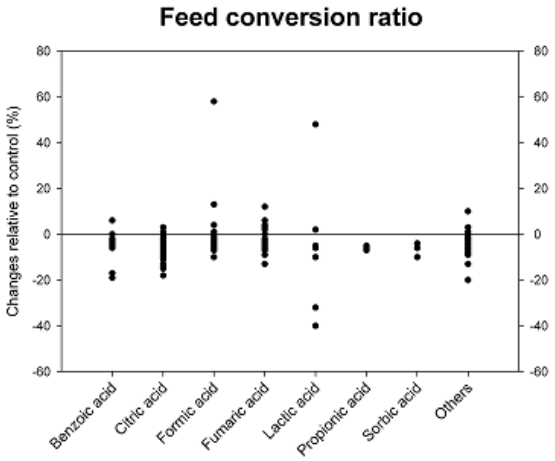
Year	Author	Species	Age/weight	Acid/Composition of blend	Dosage	Difference to control (%)	
2008	Mikulski et al.	Turkey	1 – 140 d	Blend of formic and propionic acid	0.5 %	DWG	+ 4
						FCR	- 5
						Total VFA	- 1
						Mortality	+ 20
2008	Owens et al. → Control diet supplemented with Allzyme	Broiler	7 – 28 d	Blend of propionic and formic acid and oligosaccharide mixture (50/50)	1 %	DWG	- 4
						ADFI	+ 0
						FCR	+ 3*
						Lactic acid bacteria	+ 5
						E. coli	- 1
						Villus hight (Ileum)	+ 11
						Crypt depth (Ileum)	+ 8

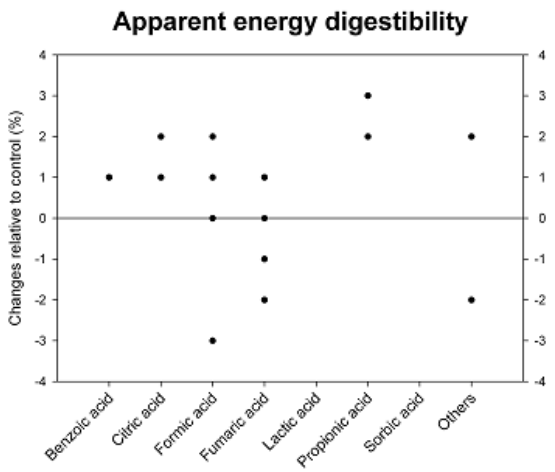
Graphical summary of selected parameters

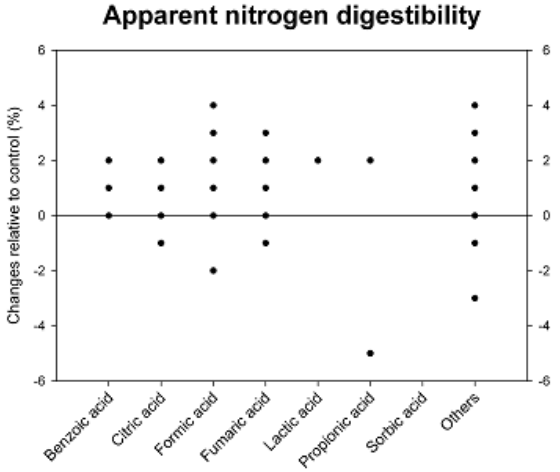
- Graphs only show the data from pig trials
- One point represents one result mentioned in the tables above → several points per study possible
- No data on energy digestibility for lactic and sorbic acid
- No data on nitrogen digestibility for sorbic acid











Appendix III: Additional remarks on the fibre study

From fibre rich diets it is known that they can have an influence on gut morphology especially on villus length and crypt depth (Jin et al. 1994). To find out whether such changes also occurred with benzoic acid in the diet, a part of the fibre study included morphological measurements.

Nine tissue samples were taken from each of the 16 animals slaughtered after the grower period: middle of duodenum, jejunum, ileum and caecum, four locations in the colon and terminal colon. The samples were then fixed in neutral buffered formalin, cut (Microtome Leica RM 2165; 0.8 µm) and stained with hematoxylin and eosin, following standard procedure.

For gut morphology, a minimum of 10 well defined villi and crypts were measured per slide (Microscope: Leica DM LBS; measurement software: LEICA IM 1000, Version 4). The parameters measured were:

- villus height: from crypt – villus junction to top
- villus area: villus height x width from crypt junction to crypt junction
- crypt height: crypt - villus junction to base
- crypt area: tracing the crypt outline

Neither benzoic acid nor fibre had any effect on gut morphology. Because of the low numbers of animals and some problems with sample preparation and sample measurement, the results were only noted but not published. This experience has resulted in several suggestions for methodical improvements when tissue samples from the gastrointestinal tract of pigs are taken:

- Tissue extraction should start with the duodenum as the gut epithelium in this area is very vulnerable to digestion by pancreas juice after death.
- The diameter of the duodenum and the jejunum is small enough to cut out 3 – 4 cm long tubular samples. For fixation the tubes should be cut in half longitudinally to make sure that all tissue comes in contact with the formalin. In case of the other parts of the gut, pieces of about 2 x 4 cm are extracted, the length of these pieces being parallel to the length of the gut.
- After extraction the tissue sample should be rinsed carefully with physiological saline or deionized water to remove all chymus and feed rests. The tissue samples taken in the fibre study were not cleaned. The remaining particles caused a lot of problems when the

samples were cut with the microtome. Of special annoyance was the destruction of the sample when the blade hit such a particle and ripped the tissue layers apart. This made exact measurements very difficult or even impossible in some cases.

- For embedding into paraffin, the tissue sample should be cut crossways into stripes of about 0.5 cm width. This ensures that enough usable sections can be obtained from the tissue sample.

Appendix IV: Straw as bedding does not influence performance and nutrient digestibility in growing-finishing pigs.

based on K. Bühler, B. Bucher and C. Wenk
published 2009 in Agrarforschung, 16(1), 34-38 (German)

During the collection of digesta samples in the fibre study it was noticed that some animals had their stomachs filled with straw. To determine the influence of this straw intake on the results and to find out whether straw consumption was comparable among the animals, this additional study was conducted:

Abstract

Thirty-two crossbred gilts (27 – 106 kg body weight) were kept in single pens provided with full-length straw (S) or a rubber mat (R) as bedding. In the grower and finisher period the pigs were restrictively fed either a low fibre diet (130 g/kg DM NDF, treatments LFS and LFR) or a high fibre diet (180 g/kg DM NDF, treatments HFS and HFR) and effects on performance and apparent total tract digestibility of nitrogen, energy, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined. Rubber mats reduced feed conversion efficiency in the grower period ($p < 0.05$) but there was no influence of type of bedding on the other parameters examined. Animals fed the HF diet showed higher daily weight gain and lower nitrogen and energy digestibility during the whole fattening period ($p < 0.01$). These results indicate that straw bedding and high fibre diets can be combined without reducing nutrient availability in the latter.

Introduction

In Switzerland and the EU pigs must be provided with material suitable for exploration (CH: directive 800.106.03; EU: directives 2001/88/EC and 2001/93/EC). In most cases straw is used to fulfil these directives and it is unclear how much of the bedding is consumed by the pigs. The intake of straw by pigs ranges from small quantities (Wenk 1984) to a considerable amount (Staals et al. 2007). Straw is rich in fibre which is known to decrease performance (Pluske et al. 2003) and nutrient digestibility (Wenk 2001) and may thus prolong the fattening period and reduce feed efficiency. Additionally to the use of straw as bedding also the use of fibre rich diets in pig fattening is increasingly of interest (Noblet and Le Goff 2001). The interaction of straw bedding and fibre rich diets is unclear. For this reason the influence of

full-length straw used as bedding on performance and nutrient digestibility in growing-finishing pigs fed diets supplemented with soy bean hulls and wheat straw meal was examined.

Material and methods

Animals and housing

The experiment was conducted with 32 Large White x Large White gilts bred at the research station "Chamau" of ETH Zurich. Initial and final bodyweight (BW) was 26.5 ± 4.9 kg and 106.4 ± 4.0 kg (mean ± SD), respectively. Bedding was full-length wheat straw in half of the pens and rubber mats in the other half. Fresh straw was daily provided by the staff after cleaning. The experimental procedure was approved by the official veterinary authority of the canton of Zug (authorization number ZG 44/06).

Diets and feeding

Animals were randomly distributed in individual pens and received either a low fibre (LF) or a high fibre (HF) diet. The combination of diet and bedding resulted in four experimental treatments: LF diet and straw (LFS), LF diet and rubber mat (LFR), HF diet and straw (LFS), HF diet and rubber mat (HFR).

The LF diet was based on cereals, peas and soybean expellers (Table 5.1). In diet HF 5 % soybean hulls and 5 % untreated wheat straw meal were added at the expense of LF.

Table 5.1. Composition of the experimental diets (g/kg).

	Grower period	**Finisher period**
Barley	200	200
Triticale	300	300
Wheat	73	203
Peas	200	160
Soybean expellers	120	50
Potato protein	33	18
Molasses	20	20
Limestone	10	10
NaCl	5	5
Dicalcium phosphate	12	8
L-lysine-HCl	1.3	1.2
DL-methionine	1.1	0.1
L-threonine	0.5	0.35
Celite 545	22.1	23.15
Vitamin/mineral premix[1)]	3	2.25
Fibre source[2)]		
Soybean hulls	50	50
Wheat straw meal	50	50

[1)] Supplied per kg of grower (finisher) diet: 8000 (6000) IU vitamin A, 1000 (750) IU vitamin D_3, 36 (27) mg vitamin E, 1110 (830) µg vitamin B_1, 3.0 (2.2) mg vitamin B_2, 2.2 (1.7) mg vitamin B_6, 18.0 (13.5) µg vitamin B_{12}, 1.3 (1.0) mg vitamin K_3, 11 (8) mg Ca-pantothenate, 22 (16) mg niacine, 570 (430) µg folic acid, 69 (52) µg biotine, 105 (79) mg choline, 105 (79) mg Fe, 37 (27) mg Mn, 9.0 (6.8) mg Cu, 540 (405) µg I, 180 (135) µg Se and 60 (45) mg Zn. [2)] Only added to the high fibre diet.

