Acidifiers in Animal Nutrition

A Guide for Feed Preservation and Acidification to promote Animal Performance

Edited by Christian Lückstädt

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Nottingham
University Press

Nottingham University Press
Manor Farm, Main Street, Thrumpton
Nottingham, NG11 0AX, United Kingdom
www.nup.com

NOTTINGHAM

First published 2007
Reprinted digitally 2009

British Library Cataloguing in Publication Data
Acidifiers in Animal Nutrition
A Guide for Feed Preservation and Acidification to Promote Animal Performance
Lückstädt, C.

ISBN-13: 978-1-904761-40-2

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Typeset by Nottingham University Press, Nottingham
Digitally printed and bound by Lightning Source

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PREFACE

This compendium provides an overview on the use of acidifiers in animal nutrition and their beneficial effects on livestock and in aquaculture. It contains a compilation of papers from research institutes and the industry worldwide related to that topic.

The potential of organic acids in forage preservation, as well as in livestock nutrition, has been known for decades and is documented by many scientific publications. Organic acids make a fundamental contribution to feed hygiene, as they suppress the growth of mould and thus restrict the potential effect of mycotoxins. Consequently feed safety is guaranteed by adding an organic acid or organic acid blend.

"The ban on antibiotic growth promoters creates significant opportunity for feed acidification" was stated in a report from Frost and Sullivan (2004). According to that report, there is a strong likelihood that the overall European animal feed acid market will grow from an estimated level of 191 million € in 2002 to more than 400 million € in 2009. This would more than double this market segment in seven years, leading to an average annual growth rate of around 16%. It was furthermore stated that this rise reflects the industry's move away from antibiotic growth promoters to acids as an alternative means for performance enhancement. Growth in this market segment is expected primarily in Southern and Eastern Europe, since the expansion of the European Union in 2004 offers suppliers the additional opportunity of getting into markets with considerable growth potential.

With this scenario in mind, the development of lesser known organic acids and acid blends, such as lactic acid and sorbic acid, to ensure product differentiation is expected to be boosted. Furthermore, novel applications, in the form of blends with non-acid ingredients, represent other potential growth sectors.

The final conclusion of the report was that "acids emerge as a cost-effective, performance-enhancing option for feed industry". This holds true for not just the European markets, but also world-wide, with companies now expanding their acidifier related businesses to Asia and America. The use of acidifiers, especially in pig production, has become a global opportunity. However, other fields of animal production are included, too, as reports of the use of acidifiers in drinking water in poultry or the inclusion of organic acid salts in aquaculture feed prove.

It is the hope of the editor and the authors that have contributed to this compendium that further scientific development in this area will boost discussions on future research, leading to more in-depth knowledge on the mode of action and benefits of acidifiers in animal nutrition.

Dr. Christian Lückstädt
Editor

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ORGANIC ACIDS AND SALTS PROMOTE PERFORMANCE AND HEALTH IN ANIMAL HUSBANDRY

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Introduction

Organic acids, such as propionic acid, have been used for more than 30 years to reduce bacterial growth and mould in feedstuffs and thus preserve hygienic quality. In feed legislation they are registered as preservatives, but their positive effects on animal health and performance, if they are added to feed in sufficient amounts, are also well documented. Acids used as feed additives are predominantly compounds that naturally occur in cell metabolism, thus they are natural products with low toxicity (Kirchgessner and Roth, 1988).

Health and performance promoting effects have been demonstrated for a number of organic acids, including formic, fumaric, citric and lactic acid and their salts. Besides improvement in hygiene and a corresponding reduction of pathogen intake, effects on feed digestion and absorption and on stabilisation of gut flora eubiosis have been demonstrated in a number of investigations. In animal husbandry, higher feed conversion rates and improved daily gain, as well as reduced incidence of diarrhoea, enhance economic return by lower feed costs and shorter time to market.

The greatest response to supplementation with acids has been recorded in piglets, especially during the weaning period. In the first three to four weeks of life gastric hydrochloric formation and pancreatic enzyme secretion is poor in the digestive tract. Moreover, piglets can be subject to stressed due to separation from the sow, and feed intake may be low for some days post-weaning. After recovery, high amounts of feed are consumed in compensation, and these volumes cannot always be acidified and digested properly, leading to diarrhoea and oedema. These problems are reduced in older pigs but the growth promoting effects of organic acids can still be achieved, though to a lower extent (Baustadt, 1993; Meyer *et al.*, 2006). However, a literature survey reveals considerable variation in effects between trials (Freitag *et al.*, 1998) that can be caused by differences in feeding, housing or hygienic conditions.

Chemical properties of selected organic acids and salts

The effects of organic acids in the reduction of pH and their antimicrobial activity vary considerably depending on their dissociation status. The amount of dissociation depends on the pH value of the environment, and is described by the specific pK (dissociation constant) value for each acid, which defines the pH at a 50% dissociation rate. The lower the pK value, the stronger the acid, which relates to its ability to lower the pH of the environment. Acids used as feed additives have pK values between 3 and 5, and are categorised as being of intermediate strength (table 1).

Table 1. Chemical properties of selected acids and salts

Acid / Salt	*pK value*	*Solubility in water*	*Molecular weight (g)*	*Gross energy (KJ/g)*	*Physical condition*
Formic acid	3.75	very good	48.0	5.8	liquid
Acetic acid	4.75	very good	60.1	14.8	liquid
Propionic acid	4.87	very good	74.1	20.8	liquid
Lactic acid	3.08	good	90.1	15.1	liquid
Fumaric acid	3.03/4.44	low	116.1	11.5	solid
Citric acid	3.14/5.95/6.39	good	210.1	10.3	solid
Ca-formate	-	low	130.1	3.9	solid
Na-formate	-	very good	68.0	3.9	solid
Ca-propionate	-	good	16.6	16.6	solid
Ca-lactate	-	low	10.2	10.2	solid

Kirchgessner and Roth, 1991

At a pK value of around 3, lactic, fumaric and citric acid are stronger than formic, acetic and propionic acid. Solubility in water is classified as good or very good for most acids and salts, except for fumaric acid, Ca-formate and Ca-lactate. The nutritive value differs considerably between acids, and is highest in propionic acid and lowest in formic acid and its salts. For handling considerations, solid acids are easy to use, while liquid forms tend to be corrosive.

