Sveriges lantbruksuniversitet
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Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

Organic acids in liquid feed for pigs

- palatability and feed intake

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Uppsala 2013

Degree project / Swedish University of Agricultural Sciences,
Department of Animal Nutrition and Management, **410**

Examensarbete, 30 hp
Masterarbete
Husdjursvetenskap
Degree project, 30 hp
Master thesis
Animal Science

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Handledare:
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Examiner: Jan Erik Lindberg
Omfattning:
Extent: 30 hp
Kurstitel:
Course title: Degree project in animal science
Kurskod:
Course code: EX0551
Program: Agronomprogrammet - Husdjur
Programme:
Nivå:
Level: Advanced A2E
Utgivningsort:
Place of publication: Uppsala
Utgivningsår:
Year of publication: 2013
Serienamn, delnr: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 410
Series name, part No:
On-line publicering:
On-line published: http://epsilon.slu.se
Nyckelord:
Key words: Organic acids, fermented liquid feed, acetic acid, lactic acid, acidifiers, palatability, growth performance, health status, pig

Organic acids in liquid feed for pigs - palatability and feed intake

1. Abstract

Fermented liquid feed is well known for its health promoting effects on piglets. High levels of lactic acid are desired in the feed together with low levels of acetic acid and certain biogenic amines. Limits for acetic acid have been suggested to be 30-40 mmol/kg to avoid a decreased palatability of the feed; however, few studies have been performed. The purpose of this trial was therefore to examine which levels of lactic acid and acetic acid that can be accepted in a fermented feed without affecting the feed intake and thus the weight gain of the pig. A total of 60 pigs (Yorkshire/ Hampshire) were used in a trial during two weeks, between 9-11 weeks of age. The trial was divided into two parts with 30 pigs in each. In the first trial, lactic acid was supplemented to the pig´s diet at levels of 0, 75, 100, 150 and 200 mmol/kg. In the second trial, acetic acid was added to the feed at levels of 0, 10, 50, 100 and 150 mmol/kg. The growth performance of the pigs was measured and a behavioral study was performed. No significant differences in feed consumption or daily weight gain could be seen between any of the levels of acetic or lactic acid. The only significant difference with lactic acid was found for feed conversion rate (FCR) between treatment L2 (100 mmol/kg) and L4 (200 mmol/kg) which had a significantly more efficient FCR on 1.77 and 1.80 respectively compared to the control group (0 mmol/kg) which had a FCR on 2.07 kg ($p=0.016$). There was also a significant difference in FCR between the pigs fed acetic acid ($p= 0.027$). The control group (0 mmol/kg) and the A2 group (50 mmol/kg) had a FCR of 2.0 kg compared to A4 (150 mmol/kg) which had an FCR of 1.77. In the behavioral study, an continues recording and one instantaneous scan sampling was performed. No differences in feeding or social behavior could be seen in the instantaneous scan sampling in either acetic or lactic acid. In the continues recording, there were some significant differences in feeding behavior in both trials but between the times of feeding and not between the inclusion levels of acids. That indicates differences in eating behavior during the day more than between the inclusion levels. Our results suggest that a fermented feed can contain lactic acid up to 200 mmol/ kg and acetic acid up to 150 mmol/ kg without affecting the feed intake or growth performance of the piglets negatively.

Sammanfattning

Fermenterat foder är känt för dess hälsofrämjande effekter på smågrisar. Höga nivåer av mjölksyra är önskade i fodret tillsammans med låga nivåer av ättiksyra och vissa biogena aminer. En gräns för ättiksyra har föreslagits av flera författare till 30- 40 mmol/kg för att undvika en minskad smaklighet på fodret, men få studier har utförts på vilka nivåer som faktiskt accepteras av grisen. Syftet med denna studie var därför att undersöka vilka nivåer av mjölksyra och ättiksyra som kan finnas i ett fermenterat foder utan att det påverkar foderintaget och därmed viktökningen hos grisar negativt. Försöket var uppdelat i två omgångar med 30 grisar i varje (Yorkshire/Hampshire). I den första omgången tillsattes mjölksyra till fodret i volymerna 0, 75, 100, 150 och 200 mmol/kg. I den andra omgången tillsattes ättiksyra till fodret i volymerna 0, 10, 50, 100 och 150 mmol/kg. Grisarnas foderkonsumtion samt tillväxt beräknades och en beteendestudie utfördes. Inga signifikanta skillnader i foderkonsumtion eller daglig viktökning kunde påvisas mellan de olika nivåerna av ättiksyra eller mjölksyra. Den enda signifikanta skillnaden som fanns hos grisarna som utfodrats med mjölksyra var i FCR där behandling L2 (100 mmol/kg) samt L4 (200 mmol/kg) hade signifikant effektivare FCR på 1,77 respektive 1,80 jämfört med kontrollgruppen (0 mmol/kg) som hade ett FCR på 2,07 (p = 0,016). Det fanns också en signifikant skillnad i FCR mellan de grisar som utfodrades med ättiksyra (p= 0,027). Kontrollgruppen (0 mmol/kg) och A2 (50 mmol/kg) hade ett foderutbyte på 2,0 kg jämfört med A4 gruppen (150 mmol/kg), som hade ett foderutbyte på 1,77 kg. I beteendestudien utfördes en kontinuerlig studie samt en frekvensstudie. I frekvensstudien kunde inga skillnader i beteendet runt utfodring eller sociala beteenden ses mellan behandlingarna hos grisar som utfodrats med vare sig mjölksyra eller ättiksyra. I den kontinuerliga studien hittades signifikanta skillnader i ätbeteende men bara mellan utfodringarna och inte mellan behandlingarna. Det tyder mer på att det finns skillnader i ätbeteende under dagen än mellan de olika nivåerna av tillsatta syror. Resultaten i denna studie indikerar därmed att ett fermenterat foder kan innehålla mjölksyra upp till 200 mmol/kg och ättiksyra upp till 150 mmol/kg utan att påverka foderintaget eller tillväxt av smågrisarna negativt.

Key words: Fermented liquid feed, organic acids, palatability, acidifiers, growth performance, health status, pig

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2. Introduction

In order to prevent bacterial resistance against antibiotics, EU banned the use of antibiotics as a growth promoter in the feed for pigs 2006. That led to an increased interest for alternative ways to improve the pigs' health and performance by using different kinds of feedstuff and additives. Producing pigs without antibiotics can be a big challenge for the producers since disease problems often increase and the general performance of the pig gets compromised. The problem is biggest during the immediate post-weaning period when the piglets are very susceptible to post-weaning diarrhea due to the sudden changes in feed composition and feed intake associated with weaning (Cranwell, 1995). Fermented liquid feed (FLF) has been suggested as an alternative to antibiotics. It is known for its good effects on the pigs' health and several reviews and papers have discussed its potential as a growth promoter to piglets (Brooks, 2008; Plumed- Ferrer & Von Wright, 2009; Missotten et al., 2010). The benefits of FLF mainly come from a reduction of the pH in the gut. A pH below 4.5 strengthens the potential of the stomach as first line of defense against possible pathogenic infections and inhibits the growth of enterobacteria such as Escherichia, Salmonella and Klebsiella in the gut. Another advantage is that FLF gives simultaneous provision of feed and water which may result in an easier transition from the sow's milk to solid feed for the piglets (Van der Wolf et al., 2001, Canibe & Jensen 2003; Brooks et al., 2003a, Canibe et al., 2007a). Enterobacteria, and especially Escherichia coli strains can be very pathogenic and cause intestinal upsets such as diarrhea, urinary tract infections, mastitis, arthritis and meningitis in animals as well as in humans. E.coli is therefore economically the most important pathogenic bacteria in the production of pigs and is not desired in the feed (Alexander, 1994; Fairbrother et al., 2005; Willey et al., 2009).

A low pH in the gut is beneficial in several ways. It will increase the activity of pepsin, which leads to a more efficient utilization of protein which is good both for the environment and for the economy of the production (Longland, 1991). It also increases the digestibility of nutrients through changes in villus height and depth in the small intestine in piglets (Scholten et al., 2002). The gastric emptying stimulus might also be delayed as a response to a low pH, which makes the food remain longer in the stomach and allows more time for digestion (Mayer, 1994; Scholten et al., 1999).

Fermented liquid feed is produced by incubating feed together with a liquid phase, normally water or a bi-product from the food or ethanol production. During the incubation, fermentative microorganisms produce different organic acids, mainly lactic and acetic acid that will reduce the pH in the gut (Beal et al., 2002; Lyberg et al., 2007; Olstorpe et al., 2008). Lactic acid and to a lesser extent acetic, generated by lactic acid bacteria (LAB) fermentations, have been shown to be the key elements in the inhibition of food-borne pathogens in fermented feed (Adams et al., 1988; Russel, 1992). If a feed is fermented spontaneously, there can be a big variation in the microflora of the gut depending on what microbes that are established in the feed. If the wrong microorganisms are established in the feed it might lead to a bad palatability and thus a decreased feed intake. Some microorganisms can be directly pathogenic for the pig like Salmonella (Russell et al., 1996; Jensen & Mikkelsen, 1998; Pedersen, 2001; Lawlor et al., 2002).