Calculated crude protein (CP) content of the diet LF was 177 g/kg in the grower and 145 g/kg in the finisher period. The digestible energy (DE) content was calculated as 13 MJ/kg with a Lys/DE ratio of 0.84 g/MJ for the grower diets and 0.63 g/MJ for the finisher diets.
The analysed nutrient content revealed a higher CP content and a higher DE than was calculated. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of the high fibre diet was about 50 g/kg DM (Table 5.2) higher than in the LF diet.

Table 5.2. Analysed nutrient content of the diets.

	Grower period		Finisher period	
	LF[1)]	**HF**	**LF**	**HF**
NDF (g/kg DM)	132.2 ± 10.4	183.5 ± 28.1	130.7 ± 0.2	180.5 ± 2.2
ADF (g/kg DM)	57.4 ± 1.9	101.2 ± 1.3	51.5 ± 2.5	95.0 ± 0.2
DM (g/kg)	896.4 ± 1.0	898.1 ± 0.8	895.0 ± 0.1	896.5 ± 2.5
CA (g/kg DM)	79.4 ± 1.3	80.6 ± 1.5	75.5 ± 0.9	74.7 ± 1.1
CP (g/kg DM)	214.2 ± 0.5	203.9 ± 2.6	178.6 ± 0.2	171.2 ± 0.01
Crude fat (g/kg DM)	30.3 ± 0.7	30.7 ± 2.2	21.3 ± 0.4	21.6 ± 0.4
DE_{exp} [2)] (MJ/kg)	13.9 ± 0.1	13.8 ± 0.03	13.6 ± 0.1	13.5 ± 0.9

[1)] LF: low fibre diet; HF: high fibre diet. Values are given as mean ± SD. DE_{exp} was calculated as $(GE_{intake} - GE_{excreted})/Feed_{intake}$.

Animals were restrictively fed and feed was offered as pellets in two equal rations per day. To obtain the same intake of energy for all pigs, animals fed on diet HF received daily 200 g * $BW^{0.569}$ of diet whereas animals fed on diet LF received 180 g diet * $BW^{0.569}$. At a body weight of 57.4 ± 6.3 kg diet was changed from grower to finisher diet for all animals. Water was available *ad libitum*.

Collection of samples

Faeces were collected every third week during four consecutive days resulting in two sampling periods for the grower and finisher period, each. Faeces of each animal were pooled for each sampling period. Feed samples were taken once in a sampling period and pooled for analysis. Animals were weighted weekly and feed refusals were measured daily for calculation of weight gain and feed conversion ratio (FCR).

Analytical methods

Faeces and feed were prepared and digestibility of all samples was estimated according to the methods described by Bühler et al. (2006). Analysis of neutral detergent fibre (NDF) and acid detergent fibre (ADF) followed the method of Robertson and Van Soest (1981).

Statistical analysis

The mixed model procedure of SAS for multiple comparisons (SAS System for Windows, SAS Institute Inc., Cary (NC), USA; Version 8.2) was used for statistical analysis. The significance of the effects of fibre level (F), bedding (BED) and fibre level x bedding interaction (F x BED) was obtained from the mixed model. Differences were considered to be significant in all statistical analyses if $p < 0.05$, which was obtained with Bonferroni-adjustment.

Results and discussion

Pig performance

Fibre content but not bedding material influenced daily weight gain (DWG) (Table 5.3). In the grower period the addition of high fibre components resulted in a DWG which was about 14 % higher than in the LF diet ($p < 0.05$). In the finisher period DWG of HF diet was 9 % higher than in LF diet ($p < 0.05$).

In this experiment feed conversion ratio (FCR) in the grower period was not influenced by fibre content but by bedding material (Table 5.3). The FCR of animals kept on rubber mats was markedly higher (2.27 vs. 2.08, $p < 0.05$) than that of animals with straw as bedding. The type of diet did not influence FCR ($p > 0.05$).

Table 5.3. Daily weight gain (DWG) and feed conversion ratio (FCR) during grower and finisher period.

	Diets[1)]					***p*-values[2)]**		
	LFS	**LFR**	**HFS**	**HFR**	**SEM**	**F**	**BED**	**F x BED**
Grower Period								
DWG (g/d)	658^{ab}	588^{b}	711^{a}	710^{a}	40	0.01	0.30	0.31
FCR (kg/kg)	2.09^{ab}	2.40^{a}	2.06^{b}	2.14^{ab}	0.12	0.11	0.03	0.22
Finisher Period								
DWG (g/d)	803^{a}	784^{a}	854^{ab}	881^{b}	40	< 0.01	0.87	0.34
FCR (kg/kg)	2.78	2.82	2.97	2.83	0.11	0.25	0.55	0.31

[1)] LF: low fibre diet; HF: high fibre diet; S: straw as bedding; R: rubber mat as bedding. [2)] Effects of fibre content (F), bedding (BED) and their interaction (F x BED). Different superscripts in a column indicate significant differences ($p < 0.05$) among diets. SEM: maximal standard error of the means.

Results show that DWG was more affected by fibre content than by bedding material whereas bedding is more important for FCR. The increase in DWG in the HF diet is in contrast to other studies (Drewry 1981; Pluske et al. 2003) where high fibre content reduced performance of growing-finishing pigs. However, this effect seems to disappear with increasing age. Galassi et al. (2007) observed that DWG of pigs fed 24 % wheat bran was lower at 70 kg BW but similar at 100 kg BW compared to control. In the current experiment the higher DWG was probably based in the different feeding regimes for LF and HF diet causing a higher energy intake in animals fed the HF diet.

The fact that FCR in the grower period was reduced when the pigs were kept on rubber mats is unexpected. It is possible that on rubber mats faeces and urine were in closer contact to each other and cleaning was more difficult. This might have led to a higher increase in ammonia emission in pens equipped with rubber mats. Although all animals were kept in the same stable, close and prolonged contact with ammonia of the animals kept on rubber mats could have affected their well-being and health (Ferket et al. 2002) and thus their FCR more than that of the animals kept on straw. However, this explanation fails to account for the fact that the DWG of treatment HFR was the second highest in the grower and the highest in the finisher period and that this difference did only occur in the grower period.

Apparent nutrient and fibre digestibility

The inclusion of soy bean hulls and straw meal reduced ($p < 0.01$) apparent nitrogen (d(N)) and energy (d(E)) digestibility in the grower and the finisher period but there was no influence of the bedding type (Table 5.4). In the whole fattening period d(N) amounted to 0.85 ± 0 for LF diet and 0.80 ± 0.01 for the high fibre diet. The corresponding values for d(E) were 0.86 ± 0.01 and 0.81 ± 0.01, respectively.

Independent of the fattening period, bedding type neither influenced ($p > 0.05$) digestibility of NDF (d(NDF)) nor that of ADF (d(ADF)) (Table 5.4). In the finisher period a slightly negative effect ($p < 0.05$) of fibre content on d(NDF) could be observed. The highest d(NDF) with a value of 0.54 was found in animals fed diet LFR which differed significantly ($p < 0.05$) from the minimal value of 0.45 found in treatment HFS (Table 5.4).

Table 5.4. Apparent total tract digestibility of crude protein (d(CP)), energy (d(E)) and neutral and acid detergent fibre (d(NDF), d(ADF)).

	Diet[1)]					*p*-values[2)]		
	LFS	**LFR**	**HFS**	**HFR**	**SEM**	**F**	**BED**	**F x BED**
Grower period								
d(N)	0.85[a]	0.85[a]	0.80[b]	0.81[b]	0.01	< 0.01	0.69	0.63
d(E)	0.85[a]	0.85[a]	0.80[b]	0.80[b]	0.04	< 0.01	0.27	0.75
d(NDF)	0.51	0.55	0.49	0.50	0.02	0.09	0.10	0.42
d(ADF)	0.41	0.47	0.48	0.49	0.03	0.17	0.28	0.37
Finisher period								
d(N)	0.85[a]	0.85[a]	0.79[b]	0.80[b]	0.01	< 0.01	0.40	0.62
d(E)	0.86[a]	0.87[a]	0.81[b]	0.81[b]	0.01	< 0.01	0.11	0.85
d(NDF)	0.51[ab]	0.54[s]	0.45[b]	0.48[ab]	0.02	0.01	0.18	0.91
d(ADF)	0.38	0.43	0.42	0.47	0.03	0.14	0.08	0.92

[1)] LF: low fibre diet; HF: high fibre diet; S: straw as bedding; R: rubber mat as bedding. [2)] Effects of fibre content (F), bedding (BED) and their interaction (F x BED). Different superscripts in a column indicate significant differences ($p < 0.05$) among diets. SEM: maximal standard error of the means.

As expected nutrient and fibre digestibility was lower in the HF diet and slightly higher in the finisher than in the grower period. This confirms the findings of Noblet and Shi (1993) that pigs adapt to high fibre diets and improve nutrient and fibre digestibility with increasing age. However, d(NDF) and d(ADF) in the finisher period of this study were numerically lower than in the grower period. This could have been caused by be the general difficulty of appropriate and comparable fibre analysis (Mertens 2003) or by the fact that the possibility to adapt to high fibre diets is dependent on the fibre source.