In pig nutrition formic and fumaric acid and their salts are most commonly used. Formic acid is a small fatty acid with the structural formula H-COOH. It is a clear liquid with a pungent smell, which is highly corrosive and hazardous to handle. Its high solubility makes it readily absorbed in the intestinal tract, and resorption may be observed in the stomach. In metabolism, formic acid is involved in the transfer of C_1 units. Antimicrobial effects are mainly against yeast and several species of bacteria, whereas activities against fungi are only observed at higher concentrations. *Lactobacilli* seem to be more resistant to formic acid. Liquid formic acid causes irritation to mucous membranes on inhalation and can be corrosive on contact with the epidermis, although the salt of formic acid, formate, is solid and less corrosive, hence it is used more widely in industrial feed processing. Because of possible detrimental effects on human and animal health, safety regulations regarding handling of acids must be observed.

Fumaric acid has the structural formula of HOOC-CH=CH-COOH. In animal metabolism fumaric salt is generated during the decomposition of aspartate, phenylalanine and tyrosine amino acids, in the ornithine cycle and during purine synthesis. It is metabolically generated as part of the citric acid cycle, and orally applied fumaric acid is involved in ATP generation in the cell. Its antimicrobial activity targets gram negative and gram-positive bacteria, and coliforms (especially *E. coli*) are particularly reduced, whereas growth of yeast varieties is hardly inhibited. Acidophilic lactobacilli tolerate fumaric acid. Fumaric acid is used as acidifier in human nutrition for bakery products and beverages and in the chemical industry, as it is less corrosive and harmless to handle.

Effects of organic acids and salts in animal nutrition

In animal nutrition, acidifiers and salts exert their performance promotion effects via three different ways: feed, intestinal tract and metabolism (table 2).

Table 2. Effects of organic acids and salts in animal nutrition

	Effective Form	*Effects*
Feed	H^+	- pH reduction - reduction of acid binding capacity
	H^+ and Anion	- reduction of microbial growth - antibacterial effects
Intestinal tract	H^+	- pH reduction in stomach and duodenum - improved pepsin activity
	Anion	- complexing agents for cations - (Ca^{++}, Mg^{++}, Fe^{++}, Cu^{++}, Zn^{++})
	H^+ and Anion	- antibacterial effects - change in microbial concentrations
Metabolism		- energy supply

Kirchgessner and Roth, 1988

Feed

Even in a hygienic environment, feed can be infected with a certain amount of fungi, bacteria or yeast. Under favourable conditions, microbes can multiply rapidly during storage, especially at higher moisture level and in a warm environment. Conserving agents reduce microbial growth and, depending on the agent, even microbial numbers, and can lower the uptake of pathogenic organisms by the animal. For conserving purposes, acid concentrations are in general lower than for performance promotion. However, for each acid its specific inhibiting effect on bacteria, yeast or mould has to be considered when recommendations for feed supplementation are made. The antimicrobial effects of organic acids in feed and their minimum inhibition concentrations are reviewed in detail elsewhere (Singh-Verma, 1973; Strauss and Hayler, 2001).

Besides its hygienic effect, reduction in the acid binding capacity of feed ingredients can promote animal health, especially in piglets. At weaning the withdrawal of milk reduces bacterial fermentation and results in production of lactic acid in the stomach while the secretion of hydrochloric acid is still low (Kamphues, 1991). Moreover, the high crude protein and mineral content of feed ensures rapid animal growth, but generates high dietary buffering capacity at the same time, thus reducing levels of free hydrochloric acid. Pepsin activation and pancreatic enzyme secretion are reduced, impairing nutrient digestion. Lowering the dietary buffering capacity has been related to beneficial effects on feed digestion (Eidelsburger, 1997).

Intestinal tract

The effects of organic acids in the intestinal tract are two-fold. They reduce pH in the stomach and small intestine. Moreover, acid dissociation in the bacterial cell and the accumulation of salt anions inhibit microbial growth.

REDUCTION OF pH

Dose response studies reveal a substantial pH reduction in feed with the most effective acid concentrations, while their salts have low effects on the pH value (Roth and Kirchgessner, 1989). Besides acid concentration, pH reduction in feed also depends on acid strength (pK value) and the buffering capacity of feed ingredients. Animal protein, extensively used in piglet feed, has a 15 fold higher buffering capacity compared to cereals such as wheat (Kirchgessner and Roth, 1988). There is also a great variation in buffering capacity of mineral sources; MgO and $CaCO_3$ buffer acids to a greater extent than mineral complexes with organic salts. These effects are especially important in view of the low hydrochloric acid output in the young piglet. Inadequate pH reduction in the stomach inhibits pepsin activity and impairs protein digestion. Effective proteolytic activity requires a pH below 4, and is increased at lower pH values. Positive effects of organic acids on protein hydrolysis have been demonstrated in many published trials (Eckel *et al.*, 1992; Eidelsburger *et al.*, 1992a; Mroz *et al.*, 2000).

Likewise duodenal secretion of pancreatic enzymes is reduced at high pH values thus impairing overall digestion. Feed supplementation with organic acids leads to lower duodenal pH, improved N retention and increased nutrient digestibility (Øverland *et al.*, 2000; Kluge *et al.*, 2004). Experimental evidence demonstrates an increase in apparent ileal lysine digestibility of up to 8% in weaned piglets and 5% in fattening pigs compared to controls. Response seems to be higher with feed that has lower digestibility (Partanen and Mroz, 1999). In growing pigs, formic acid supplementation resulted in improved fat digestibility (Partanen *et al.*, 1998) and improved nutrient retention leads to higher feed conversion rate and daily gain (Paulicks *et al.*, 1999; Hasselmann, 2003; Ettle *et al.*, 2004). These effects are most pronounced in piglets and young pigs (Roth *et al*., 1998a, Eidelsburger *et al.*, 2000; Øverland *et al.*, 2000). The effects of various acids, salts and acid-salt combinations on feed intake, daily gain, feed conversion rate and incidence of diarrhoea have been extensively reviewed (Freitag *et al.*, 1998). Improved apparent digestibility and nutrient retention applies also to minerals, either due to homoeostatic effects in faster growing animals or to the formation of mineral and acid complexes, e.g. with Cu (Roth *et al.*, 1998b; Partanen, 2001) leading to reduced mineral excretion and beneficial environmental effects.