Organic acids can be added directly to a liquid diet, called an acidified diet. This gives the same anti-microbial activity through a low pH but without the risk for unsuccessful fermentation (Henry et al., 1985). Organic acids are rather expensive to purchase and the work is time consuming for the farmer since the acids need to be added every time of feeding. Organic acids can be both bacteriostatic and bactericidal depending on what concentrations they are added. They can effectively be used with other additives (Lückstädt & Mellor, 2011).

Fermented feed has several advantages over organic acids such as its probiotic qualities and increased digestibility of protein (Longland, 1991). It also gives an opportunity to utilize cheaper bi-products as feed instead of discarding them which is good both environmentally and economically (Scholten et al., 1999; Brooks et al., 2003a). A well fermented feed with lactic acid bacteria is therefore a very cost-effective mechanism to generate organic acids directly instead of adding them to a liquid diet which is both expensive and time consuming.

One of the main problems with FLF is the production of off-flavors since it generates in various results in weight gain of the pigs. High inclusion levels of acetic acid are suggested to be the main factor in lowering the palatability of the feed by several authors (Brooks et al., 2001; Beal et al., 2005) together with certain biogenic amines. However, there are no clear guidelines on what inclusion levels that are accepted without having a negative effect on the feed intake (Winsen et al., 2001; Brooks, 2003; Brooks et al., 2003 b; Brooks, 2008). The balance between what inclusion levels of acids that are needed for gaining the desired effects of the acids, without affecting the feed intake negatively is not known either.

If we get a better understanding of what affects the palatability of a feed negatively, and what levels of the substance/substances that may not be exceeded, it will hopefully reduce the variations in growth performance and make it easier and safer to use FLF as a health promoting feed. The purpose of this trial was therefore to examine what levels of lactic acid and acetic acid that can be accepted in a feed without affecting the feed intake and weight gain of the pig negatively. The levels of organic acids that were tested have been chosen from the levels that can occur naturally in a fermented feed.

3. Literature review

3.1 Liquid feed

Liquid feed (LF) is made by mixing a dry feed with a liquid just before feeding. Liquid bi-products from the human food industry such as skim milk and whey are often used in areas where it is abundant and cheap. Several million tons of liquid bi-products from the human food industry are annually used for pig feed in LF/FLF instead of being disposed as garbage and burned. This is good both environmentally and economically for the farmer (Scholten & Verdoes, 1997; Scholten et al., 1999; Brooks et al., 2003a). Commonly used bi-products come from the sugar industry, beer industry, wheat starch industry, fermentation industry, potato processing industry or dairy industry (Scholten et al., 1999). The most commonly used bi-products are stillage and whey. Some producers mix the feed with water for a better palatability and less dusting in the stables (McDonald et al., 2002).

Liquid feed demands a wet feeding system in the stables. It is an automatic system with one tank for the dry feedstuff and another tank for the liquid. Liquid and feed is mixed before feeding at a ratio of between 1:1.5 to 1:4 (Chae, 2000; Brooks et al. 2003a). Since the feed and liquid is mixed just before feeding, there is no time for the feed to ferment. Feed that is mixed with water and non-fermented bi-products, normally has a pH >6 which will allow a rapid proliferation of unwanted enterobacteria like salmonellas and coliforms and can spoil the feed or lead to disease. The recommendation is that the pH of the feed should be stabilized between 3.5-4.5 by acidification, fermentation or a combination of both (Russel et al., 1996; Geary et al., 1999; Brooks et al., 2001b; Plumed-Ferrer et al., 2005). If the feed is mixed with a bi-product that already is fermented, the pH of the feed will be lower.

One major problem for nutritionists and pig producers is the variations in nutritional composition of the bi-products, which can make it difficult to calculate an optimal diet. If bi-products are going to be used efficiently, the diets have to be reformulated from one batch to another to compensate for the changes that can occur in composition. Despite the variability of liquid products, they can be used efficiently and without restraining to the pigs performance if diets are formulated accurately (Brooks & McGill, 1995).

3.2 Fermented liquid feed

Almost all combinations of feed ingredients will ferment if soaked in water. Most raw materials have a natural flora that mainly consists of LAB and yeast but many also have an undesired microflora of moulds, salmonellas and coliforms. The quality of a feed depends on what microorganisms that are established and what fermentation products they produce. Normally LAB is the dominant microflora in a fermented feed but some feed components, such as whey or bi-products from the brewing and ethanol industry can be dominated by yeast, which might have an adverse effect on the pigs' health and performance. The risk for domination of yeast is even larger if the feed is kept at low temperatures (Brooks et al., 2001). During fermentation, sugar and starch in the feed are transformed by microbes in the gut to fermentation products like organic acids and ethanol (Prescott et al., 1996). For each feed, characteristic species of LAB and yeast are developed. The composition and growth rate of the species can be various depending on: substrates, temperatures, fermentation periods, feed components, acid concentration, pH and buffering capacity of the feed (Canibe et al., 2001; Canibe et al., 2007a; Olstorpe et al., 2008).

In FLF, the fermentation can start spontaneously, through backslopping or be induced. A feed that is fermented spontaneously is mixed together with a liquid and left to ferment a certain time before feeding. The fermentation process can be seen in figure 1. It starts the moment liquid and feed is mixed and the process consists of two phases (Canibe & Jensen, 2003). The first phase of the fermentation is characterized by low concentrations of LAB and therefore low levels of lactic acid and yeast. There is normally a strong inflorescence of enterobacteria and the pH is high. The second phase is characterized by higher levels of LAB, yeast and other bacteria that produce organic acids such as acetic acid, lactic acid, propionic acid and butyric acid. These lower the pH in the gut and reduce or inhibit the growth of enterobacteria (Mikkelsen & Jensen., 1997, Winsen et al., 2001; Canibe et al., 2007a). There are different properties of acids depending on what microorganisms that are established. High levels of lactic acids are most desired since they bring the biggest threat against enterobacteria including salmonella through a low pH. Studies have shown that a concentration of 70 mmol/kg lactic acid is bacteriostatic to salmonella and concentrations above 100 mmol/kg are bactericidal (Beal et al., 2005). The desired characteristics of a fermented feed can be seen in figure 2. Brooks (2008) wanted to add a third phase to the fermentation process (the steady phase) in which the LAB population and pH stabilizes (figure 1).

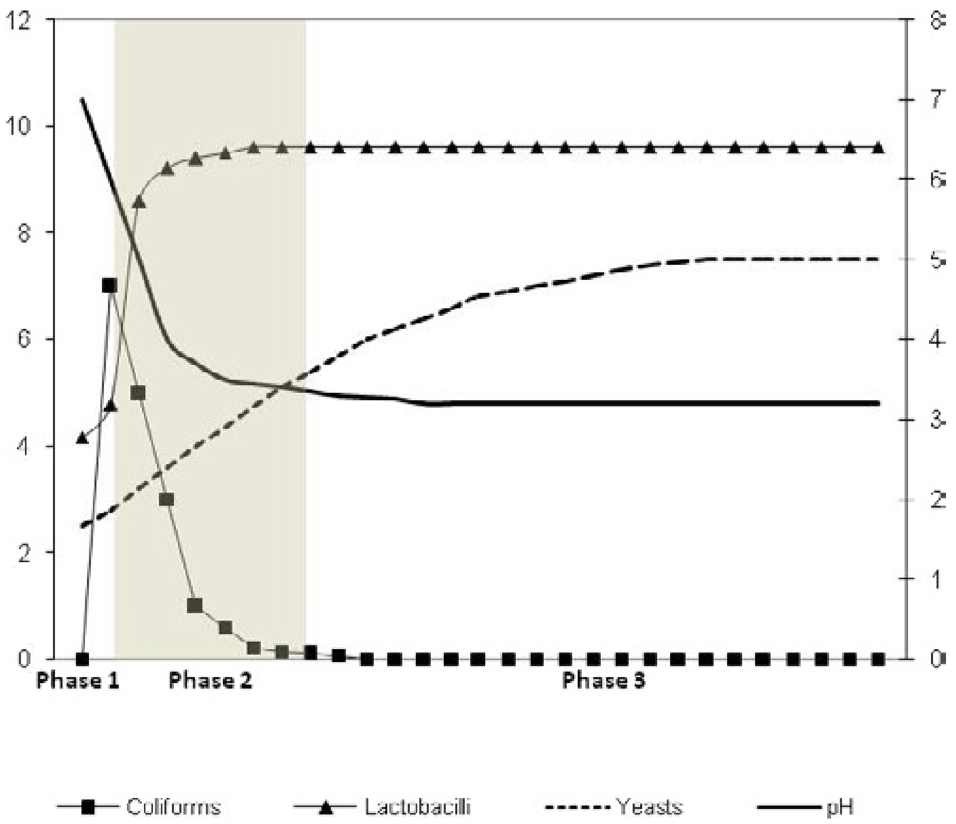

Figure 1. Phases in fermentation of FLF (Brooks et al., 2008)

Uncontrolled spontaneous fermentation is quite unreliable and can lead to high concentrations of undesired fermentation products such as acetic acid and biogenic amines that might reduce the palatability of the feed and thus the feed intake (Brooks et al., 2003b; Niven et al., 2006). It can also result in a big variation of lactic acid concentrations between 0-140 mmol/kg and can therefore not be relied upon to produce bactericidal levels of lactic acid (>100 mmol/kg; Beal et al., 2005).