Digestibility parameters of the animals kept on straw were similar to those kept on rubber mats indicating that animals fed diet LF did not consume more straw as animals fed the diet HF. It also indicates that the negative effects of high fibre content in pig diets are not increased when straw is provided. This is in contrast to Staals et al. (2007) who reported decreased digestibility and diet-depending straw intake. On the other hand, Wenk (1984) described that the amount of straw consumed was only marginal and did not influence digestibility. Similar to the age dependent adaptation to high fibre diets, the variability of results in studies dealing with straw intake and its influence on digestibility could be based on the variety of diets and fibre sources used.

Conclusion

From this study it can be concluded that straw bedding has no influence on performance and nutrient digestibility in growing-finishing pigs and it even seems to improve feed conversion ratio. For similar studies in the future it would be helpful to develop a simple and easy method to perform measurement of straw intake and to qualify straw quality. This could be used to analyse which parameters influence straw intake and whether the effects of straw are quality dependant.

References

Aarnink AJA and Verstegen MWA. 2007. Nutrition, key factor to reduce environmental load from pig production. Livestock Science. 109:194-203.

Adjiri-Awere A and Van Lunen TA. 2005. Subtherapeutic use of antibiotics in pork production: risks and alternatives. Canadian Journal of Animal Science. 85:117-130.

Agroscope Liebefeld-Posieux (ALP). 2004. Fütterungsempfehlungen und Nährwerttabellen für Schweine. Landwirtschaftliche Lehrmittelzentrale, Zollikofen, Switzerland.

Alexander TW, Yanke LJ, Topp E, Olson ME, Read RR, Morck DW, and McAllister TA. 2008. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. Applied and Environmental Microbiology. 74:4405-4416.

Amory JR, Mackenzie AM, and Pearce GP. 2006. Factors in the housing environment of finisher pigs associated with the development of gastric ulcers. The Veterinary Record. 158:260-264.

Anguita M, Canibe N, Perez JF, and Jensen BB. 2006. Influence of the amount of dietary fiber on the available energy from hindgut fermentation in growing pigs: Use of cannulated pigs and in vitro fermentation. Journal of Animal Science. 84:2766-2778.

Argenzio RA and Southworth M. 1975. Sites of organic acid production and absorption in gastrointestinal tract of the pig. American Journal of Physiology. 228:454-460.

Asp NG, Schweizer TF, Southgate DAT, and Theander O. 1992. Dietary fibre analysis. In: Schweizer TF and Edwards CA. Dietary fibre - A component of food. Springer Verlag, Berlin, Germany. Chapter 4: 57-101.

Augspurger NR, Webel DM, Lei XG, and Baker DH. 2003. Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. Journal of Animal Science. 81:474-483.

Bach Knudsen KE. 2001. The nutritional significance of dietary fibre analysis. Animal Feed Science and Technology. 90:3-20.

Bach Knudsen KE and Jensen BB. 1991. Effect of source and level of dietary fibre on microbial fermentation in the large intestine of pigs. Proceedings of the Vth International Symposium on Digestive Physiology in Pigs. EAAP Publication Wageningen, The Netherlands: 389-394.

Bach Knudsen KE, Serena A, Canibe N, and Juntunen KS. 2003. New insight into butyrate metabolism. Proceedings of the Nutrition Society. 62:81-86.

Barton MD. 2000. Antibiotic use in animal feed and its impact on human health. Nutrition Research Reviews. 13:279-299.

Bates J, Jordens Z, and Selkon JB. 1993. Evidence for an animal origin of vancomycin-resistant *Enterococci*. Lancet. 342:490-491.

Baustad B and Nafstad I. 1969. Gastric ulcers in swine. 4. Effects of dietary particle size and crude fiber contents on ulceration. Pathologia Veterinaria. 6:546-556.

Beers S and Jongbloed AW. 1992. Effect of supplementary *Aspergillus niger* phytase in diets for piglets on their performance and apparent digestibility of phosphorus. Animal Production. 55:425-430.

Bergman EN. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews. 70:567-590.

Biagi G and Piva A. 2005. Modulation of swine cecal microflora by some organic acids. In: Plitzner Ch, Wetscherek-Seipelt D, Schedle K, Kraft M, and Windisch WM. 4. BOKU Symposium Tierernährung. Wien, Austria: 182-185.

Biagi G, Piva A, Hill T, Schneider D, and Crenshaw T. 2003. Bone mineral content gain is reduced in weaned pigs fed diets with low-buffer capacity and organic acids. Journal of Animal Science. 81:202-202.

Biagi G, Piva A, Moschini M, Vezzali E, and Roth FX. 2006. Effect of gluconic acid on piglet growth performance, intestinal microflora, and intestinal wall morphology. Journal of Animal Science. 84:370-378.

Biagi G, Piva A, Moschini M, Vezzali E, and Roth FX. 2007. Performance, intestinal microflora, and wall morphology of weanling pigs fed sodium butyrate. Journal of Animal Science. 85:1184-1191.

Blank R, Mosenthin R, Sauer WC, and Huang S. 1999. Effect of fumaric acid and dietary buffering capacity on ileal and fecal amino acid digestibilities in early-weaned pigs. Journal of Animal Science. 77:2974-2984.

Blottiere HM, Champ M, Hoebler C, Michel C, and Cherbut C. 1999. Production and digestive effects of short chain fatty acids. Sciences des Aliments. 19:269-290.

Bolduan G, Jung H, Schneider R, Block J, and Klenke B. 1988a. Die Wirkung von Fumarsäure und Propandiol-Formiat in der Ferkelaufzucht. Journal of Animal Physiology and Animal Nutrition. 59:143-149.

Bolduan G, Jung H, Schneider R, Block J, and Klenke B. 1988b. Die Wirkung von Propion- und Ameisensäure in der Ferkelaufzucht. Journal of Animal Physiology and Animal Nutrition. 59:72-78.

Bolhuis JE, van den Brand H, Staals S, and Gerrits WJJ. 2007. Effects of pregelatinized vs. native potato starch on intestinal weight and stomach lesions of pigs housed in barren pens or on straw bedding. Livestock Science. 109:108-110.

Boling SD, Webel D, Mavromichalis I, Parsons C, and Baker D. 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. Journal of Animal Science. 78:682-689.

Bosi P, Jung HJ, Han IK, Perini S, Cacciavillani JA, Casini L, Creston D, Gremokolini C, and Mattuzzi S. 1999. Effects of dietary buffering characteristics and protected or unprotected acids on piglet growth, digestibility and characteristics of gut content. Asian-Australasian Journal of Animal Sciences. 12:1104-1110.

Boyd RD, Hall D, and Wu JF. 1983. Plasma alkaline-phosphatase as a criterion for determining biological availability of phosphorus for swine. Journal of Animal Science. 57:396-401.

Bridges JW, French MR, Smith RL, and Williams RT. 1970. The fate of benzoic acid in various species. Biochemical Journal. 118:47-51.

Briot K and Roux C. 2005. What is the role of DXA, QUS and bone markers in fracture prediction, treatment allocation and monitoring? Best Practice and Research Clinical Rheumatology. 19:951-964.

Broz J and Schulze J. 1987. Efficacy of citric acid as a feed additive in early weaned piglets. Journal of Animal Physiology and Animal Nutrition. 58:215-223.

Brul S and Coote P. 1999. Review: Preservative agents in foods - Mode of action and microbial restistance mechanisms. International Journal of Food Microbiology. 50:1-17.

Budde RA and Crenshaw TD. 2003. Chronic metabolic acid load induced by changes in dietary electrolyte balance increased chloride retention but did not compromise bone in growing swine. Journal of Animal Science. 81:197-208.

Bühler K, Bucher B, and Wenk C. 2009. Auswirkungen von Stroh und faserreichem Futter auf Mastschweine. Agrarforschung. 16:34-38.

Bühler K, Liesegang A, Gebert S, and Wenk C. 2007. Influence of dietary benzoic acid on bone stability and bone metabolism in growing/finishing pigs. Proceedings of the Society of Nutrition Physiology. 16:97.

Bühler K, Wenk C, Broz J, and Gebert S. 2006. Influence of benzoic acid and dietary protein level on performance, nitrogen metabolism and urinary pH in growing-finishing pigs. Archives of Animal Nutrition. 60:382-389.

Burnell TW, Cromwell GL, and Stahly TS. 1988. Effects of dried whey and copper sulfate on the growth responses to organic acid in diets for weanling pigs. Journal of Animal Science. 66:1100-1108.

Canh TT, Aarnink AJA, Mroz Z, Jongbloed AW, Schrama JW, and Verstegen MWA. 1998a. Influence of electrolyte balance and acidifying calcium salts in the diet of growing-finishing pigs on urinary pH, slurry pH and ammonia volatilisation from slurry. Livestock Production Science. 56:1-13.

Canh TT, Aarnink AJA, Schutte JB, Sutton A, Langhout DJ, and Verstegen MWA. 1998b. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livestock Production Science. 56:181-191.

Canh TT, Sutton AL, Aarnink AJA, Verstegen MWA, Schrama JW, and Bakker GC. 1998c. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. Journal of Animal Science. 76:1887-1895.

Canibe N and Bach Knudsen KE. 2001. Degradation and physicochemical changes of barley and pea fibre along the gastrointestinal tract of pigs. Journal of the Science of Food and Agriculture. 82:27-39.