ANTIMICROBIAL ACTIVITY

Organic acids and salts exert their growth inhibiting effects on stomach and gut microbes through pH reduction and anion and proton effects in the microbial cell. Growth rates of many microbes like *Cl. perfringens*, *E. coli* or *Salmonella* ssp. are reduced below pH 5, while acid tolerant microbes are unharmed. Low pH also provides a barrier against microbes ascending from the ileum and large intestine. Moreover, small acids are lipophilic and can diffuse across the cell membrane, where, in the more alkaline cytoplasm, they dissociate and the released protons will subsequently lower the internal pH. This reduction in pH alters cell metabolism and enzyme activity, thus inhibiting growth of intra-luminal microbes, especially pathogens. Several investigations have demonstrated a reduction in bacterial count in the stomach (Kluge *et al*., 2004) and the duodenum (Kirchgessner and Roth, 1991; Hebeler *et al*., 2000; Hellweg *et al*., 2006). However *Lactobacillus* spp. numbers seem to be unaffected, or may even be enhanced (Hellweg *et al.*, 2006). This will help promote eubiosis in the piglet's intestinal microflora during the post-weaning period. However, the rate at which organic acids kill bacteria depends on the time of exposure, ambient temperature and specific properties of the acid used. Minimum inhibiting concentrations of organic acids are specific for each acid (Strauss and Hayler, 2001), for example, gram negative bacteria are only sensitive to acids with less than eight carbon atoms, whereas gram positive bacteria are sensitive to longer chain acids (Partanen, 2001). Formic acid seems to be more effective than propionic or lactic acids against the majority of bacteria (Strauss and Hayler, 2001).

Since salts cannot reduce environmental pH, their effects can only be asserted via acid anions. The importance of anions in overall benefits for performance can be demonstrated by comparison of acids and salts in feeding trials, as demonstrated by Kirchgessner and Roth (1987) with formic acid and Na-formate, where equal amounts of anions were added to both groups. Growth promoting effects, such as daily gain and feed conversion ratio, were around 50% in the salt group compared with those receiving formic acid. This appears to be due to the effects of anions, whereas the higher effects observed for the formic acid group were due to pH reduction. Conversely, reduction of gut microbe counts seems to be due to the anionic effect. Experimental evidence suggests that numbers of *E. coli* and *Enterococcus* ssp. can be reduced to similar levels with either Ca-formate or formic acid (Kirchgessner *et al.*, 1992).

Data from feed trials demonstrate the effectiveness of anions in the regulation of gut microbe composition and the performance promoting effects of organic salts. Changes in the gut microflora cause changes in the intestinal environment, resulting in reduced ammonia and lactic acid concentrations in stomach and small intestine (Eckel, 1990; Hebeler *et al.*, 2000). An overall reduction in gut bacteria reduces metabolic needs of microbes and enhances absorption rates of nutrients, especially energy and amino acids (Hebeler *et al.*, 2000; Øverland *et al.*, 2000), leading to better feed efficiency and improved daily gain (Paulicks *et al.*, 1999; Eidelsburger *et al.*, 2000; Øverland *et al.*, 2000; Hasselmann *et al.*, 2003; Ettle *et al.*, 2004). This effect is most distinctive in younger animals, but recent studies demonstrate similar benefits in finishing pigs (Meyer *et al.*, 2006). However, sufficient amounts of anions are essential, as acids with low molecular weight, e.g. formic acid, seem to have more pronounced activities than those with higher molecular weights, e.g. fumaric or citric acid (Eidelsburger, 1997).

Eubiosis of intestinal flora and the reduction in the activity of microbes relieves the metabolism of the host animal due to reduced intestinal ammonia, amine and toxin concentrations (Hebeler *et al.*, 2000), leading to less digestive disorders, e.g. diarrhoea. In a recent study, feed supplementation with potassium diformate significantly enhanced dry matter content in the caecum (Hellweg *et al.*, 2006). In this respect, salts in sufficient concentrations seem to be as effective as acids (Eidelsburger *et al.*, 1992b). Diarrhoea can also be prevented by inhibition of pathogens like *E. coli* to adhere to the gut wall (Gedek, 1993). Most microbes can only proliferate, alter epithelial function or produce toxins after binding to specific receptors on the gut wall. Again, these effects seem to be more closely related to anions rather than to undissolved acids (Diebold and Eidelsburger, 2006).

Impact on metabolism

Most organic acids contribute a considerable amount of energy (table 1). Organic acids are absorbed through the intestinal epithelia by passive diffusion. Short chain acids can be used for ATP generation in the citric cycle, for example, 1M of fumaric acid generates 18M ATP. If ATP formation is compared to the energy content of 1340 kJ/M fumaric acid, it becomes obvious that 74.3 kJ are required per M ATP, approximately the same amount of energy that is involved in ATP generation from glucose. Thus fumaric acid can be compared with glucose in energetic terms. The same is true for citric acid, while acetic and propionic acid need 18% and 15% more energy for 1M ATP synthesis (Kirchgessner and Roth, 1988). Sorbic acid is metabolised via ß-oxidation, as are long chain fatty acids (Sofos and Busta, 1993). As the energy content of organic acids is made completely available during metabolism, it should be considered in the energy calculation of feed rations. For example, propionic acid contains up to five times more energy than wheat (Diebold and Eidelsburger, 2006).