In order to get a well fermented feed from the beginning and avoid the first phase of fermentation, many authors propose the addition of a LAB strain as a starter culture to the feed (Canibe et al., 2001; Van Winsen et al., 2001; Beal et al., 2002). The starter culture should preferably be a strain of LAB with a high capacity for lactic acid production and active against enteric pathogens (Van Winsen et al., 2001, Plumed-Ferrer & Von Wright 2009). The most commonly used strains are Lactobacillus plantarum and Pediococcus spp. (Van Winsen et al., 2001; Plumed-Ferrer et al., 2005; Missotten et al., 2007). Lactobacillus plantarum grow very well in pig feed, likely since the species usually is isolated from cereals and other plant materials (Plumed-Ferrer & Von Wright, 2009).

The fermentation of a feed can be started by leaving a certain amount of feed in the tank to work as a starter culture for the next batch (backslopping). There is almost always some backslopping in LF systems due to difficulties in getting the pipes clean and sterilized. Most LF systems can be cleaned by flushing water through the pipes, but it is hard to get them properly cleaned and there is almost always some feed left in the pipes. If the remaining feed goes bad or contains undesired microorganisms, it can work as a bad starter culture for the feed and can result in off-tastes, growth of pathogenic bacteria or give the wrong properties to the feed. If backslopping comes from a well fermented feed, it can work excellent as a starter culture for the next batch (Plumed- Ferrer & von Wright, 2009). Studies suggest that a backslopping of between 20% (Moran et al., 2006) and 25% (Plumed- Ferrer et al., 2005) of the feed should be enough to maintain a proper fermentation. However, the effect depends on several factors such as temperature, turnover etc.

1. **pH < 4.5**
   (Winsen et al., 2001; Mcdonald et al., 2002; Plumed- Ferrer et al., 2005).
2. **LAB concentration above 9 log 10** (Winsen et al., 2001).
3. **Lactic acid concentration >100 mmol/kg**
   (Beal et al., 2002; Brooks et al., 2003b).
4. **Acetic acid < 40 mmol/kg** (Winsen et al., 2001)
   < 30 mmol/kg (Brooks; 2003, 2003b, 2008)
5. **Ethanol concentration < 0.8 mmol/kg** (Winsen et al., 2001)

Figure 2. Desired characteristics for FLF

In some cases, the fermentation has never reached the second phase and no reduction of coliform bacteria has been seen when comparing LF to FLF (Mikkelsen & Jensen, 1998; Scholten et al., 2002). The reason for this could be due to insufficient amounts of organic acids from the fermentation and thus a pH to high to reach bactericidal levels (Russel et al., 1996). It could also mean that the feed has been dominated by yeast (Johannsen et al., 2000). Yeast can adapt to an acidic environment and can therefore grow well in fermented feed. Some yeast species are beneficial for the pig's gastro- intestinal health and are used as prebiotica in the feeding of pigs (Jensen & Mikkelsen, 1998; Bontempo et al., 2006), but other species can reduce the palatability of the feed (Piper et al., 1998; Schaller et al., 2005). Yeast fermentation converts carbohydrates to alcohol and produces carbon dioxide as a bi-product.

This can reduce the energy value of the feed and affect the growth performance of the pigs negatively (Binder et al., 1990). Another explanation is that the enterobacteria have produced stress-proteins for protection during the first phase and therefore remain in the feed (Brooks et al., 2001).

As mentioned earlier, different fermented feeds contain different levels of organic acids. Analyses of FLF from 15 Danish units showed an average lactic acid concentration of 62± 39 mmol/kg with only 1 of 15 units exceeding 100 mmol/kg (the limit needed to exclude pathogens (Anon, 2005)). A study of the spontaneous fermentation of a mixture between barley and wheat resulted in only 40 mmol/kg lactic acid and 13 mmol/kg acetic acid with pH 5.0 (Canibe et al., 2007). The fermentation of 100 wheat and barley samples in the UK produced lactic acid concentrations with a mean of 59.6± 40 mmol/kg. Only 3% of the fermentations produced more than 75 mmol/kg lactic acid after 24 h fermentation (Beal et al., 2005). A feed fermented with wet wheat-distillers grains had very low concentrations of lactic acid of 24 mmol/kg and contained 117 mmol/kg acetic acid and 33 mmol/kg succinic acid (Lyberg et al., 2007). The typical concentration of acetic acid in FLF prepared with compound pig feed is however between 20-40 mmol/kg feed (Mikkelsen & Jensen, 2000; Scholten et al., 2001; Van Winsen et al., 2001; Canibe & Jensen, 2003; Canibe et al., 2007). However, levels of 54 mmol/kg feed have been reported (Pedersen, 2001).

3.3 Organic acids

Organic acids are wildly distributed in nature as constitutes of plants or animals. They have been used for decades in commercial compound feeds as effective preservatives of feedstuff due to their ability to acidify feed and digesta and its ability to inhibit the growth of microbes (Schutte, 2011). The addition of acidifiers to liquid feed or drinking water is a rather common practice in production units. The acidification has to be repeated each feeding which can be expensive for the farmer, both in terms of work and in purchase of the acids (Geary et al., 1999). The supplement of organic acids was initially targeted for weaned piglets since they often have problems with post-weaning diarrhea. Several studies indicate that dietary acidification also might be beneficial for the performance of fattening pigs. Research implies an improved apparent ileal digestibility of protein and amino acids (Mosenthin et al., 1992; Mroz et al., 1997) and an improved absorption of minerals (Jongbloed & Jongbloed, 1996).

Acidifying products used in pig diets can be organic or inorganic acids and their salts. The following acidifiers are officially approved in the EU: Na-sorbate, Ca-sorbate, K-sorbate, tartaric acid, Na-tartarate, K-tartarate, NaK-tartarate, NH3-formate, Na-formate, NH3-propionate, Na-propionate, K-acetate, Ca-acetate, Na-diacetate, Na-citrate, K-citrate, K-lactate, benzoic acid and Na-benzoate. These acidifiers can be administered individually or as a mix in the feed or the drinking water (Mroz, 2005). Combinations of acids are generally giving better results than single acids since it broadens the spectrum of antimicrobial activity. This is due to the different dissociation properties of these acids at various locations in the pig's digestive tract. Acids can be in a solid or a liquid phase. Solid acidifiers are easier to handle, whereas the liquid forms may be volatile during spraying (up to 20%). They can also give an unpleasant odor and be corrosive (Hardy, 2002; Mroz, 2005). There is a discussion about what acids are most effective as acidifiers. Formic (Bolduan et al., 1988) and lactic acid (Sutton et al., 1991; Knarreborg et al. 2002) are effective against E. coli and salmonella and it seems that supplementing lactic acid to dry diets increase the feed intake in pigs. Several authors claim that the addition of organic acids exert a small, positive influence on the apparent total tract digestibility of crude protein and energy (Thomlinson & Lawrence, 1981; Ratcliffe et al., 1986; Partanen & Mroz, 1999). Among the organic acids, fumaric, lactic, citric, and phosphoric acids the most frequently used in mixed acid products (Tung & Pettigrew, 2006). Some of the most recently used inorganic acids are hydrochloric, sulfuric and phosphoric acids. They are cheaper than organic acidifiers, but are very corrosive and hazardous liquids in their pure state. Salts from organic acids such as Ca-formate and Ca-propionate are also used under the classification feed preservatives.

In pig diets, organic acids show effect in the gastrointestinal tract, mainly the stomach and small intestine. Their most important quality is to inhibit microorganisms through a decreased pH in the stomach but they also stabilize the hygiene as well as the nutritional quality of the feed. They also reduce the buffering capacity of the feed and improve the intestinal flora of the pig (Geary et al., 1999; Mroz, 2005; Plumed-Ferrer et al., 2005). Organic acids consist of one proton and one anion. The effect of the proton of an organic acid is an acidification of the feed and digesta while the anion inhibits the growth of microbes (Schutte, 2011). Organic acids have the ability to change from undissociated form to dissociated form, depending on the pH around them, which makes them effective antimicrobial agents. When an acid is in its undissociated form, it can diffuse through the membrane of bacteria into their cell cytoplasm. The acid dissociates inside the cell and suppresses cell enzymes such as decarboxylases and catalase. The nutrient transport systems of the microorganism will also be inhibited (Lueck, 1980). Depressed enzyme activity together with inefficient nutrient transport will slow down the metabolism of the microbes, which either kills or inhibits them (Lückstädt & Mellor, 2011).

Another important effect of a low gastric pH is that it optimizes the pepsin activity which improves the digestibility of protein and decrease the rate of gastric emptying. Organic acids also stimulate exocrine pancreatic secretion of enzymes and bicarbonate, which will improve the protein and fat digestion (Lückstädt & Mellor, 2011).

3.3.1 Lactic acid

Lactic acid occurs naturally in several feedstuffs and is one of the oldest preservatives. It is an end product from the fermentation of sugar (Stryer, 1988). Many members of the order lactobacillales produce lactic acid as their major or only fermentation product and are therefore called LAB. Some of the major members of this group are Streptococcus, Pediococcus, Enterococcus and Lactobacillus.