Canibe N, Engberg RM, and Jensen BB. 2001a. an overview of the effect of organic acids on gut flora and gut health. Available from: http://www-afac.slu.se/Workshop%20Norge/organic_acids_canibe_et_al.pdf. Last accessed: March 2009.

Canibe N, Hojberg O, Hojsgaard S, and Jensen BB. 2005. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. Journal of Animal Science. 83:1287-1302.

Canibe N, Steien SH, Overland M, and Jensen BB. 2001b. Effect of K-diformate in starter diets on acidity, microbiota, and the amount of organic acids in the digestive tract of piglets, and on gastric alterations. Journal of Animal Science. 79:2123-2133.

Carter SD, Cromwell GL, Combs TR, Colombo G, and Fanti P. 1996. The determination of serum concentrations of osteocalcin in growing pigs and its relationship to end-measures of bone mineralization. Journal of Animal Science. 74:2719-2729.

Castillo M, Martin-Orue SM, Nofrarias M, Manzanilla EG, and Gasa J. 2007. Changes in caecal microbiota and mucosal morphology of weaned pigs. Veterinary Microbiology. 124:239-247.

Cherrington CA, Hinton M, Mead GC, and Chopra I. 1991. Organic-acids - Chemistry, antibacterial activity and practical applications. Advances in Microbial Physiology. 32:87-108.

Christenson RH. 1997. Biochemical markers of bone metabolism: An overview. Clinical Biochemistry. 30:573-593.

CICA (Concise international chemical assessment). 2000. CICA document 26. Available from: http://www.inchem.org/documents/cicads/cicads/cicad26.htm. Last accessed: March 2009

Cole DJA, Beal RM, and Luscombe JR. 1968. Effect on performance and bacterial flora of lactic acid, propionic acid, calcium propionate and calcium acrylate in drinking water of weaned pigs. Veterinary Record. 83:459-463.

DANMAP. 2004. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.

Den Brok GM, Hendriks JGL, Vrielink MGM, and van der Peet-Schwering CMC. 1999. Urinary pH, ammonia emission and performance of growing/finishing pigs after the addition of a mixture of organic acids, mainly benzoic acid, to the feed. Rosmalen: Research Institute for Pig Husbandry. pp. 36. Report No.: P 5.7

Deunert F, Lennartz B, and Tiemeyer B. 2007. Legislative effects on the development of surface water quality in rural areas in Northern Germany. Journal of Cleaner Production. 15:1507-1513.

Dierick NA, Michiels J, and van Nevel Ch. 2004. Effect of medium chain fatty acids and benzoic acid, as alternatives for antibiotics, on growth and some gut parameters in piglets. Communications in Agricultural and Applied Biological Sciences. 69:187-190.

Dierick NA, Vervaeke IJ, Demeyer DI, and Decuypere JA. 1989. Approach to the energetic importance of fibre digestion in pigs. I. Importance of fermentation in the overall energy supply. Animal Feed Science and Technology. 23:141-167.

Doane PH, Nell AN, and Schofield P. 1998. Ensiling effects of the ethanol fractionation of forages using gas production. Journal of Animal Science. 76:888-895.

Dourmad JY, Guingand N, Latimier P, and Seve B. 1999a. Nitrogen and phosphorus consumption, utilisation and losses in pig production: France. Livestock Production Science. 58:199-211.

Dourmad JY and Jondreville C. 2007. Impact of nutrition on nitrogen, phosphorus, Cu and Zn in pig manure, and on emissions of ammonia and odours. Livestock Science. 112:192-198.

Dourmad JY, Seve B, Latimier P, Boisen S, Fernandez J, van der Peet-Schwering CMC, and Jongbloed AW. 1999b. Nitrogen consumption, utilisation and losses in pig production in France, The Netherlands and Denmark. Livestock Production Science. 58:261-264.

Drewry KJ. 1981. Postweaning performance of crossbred pigs fed normal and high fiber diets. Journal of Animal Science. 52:197-209.

Ebrahimnezhad Y, Shivazad M, Taherkhani R, and Nazerad K. 2008. Effects of citric acid and microbial phytase supplementation on performance and phytate phosphorus utilization in broiler chicks. Journal of Poultry Science. 45:20-24.

Eckel B, Kirchgessner M, and Roth FX. 1992. Influence of formic-acid on daily weight-gain, feed-intake, feed conversion rate and digestibility. 1. Communication. Investigations about the nutritive efficacy of organic-acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 67:93-100.

Edmonds MS, Izquierdo OA, and Baker DH. 1985. Feed additive studies with newly weaned pigs: Efficacy of supplemental copper, antibiotics and organic acids. Journal of Animal Science. 60:462-469.

Eeckhout W and De Paepe M. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology. 47:19-29.

Ehrlich GG, Goerlitz DF, Bourell JH, Eisen GV, and Godsy EM. 1981. Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. Applied and Environmental Microbiology. 42:878-885.

Eidelsburger U, Kirchgessner M, and Roth FX. 1992a. Influence of formic acid, calcium formate and sodium hydrogencarbonate on dry matter content, pH value, concentration of carbonic acids and ammonia in different segments of the gastrointestinal tract. 8. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 68:20-32.

Eidelsburger U, Kirchgessner M, and Roth FX. 1992b. Influence of fumaric acid, hydrochloric acid, sodium formate, tylosin and toyocerin on daily weight gain, feed intake, feed conversion rate and digestibility. 11. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 68:82-92.

Eidelsburger U, Roth FX, and Kirchgessner M. 1992c. Influence of formic acid, calciumformate and sodiumhydrogencarbonate on daily weight-gain, feed-intake, feed conversion rate and digestibility. 7. Investigations about the nutritive efficacy of organic-acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 67:258-267.

Eidelsburger U, Wald Ch, and Looft Ch. 2005. Zum Einfluss von Kaliumdiformiat auf die Mast- und Schlachtleistung von Schweinen. 4. BOKU-Symposium Tierernährung. 176-180.

Eisemann JH and van Heugten E. 2007. Response of pigs to dietary inclusion of formic acid and ammonium formate. Journal of Animal Science. 85:1530-1539.

Epstein S. 1988. Serum and urinary markers of bone remodeling - Assessment of bone turnover. Endocrine Reviews. 9:437-449.

Ettle T, Mentschel K, and Roth FX. 2004. Dietary self-selection for organic acids by the piglet. Archives of Animal Nutrition. 58:379-388.

Falkowski JF and Aherne FX. 1984. Fumaric and citric acid as feed additives in starter pig nutrition. Journal of Animal Science. 58:935-938.

Ferket PR, van Heugten E, van Kempen TATG, and Angel R. 2002. Nutritional strategies to reduce environmental emissions from nonruminants. Journal of Animal Science. 8(Suppl. 2):E168-E182.

Fernandez JA and Jorgensen JN. 1986. Digestibility and absorption of nutrients as affected by fibre content in the diet of the pig. Quantitative aspects. Livestock Production Science. 15:53-71.

Franco L, Fondevila M, Lobera M, and Castrillo C. 2005. Effect of combinations of organic acids in weaned pig diets on microbial species of digestive tract contents and their response on digestibility. Journal of Animal Physiology and Animal Nutrition. 89:88-93.

Franklin MA, Mathew AG, Vickers JR, and Clift RA. 2002. Characterization of microbial populations and volatile fatty acid concentrations in the jejunum, ileum, and cecum of pigs weaned at 17 vs 24 days of age. Journal of Animal Science. 80:2904-2910.

Freire JPB, Guerreiro AJG, Cunha LF, and Aumaitre A. 2000. Effect of dietary fibre source on total tract digestibility, caecum volatile fatty acids and digestive transit time in the weaned piglet. Animal Feed Science and Technology. 87:71-83.

Friend DW, Cunningham HM, and Nicholson JWG. 1963. The production of organic acids in the pig. II. the effect of diet on the levels of volatile fatty acids and lactic acid in sections of the alimentary tract. Canadian Journal of Animal Science. 43:156-168.

Fuller MF, Mcwilliam R, Wang TC, and Giles LR. 1989. The optimum dietary amino-acid pattern for growing-pigs. 2. Requirements for maintenance and for tissue protein accretion. British Journal of Nutrition. 62:255-267.

Gabert VM and Sauer WC. 1994. The effects of supplementing diets for weanling pigs with organic acids. A review. Journal of Animal and Feed Sciences. 3:73-87.

Gabert VM, Sauer WC, Schmitz M, Ahrens F, and Mosenthin R. 1995. The effect of formic acid and buffering capacity on the ileal digestibilities of amino acids and bacterial populations and metabolites in the small intestine of weanling pigs fed semipurified fish meal diets. Canadian Journal of Animal Science. 75:615-623.

Galassi G, Malagutti L, and Crovetto GM. 2007. Growth and slaughter performance, nitrogen balance and ammonia emission from slurry in pigs fed high fibre diets. Italian Journal of Animal Science. 6:227-239.

Gassner A. 2006. Gewässerschutzbestimmungen in der Landwirtschaft. Ein internationaler Vergleich. Umwelt-Wissen Nr. 0618, ed. Bundesamt für Umwelt, Bern, Switzerland.

Gatel F and Grosjean F. 1992. Effect of protein content of the diet on nitrogen excretion by pigs. Livestock Production Science. 31:109-120.

Geary TM, Brooks PH, Beal JD, and Campbell A. 1999. Effect of weaner pig performance and diet microbiology of feeding a liquid diet acidified to pH 4 with either lactic acid or through fermentation with *Pediococcus acidilactici*. Journal of the Science of Food and Agriculture. 79:633-640.