Conclusions

Organic acids and their salts can be used to maintain animal health and performance, and as such can be used to replace sub-therapeutic antibiotic growth promoters. Their mode of action includes stimulating gastric enzyme secretion, nutrient digestibility and retention, leading to improved feed conversion rate and daily gain. Economic benefits result from a reduction in feed costs and a shorter time to market. Acids and salts also reduce microbial growth in feed as well as in the intestinal tract, and the combination of improved hygienic standards in feed and eubiosis of intestinal flora reduces the incidence of diarrhoea. An overall reduction in gut microflora reduces the nutrient and metabolic requirement generated by bacteria, leading to better feed efficiency and improved daily gain. Effects are most pronounced in younger piglets, especially during the weaning period. However, several studies have also demonstrated growth promoting effects in fattening pigs.

References

Baustadt, B. (1993) Effects of formic acid on performance in growing pigs. *Norwegian Journal of Agricultural Sciences* **7**: 61-69.

Diebold, G. and Eidelsburger, U. (2006) Acidification of diets as an alternative to antibiotic growth promoters. In: *Antimicrobial growth promoters*. Edited by D. Barug, J. de Jong, A.K. Kies and M.W.S. Verstegen. pp. 311-327. Wageningen Academic Publishers, Wageningen, The Netherlands.

Eckel, B. (1990) Untersuchungen zur wachstumsfördernden Wirksamkeit von Ameisensäure in der Ferkelaufzucht. [Studies on growth promoting effects of formic acid in piglet rearing] *Dissertation, Fakultät für Landwirtschaft und*

Gartenbau der Technischen Universität München.

Eckel, B., Kirchgessner, M. and Roth, F.X. (1992) Zum Einfluß von Ameisensäure auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 1. Mitteilung: Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Effects of formic acid on daily gain, feed uptake, feed conversion and digestibility. 1st communication: Studies on nutritive effects of organic acids in piglet rearing] *Journal of Animal Physiology and Animal Nutrition* **67**: 93-100.

Eidelsburger, U. (1997) Optimierung der Futterqualität ist nur ein Teilaspekt. [Optimising feed quality] *Schweinewelt* **January**: 18-21.

Eidelsburger, U., Roth, F.X. and Kirchgessner, M. (1992a) Zum Einfluß von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 7. Mitteilung: Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Effects of formic acid, Ca-formate and Na-hydrogen-carbonate on daily gain, feed uptake, feed conversion and digestibility. 7th communication: Studies on nutritive effects of organic acids in piglet rearing] *Journal of Animal Physiology and Animal Nutrition* **67**: 258-267.

Eidelsburger, U. Kirchgessner, M. and Roth, F.X. (1992b) Zum Einfluß von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf pH-Wert, Trockenmassegehalt, Konzentration an Carbonsäuren und Ammoniak in verschiedenen Segmenten des Gastrointestinaltraktes. 8. Mitteilung: Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Effects of formic acid, Ca-formate and Na-hydrogen-carbonate on pH, dry matter, concentration of carbon acids and ammonia in different parts of the gastrointestinal tract. 8th communication: Studies on nutritive effects of organic acids in piglet rearing] *Journal of Animal Physiology and Animal Nutrition* **68**: 20-32.

Eidelsburger, U., Hoppe, P.P. and Krennrich, G. (2000) Zum Einfluss einer Mischung von Ameisensäure und Propionsäure auf Kotkonsistenz und Leistungsparameter in der Ferkelaufzucht und Schweinemast. [Effects of a blend of formic and propionic acids on faecal consistency and performance in pigs from weaning to slaughter] *Proceedings of the Society of Nutrition Physiology* **9**: 73.

Ettle, T., Mentschel, K. and Roth, F.X. (2004) Effect of organic acids on dietary self-selection by the piglet. *Proceedings of the Society of Nutrition Physiology* **13**: 125.

Freitag, M., Hensche, H.U., Schulte-Sienbeck, H. and Reichel, B. (1998) Kritische Betrachtung des Einsatzes von Leistungsförderern in der Tierernährung. [Critical review on the use of growth promoters in animal nutrition] *Forschungsberichte des Fachbereichs Agrarwirtschaft Soest der Universität Gesamthochschule Paderborn* **8**: 100-161.

Gedek, B. (1993) Probiotika als Bioregulatoren. [Probiotics as bioregulators] In: *Vitamine und weitere Zusatzstoffe in der Ernährung von Mensch und Tier*. Edited by G. Flachowsky and R. Schubert. pp. 253-262. 4. Symposium, 30.09./01.10. Jena/Thüringen: 253-262.

Hasselmann, L., Münchow, H., Rühle, J. and Nattermann, H. (2003) Influence of formic acid, sorbic acid and hydrochloride acid on selected gastrointestinal and microbiological parameters in early weaned piglets. *Proceedings of the Society of Nutrition Physiology* **12**: 78.

Hebeler, D., Kulla, S., Winkenwerder, F., Kamphues, J., Zentek, J. and Amtsberg, G. (2000) Einfluss eines Ameisensäure-Kaliumformiat-Komplexes auf die Zusammensetzung des Chymus sowie die Mikroflora im Darmkanal von Absetzferkeln. [Influence of a formic acid-potassiumformate-complex on chyme composition as well as on the intestinal microflora of weaned piglets] *Proceedings of the Society of Nutrition Physiology* **9**: 63.

Hellweg**,** P., Tats, D., Männer, K., Vahjen, W. and Zentek, J. (2006) Impact of potassium diformate on gut flora of weaned piglets. *Proceedings of the Society of Nutritional Physiology* **15**: 63.

Kamphues, J. (1991) Fütterungsbedingte Gesundheitsstörungen in Nutztierbeständen. [Feed related health disturbances in livestock] *Übersichten zur Tierernährung* **19**: 247-272.

Kirchgessner, M. and Roth, F.X. (1987) Einsatz von Formiaten in der Ferkelfütterung. 2. Mitteilung: Natriumformiat. [Effects of formates in piglet feeding. 2nd communication: Na-formate] *Landwirtschaftliche Forschung* **40**: 287-294.