The main advantage of having LAB in the feed is because they reduce the pH in the gut (Willey et al., 2009). They grow optimally under acidic conditions at a pH between 4.5- 6.4 and are therefore often the major bacteria left at the end of a fermentation process due to their ability to live in an acidic environment. They are less sensitive to the pH differential across the cell membrane than other bacteria and can therefore remain unaffected by it. Lactic acids antimicrobial action is mainly directed against bacteria since many moulds and yeasts can metabolize it (Foegeding & Busta, 1991).

3.3.2 Acetic acid

Acetic acid is produced through an oxidation of alcohol mainly by yeast but also heterofermentative LAB. It is believed to cause the biggest reduction in palatability of a feed and is therefore wished to be low. Acetic acid inhibits the growth of many species of bacteria, and to a lesser extent of yeasts and moulds (Foegeding & Busta, 1991). The minimum concentration of acetic acid that prevents E. coli is 0.5%, which is five times higher than that of formic acid (Frank, 1994).

Table 1. Energy content and pKa of lactic and acetic acid.

Organic acid	pKa	GE (MJ/kg)
Lactic acid	3.83	15.1
Acetic acid	4.76	14.6

3.3.3 Factors affecting the effect of organic acids

The impact from organic acids on the animal can be very various due to several factors such as: type and pKa of acid, inclusion rate and dose of supplemented acids, composition of the diet, buffering capacity, palatability of the feed, intrinsic acid activity, hygiene and welfare standards of animals, maternal immunity by vaccinations against pathogens etc. (Partanen & Mroz, 1999; Strauss & Hayler, 2001; Decuypere & Dierick, 2003; Morz, 2005).

The composition of the diet affects the response to acidifiers. It seems that the response is better when pigs are fed simple diets, rather than complex diets containing milk products. This is probably since the LAB species convert lactose from the milk products to lactic acid in the stomach, thus creating an acidic environment anyway which reduce the need for acidifiers (Weeden et al., 1990).

The buffering capacity of a feed affects the impact of the acids. Buffering capacity is the ability of a liquid to absorb and neutralize the added acid without significantly changing the pH. It varies substantially between different feedstuffs and is low in cereals and high in diets containing high concentrations of proteins and minerals. A high buffering capacity limits the acids secretory capacity in the pig. That means that a feed with a high buffering capacity demands an increased addition of acids to the diet in order to change the pH. It is difficult to decide the buffering capacity of a feed, which is one reason for the difficulties of deciding the proper amount of acid needed. The pH-lowering effect of different organic acids is reduced in the following order: tartaric acid >citric-acid >malic acid >fumaric acid >lactic and formic acids >acetic acid >propionic acid (Partanen & Mroz, 1999; Tung & Pettigrew, 2006).

Acids efficiency is decided by their pKa-value. The pKa-value is a quantitative measure that indicates how much an acid can be protolized in a solution. It is equivalent to the pH at which 50% of the acid is dissociated. The absorption rate of the acid depends on the pH together with the pKa. When the luminal pH is below the pKa-value, short-chained fatty-acids are absorbed very quickly. Normally the pH in ileum, caecum and colon is higher than 6.5, which means that most short-chained fatty acids remain in their dissociated form and are poorly absorbed (Chang & Rao, 1994). Organic acids with high pKa-values are generally more efficient antimicrobial acids than acids with a lower pKa (Foegeding & Busta, 1991). The pKa-value of lactic and acetic acid can be found in table 1. Since acetic acid has a higher pKa than lactic acid, acetic acid is expected to be more antimicrobial efficient in a well buffered feed with a rather low pH (6-4). This since a greater proportion of the acid would be undissociated. Lactic acid on the other hand, is the stronger acid which will produce a lower pH and thus increase the undissociated fraction and make acetic acid a stronger antimicrobial agent. The balance between these factors depends on the buffering capacity of the feed and the amount of acid used (Adams & Hall, 1988).

The effects of an acidification on a diet seem to differ with the age of the pig. Results show that it seems most beneficial during the first days after weaning. The stomach of a newly weaned piglet has not matured yet physiologically and may not secrete enough acid to aid the digestion of solid feed. The response to an acidification is therefore often most evident immediately after early weaning and declines with age (Ravindran & Kornegay, 1993). Giesting et al. (1991) claim that acidification is most effective during the first 2 to 4 weeks after weaning.

3.4 Possible effects of fermented liquid feed and organic acids

3.4.1 Health

When FLF has been fed to growing pigs and piglets, it has repeatedly been reported to improve the gastrointestinal health of the animals compared to dry feed (DF) or non fermented liquid feed (NFLF) (Van Winsen et al., 2001 b; Canibe & Jensen, 2003; Lindecrona et al., 2003). As mentioned earlier, the most important health benefits from feeding pigs with an acidified diet or FLF come through the decrease in pH. Many studies has shown that pigs fed with FLF have a lower pH in the stomach together with a reduced amount of enterobacteria in the whole gastrointestinal tract compared to pigs fed with dry feed or non fermented liquid feed (Mikkelsen & Jensen 1998; Van Winsen et al., 2001b; Scholten et al., 2002; Canibe & Jensen, 2003). The pH in the small intestine is normally higher when feeding FLF compared to NFLF. This could depend on the increased production of pancreatic juice as a consequence of the low pH in the gut (Jensen & Mikkelsen, 1998; Canibe & Jensen 2003; Plumed- Ferrer & von Wright, 2009).

Both fermented feed and acidified feed reduces the incidence of salmonella (Van der Wolf et al., 1999). Salmonella control is of high priority in the European pig production and can cause major economical losses through veterinary and hygiene costs as well as lower productivity. It is therefore in both the producer's and consumer's interest to prevent the spreading of salmonella. Several serotypes of salmonella are resistant to antibiotics which have made it even more important to prevent contamination. Biosecurity is the most important factor, but a good gut health is increasingly being shown to be very effective against salmonella. Van Winsen et al. (2001) showed that the concentration of lactic and acetic acid was responsible for the reduction of salmonella in fermented pig feed. When the concentration of lactic acid was 200 mmol/kg there was a reduction in Salmonella typhimurium as acetic acid concentration increased from 10 to 30 mmol/kg. The author suggested that in order to reduce/eliminate salmonella from a feed it was necessary to have a concentration of 150 mmol/kg lactic acid or 80 mmol/kg acetic acid (with an appropriate pH <4.5). Beal et al. (2002) found that salmonella died when the levels of lactic acid were above 75 mmol/kg and the pH <4.5. The low pH is crucial, and if a feed is not acidified, either by fermentation or by acidification, salmonella will multiply in liquid pig feed. It is therefore important to acidify the feed even at low temperatures in the control of salmonella (Van Winsen et al., 2001). It is worth noting that the European Food Safety Authority Scientific Panel on Biological Hazards has acknowledged the contribution that fermented feed can limit the risk of salmonella in pig production (EFSA, 2006).

Weaning is associated with both environmental and nutritional stress for the piglets and often results in a reduced feed intake and weight gain. The immunological status of a piglet is also low after weaning since its active immune system only is beginning to develop, and the colostrum from the sow with its passive immunity drastically is decreased (Gaskin & Kelley, 1995). The stress at weaning often disturbs the intestinal microflora and lead to an increased amount of Enterotoxigenic strains of E. coli (ETEC). This can give the piglet's problem with post-weaning diarrhoea syndrome (PWDS) (Tsiloyiannis et al., 2001). A lower pH in the gut is therefore especially important for piglets since their gastric pH not is low enough, which makes them more susceptible to bacterial infections. A low pH together with a good gastro intestinal tract (GIT) health and less enterogenic bacteria can result in fewer diarrheas for the piglets (Højberg et al., 2003). Several studies claim that LAB is the most useful tool to control PWDS (Thomlinson & Lawrence, 1981; Tsiloyiannis et al., 2001). Newly weaned piglets also seem to find liquid feed more palatable and an easier transition from the sow's milk to solid food. It is also a good way to make sure the piglets get a high and regular intake of both feed and water after weaning (Van Winsen et al., 2001b; Scholten et al., 2002; Canibe & Jensen, 2003).

The villus in the small intestine of piglets is also affected by FLF. When the piglets are weaned, there is a reduction of the villus height and depth of the crypts in the small intestine. This gives a reduced ability to absorb and digest nutrients, which causes a reduction in growth performance (Nabuurs, 1995; Pluske et al., 1996). Feeding piglets with FLF has shown a higher villus height, a better villus shape and a higher villus/crypt ratio which instead will increase the digestibility of nutrients (Scholten et al., 2002). No information has been found whether organic acids will bring similar effects on the villus or not. But it is well known that short chained fatty acids such as acetic and propionic acid, produced by microbial fermentation of carbohydrates stimulate epithelial cell proliferation (Schutte, 2011).

3.4.2 Performance

There have been quite variable results on the effect of FLF on growth performance on pigs (Russell et al., 1996; Mikkelsen & Jensen, 2000; Pedersen, 2001; Canibe & Jensen, 2003). Many studies have focused on piglets between 7 and 32 days due to their limited ability to maintain a low gastric pH. Feeding FLF to piglets has generally been reported to improve their growth performance compared to a dry diet (Russell et al., 1996). Jensen & Mikkelsen (1998) summarized the results from 17 different feeding trials on newly weaned piglets and came to the result that fermented liquid diets increased the daily gain by 13.4% compared with non fermented liquid diets. There was no difference in FCR.