Gebert S. 1998. Effects of microbial phytase and vitamin E in fat supplemented diets for growing pigs and laying hens on performance, nutrient utilization and product stability against oxidative alterations (Dissertation). Zurich: ETH Zurich.

Gebert S, Bee G, Pfirter HP, and Wenk C. 1999. Phytase and vitamin E in the feed of growing pigs. 1. Influence on growth, mineral digestibility and fatty acids in digesta. Journal of Animal Physiology and Animal Nutrition. 81:9-19.

Gedek B, Kirchgessner M, Eidelsburger U, Wiehler S, Bott A, and Roth FX. 1992a. Influence of formic acid on the microflora in different segments of the gastrointestinal tract. 5. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 67:206-214.

Gedek B, Roth FX, Kirchgessner M, Wiehler S, Bott A, and Eidelsburger U. 1992b. Influence of fumaric acid, hydrochloric acid, sodium formate, tylosin und toyocerin on the microflora in different segments of the gastrointestinal tract. 14. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 68:209-217.

Gentile JM, Roneker KR, Crowe SE, Pond WG, and Lei XG. 2003. Effectiveness of an experimental consensus phytase in improving dietary phytate-phosphorus utilization by weanling pigs. Journal of Animal Science. 81:2751-2757.

Gerrits WJJ, Rijnen MMJA, Bolhuis JE, and Schrama JW. 2003. Influences of dietary fibre on physical activity and behaviour, and on its energy value for growing pigs. In: Wiseman J, Varley MA, and Kemp B. Perspectives in Pig Science. Nottingham University Press, Nottingham (UK): 475-490.

Ghosh S and Lapara TM. 2007. The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME Journal. 1:191-203.

Giesting DW and Easter RA. 1985. Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. Journal of Animal Science. 60:1288-1294.

Giesting DW and Easter RA. 1991. Effect of protein source and fumaric acid supplementation on apparent ileal digestibility of nutrients by young pigs. Journal of Animal Science. 69:2497-2503.

Greiner R and Farouk AE. 2007. Purification and characterization of a bacterial phytase whose properties make it exceptionally useful as a feed supplement. Protein Journal. 26:467-474.

Greiner R, Konietzny U, and Jany KD. 1993. Purification and characterization of 2 phytases from *Escherichia coli*. Archives of Biochemistry and Biophysics. 303:107-113.

Guggenbuhl P, Quintana AP, and Nunes CS. 2007a. Comparative effects of three phytases on phosphorus and calcium digestibility in the growing pig. Livestock Science. 109:258-260.

Guggenbuhl P, Seon A, Quintana AP, and Nunes CS. 2007b. Effects of dietary supplementation with benzoic acid (VevoVitall $^{(r)}$) on the zootechnical performance, the gastrointestinal microflora and the ileal digestibility of the young pig. Livestock Science. 108:218-221.

Guilfoyle DE and Hirshfield IN. 1996. The survival benefit of short-chain organic acids and the inducible arginine and lysine decarboxylase genes for *Escherichia coli*. Letters in Applied Microbiology. 22:393-396.

Guo CY, Ward W, Cairns P, and Atkinson S. 2000. Comparative response in growth and bone status to three dexamethasone treatment regimens in infant piglets. Pediatric Research. 48:238-243.

Gutzwiller A, Hess HD, Stoll P, Guggisberg D, and Liesegang A. 2007a. Einfluss von NPr-Futter und Benzoesäure auf den Knochenstoffwechsel des wachsenden Schweines. In: Kreuzer M, Wenk C, and Lanzini T. Schriftenreihe Institut für Nutztierwissenschaften. ETH Zurich: 201-203.

Gutzwiller A, Stoll P, and Guggisberg D. 2007b. Phosphorus intake and leg weakness in growing pigs. Agrarforschung. 14:312-317.

Hampson DJ. 1986. Alterations in piglet small intestinal structure at weaning. Research in Veterinary Science. 40:32-40.

Han YM, Roneker KR, Pond WG, and Lei XG. 1998. Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. Journal of Animal Science. 76:2649-2656.

Hansen CF, Sorensen G, and Lyngbye M. 2007. Reduced diet crude protein level, benzoic acid and inulin reduced ammonia, but failed to influence odour emission from finishing pigs. Livestock Science. 109:228-231.

Hansen MJ, Chwalibog A, Tauson AH, and Sawosz E. 2006. Influence of different fibre sources on digestibility and nitrogen and energy balances in growing pigs. Archives of Animal Nutrition. 60:390-401.

Hansson LI, Stenstro A, and Thorngre KG. 1974. Diurnal variation of longitudinal bone-growth in rabbit. Acta Orthopaedica Scandinavica. 45:499-507.

Harper AF, Kornegay ET, and Schell TC. 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. Journal of Animal Science. 75:3174-3186.

Hassager C, Risteli J, Risteli L, Jensen SB, and Christiansen C. 1992. Diurnal variation in serum markers of type-I collagen-synthesis and degradation in healthy premenopausal women. Journal of Bone and Mineral Research. 7:1307-1311.

Hedemann MS, Eskildsen M, Laerke HN, Pedersen C, Lindberg JE, Laurinen P, and Bach Knudsen KE. 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. Journal of Animal Science. 84:1375-

Henry RW, Pickard DW, and Hughes PE. 1985. Citric-acid and fumaric-acid as food additives for early-weaned piglets. Animal Production. 40:505-510.

Hernandez F, Garcia V, Madrid J, Orengo J, Catala P, and Megias MD. 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. British Poultry Science. 47:50-56.

Hess HD and Gutzwiller A. 2008. Effect of benzoic acid on urinary mineral excretion in growing pigs. Proceedings of the Society of nutrition Physiology. 17:83.

Höhler D and Pallauf J. 1993. Effect of citric acid added to a maize-soya-diet with or without zinc supplementation on the availability of minerals. Journal of Animal Physiology and Animal Nutrition. 69:132-142.

Höhler D and Pallauf J. 1994. Effects of Zn-supply and addition of citric acid to a maize-soya-diet on the nutritional efficiency and absorption of minerals in piglets. Journal of Animal Physiology and Animal Nutrition. 71:75-82.

Igbasan FA, Manner K, Miksch G, Borriss R, Farouk A, and Simon O. 2000. Comparative studies on the in vitro properties of phytases from various microbial origins. Archives of Animal Nutrition. 53:353-373.

Jendza JA, Dilger RN, Adedokun SA, Sands JS, and Adeola O. 2005. *Escherichia coli* phytase improves growth performance of starter, grower, and finisher pigs fed phosphorus-deficient diets. Journal of Animal Science. 83:1882-1889.

Jensen BB. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. Journal of Animal and Feed Sciences. 7:45-64.

Jensen MS, Jensen SK, and Jakobsen K. 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. Journal of Animal Science. 75:437-445.

Jin L, Reynolds LP, Redmer DA, Caton JS, and Crenshaw JD. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. Journal of Animal Science. 72:2270-2278.

Johnston SL, Williams SB, Southern LL, Bidner TD, Bunting LD, Matthews JO, and Olcott BM. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. Journal of Animal Science. 82:705-714.

Jongbloed AW. 2007. Environmental impact of pig production and nutritional strategies to reduce nitrogen, ammonia, odor and mineral excretion and emission. 58th Annual Meeting of EAAP. Wageningen Academic Publishers, Wageningen, The Netherlands: 27.

Jongbloed AW, Jongbloed R, Kies A, and Lenis N. 1996. The effect of organic acids in diets for growing pigs on enhancement of microbial phytase efficacy. Journal of Animal Science. 74:185-185.

Jongbloed AW and Lenis NP. 1992. Alteration of nutrition as a means to reduce environmental pollution by pigs. Livestock Production Science. 31:75-94.

Jongbloed AW, Mroz Z, van der Weij-Jongbloed R, and Kemme PA. 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livestock Production Science. 67:113-122.

Jongbloed AW, Poulsen HD, Dourmad JY, and van der Peet-Schwering CMC. 1999. Environmental and legislative aspects of pig production in The Netherlands, France and Denmark. Livestock Production Science. 58:243-249.

Jorgensen H, Larsen T, Zhao XQ, and Eggum BO. 1997. The energy value of short-chain fatty acids infused into the caecum of pigs. British Journal of Nutrition. 77:745-756.

Kass ML, Vansoest PJ, and Pond WG. 1980. Utilization of dietary fiber from alfalfa by growing swine. 2. Volatile fatty-acid concentrations in and disappearance from the gastrointestinal-tract. Journal of Animal Science. 50:192-197.

Kemme PA, Jongbloed AW, Mroz Z, Kogut J, and Beynen AC. 1999a. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. 1. Apparent ileal digestibility of amino acids. Livestock Production Science. 58:107-117.

Kemme PA, Jongbloed AW, Mroz Z, Kogut J, and Beynen AC. 1999b. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livestock Production Science. 58:119-127.

Kil DY, Piao LG, Long HF, Lim JS, Yun MS, Kong CS, Ju WS, Lee HB, and Kim YY. 2006. Effects of organic or inorganic acid supplementation on growth performance, nutrient digestibility and white blood cell counts in weanling pigs. Asian-Australasian Journal of Animal Sciences. 19:252-261.

Kim Y, Kil D, Oh H, and Han iK. 2005. Acidifier as an alternative material to antibiotics in animal feed. Asian-Australasian Journal of Animal Sciences. 18:1048-1060.

Kirchgessner M, Gedek B, Wiehler S, Bott A, Eidelsburger U, and Roth FX. 1992. Influence of formic acid, calcium formate and sodium biscarbonate on the microflora in different segments of the gastrointestinal tract. 10. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 68:73-81.