Kirchgessner, M. and Roth, F.X. (1988) Ergotrope Effekte durch organische Säuren in der Ferkelaufzucht und Schweinemast. [Nutritive effects of organic acids in piglet rearing and pig fattening] *Übersichten zur Tierernährung* **16**: 93-108.

Kirchgessner, M. and Roth, F.X. (1991) Ergotrope Effekte durch nutritiven Einsatz von organischen Säuren. [Nutritive effects of organic acids] *Zentralblatt für Hygiene und Umweltmedizin* **191**: 265-276.

Kirchgessner, M., Gedek, B., Wiehler, S., Bott, A., Eidelsburger, U. and Roth, F.X. (1992) Zum Einfluß von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf die Keimzahlen der Mikroflora und deren Zusammensetzung in verschiedenen Segmenten des Gastrointestinaltraktes. 10. Mitteilung: Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Effects of formic acid, Ca-formate and Na-hydrogen-carbonate on bacterial counts and composition in different parts of the gastrointestinal tract. 10th communication: Studies on the nutritive efficacy of organic acids in piglet rearing] *Journal of Animal Physiology and Animal Nutrition* **68**: 73-81.

Kluge, H., Broz, J. and Eder, K. (2004) Untersuchungen zum Einfluss von Benzoesäure als Futterzusatz auf Leistungsparameter, Nährstoffverdaulichkeit, N-Bilanz, Mikroflora und Parameter des mikrobiellen Stoffwechsels im Verdauungstrakt von Absetzferkeln. [Studies on the influence of benzoic acid as a feed additive on growth performance, digestibility of nutrients, nitrogen balance, microflora and parameters of the microbial metabolism in the gastrointestinal tract of weaned piglets] *8. Tagung für Schweine und Geflügelernährung Halle (Saale) Germany*: 42-45.

Meyer, A., Schön, A. and Brade, W. (2006) Einsatz des Leistungsförderers Formi in der Mast. [Use of the growth promoter Formi in pig fattening] *Forum angewandte Forschung in der Rinder- und Schweinefütterung Fulda 05/06.04.*: 186-187.

Mroz, Z., Krasucki, W., Grela, E., Matras, J. and Eidelsburger, U. (2000) The effects of propionic and formic acids as a blend (Lupro-Cid®) in graded dosages on the health, performance and nutrient digestibility (ileal/overall) in sows. *Proceedings of the Society of Nutrition Physiology* **9**: 72.

Øverland, M., Granli, T., Kjos, N.P., Fjetland, O., Steien, S.H. 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.

Partanen, K. (2001) Organic acids – their efficacy and modes of action in pigs. In: *Gut environment of pigs*. Edited by A. Piva, K.E. Bach Knudsen and J.E. Lindberg. pp. 201-218. Nottingham University Press, Nottingham, UK.

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

Partanen, K., Valaja, J., Siljander-Rasi, H., Jalava, T. and Panula, S**.** (1998) Effects of Carbadox or formic acid and diet type on ileal digestion of amino acids by pigs. *Journal of Animal and Feed Sciences* **7**: 199-203.

Paulicks, B.R., Roth, F.X. and Kirchgessner, M. (1999) Nutritive Wirksamkeit eines Ameisensäure-Formiat Komplexes (Formi™ LHS) auf die Leistung von Absetzferkeln in Abhängigkeit von der Rationszusammensetzung. [Nutritive efficacy of a formic acid –formate-complex (Formi™ LHS) on the performance of weaned piglets depending on the feed composition] *Proceedings of the Society of Nutrition Physiology* **8**: 127.

Paulicks, B.R., Roth, F.X. and Kirchgessner, M. (2000) Effects of potassium diformate (Formi® 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.

Roth, F.X., Windisch, W. and Kirchgessner, M. (1998a) Effects of potassium diformate (Formi™ LHS) on nitrogen metabolism and nutrient digestibility in piglets at graded dietary lysine supply. *Agribiological Research* **49**: 167-175.

Roth, F.X., Windisch, W. and Kirchgessner, M. (1998b) Mineral metabolism (P, K, Ca, Mg, Zn, Mn, Cu) of piglets supplied with potassium diformate (Formi™ LHS). *Agribiological Research* **51**: 177-183.

Singh-Verma, S.B. (1973) Wirkung verschiedener organischer Säuren in der Konservierung von Feuchtgetreide und Futtermitteln aus mikrobiologischer

Sicht. [Effects of different organic acids in the conservation of moist grain and feedstuffs from a microbial viewpoint] *Landwirtschaftliche Forschung* **26**, **Sonderheft 28/II**: 95-114.

Sofos, J.N. and Busta, F.F. (1993) Sorbic acid and sorbates. In: *Antimibrobials in foods*. Edited by P.M. Davidson and A.L. Branen. pp. 49-94 Marcel Dekker, Inc., New York, USA.

Strauss, G. and Hayler, T. (2001) Effects of organic acids on microorganisms. *Kraftfutter* **4**: 147-151.

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POSSIBILITIES OF E. COLI CONTROL BY USING ACIDIFIER IN LIVESTOCK PRODUCTION

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Escherichia coli is a gram-negative bacillus. Important strains of *E. coli* in animal production are K88, K99, 987P and F41. Infection in neonates is commonly caused by K88 and 987P strains, whereas post weaning colibacillosis is nearly always due to the impact of K88 strains.

E. coli is an important cause of enteric diseases in the piglet, from birth until after weaning. Immunization of sows using commercially available vaccines may effectively control neonatal diarrhoea but not the post weaning diarrhoea or oedema disease. In the past, *E. coli* has been traditionally associated with severe, watery diarrhoea, dehydration, and often death in piglets during the first week of life. These *pathogens* colonize the intestinal epithelium by means of various fimbrial adhesins including F4 (also known as K88), F5, F6, and F41. They produce enterotoxins which induce an influx of water and electrolytes into the intestine, resulting in the characteristic clinical symptoms. Certain combinations of adhesins and enterotoxins (pathotypes) have been associated more frequently with neonatal diarrhoea, and these may vary from one geographical region to another.