Some studies have not shown any differences in growth performance on piglets, which can depend on the differences in feeding behavior between older and newly weaned piglets (Lawlor et al., 2002; Pederson et al., 2005). Newly weaned piglets normally leave more feed waste when fed with LF than the older piglets. Scholten et al., (2002) could show some improvement when feeding FLF compared to NFLF but some authors have not seen a difference at all (Mikkelsen & Jensen, 1998; Geary et al., 1999).

The effect of FLF on growth performance during the growing period has also resulted in various results. Many researchers have seen an improvement in growth when feeding LF (NFLF or FLF) compared to DF (Canibe & Jensen, 2003; Dung et al., 2005). Data from Dutch pig farms showed that farms feeding liquid diets with fermented bi-products to pigs showed an improved daily gain and feed conversion ratio compared to pigs fed liquid diets without these bi-products (Scholten et al., 1998). A Danish study fed growing pigs fermented grain and increased the productivity due to a higher daily gain (33 g/day) and an improved feed conversion (0.15 FUp/kg gain) (Pedersen et al., 2002). On the other hand, a study from

UK fed a fermented barley/wheat mixture in growing–finish diets reduced the growth rate from 844 to 818 g/d, which also increased the production costs (MLC, 2005).

Negative results on performance have been suggested to depend mainly on the production of high levels of off-flavors and the disappearance of amino acids during fermentation of liquid feed. Microbial fermentation in FLF can result in a loss of free amino acids such as lysine, threonine and methionine, which can give a negative effect on growth performance (Brooks et al., 2001; Pedersen, 2001; Pedersen & Jensen, 2005; Canibe et al. 2007). These losses are mostly depending on the utilization of amino acids by endogenous microorganisms that are present in fermented liquid feed, especially coliform bacteria (Niven et al., 2006). A Danish study showed that 17% of added lysine might be lost from fermented liquid feed after 24 h of storage (Pedersen et al., 2002). Pedersen (2001) on the other hand, did not see any change in composition of amino acids bound in feed protein during fermentation. He came to the conclusion that only synthetic lysine was decomposed during fermentation. Niven et al. (2006) did not see any decrease of free lysine during the first 4 hours of incubation of a liquid feed. However, after 21 hours of incubation, 86% of the free lysine was disappeared. They claim that the losses of synthetic lysine and possibly other amino acids can be minimized if the lactic acid concentration is >100 mmol/kg quickly enough or if the amino acids are added after the pH has dropped to <4.5. However, some authors have found an increased uptake of some amino acids. Lyberg et al. (2006) found that the utilization of several amino acids such as leucine, lysine, methionine and phenylalanine was more efficient in pigs eating FLF than in pigs fed with DF or LF.

The results on growth performance of organic acids to piglets are also very various. According to a review by Partanen and Mroz (1999), there is evidence that the addition of organic acids improves the performance of young piglets in form of an improvement in feed conversion rate (FCR) of the pig. They also came to the conclusion that formic acid and formats are the most effective acids in promoting growth, followed by fumaric acid. But Kil (2004) and Tsiloyiannis et al. (2001) observed that lactic acid gave the best results on growth performance on weaner piglets compared to piglets fed other acidifiers. Several researchers have found positive results on the addition of acids to a diet (Burnell et al., 1988; Boling et al., 2000; Tsiloyiannis et al., 2001; Kil, 2004). Some author's found no or negative responses (Eidelsburger et al., 1992, Manzanilla et al., 2004). The highly inconsistent responses to acids are due to the different factors mentioned earlier but according to Partanen & Mroz (1999), the feed consumption explains 74% of the variations in growth performance.

Janson et al. (2011) studied the effect of organic acids as a feed complement on FCR and found that it improved by 4.2% compared to pigs without supplemented acids. The daily live weight gain was improved by 5.8%. When acetic acid has been supplemented to the diet it has not resulted in either an increased average daily gain or FCR (Zhang et al., 1986; Roth & Kirchgessner, 1988).

3.4.3 Digestibility

Studies suggest that fermentation has a positive effect on the utilization of the feed in several ways. A low gastric pH optimizes the pepsin activity through a stimulation of the conversion of inactive pepsinogen to active pepsin. A higher activity of pepsin leads to an improved digestibility of protein (Longland, 1991). Pepsin has two pH optima, at 2 and 3.5. The activity decreases when pH is above 3.6 and is inactive at pH 6 (Kidder & Manners, 1978). Fermentation of a feed generally improves the dry matter and protein digestibility by 3-8%

according to Hong & Lindberg (2007) and Dung et al. (2005). The gastric emptying stimulus might also be delayed as a response to a low pH. That means that the feed remains longer in the stomach, which allows more time for digestion and a higher utilization of the feed (Mayer, 1994; Scholten et al., 1999).

Lyberg et al. (2006) and Pedersen et al. (2010) showed that fermentation improves the apparent total tract digestibility (ATTD) of organic matter. The digestibility of a feed is largely depending on the type and amount of fiber it contains. Pigs have a limited ability to break down and utilize the fibers and especially young piglets where the starch-degrading enzymes have a low activity level. Lyberg et al. (2006) found a decreased proportion of neutral detergent fiber (NDF) in fermented feed. NDF consist of ligning, cellulose and hemicelluslose. They also found an increased ileal digestibility of organic matter and NDF. Hong & Lindberg (2007), also found a higher ileal digestibility of NDF in fermented feed compared to untreated feed and cooked feed. The reason for this could be that an increased microbial activity may increase the fiber solubility and therefore decrease the appearance of fibers in the gastro- intestinal tract GIT (Pedersen & Lindberg, 2003).

Organic acid anions can improve the digestibility and absorption of Ca, P, Mg and Zn by binding to them and thereby reducing the emission of supplemental minerals and nitrogen (Roth et al., 1998a, b; Partanen & Mroz, 1999). The acids can form complexes with different cations and can therefore act as chelating agents and might increase the absorption of minerals. Some data indicates that acidifiers can act together with phytase to improve P and Mg digestibility (Jongbloed et al., 2000; Omogbenigun et al., 2003). About 60-70% of the P in plant based ingredients in pig feed occurs as phytat P. Phytat is only partly available for pigs due to their inability to produces phytase, which is needed to hydrolyse the phytate-molecule. A big part of the P will therefore be excreted in the feces which is both economically and environmentally inefficient (Cromwell, 1992; Ravindran et al., 1994; 1995). Lyberg et al. (2006) found that the intestinal uptake of P were higher in pigs fed with FLF compared to pigs fed with DF or LF. The fermentation made 80% of the feed-bound P available for the pig. LF made only 10% of the P available. Lückstädt & Mellor (2011), claim that organic acids stimulate the exocrine pancreatic secretion of enzymes and bicarbonate which will improve the protein and fat digestion.

Results from just a few experiments indicate that organic acids also improve the apparent ileal digestibility of amino acids in growing pigs, but not in weaned piglets. They might also influence gut morphology, increasing villous height and therefore the absorptive capacity, which could contribute to improved protein, energy and mineral absorption (Partanen & Mroz, 1999). Lyberg et al. (2006) found that the utilization of several amino acids such as leucine, lysine, methionine and phenylalanine was more efficient in pigs eating FLF than in pigs fed with DF or LF.

3.4.4 Palatability

One key factor for a profitable pig production is of course to maintain a high growth rate and feed intake of the pig. A good health status is very important when it comes to feed consumption but the palatability of the diet is crucial. It is therefore very important to have the accurate knowledge of the different dietary factors that can affect the palatability of diets for pigs. Even though the growth- promoting effects of organic acids are considered to depend to a big extent on how they improve the feed intake there are few studies on the effect they have on the palatability of the feed. It is considered that high dietary levels of certain organic acids can reduce the palatability of the diet substantially and affect the pigs feed intake. In a study when pigs had access to both an acidified (citric and fumaric acid) and non-acidified diet, the consumed significantly more of the non-acidified diet (Partanen & Mroz, 1999).

In general, lactic acid is considered to enhance the palatability of the feed even at higher inclusion levels (Van Winsen et al., 2001, Prohaszka et al., 1990). Brooks et al. (2001) showed that pigs are tolerant for dietary lactic acid concentrations up to 200 mmol/kg. In general, acids that are metabolized via the citric cycle (e.g. lactic, citric and fumaric acids) are considered to have a positive effect on feed intake even at relatively high inclusion levels. The palatability always depends on type and dose of the acid. It has been suggested that an improved palatability of a diet can be an important factor for the growth performance of the pigs fed organic acids (Brooks, 2008).

The chemical composition of the feed is different depending on where in the fermenting process the feed is, and it affects both pH and palatability (Scholten et al., 1999). Certain fermentation metabolites such as acetic acid and biogenic amides have been suggested to be the reason for lowering the palatability of a feed, especially to piglets (Brooks et al., 2001; Moran, 2001; Brooks, 2008). Limits have been suggested by several authors. Winsen et al. (2001) claimed that the concentration of acetic acid should be below 40 mmol/kg, but Brooks (2003, 2008) and Brooks et al., (2003b) claim that a concentration of more than 30 mmol/kg can have a negative effect on palatability. However, Canibe et al. (2010) added acetic acid at 0, 30, 60 and 120 mmol/kg feed and could not see a difference in daily weight gain or daily feed intake.