Kirchgessner M and Müller HL. 1991. Energy utilization via hindgut fermentation in pigs. Journal of Animal Physiology and Animal Nutrition. Supplement 22:41-49.

Kirchgessner M and Roth FX. 1978. Nährstoff- und Energieverdaulichkeit bei Fumarsäure-Zulage. Journal of Animal Physiology and Animal Nutrition. 41:71-76.

Kirchgessner M and Roth FX. 1980. Verdaulichkeit und Bilanz von Protein, Energie und einigen Mineralstoffen bei Fumarsäurezulagen an Ferkel. Journal of Animal Physiology and Animal Nutrition. 44:239-246.

Kirchgessner M, Roth FX, and Eidelsburger U. 1993. Nutritive efficiency of tartaric acid and malic acid in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 70:216-224.

Kirchgessner M, Roth FX, and Paulicks BR. 1995. Nutritive efficacy of sorbic acid in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 74:235-242.

Kluge H, Broz J, and Eder K. 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism in piglets. Journal of Animal Physiology and Animal Nutrition. 90:316-324.

Knarreborg A, Miquel N, Granli T, and Jensen BB. 2002. Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. Animal Feed Science and Technology. 99:131-140.

Koch ME and Mahan DC. 1986. Biological characteristics for assessing low phosphorus intake in finishing swine. Journal of Animal Science. 62:163-172.

Kornegay ET, Evans JL, and Ravindran V. 1994. Effects of diet acidity and protein level or source of calcium on the performance, gastrointestinal content measurements, bone measurements, and carcass composition of gilt and barrow weanling pigs. Journal of Animal Science. 72:2670-2680.

Krause DO, Harrison PC, and Easter RA. 1994. Characterization of the nutritional interactions between organic acids and inorganic bases in the pig and chick. Journal of Animal Science. 72:1257-1262.

Kraut JA, Mishler DR, Singer FR, and Goodman WG. 1986. The effects of metabolic-acidosis on bone-formation and bone-resorption in the rat. Kidney International. 30:694-700.

Lambert LA, Abshire K, Blankenhorn D, and Slonczewski JL. 1997. Proteins induced in *Escherichia coli* by benzoic acid. Journal of Bacteriology. 179:7595-7599.

Lambert RJ and Stratford M. 1999. Weak-acid preservatives: Modelling microbial inhibition and response. Journal of Applied Microbiology. 86:157-164.

Le Goff G, Dubois S, van Milgen J, and Noblet J. 2002a. Influence of dietary fibre level on digestive and metabolic utilisation of energy in growing and finishing pigs. Animal Research. 51:245-259.

Le Goff G, van Milgen J, and Noblet J. 2002b. iInfluence of dietary fibre on digestive utilization and rate of passage in growing pigs, finishing pigs and adult sows. British Society of Animal Science. 74:503-515.

Lei XG and Stahl CH. 2000. Nutritional benefits of phytase and dietary determinants of its efficacy. Journal of Applied Animal Research. 17:97-112.

Lepage OM, Descoteaux L, Marcoux M, and Tremblay A. 1991. Circadian rhythms of osteocalcin in equine serum. Correlation with alkaline phosphatase, calcium, phosphate and total protein-levels. Canadian Journal of Veterinary Research. 55:5-10.

Li D, Che X, Wang Y, Hong C, and Thacker PA. 1998. Effect of microbial phytase, vitamin D3, and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. Animal Feed Science and Technology. 73:173-186.

Li D, Liu S, Qiao S, Yi G, Liang C, and Thacker P. 1999. Effect of feeding organic acid with or without enzyme on intestinal microflora, intestinal enzyme activity and performance of weaned pigs. Asian-Australasian Journal of Animal Sciences. 12:411-416.

Li ZJ, Yi GF, Yin JD, Sun P, Li D, and Knight C. 2008. effects of organic acids on growth performance, gastrointestinal pH, intestinal microbial populations and immune responses of weaned pigs. Asian-Australasian Journal of Animal Sciences. 21:252-261.

Liem A, Pesti GM, and Edwards HM. 2008. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. Poultry Science. 87:689-693.

Liesegang A, Loch L, Burgi E, and Risteli J. 2005. Influence of phytase added to a vegetarian diet on bone metabolism in pregnant and lactating sows. Journal of Animal Physiology and Animal Nutrition. 89:120-128.

Liesegang A, Reutter R, Sassi ML, Ristelli J, Kraenzlin M, Riond JL, and Wanner M. 1999. Diurnal variation in concentrations of various markers of bone metabolism in dogs. American Journal of Veterinary Research. 60:949-953.

Liesegang A, Sassi ML, and Risteli J. 2003. Diurnal variation in concentrations of various markers of bone metabolism in growing female goats and sheep. Animal Science. 77:197-203.

Liesegang A, Ursprung R, Gasser J, Sassi ML, Risteli J, Riond JL, and Wanner M. 2002. Influence of dietary phosphorus deficiency with or without addition of fumaric acid to a

diet in pigs on bone parameters. Journal of Animal Physiology and Animal Nutrition. 86:1-16.

Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, and Foster JW. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. Applied and Environmental Microbiology. 62:3094-3100.

Lipiec A, Grela E, Zürcher U, and Wenk C. 1994. Estimation of the fiber content of feedstuffs. Archives of Animal Nutrition. 47:53-62.

Lynch MB, Sweeney I, Callan IJ, and O'Doherty IV. 2007. Effects of increasing the intake of dietary beta-glucans by exchanging wheat for barley on nutrient digestibility, nitrogen excretion, intestinal microflora, volatile fatty acid concentration and manure ammonia emissions in finishing pigs. Animal. 1:812-819.

Mallin MA and Cahoon LB. 2003. Industrialized animal production - A major source of nutrient and microbial pollution to aquatic ecosystems. Population and Environment. 24:369-385.

Maribo H, Olsen LE, Mishler DR, Jensen BB, and Miquel N. 2000. Products for weaners: Benzoic acid or the combination of lactic acid and formic acid. Copenhagen: The National Committee for Pig Production. pp. 16. Report No.: 490

Mathew AG, Sutton AL, Scheidt AB, Forsyth GW, Patterson JA, and Kelly DT. 1991. Effects of a propionic acid containing feed additive on performance and intestinal microbial fermentation of the weanling pig. In: Verstegen MWA, Huisman J, and den Hartog LA. EAAP Publication. Pudoc, Wageningen, The Netherlands: 464-469.

Mertens DR. 2003. Challenges in measuring insoluble dietary fiber. Journal of Animal Science. 81:3233-3249.

Metzler B, Bauer E, and Mosenthin R. 2005. Microflora management in the gastrointestinal tract of piglets. Asian-Australasian Journal of Animal Sciences. 18:1353-1362.

Mikulski D, Zdunczyk Z, Jankowski J, and Juskiewicz J. 2008. Effects of organic acids or natural plant extracts added to diets for turkeys on growth performance, gastrointestinal tract metabolism and carcass characteristics. Journal of Animal and Feed Sciences. 17:233-246.

Min T, Kim J, Hyun Y, Sohn K, Heo K, and Han iK. 2001. Effects of environmentally friendly agents on growth performance, nutrient digestibility, nutrient excretion and carcass characteristics in growing-finishing pigs. Asian-Australasian Journal of Animal Sciences. 14:540-547.

Mosenthin R, Hambrecht E, and Sauer WC. 1999. Utilisation of different fibres in piglet feeds. In: Garnsworthy PC and Wiseman J. Recent advances in animal nutrition, 1999. Nottingham University Press, Nottingham (UK). Chapter 11: 227-256.

Mosenthin R, Sauer WC, Ahrens F, Delange CFM, and Bornholdt U. 1992. Effect of dietary-supplements of propionic acid, siliceous earth or a combination of these on the energy, protein and amino acid digestibilities and concentrations of microbial metabolites in the digestive-tract of growing pigs. Animal Feed Science and Technology. 37:245-255.

Mroz Z, Jongbloed AW, and Kemme PA. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. Journal of Animal Science. 72:126-132.

Mroz Z, Jongbloed AW, Partanen KH, Vreman K, Kemme PA, and Kogut J. 2000. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients, and manure characteristics in swine. Journal of Animal Science. 78:2622-2632.

Muhlbauer RC and Fleisch H. 1995. The diurnal rhythm of bone resorption in the rat - Effect of feeding habits and pharmacological inhibitors. Journal of Clinical Investigation. 95:1933-1940.

Namkung H, Li M, Gong J, Yu H, Cottrill M, and de Lange CFM. 2004. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Canadian Journal of Animal Science. 84:697-704.

Naumann C and Bassler R. 1997. Die chemische Untersuchung von Futtermitteln. Volume 3, ed. 3. VDLUFA-Verlag, Darmstadt, Germany.

Nehring K, Zelck U, and Schiemann R. 1965. Über die Zusammensetzung des Harns an organischen Inhaltsstoffen bei Rindern, Schafen und Schweinen. 2. Mitteilung. Zusammensetzung der organischen Harninhaltsstoffe bei Rindern, Schafen und Schweinen bei Verfütterung einiger gemischter Rationen. Archiv Für Tierernährung. 15:45-52.

Nicodemo MLF, Scott D, Buchan W, Duncan A, and Robins SP. 1998. Effects of variations in dietary calcium and phosphorus supply on plasma and bone osteocalcin concentrations and bone mineralization in growing pigs. Experimental Physiology. 83:659-665.

Noblet J and Le Goff G. 2001. Effect of dietary fibre on the energy value of feeds for pigs. Animal Feed Science and Technology. 90:35-52.