Most commercially available vaccines for *E. coli* diarrhoea are directed against the fimbrial adhesins. Immunization of sows near the end of gestation with such vaccines results in the production of specific antibodies which are passed to the piglets via colostrum, and effectively block intestinal colonization by the pathogenic *E. coli*; hence preventing the development of the more severe form of the disease observed in piglets during the first week of life. However, this type of immunization does not usually stimulate a high level of specific lactogenic antibodies which would be present in the sow's milk until weaning. Occasionally, immunization of the sow with a commercial *E. coli* vaccine does not appear to effectively protect piglets against the development of neonatal diarrhoea. It is important to realize that *E. coli* diarrhoea may be clinically indistinguishable from diarrhoea of other causes or may be present as a mixed infection with other organisms. Hence, a thorough and accurate diagnosis should be made following submission of intestinal samples from autopsied piglets or rectal swabs from live piglets. Diagnostic tests based on the detection of fimbrial adhesins and enterotoxins are required to accurately identify the causative *E. coli*. Another possible reason for vaccination may be that the causative *E. coli* produces an adhesin which is not present in the vaccine. In such cases, the use of an autogenous bacterin for vaccination may be advised.

F4 *E. coli* diarrhoea can affect older suckling piglets, although it tends to be less severe. Routine intra-muscular or subcutaneous immunization of the sow, either with a killed bacterin or subunit commercial vaccine, stimulates the production of specific colostral antibodies that are mostly effective in the first week of life and hence may not protect the older suckling piglets. In such cases, the use of an oral, preferably live, vaccine which would stimulate the production of more long-lasting lactogenic antibodies may be advisable.

The development of multiple bacterial resistances to a wide range of commonly used antibiotics, and a recent increase in the prevalence of post-weaning syndromes will necessitate the use of alternative measures, such as piglet vaccination or the use of functional additives like acidifiers, for their control.

E. coli is considered to be the most dangerous bacteria in the gut of pigs. It can survive inactively or can cause an outbreak of diarrhoea and other diseases in animals. Traditionally, low-dose antibiotics given to the feed were used to control *E. coli*. However, this procedure doesn’t bring long-term benefits to animal producers since the bacteria are able to rapidly develop resistances.

Main consequences of frequently used in-feed antibiotics for diarrhoea treatment are:

- an imbalance of the gut micro-flora
- the reduced efficacy of antibiotic therapeutics
- the development of resistant bacteria strains, therefore leading to a lower efficacy of therapeutics and finally
- the increased spread of salmonella and clostridium in faeces and thereby an increased risk of epidemic diseases

Results from investigations into antibiotic resistance to pathogenic bacteria *E. coli* (α-haemolysis) in piglets conducted in Vietnam clearly proved these points (table 1).

The data showed that *E. coli* was almost 100% resistant to therapeutic antibiotics such as penicillin, erythromycin, tetracycline and streptomycin, which have been in use for a long time. Newer antibiotics are still effective but *E. coli* may become resistant to them as well over time. Due to this rapid development of resistance, *E. coli* diarrhoea in piglets, MMA in sows and umbilicus inflammation in chicks may become more severe, leading to higher economic losses in livestock production. Some potential problems in layers are displayed in table 2.

Table 1. Analysis of antibiotic resistance to *E. coli* in piglets as determined by antibiogram (α-haemolysis), (n = 155)

Antibiotic	*No. of resistant samples*	*Percentage*
Penicillin	155	100.0
Erythromycin	155	100.0
Tetracycline	154	99.4
Streptomycin	142	91.6
Lincomycin	120	77.4
Ampicillin	112	72.3
Bacterium	110	71.0
Amoxicillin	101	65.2
Chloramphenicol	99	63.9
Cephalexin	86	55.5
Flumequine	68	43.9
Neomycin	58	37.4
Kanamycin	55	35.5
Colistin	46	29.7
Gentamycin	34	21.9
Norfloxacine	27	17.4

Table 2. Antibiotic resistance in laying hens - results from antibiogram tests to *E.coli* (haemolysis) isolated from a flock in Vietnam, (n = 45)

Antibiotic	*No. of resistant samples*	*Percentage*
Penicillin	45	100.0
Erythromycin	44	97.8
Chloramphenicol	43	95.6
Streptomycin	41	91.1
Ampicillin	40	88.9
Amoxicillin	37	82.2
Bactrim	24	53.3
Cephalexin	22	48.9
Gentamycin	17	37.8
Kanamycin	14	31.1
Neomycin	12	26.7
Tobramycin	12	26.7
Norfloxacine	11	24.4
Colistin	10	22.2

Although the laying hen flock was treated with antibiotics via a pulsed program administered over 3 days per month in drinking water, *E. coli* (α-haemolysis) was resistant to many therapeutic antibiotics.

Lower efficacy of antibiotics will not only effect livestock production, as several studies have shown development of antibiotic resistance in humans. Antibiotic residues in animal products may be linked to consumer health and have been associated with the following problems:

- allergic reaction when eating meat with antibiotic residues
- some synthetic antibiotics derived from Quinolon like olaquindox, carbadox and norfloxacine are carcinogenic
- consumption of products containing antibiotic residues frequently results in the disorder of the gut micro-flora and creates resistant strains of bacteria, causing problems with therapeutics

Research by Chinh (2000) proved that human *E. coli* (haemolysis) is also resistant to many popular antibiotics. This causes difficulties for therapeutic treatment of infectious diseases and food toxins (table 3).