Biogenic amines are nitrogenous substances that are produced by some LAB strains through a decarboxylation of amino acids. Several authors have seen a higher content of biogenic amines in FLF compared to DF and NFLF which indicates that they are formed during fermentation (Pedersen, 2001). The influence of biogenic amines on the pig's health is unknown, but they are known to cause poisoning in humans at a high consumption. Histamine is one of the most well documented poisonous biogenic amines (Santos, 1996; Spano et al., 2010). The amines are believed to lower the palatability by several authors (Pedersen et al., 2001, 2002) but the effect seem to be poorly investigated. Biogenic amines have been found to affect the palatability of a feed negatively for sheep (Van Os et al., 1997)

Suarez et al. (2010) made a preference test to see if the pigs preferred some of the acids to the others. They found that the lowest preference was for propionic, acetic, caprilic, formic and butyric acids at the highest inclusion level of their trial, 1.5% (250 mmol/kg).

3.4.5 Environment

The pig industry has been under a lot of pressure during the recent years to decrease its emissions to the environment and make the production more sustainable. As mentioned under digestibility, the addition of organic acids to a diet might improve the digestibility and utilization of N, P, Ca, Mg and Z. They can thereby reduce the emission of nutrients and minerals to the environment (Roth et al., 1998a, 1998b, Partanen & Mroz., 1999). This is especially useful in the European pig production which is under increasing inspection from legislators because of their emissions into the environment. Liquid feeding has made a significant contribution to the environment in some countries through the recycling of human food bi-products. But some are concerned that LF might increase environmental loading. Liquid diets tend to increase effluent volume, but even if that means a reduction in nutrient load per liter it might still be more efficient if looking at the environmental load in terms of nutrients voided per kg growth or preferably per kg meat produced (Brooks, 1999). The transportation of liquid biproducts increases as more water is transported so the use of them might only be efficient both environmentally and economically if the pig production is close to the source of supply. However, in Europe many products are transported considerable distances as ‘back loads’ in tankers that would otherwise travel empty. In that case, the only increase in fuel cost is the difference between running the tanker empty and full (Brooks et al., 2003).

Since many bi-products have a high content of minerals they might increase the water consumption of the pig in order to maintain its homeostatic balance (Brooks et al., 1990). On the other hand, pigs are able to utilize more of the nutrients from liquid diets than from dry ones (Jensen & Mikkelsen, 1998).

4. Material and method

4.1 Experimental design

A total of 60 pigs (Yorkshire/ Hampshire) were used in the experiment. The experiment was divided into two trials with 30 pigs in each. In the first trial the pigs were fed different levels of lactic acid and in the second trial the pigs were fed different levels of acetic acid. The stable was cleaned and empty for a week between the trials. The pigs were in the stable from 9 weeks of age, one week before the start of the trial to acclimatize. The trial was performed during 2 weeks for each acid, i.e. from 10-12 weeks of age. The pigs were divided in pairs, one castrate and one female from different litters in each pen, with as similar weights as possible. The pigs were already familiar with each other since they were kept in the same pens after weaning. The pigs were randomly assigned to five treatments with four different levels of lactic acid or acetic acid and one control group with water only (table 2). At the start of the trial they had a mean weight of 27 ± 3.5 kg.

Table 2. Acid concentrations in treatments (mmol/ kg feed) in part one and part two of the trial.

Lactic acid	L0	L1	L2	L3	L4
	0	75	100	150	200
Acetic acid	A0	A1	A2	A3	A4
	0	10	50	100	150

There were three pens, with two pigs per pen, on each treatment and the same proportion of acid was kept throughout the whole trial. The feed was ORIGO, a conventional feed based on cereals. The feed was given *semi ad lib*, twice a day (8.30 and 15.00). The feed was mixed with water and acid to a DM of approximately 40%. Before each feeding, the remaining feed was removed, weighed and frozen to be able to calculate the DM intake of the pigs. Temperature and pH of the feed was measured with an electronic pH meter every morning at feeding. Feed samples were collected each day from all treatments. The pigs were weighed in the beginning and the end of the trial.

4.2 Behavior

A video system (MSH video client™) was used to monitor the behaviors of the pigs. The behavior was recorded during the first three days and the last day of each trial and consisted of two kinds: one instantaneous scan sampling of behavior and a continuous recording of behavior frequencies.

Table 3. Definitions of behavior parameters during the instantaneous scan sampling.

Behavior parameter	Definition
Resting	Lying or sitting down
Standing	Standing, walking or running
Eating	Head in the trough
Contact	Trout against another pig
1 m	Pigs head is within 1 meter from the trough
Chewing	Chewing on interior
Out of sight	Pig cannot be seen in picture

The instantaneous scan sampling was performed during the 4 days mentioned above. The behaviors were recorded per pen and every 9 minute during 24 hours and the behaviors are defined in table 3.

During the continuous recording, all behaviors (defined in table 4) were recorded for each pig during one hour after each feeding occasion.

Table 4. Definitions of behavior parameters used in the continuous recording.

Behavior parameter	Definition
Eating	Head in trough
Break	The pigs head is outside the trough longer than 5 minutes
Contact	Trout against another pig

4.3 Statistical analysis

Data were analysed with the Statistical Analysis System, version 9.2 (SAS Institute, Cary, NC, USA). The effect of treatment on performance was evaluated with Proc Mixed. The model included the fixed factor of treatment. Pig was the experimental unit for daily weight gain and pen for feed conversion ratio. When analysing daily weight gain, initial weight was used as a covariate.

The behaviors recorded at the scan are presented as the percentage of pigs performing a particular behavior on each observation occasion. The continuous recording behaviors are presented as the total number of interactions performed per pen on each observation occasion. Pen was the statistical unit for all behavior analyses. These behavior parameters were evaluated within each observation occasion with Proc Mixed. The model included treatment as fixed factor. The results are presented as Least Square means ± pooled S.E.M. and the statistical significance level is set to $P<0.05$.

5. Results

5.1 Feed and pig performance

All pigs remained throughout the whole trials since they ate properly and remained healthy.

Table 5. Performance of pigs fed lactic acid treatments*.

	L0	L1	L2	L3	L4	S.E.M	p-value
Initial weight (kg)	31.4	31.4	30.2	29.0	31.1	1.61	0.788
Final weight (kg)	46.0	48.1	46.7	44.3	45.5	2.25	0.818
Daily weight gain (g)	1023	1169	1186	1157	1015	51.6	0.069
Total feed consumption/ pig and period (kg DM)	30.1	30.7	29.2	28.9	26.0	2.11	0.574
Feed consumption day 1 (g)	1149	1114	1146	970	1004	92.4	0.540
Kg feed/ kg weight gain	2.07[a]	1.85[ab]	1.77[b]	1.88[ab]	1.80[b]	0.05	0.016

*L0= 0 mmol/kg, L1= 75 mmol/kg, L2= 100 mmol/kg, L3= 150 mmol/kg, L4= 200 mmol/kg
Data are presented as least square means. S.E.M. = pooled standard error of means.
Means with different superscripts within the rows differ at $p<0.05$.

Table 6. Performance of pigs fed acetic acid treatments*.

	A0	A1	A2	A3	A4	S.E.M.	p-value
Initial weight (kg)	30.0	31.7	29.1	27.9	30.7	1.36	0.686
Final weight (kg)	45.6	47.0	45.5	43.7	48.6	1.85	0.679
Daily weight gain (g)	1108[ab]	1065[a]	1185[ab]	1167[ab]	1266[b]	49.1	0.034
Total feed consumption/ pig (kg DM)	31.1	30.2	32.7	29.3	31.8	1.47	0.551
Feed consumption day 1 (g)	1564	1556	1593	1414	1454	52.8	0.149
Kg feed/kg weight gain	2.00[a]	1.96[ab]	2.00[a]	1.86[ab]	1.77[b]	0.05	0.027

*A0= 0 mmol/kg, A1=10 mmol/kg, A2= 50 mmol/kg, A3= 100 mmol/kg, A4= 150 mmol/kg
Data are presented as least square means. S.E.M. = pooled standard error of means.
Means with different superscripts within the rows differ at $p<0.05$.

The performance of the pigs can be seen in table 5 (for lactic acid) and table 6 (for acetic acid). The pigs fed lactic acid had a mean weight of 30.6 kg (S.D. = 3.8 kg) when they started the trial and 46.1 kg (S. D. = 5.3 kg) at the end. They gained on average 1054 g per day (S.D. = 51.6 g). There were no significant differences in final weight, total feed consumption, feed consumption during the first day or daily weight gain between the different treatments of supplemented acid. A significant difference in feed conversion rate (FCR) was found between the different treatments. The control group (L0) had a significantly higher FCR (2.07 kg feed per kg weight gain) than treatment L2 and L4, which had a FCR of 1.77 and 1.80 respectively.