Noblet J and Shi XS. 1993. Comparative digestibility of energy and nutrients in growing pigs fed ad-libitum and adults sows fed at maintenance. Livestock Production Science. 34:137-152.

Omogbenigun FO, Nyachoti CM, and Slominski BA. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. Journal of Animal Science. 81:1806-1813.

Overland M, Granli T, Kjos NP, Fjetland O, Steien SH, and Stokstad M. 2000. Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing-finishing pigs. Journal of Animal Science. 78:1875-1884.

Overland M, Kjos NP, Borg M, Skjerve E, and Sorum H. 2008. Organic acids in diets for entire male pigs. Effect on skatole level, microbiota in digesta, and growth performance. Livestock Science. 115:169-178.

Overland M, Kjos NP, Borg M, and Sorum H. 2007. Organic acids in diets for entire male pigs. Livestock Science. 109:170-173.

Owens B, Tucker L, Collins MA, and McCracken KJ. 2008. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. British Poultry Science. 49:202-212.

Pallauf J and Rimbach G. 1997. Nutritional significance of phytic acid and phytase. Archives of Animal Nutrition. 50:301-319.

Partanen K. 2001. Organic acids - Their efficacy and mode of action in pigs. In: Piva A, Bach Knudsen KE, and Lindberg JE. Gut environment of pigs. The Nottingham University Press, Nottingham (UK). Chapter 13: 201-217.

Partanen K, Jalava T, and Valaja I. 2007. Effects of a dietary organic acid mixture and of dietary fibre levels on ileal and faecal nutrient apparent digestibility, bacterial nitrogen flow, microbial metabolite concentrations and rate of passage in the digestive tract of pigs. Animal. 1:389-401.

Partanen K, Jalava T, Valaja J, Perttila S, Siljander-Rasi H, and Lindeberg H. 2001. Effect of dietary carbadox or formic acid and fibre level on ileal and faecal nutrient digestibility and microbial metabolite concentrations in ileal digesta of the pig. Animal Feed Science and Technology. 93:137-155.

Partanen K and Mroz Z. 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews. 12:117-145.

Partanen K, Siljander-Rasi H, Alaviuhkola T, Suomi K, and Fossi M. 2002a. Performance of growing-finishing pigs fed medium- or high-fibre diets supplemented with avilamycin, formic acid or formic acid-sorbate blend. Livestock Production Science. 73:139-152.

Partanen K, Siljander-Rasi H, and Suomi K. 2002b. Dietary preferences of weaned piglets offered diets containing organic acids. Agricultural and Food Science in Finland. 11:107-119.

Pastuszewska B, Tomaszewska-Zaremba D, Buraczewska L, Swiech E, and Taciak M. 2007. Effects of supplementing pig diets with tryptophan and acidifier on protein digestion and deposition, and on brain serotonin concentration in young pigs. Animal Feed Science and Technology. 132:49-65.

Paulicks BR, Roth FX, and Kirchgessner M. 2000. Effects of potassium diformate (Formi (r) LHS) in combination with different grains and energy densities in the feed on growth performance of weaned piglets. Journal of Animal Physiology and Animal Nutrition. 84:102-111.

Payne RL, Lavergne TK, and Southern LL. 2005. A comparison of two sources of phytase in liquid and dry forms in broilers. Poultry Science. 84:265-272.

Philippe FX, Laitat M, Canart B, Farnir F, Massart L, Vandenheede M, and Nicks B. 2006. Effects of a reduction of diet crude protein content on gaseous emissions from deep-litter pens for fattening pigs. Animal Research. 55:397-407.

Pierce KM, Sweeney T, Brophy PO, Callan JJ, McCarthy P, and O'Doherty JV. 2005. Dietary manipulation post weaning to improve piglet performance and gastro-intestinal health. Animal Science. 81:347-356.

Piper P, Calderon CO, Hatzixanthis K, and Mollapour M. 2001. Weak acid adaptation: The stress response that confers yeasts with resistance to organic acid food preservatives. Microbiology. 147:2635-2642.

Pirgozliev V, Murphy TC, Owens B, George J, and McCann MEE. 2008. Fumaric and sorbic acid as additives in broiler feed. Research in Veterinary Science. 84:387-394.

Pisarikova B, Peterka J, Trckova M, Moudry J, Zraly Z, and Herzig I. 2007. The content of insoluble fibre and crude protein value of the aboveground biomass of *Amaranthus cruentus* and *A. hypochondriacus*. Czech Journal of Animal Science. 52:348-353.

Piva A, Anfossi P, Meola E, Pietri A, Panciroli A, Bertuzzi T, and Formigoni A. 1997. Effect of microcapsulation on absorption processes in the pig. Livestock Production Science. 51:53-61.

Piva A, Casadei G, and Biagi G. 2002. An organic acid blend can modulate swine intestinal fermentation and reduce microbial proteolysis. Canadian Journal of Animal Science. 82:527-532.

Plitzner C, Schedle K, Wagner V, Ettle T, and Windisch W. 2006. Influence of adding 0.5 % or 1.0% of benzoic acid on growth performance and urinary parameters of fattening pigs. Slovakian Journal of Animal Science. 39:69-73.

Pluske JR, Black B, Pethick DW, Mullan BP, and Hampson D. 2003. Effects of different sources and levels of dietary fibre in diets on performance, digesta characteristics and antibiotic treatment of pigs after weaning. Animal Feed Science and Technology. 107:129-142.

Poulsen HA. 2000. Phosphorus utilization and excretion in pig production. Journal of Environmental Quality. 29:24-27.

Radcliffe JS, Zhang Z, and Kornegay ET. 1998. The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. Journal of Animal Science. 76:1880-1886.

Radecki SV, Juhl MR, and Miller ER. 1988. Fumaric and citric acids as feed additives in starter pig diets - Effect on performance and nutrient balance. Journal of Animal Science. 66:2598-2605.

Rafacz-Livingston KA, Parsons CM, and Jungk RA. 2005. The effects of various organic acids on phytate phosphorus utilization in chicks. Poultry Science. 84:1356-1362.

Ramonet Y, Robert S, Aumaitre A, Dourmad JY, and Meunier-Salaun MC. 2000. Influence of the nature of dietary fibre on digestive utilization, some metabolite and hormone profiles and the behaviour of pregnant sows. Animal Science. 70:275-286.

Ravindran V and Kornegay ET. 1993. Acidification of weaner pig diets: A review. Journal of the Science of Food and Agriculture. 62:313-322.

Richard TH and Foster JW. 2003. Acid restistance in *Escherichia coli*. Advances in Applied Microbiology. 52:167-186.

Richards JD, Gong J, and de Lange CFM. 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs: Current understanding, possible modulations, and new technologies for ecological studies. Canadian Journal of Animal Science. 85:421-435.

Ricke SC. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Science. 82:632-639.

Risley CR, Kornegay ET, Lindemann MD, and Weakland SM. 1991. Effects of organic acids with and without a microbial culture on performance and gastrointestinal tract measurements of weanling pigs. Animal Feed Science and Technology. 35:259-270.

Risley CR, Kornegay ET, Lindemann MD, Wood CM, and Eigel WN. 1993. Effect of feeding organic acids on gastrointestinal digesta measurements at various times postweaning in pigs challenged with entertoxigenic *Escherichia coli*. Canadian Journal of Animal Science. 73:931-940.

Robertson JB and Van Soest PJ. 1981. The detergent system of analysis and its application to human food. In: James WPT and Theander O. The analysis of dietary fiber in food. Marcel Dekker, New York and Basel, USA and Switzerland. Chapter 8: 123-158.

Rodehutscord M, Abel HJ, Friedt W, Wenk C, Flachowsky G, Ahlgrimm HJ, Johnke B, Kuhl R, and Breves G. 2002. Review article - Consequences of the ban of by-products from terrestrial animals in livestock feeding in germany and the european union: Alternatives, nutrient and energy cycles, plant production, and economic aspects. Archives of Animal Nutrition. 56:67-91.

Roe AJ, McLaggan D, Davidson I, O'Byrne C, and Booth IR. 1998. Perturbation of anion balance during inhibition of growth of *Escherichia coli* by weak acids. Journal of Bacteriology. 180:767-772.

Roth FX, Eckel B, Kirchgessner M, and Eidelsburger U. 1992a. Influence of formic acid on pH-value, dry matter content, concentrations of volatile fatty acids and lactic acid in the gastrointestinal tract. 3. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 67:148-156.

Roth FX, Eidelsburger U, and Kirchgessner M. 1992b. Influence of fumaric acid, hydrochloric acid, sodium formate, tylosin and toyocerin on dry matter content, pH value, concentration of carbonic acids and ammonia in different segments of the gastrointestinal tract. 12. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. Journal of Animal Physiology and Animal Nutrition. 68:93-103.

Roth FX and Kirchgessner M. 1998. Organic acids as feed additives for young pigs: Nutritional and gastrointestinal effects. Journal of Animal and Feed Sciences. 7:25-33.

Russell JB. 1992. A review: Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. Journal of Applied Bacteriology. 73:363-370.

Sacakli P, Sehu A, Ergun A, Genc B, and Selcuk Z. 2006. The effect of phytase and organic acid on growth performance, carcass yield and tibia ash in quails fed diets with low levels of non-phytate phosphorus. Asian-Australasian Journal of Animal Sciences. 19:198-202.

Sauer WC, Mosenthin R, Ahrens F, and den Hartog LA. 1991. The effect of source of fiber on ileal and fecal amino acid digestibility and bacterial nitrogen excretion in growing pigs. Journal of Animal Science. 69:4070-4077.

Schroder JJ and Neeteson JJ. 2008. Nutrient management regulations in The Netherlands. Geoderma. 144:418-425.