Table 3. Antibiotic resistance to *E. coli* (haemolysis) in humans from studies conducted inVietnam

Antibiotic	*No. of tested samples*	*Percentage of resistance*
Chloramphenicol	129	94.7
Ampicillin	129	84.5
Bactrim	53	79.2
Cephalothin	60	73.3
Gentamycin	115	39.1
Ceftriazone	120	31.7
Netromycin	125	29.6
Amikacine	90	26.7
Cefobis	50	20.0

Such issues have heightened public concern regarding the development of resistant pathogen strains and antibiotic residues in animal products. This has led to pressure for alternative means of controlling scours in animals with no hazard for consumers. In this context, organic acids, which form natural constituents of some feeds and are generated during metabolism and in the gastrointestinal tract, are attractive alternatives. They can be used as an in-feed prophylactic measure in a manner similar to that provided by antimicrobial feed additives. Organic acids are also known as effective preservatives which protect stored feeds against undesirable bacterial or fungal growth (Frank, 1994), and improved quality of feeds over time may further contribute to improved animal performance.

Acidifiers used in feed have two modes of action. The first one is reduction of pH in the digestive tract, while the second is the direct anti-microbial effect against gram-negative bacteria like *E. coli*.

A row-dilution test (Sadler and Binder, 1996) was carried out in order to determine the anti-bacterial effect of different test substances (organic acids). This trial design made it possible to determine the minimum inhibitory concentration (MIC) required for bacteriostasis (Drews, 1983). The substances that were used in the test are listed in table 4.

Table 4. Test substances used in row-dilution test for bacteriostasis assessments

Substance	*Concentration (%)*
acetic acid	99
formic acid	98
lactic acid	90
ortho-phosphoric acid	85
citric acid	100
propionic acid	100
fumaric acid	100
ammonium acetate	100
ammonium formate	100
calcium formate	100

Furthermore, the following substance mixtures were included into the trial too (as a 1:1 mixture) (table 5).

Table 5. Substance mixtures used in row-dilution test

Substance mixtures	*Concentration (%)*
acetic acid / lactic acid	99 / 90
formic acid / lactic acid	98 / 90
formic acid / ortho-phosphoric acid	98 / 85

The test to identify the minimal inhibitory concentration of *E. coli* was conducted using the strain *E. coli* IFA-No. 00-058,00.

The experimental design used 2% solutions of the test substances and mixtures, which were prepared in a PBS buffer and sterile filtered, except for fumaric acid which was prepared in a 1% solution, since its solubility is limited. Calcium formate was dissoved in normal tap water, since a precipitation was visible in PBS.

Substance mixtures were provided by mixing the substances in a ratio of 1:1, and 3 ml of the 2% solutions were added to 3 ml of sterile culture medium and 1:2 ratio dilution rows were prepared. Dilution steps were 1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.0313% and 0.0156%. The inoculation was done with an overnight culture of *E. coli*, washed with 200 µl PBS, and using an OD of 600 nm with 0.2±0.01 accuracy.

Due to the volume of the inoculation the real substance concentrations were 0.9375%, 0.4688%, 0.2344%, 0.1172%, 0.0586%, 0.0293% and 0.0147% respectively.

Samples were incubated at 35°C for a period of 16 hours under light rotation. Results were taken by optical analysis of the culture pipes after 16 h of incubation (table 6).

Table 6. MIC of *E. coli* for single acids and mixtures of test substances

Test substance	*MIC of E. coli (%)*
acetic acid	0.0586
formic acid	0.0293
lactic acid	0.1172
ortho-phosphoric acid	0.0586
citric acid	0.1172
propionic acid	0.0586
fumaric acid	0.0586
ammonium acetate	>1
ammonium formate	>1
calcium formate	>1
acetic / formic acid	0.0586
formic / ortho-phosphoric acid	0.0293
formic / lactic acid	0.0293

As shown by the results of the MIC-test, each organic acid performed differently, in particular formic acid and its combinations (e.g. formic acid + ortho-phosphoric acid), the latter having the strongest impact on *E. coli* in a laboratory trial.

These findings were supported by studies from Hsiao and Siebert (1999) and Nakai and Siebert (2003), who found much lower MIC levels for formic acid compared to fumaric acid in *E. coli* strain ATCC 25922. They reported MIC levels of 5.16 g/litre and 2.20 g/litre of formic acid compared to 98 and 122 g/litre fumaric acid respectively.

The efficacy of different acidifiers against *E. coli* under field conditions in animal production and their potential activity *in vivo* has been reported in several studies. Hebeler (2000) found significantly reduced numbers of *E. coli* in piglets one week after weaning when fed diets containing 18 g formic acid salt per kg. In segments of the small intestine, the micro-organism counts of *Lactobacillus*, *Bifidobacterium*, *Eubacterium* and *Bacteroidaceae* spp. were slightly decreased by the addition of 6-24 g formic acid per kg diet (Gedek *et al.*, 1992a). The effects on *E. coli* counts in ileal digesta were not consistent, the highest counts being observed for diets supplemented with 18 g formic acid per kg diet. In the caecum and colon, the counts of *Lactobacillus*, *Bifidobacterium* and *E. coli* were decreased significantly ($P<0.05$) irrespective of the level of formic acid supplementation. Counts of *Lactobacillus*, *Bifidobacterium*, *Eubacterium* spp. and also the total populations of microflora in the duodenum, jejunum and ileum were significantly reduced by the addition of 18 g fumaric acid per kg feed (Gedek *et al.*, 1992b), with the influence of sodium formate being less profound. Fumaric acid also reduced the *Lactobacillus*, *Bifidobacterium* counts in the caecum and colon. *E. coli* counts were reduced in the jejunum by fumaric acid treatment, but not in other segments of the gastrointestinal tract.

Walsh *et al*. (2003) found that the *E. coli* counts for pigs being fed an antibiotic diet (carbadox) were significantly ($P<0.05$) higher than pigs fed a diet containing a combination of organic (0.4%) and inorganic acids (0.2%). Also, pigs fed the combined acidifier diet and an organic acid blend (0.4%) diet tended to have lower *E. coli* fecal shedding ($P<0.10$) than pigs fed the positive antibiotic growth promoter treatment or the negative control diets. The inorganic acid based blend (0.2%) diet gave intermediate results.