The pigs fed acetic acid (table 6) had a mean initial weight of 26.0 kg (S.D. = 3.12 kg) and a final weight of 46 kg (S.D.= 4.5 kg). They gained on average 1079 g per day (S. D.= 49.1 g). Pigs feed the highest inclusion of acetic acid (A4) had a significantly higher weight gain than pigs fed the lowest inclusion level (A1). There was a significant difference in FCR between the inclusion levels of acetic acid (p= 0.027). A0 and A2 both had FCR on 2.0 kg feed per weight gain, compared to A4, which had a significantly more efficient FCR on 1.77. There were no significant differences in total feed consumption (p= 0.551).

5.2 Behavior

5.2.1 Instantaneous scan sampling

Table 7. Percent of time during 24h used on scan sampling behaviors# of pigs fed lactic acid treatments*.

Parameter	L0	L1	L2	L3	L4	S.E.M	p-value
Day 1							
Resting	35.4	53.6	35.6	51.9	48.8	10.25	0.605
Standing	5.5	12.3	4.3	5.3	4.5	2.98	0.354
Eating	18.9	10.6	17.1	21.4	13.3	2.77	0.158
Contact	0	0.4	0.1	0	0.3	0.19	0.512
1 m	2.7	2.9	2.6	3.8	2.2	0.71	0.729
Chewing	0	0.4	0.1	0	0.3	0.19	0.512
Out of sight	37.3	20.0	40.0	17.6	30.9	11.42	0.622
Day 2							
Resting	47.3	74.5	54.8	70.6	59.1	16.18	0.750
Standing	5.8	4.3	3.8	2.3	4.9	1.14	0.313
Eating	11.7	12.2	12.4	15.9	12.4	2.07	0.632
Contact	0.2	0.1	0.1	0.1	0	0.10	0.655
1 m	1.8	2.5	2.3	2.2	2.4	0.74	0.975
Chewing	0.2	0.1	0.1	0.1	0	0.10	0.655
Out of sight	33.0	6.4	26.6	8.9	21.0	15.85	0.725
Day 3							
Resting	47.3	69.2	51.0	60.2	58.7	16.00	0.882
Standing	4.8	2.8	4.7	3.2	3.1	1.35	0.765
Eating	13.5	13.7	13.1	14.4	11.6	1.82	0.849
Contact	0.3	0.1	0.4	0	0	0.25	0.649
1 m	2.4	1.3	2.2	2.8	1.4	0.48	0.207
Chewing	0.3	0.1	0.4	0	0	0.25	0.649
Out of sight	31.7	12.9	28.7	19.4	25.2	16.06	0.921
Day 14							
Resting	54.7	65.1	53.6	48.5	59.8	14.06	0.931
Standing	4.0	4.2	3.4	3.0	4.3	0.82	0.751
Eating	11.6	7.9	10.9	12.7	10.7	1.41	0.256
Contact	0	0.2	0.4	0.4	0	0.19	0.322
1 m	1.5	1.0	1.8	1.6	1.8	0.40	0.561
Chewing	0	0.2	0.4	0.4	0	0.19	0.322
Out of sight	28.2	21.6	29.9	33.8	23.3	14.05	0.971

#See table 3 for definitions of behavior parameters
*L0= 0 mmol/kg, L1= 75 mmol/kg, L2= 100 mmol/kg, L3= 150 mmol/kg, L4= 200 mmol/kg

The results from the instantaneous scan sampling can be seen in table 7 and table 8. Inclusion of different levels of either lactic acid (table 7) or acetic acid (table 8) did not affect the time pigs spent on different behaviors.

Table 8. Percent of time during 24h used on scan sampling behaviors# of pigs fed acetic acid treatments*.

Parameter	A0	A1	A2	A3	A4	S.E.M	p-value
Day 1							
Resting	43.1	52.2	46.2	34.1	45.5	10.68	0.871
Standing	4.1	6.7	5.2	4.4	7.3	0.96	0.199
Eating	24.9	25.2	31.3	22.6	13.3	9.31	0.689
Contact	0.2	0	0	0	0	0.11	0.714
1 m	3.2	3.0	2.7	1.5	3.2	0.74	0.603
Chewing	0.2	0	0	0	0	0.11	0.714
Out of sight	24.5	12.9	14.4	37.5	37.7	9.26	0.436
Day 2							
Resting	64.3	53.2	46.0	33.7	55.0	12.54	0.491
Standing	3.7	4.7	3.8	3.8	3.9	0.72	0.874
Eating	12.0	23.2	27.4	14.2	12.2	4.86	0.154
Contact	0.1	0	0	0	0	0.05	0.664
1 m	2.8	2.6	3.4	1.8	2.3	0.56	0.477
Chewing	0.1	0	0	0	0	0.05	0.644
Out of sight	17.1	16.3	19.5	46.5	26.6	11.70	0.372
Day 3							
Resting	66.0	64.0	62.3	45.2	46.1	11.88	0.608
Standing	2.7	3.7	3.7	2.8	5.7	1.44	0.702
Eating	13.1	16.7	19.3	12.4	10.2	2.47	0.189
Contact	0	0.2	0	0	0	0.10	0.452
1 m	1.9	4.3	2.5	1.4	2.9	0.67	0.078
Chewing	0	0.2	0	0	0	0.10	0.452
Out of sight	16.3	11.1	12.2	38.1	35.1	12.58	0.456
Day 14							
Resting	57.2	64.1	64.7	51.3	52.4	10.11	0.830
Standing	2.3	3.5	2.2	2.9	5.0	1.13	0.520
Eating	12.4	10.3	9.7	12.4	11.4	0.82	0.123
Contact	0	0	0	0.2	0	0.10	0.452
1 m	1.5	1.2	1.6	1.7	1.9	0.37	0.719
Chewing	0	0	0	0.2	0	0.10	0.452
Out of sight	26.6	21.0	21.8	31.4	29.2	10.69	0.947

#See table 3 for definitions of behavioral parameters
*A0= 0 mmol/kg, A1=10 mmol/kg, A2= 50 mmol/kg, A3= 100 mmol/kg, A4= 150 mmol/kg

5.2.2 Continues recording

Inclusion of different levels of lactic acid (table 9) did not significantly influence ($p>0.05$) any of the recorded behaviors (eating, break and contact) during 1 h after feeding. However, feeding occasion affected the time to first break from feeding that was longer than 5 minutes at day 1. The break was shorter in the morning than in the afternoon ($p<0.001$). There was also a significant difference in social contact between the times of the feeding occasions, day 1 and day 14. The pigs had more contact with each other during the morning than in the afternoon on day 1 and it was the opposite situation on day 14.

Table 9. Total number of observed behaviors# per pig during 1 h after feeding, when fed lactic acid treatments.

	Treatment*							Feeding ¤			
	L0	L1	L2	L3	L4	S.E.M	p-value	1	2	S.E.M	p-value
Day 1											
Eating	13.8	18.8	19.6	14.7	12.5	3.51	0.542	14.2	17.6	2.22	0.290
Break	28	30	23	37	15	5.71	0.132	16	38	3.61	<0.001
Contact	6.5	3.9	8.1	4.8	7.5	1.75	0.420	8.3	4.0	1.11	0.011
Day 2											
Eating	12.7	15.6	11.4	12.5	11.1	2.04	0.563	14.0	11.3	1.29	0.142
Break	32	37	30	43	26	4.50	0.134	36	31	2.85	0.206
Contact	2.1	1.7	9.1	1.4	4.0	2.60	0.230	5	2.3	1.65	0.270
Day 3											
Eating	12.3	9.5	14.0	10.4	10.6	1.46	0.228	11.8	11	0.92	0.545
Break	36	28	34	32	29	4.40	0.620	34	29	2.78	0.217
Contact	2.8	2.8	4.6	4.3	4.0	1.87	0.924	3.6	3.7	1.18	0.953
Day 14											
Eating	8.3	5.5	8.8	8.1	9.1	1.47	0.450	8	8.0	0.93	0.980
Break	30	25	22	30	29	3.86	0.529	28	26	2.43	0.495
Contact	1.8	0.4	2.4	3.1	3.1	1.04	0.359	0.7	3.6	0.66	0.006

#See table 4 for definitions of behavioral parameters.
*L0= 0 mmol/kg, L1= 75 mmol/kg, L2= 100 mmol/kg, L3= 150 mmol/kg, L4= 200 mmol/kg
¤1=Morning feed;2=Evening feed

Table 10. Total number of observed behaviors# per pig during 1h after feeding, when fed acetic acid treatments.