Serena A, Hedemann MS, and Bach Knudsen KE. 2008. Influence of dietary fiber on luminal environment and morphology in the small and large intestine of sows. Journal of Animal Science. 86:2217-2227.

Simon O and Igbasan F. 2002. In vitro properties of phytases from various microbial origins. International Journal of Food Science and Technology. 37:813-822.

Simons PCM, Versteegh HAJ, Jongbloed AW, Kemme PA, Slump P, Bos KD, Wolters MGE, Beudeker RF, and Verschoor GJ. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. British Journal of Nutrition. 64:525-540.

Snow JL, Baker DH, and Parsons CM. 2004. Phytase, citric acid, and 1-alpha-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poultry Science. 83:1187-1192.

Souffrant WB. 2001. Effect of dietary fibre on ileal digestibility and endogenous nitrogen losses in the pig. Animal Feed Science and Technology. 90:93-102.

Staals STM, Bolhuis JE, van den Brand H, and Gerrits WJJ. 2007. Contribution of a straw bedding to digestible nutrient intake of pigs fed diets based on either native or pregelatinized potato starch. Livestock Science. 109:104-107.

Stanogias G and Pearce GR. 1985a. The digestion of fibre by pigs. 1. The effects of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage. British Journal of Nutrition. 53:513-530.

Stanogias G and Pearce GR. 1985b. The digestion of fibre by pigs. 2. Volatile fatty acid concentrations in large intestine digesta. British Journal of Nutrition. 53:531-536.

Starr MP and Reynolds DM. 1951. Streptomycin resistance of coliform bacteria from turkeys fed streptomycin. American Journal of Public Health. 41:1375-1380.

Stopforth JD, Sofos JN, and Busta FF. 2005. Sorbic acids and sorbates. In: Davidson PM, Sofos JN, and Branen AL. Antimicrobials in food. Taylor and Francis, Boca Raton, FL (USA). Chapter 3: 49-90.

Sutton AL, Mathew AG, Scheidt AB, Patterson JA, and Kelly DT. 1991. Effects of carbohydrate sources and organic acids on intestinal microflora and performance of the weanling pig. Verstegen MWA, Huisman J, and den Hartog LA. EAAP Publication. Pudoc Wageningen, The Netherlands: 422-427.

Swann MM, Baxter KL, and Field HI. 1969. Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. HMSO, London (UK).

Thacker PA, Campbell GL, and Groot Wassink J. 1992. The effect of organic acids and enzyme supplementation on the performance of pigs fed barley-based diets. Canadian Journal of Animal Science. 72:395-402.

Thacker PA, Rossnagel BG, and Raboy V. 2006. The effects of phytase supplementation on nutrient digestibility, plasma parameters, performance and carcass traits of pigs fed diets based on low-phytate barley without inorganic phosphorus. Canadian Journal of Animal Science. 86:245-254.

Theron MM and Lues JFR. 2007. Organic acids and meat preservation: A review. Food Reviews International. 23:141-158.

Thomlinson JR and Lawrence TLJ. 1981. Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs - Some field observations. Veterinary Record. 109:120-122.

Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, Tuckova L, Cukrowska B, Lodinova-Zadnikova R, Kozakova H, Rossmann P, Bartova J, Sokol D et al. 2004. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunology Letters. 93:97-108.

Tomokuni K and Ogata M. 1972. Direct colorimetric determination of hippuric acid in urine. Clinical Chemistry. 18:349-351.

Torrallardona D, Badiola I, and Broz J. 2007. Effects of benzoic acid on performance and ecology of gastrointestinal microbiota in weanling piglets. Livestock Science. 108:210-213.

Tsiloyiannis V, Kyriakis S, Vlemmas J, and Sarris K. 2001a. The effect of organic acids on the control of porcine post-weaning diarrhoea. Research in Veterinary Science. 70:287-293.

Tsiloyiannis V, Kyriakis S, Vlemmas J, and Sarris K. 2001b. The effect of organic acids on the control of post-weaning oedema disease of piglets. Research in Veterinary Science. 70:281-285.

Ullah AHJ and Sethumadhavan K. 1998. Differences in the active site environment of *Aspergillus ficuum* phytases. Biochemical and Biophysical Research Communications. 243:458-462.

Ullah AHJ and Sethumadhavan K. 2003. PhyA gene product of *Aspergillus ficuum* and *Peniophora lycii* produces dissimilar phytases. Biochemical and Biophysical Research Communications. 303:463-468.

Valencia Z and Chavez ER. 2002. Phytase and acetic acid supplementation in the diet of early weaned piglets. Effect on performance and apparent nutrient digestibility. Nutrition Research. 22:623-632.

van Beers-Schreurs HM, Nabuurs MJ, Vellenga L, Valk HJ, Wensing T, and Breukink HJ. 1998. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. Journal of Nutrition. 128:947-953.

van der Eerden L. 1998. Nitrogen on microbial and global scales. New Phytologist. 139:201-204.

van der Peet-Schwering CMC, Jongbloed AW, and Aarnink AJA. 1999a. Nitrogen and phosphorus consumption, utilisation and losses in pig production: The Netherlands. Livestock Production Science. 58:213-224.

van der Peet-Schwering CMC, Verdoes N, and Plagge JG. 1999b. Influence of benzoic acid in the diet on performance and urine pH of growing and finishing pigs. Raalte: Research Institute for Pig Husbandry, The Netherlands. pp. 24. Report No.: P 5.8, English translation of Report P1.212

van Kempen TATG. 2001. Dietary adipic acid reduces ammonia emission from swine excreta. Journal of Animal Science. 79:2412-2417.

Varel VH and Yen JT. 1997. Microbial perspective on fiber utilization by swine. Journal of Animal Science. 75:2715-2722.

Vasikaran SD. 2008. Utility of biochemical markers of bone turnover and bone mineral density in management of osteoporosis. Critical Reviews in Clinical Laboratory Sciences. 45:221-258.

Vats P and Banerjee UC. 2004. Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): An overview. Enzyme and Microbial Technology. 35:3-14.

Vervaeke IJ, Dierick NA, Demeyer DI, and Decuypere JA. 1989. Approach to the energetic importance of fibre digestion in pigs. II. An experimental approach to hindgut digestion. Animal Feed Science and Technology. 23:169-194.

Veum TL and Ellersieck MR. 2008. Effect of low doses of *Aspergillus niger* phytase on growth performance, bone strength, and nutrient absorption and excretion by growing and finishing swine fed corn-soybean meal diets deficient in available phosphorus and calcium. Journal of Animal Science. 86:858-870.

Wegener HC, Aarestrup FM, Jensen LB, Hammerum AM, and Bager F. 1998. The association between the use of antimicrobial growth promoters and development of resistance in pathogenic bacteria towards growth promoting and therapeutic antimicrobials. Journal of Animal and Feed Sciences. 7:7-14.

Wenk C. 1984. Einfluss der Einstreu auf die Verdaulichkeit des Futters bei wachsenden Schweinen. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde. 52:70-72.

Wenk C. 2000. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals - Review. Asian-Australasian Journal of Animal Sciences. 13:86-95.

Wenk C. 2001. The role of dietary fibre in the digestive physiology of the pig. Animal Feed Science and Technology. 90:21-33.

Whittaker X, Edwards SA, Spoolder HAM, Lawrence AB, and Corning S. 1999. Effects of straw bedding and high fibre diets on the behaviour of floor fed group-housed sows. Applied Animal Behaviour Science. 63:25-39.

Yen JT, Nienaber JA, Hill DA, and Pond WG. 1991. Potential contribution of absorbed volatile fatty acids to whole-animal energy requirement in conscious swine. Journal of Animal Science. 69:2001-2012.

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Curriculum vitae

Kathrin Bühler

Born May 2, 1979

Citizen of Winterthur ZH

1986 – 1994	Primary and secondary school in Uzwil SG
1994 – 1998	High school in Mörschwil (Gymnasium Untere Waid)
1998	Matura Typus B (Latin and English)
1998 – 2003	Study of Systematic and Ecological Biology at the Department of Biology at ETH Zurich, Switzerland
2003	Graduation as Dipl. Sc. Nat. ETH Diploma thesis: Die Nutzung verschiedener Habitatstypen durch das Reh *(Capreolus capreolus)* in einem vom Orkan 'Lothar' betroffenen Wald (The use of different habitat types by roe deer *(Capreolus capreolus)* in a forest affected by the storm 'Lothar') Swiss Federal Institut for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland
2003	Internship at the Macaulay Land Use Research Institute, Aberdeen (UK) Certificate of Proficiency in English in Cambridge
2004	Internship as curator at the Zurich Zoo Internship at the Research Institute of Organic Agriculture (FIBL), group of Veterinary Parasitology
2004 – 2009	Research Assistant at the Institute of Animal Sciences, ETH Zurich, in the group of Prof. Dr. C. Wenk (Nutrition Biology) Since 2006 Doctoral studies under the guidance of Prof. Dr. C. Wenk

Publications:

- Bühler, K. (2006) Workshop Summary (October 13, 2006). In: Sustainable animal health through eubiosis – Relevance for man. International Conference, 8. – 13. October 2006, Centro Stefano Franscini, Ascona, Switzerland:1-4.
- Bühler K., Liesegang A., Gebert S., Wenk C. (2007). Influence of dietary benzoic acid on bone stability and bone metabolism in growing-finishing pigs. Proceedings of the Society of Nutrition Physiology 16:97.

- Bühler K, Bucher B, Wenk C. (2009): Auswirkungen von Stroh und faserreichem Futter auf Mastschweine. Agrarforschung 16:34-38.