	Treatment*							Feeding ¤			
	A0	A1	A2	A3	A4	S.E.M	p-value	1	2	S.E.M	p-value
Day 1											
Eating	10.9	10.8	13.3	10.7	10.7	2.32	0.907	14.8	7.7	1.47	0.002
Break	29	25	27	27	27	2.44	0.913	28	26	1.43	0.307
Contact	2.3	5.3	3.5	1.8	3.7	2.25	0.837	5.6	1.0	1.42	0.030
Day 2											
Eating	8.7	9.6	11.6	9.2	8.5	1.29	0.483	11	8.1	0.82	0.019
Break	31^b	29^b	34^a	28^b	28^b	3.29	0.761	29	32	2.13	0.366
Contact	0.1	0.3	0.3	0.3	1	0.21	0.055	0.2	0.6	0.13	0.047
Day 3											
Eating	8.7	7.2	10.4	5.8	7.8	1.38	0.220	7.9	8.1	0.87	0.872
Break	33	33	42	26	26	2.41	0.002	28	37	1.42	0.004
Contact	0.2	1.3	1.4	0.3	0.9	0.55	0.398	0.8	0.9	0.35	0.894
Day 14											
Eating	11.6	8.2	12.4	10.8	9.8	1.28	0.191	11.3	9.8	0.81	0.203
Break	27	26	23	26	27	4.08	0.943	26	25	2.37	0.759
Contact	2.0	0.8	2.3	0.9	1.3	0.58	0.299	2.0	0.9	0.37	0.039

#See table 4 for definitions of behavioral parameters.
*A0= 0 mmol/kg, A1=10 mmol/kg, A2= 50 mmol/kg, A3= 100 mmol/kg, A4= 150 mmol/kg
Means with different superscripts within the rows differ at p<0.05
¤1=Morning feed; 2=Evening feed

The behavior of the pigs fed acetic acid (table 10) was very similar between the inclusion levels. The pigs with A2 ate for a longer time before their first break compared to pigs with all other levels (p= 0.002). There were also some significant differences between the times of feeding. There was a difference in eating behavior ($p < 0.05$) between the first and second feeding occasion during the two first days with a higher frequency in the morning

A significant difference in contact between the pigs could also be seen day 1 ($p=0.030$), day 2 ($p= 0.047$) day 14 ($p= 0.039$) between the feeding occasions. The pigs had more contact with each other during the morning compared to the afternoon on the first and last day. It was the opposite relation the second day. However, the number of registrated contact behavior was very low.

Table 11. pH in diets* with supplemented lactic and acetic acid.

Treatment	L0	L1	L2	L3	L4	S.E.M	p-value
pH	5.8	4.7	4.5	4.2	4.1	0.048	<0.0001
Treatment	A0	A1	A2	A3	A4	S.E.M	p-value
pH	5.9	5.6	5.0	4.7	4.5	0.021	<0.0001

*L0= 0 mmol/kg, L1= 75 mmol/kg, L2= 100 mmol/kg, L3= 150 mmol/kg, L4= 200 mmol/kg
A0= 0 mmol/kg, A1=10 mmol/kg, A2= 50 mmol/kg, A3= 100 mmol/kg, A4= 150 mmol/kg
Data are presented as least square means. S.E.M. = pooled standard error of means.

The pH of the different diets can be seen in table 11. There was a significant difference between all diets ($p<0.001$). The lactic acid concentration had to be >100 mmol/kg in order to reach the desired pH of <4.5. Acetic acid had to be supplemented with 150 mmol/kg in order to decrease the pH to < 4.5. The mean temperature of the feed was 20^{o} C.

6. Discussion

Fermented feed and organic acids have been recognized as potential alternatives to antibiotics in order to improve the performance of pigs, although, there have been inconsistent results on the effects of fermented feed and organic acids on performance and feed intake. The variations in weight gain from feeding pigs with FLF are believed to be due to a bad palatability of the feed through high levels of acetic acid, biogenic amines or an unsuccessful fermentation. A common belief seem to be that a high inclusion levels of acetic acid will cause a bad palatability to the feed and limits have been set by some authors of a maximum inclusion level of 30-40 mmol/kg (Brooks et al., 2003, 2003 b, 2008; Winsen et al., 2001). Lactic acid is considered to enhance the palatability of a feed (Brooks et al., 2001b) but there has not been a lot of research on until what level. Since there were no differences in feed intake or weight gain between any levels of supplemented lactic acid in this trial, the pigs seem to accept the taste of lactic acid to a very high extent. This agrees with previous studies which also have shown an acceptance for lactic acid up to 200 mmol/kg (Brooks et al., 2001).

We added acetic acid at much higher levels than recommended, (up to 150 mmol/kg feed) and did not see any significant differences in feed consumption, behavior or weight gain between the pigs. This suggests that acetic acid is accepted at much higher levels than previously believed. The typical concentrations of acetic acid in FLF with compound feed are between 20-40 mmol/kg feed (Mikkelsen & Jensen, 2000; Scholten et al., 2001; Van Winsen et al., 2001; Canibe & Jensen, 2003; Canibe et al., 2007) and even when high levels have been reported (54 mmol/ kg feed (Pedersen, 2001)) it is still a lot lower than the concentrations we added. Therefore, our results suggest that the levels of acetic acid typically measured in FLF prepared with standard compound feed would not profoundly affect its palatability, and thereby feed intake by piglets. Our results are supported by a study performed by Canibe et al. (2010). They added acetic acid to diets for newly weaned piglets (4 weeks old), at 30, 60 and 120 mmol/kg feed during 6 weeks and did not find any differences in daily weight gain or daily feed intake between the groups.

Our trial was performed during only two weeks for each acid; a longer period might have given different results. The fact that the pigs received wet feed instead of dry feed might have caused an increase in feed intake due to a better palatability. This factor might decrease after a longer period when they have acclimatized properly to the wet feed and might have resulted in different results. Canibe et al. (2010) saw a tendency to a lower feed intake with increasing levels of acetic acid during the last 4 weeks of their 6 week trial, but the difference was not significant ($p=0.09$). It could therefore be of interest to make a longer trial to investigate the long term effects from acetic acid on the palatability of a feed.

The pigs in our trial were of mixed breeds Yorkshire×Hampshire. Both breeds are bred for a good appetite and feed consumption. Other breeds might have other preferences of taste or are more or less selective.

Since our results indicate a relatively high acceptance for acetic acid, it could be interesting to investigate the effects of biogenic amines on the palatability. The influence of biogenic amines on pig health is unknown but Pedersen et al., (2001, 2002) claim that they lower the palatability of a feed. Biogenic amines are known to cause poisoning in humans at a high consumption (Santos, 1996; Spano et al., 2010) and a decrease in feed intake and palatability have been found in a trial on sheep. Several authors have seen a higher content of biogenic amines in FLF compared to DF and NFLF which indicates that they are formed during fermentation but the effect of them seems poorly investigated (Pedersen, 2001).

The only significant differences in performance among the pigs fed lactic acid and acetic acid was found in FCR and in favor of the higher levels of supplemented acids. Several previous studies have also resulted in a higher FCR when supplementing organic acids to a diet. Janson et al. (2011) added organic acids to a diet which resulted in higher FCR than in pigs without supplemented acids. Tsiloyiannis et al. (2001) also found an improvement in weight gain and feed intake when supplementing lactic acid to a diet. The improvement in performance was similar to that from antibiotic treatment. Studies by Zhang et al. (1986) and Roth & Kirchgessner, (1988) did not result in either an increased average daily gain or FCR when acetic acid was added to the diet.

An improvement in FCR with increasing levels of acids could be due to a more efficient utilization of the feed. Fermented feed have resulted in an increased ilial digestibility of organic matter and NDF compared to untreated or cooked feed (Lyberg et al., 2006; Hong & Lindberg, 2007). A low pH also results in an increased digestibility of protein, P and several amino acids in a feed (Jongbloed et al., 2000; Omogbenigun et al., 2003; Lyberg et al., 2006). A more efficient utilization and absorption of nutrients could therefore be the explanation for an improved FCR with increasing levels of organic acids.

The improved utilization of nutrients as well as the feed antimicrobial qualities is mainly connected to a low pH in the feed. Since pH is important for the response of FLF and acidifiers, it is important to add the proper amount of acids or make sure that enough LAB are produced in FLF to lower the pH below 4.5 for the desired effects (Van der Wolf et al., 2001, Canibe & Jensen 2003; Brooks et al. 2003, Canibe et al., 2007a). When lactic acid was supplemented to the diet in our trial, a minimum of 100 mmol/kg feed was needed to lower the pH to <4.5. This result is supported by several other studies that have shown that lactic acid concentration needs to be >100 mmol/kg feed in order to have a bactericidal effect (Beal et al., 2002; Brooks et al., 2003b). When adding acetic acid to the diet, 150 mmol/kg feed (the highest inclusion level) was needed in order to get the pH to < 4.5. However, a fermented feed contain both acids (along with others) which will work together to lower the pH.

The supplemented acids contained some energy that could have affected the performance of the pigs. The pigs consumed a total mean of 26.7 MJ (±1.9) per pig and day through the feed. Both acetic and lactic acid contains a small amount of energy (table 1). The pigs that were given the highest inclusion level of lactic acid received a 1.6 MJ extra per pig and day. The pigs with the highest inclusion level of acetic acid were given 0.8 MJ extra.

The results from the instantaneous scan sampling and continues recordings did not show any differences in eating behavior of relevance between the different treatments of supplemented acids. Some differences were found between the times of feeding but these differences are more connected to pigs activity during the day in general and the results are therefore not relevant for this trial since no differences between the levels of supplemented acids were found.

7. Conclusion

Our results indicate that pigs will accept inclusion levels of lactic acid up to 200 mmol/kg feed and acetic acid up to 150 mmol/kg feed without affecting the palatability of a feed negatively. This means that fermented feeds that previously have been discarded due to what has been considered too high amounts of acids can be used

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