No *E. coli* were found in the stomach of piglets fed on diets supplemented with 10 g propionic acid or 3.5 - 12 g formic acid per kg diet (Bolduan *et al.*, 1988). A rise in gastric pH with inefficient digestion may provide an optimal environment for the colonization of enterotoxigenic haemolytic bacteria on the surface of villi, resulting in the initiation of scours and/or oedema disease in young pigs, particularly after weaning (Smith and Jones, 1963). Alternately, a reduced buffering capacity of diets containing organic acids may also be expected to slow down the proliferation and/or colonization of undesirable microbes, in the gastro-ileal region. As particular organic acids have strong antimicrobial activities, it seems appropriate that their positive influence on digestion may be related to indirect effects on gastrointestinal microbes.

It can be concluded from this research that increased use of antibiotics as growth promoters (AGPs) for livestock production, both in pig and poultry farming, can result in a high percentage of resistant bacterial strains, especially *E. coli*. This not only affects animal production, but may have an impact on human medicine too. As a result, a cutback in the use of AGPs in animal production has been recommended and since January 2006 in EU countries, they have been banned.

Possible solutions include new feeding concepts, including pro- and prebiotics as well as acidifiers, as they are commercial available and scientifically proven within a wide range of trials. Implimenting the use of acidifiers in-feed can improve animal welfare and performance, and acid combinations can provide a new feeding concept leading to cost-effective as well as sustainable livestock production without AGPs, whilst allowing control of undesired *E. coli* growth.

References

Bolduan, G., Jung, H., Schneider, R., Block, J. and Klenke, B. (1988) Influence of propionic and formic acids on piglets. *Journal of Animal Physiology and Animal Nutrition* **59**: 72-78.

Drews, G. (1983) *Mikrobiologisches Praktikum*. Springer Verlag, Germany. pp. 176-177.

Liem, D.T. (2004) E.coli resistant to most antibiotics in Vietnam. *Asian Pork* **8/9**: 22-24.

Frank, K. (1994) Measures to preserve food and feeds from bacterial damage. *Übersichten zur Tierernährung* **22**: 149-163.

Gedek, B., Kirchgessner, M., Eidelsburger, U., Wiehler, S., Bott, A. and Roth, F.X. (1992a) Influence of formic acid on the microflora in different segments of the gastrointestinal tract. 5. Nutritive value of organic acids in piglet rearing. *Journal of Animal Physiology and Animal Nutrition* **67**: 206-214.

Gedek, B., Roth, F.X., Kirchgessner, M., Wiehler, S., Bott, A. and Eidelsburger, U. (1992b) Influence of fumaric acid, hydrochloride acid, sodium formate, tylosin and toyocerin on the microflora in different segments of the gastrointestinal tract. 14. Nutritive value of organic acids in piglet rearing. *Journal of Animal Physiology and Animal Nutrition* **68**: 209-217.

Hebeler, D., Kulla, S., Winkenwerder, F., Kamphues, J., Zentek, J. and Amtsberg, G. (2000) The antimicrobial effect of KDF in piglets one week after weaning. *54th Proceedings of the Society of Nutrition Physiology*. Göttingen, Germany. March 7-9.

Hsiao, C.P., Siebert, K.J. (1999) Modeling the inhibitory effects of organic acids on bacteria. *International Journal of Food Microbiology* **47**: 189-201.

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

Nakai, S.A. and Siebert, K.J. (2003) Validation of bacterial growth inhibition models based on molecular properties of organic acids. *International Journal of Food Microbiology* **86**: 249-255.

Chinh, N.T. (1999 – 2000) The Central Army Hospital # 108.

Sadler, M. and Binder, J. (1996) Determination of the minimal inhibiting concentration of different substances and substance mixtures with the aid of a row-dilution-test. IFA Tulln, In-house report: 1-5.

Smith H.W. and Jones, J.E. (1963) Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. *Journal of Pathology and Bacteriology* **86**: 387-412.

Walsh, M., Sholly, D., Kelly, D., Cobb, M., Trapp, S., Hinson, R., Hill, B., Sutton, A., Radcliffe, S., Harmon, B., Smith, J. and Richert, B. (2003) The effects of supplementing weanling pigs diets with organic and inorganic acids on growth performance and microbial shedding. *2003 Swine Research Report*. Purdue University. pp. 89-98.

3

POSSIBILITIES OF SALMONELLA CONTROL WITH THE AID OF ACIDIFIERS

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Salmonella in animal husbandry

In 1885, a veterinarian, Daniel E. Salmon, discovered the first strain of salmonella from the intestine of a pig, which was named *Salmonella choleraesuis*. When infection by salmonella organisms occurs in the digestive tract, it can lead to diarrhoea, septicemia, and death. Infection by the respiratory route leads to pneumonia, septicemia, and eventually death.

Salmonella is a gram-negative, motile, rod. More than 2,500 strains of *Salmonella* have been identified and some are species specific. It is non-spore forming, facultative anaerobe. This bacterium causes typhoid fever and other infections of intestinal origin in humans. In recent years poultry breeders have nearly eradicated *Salmonella pullorum* from parent stock, however, *Salmonella enteritidis* and *Salmonella typhimurium* remain as major concerns in food health safety for humans, as their serotypes are associated with food poisoning. In poultry, *Salmonella typhimurium* damages the intestinal mucosa of animals and causes pericarditis and perihepatitis. *Salmonella enteridis* results in liver necrosis and the caeca develops yellowish cheese-like plugs.

Control of salmonella at the farm level

Because *Salmonella* spp. are ubiquitous, transmission can occur through contact with soil, wild birds, rodents, insects, pet animals, and water. In humans, a simple handshake can spread the bacterium. Feed (figure 1) is always a main suspect, since ingredients are harvested with little regard to possible contamination by wildlife. In recent years, concerns have been raised because many strains of *Salmonella* spp. have become resistant to several of the antibiotics traditionally used for treatment, in both animals and humans. An effective prevention strategy is required to limit exposure to this pathogen on the farm. It is necessary to monitor animals, visitors, equipment, water supply, pests, and feed with appropriate actions. Vaccination is also available, while use of antibiotics is more questionable due to increased resistance and recent legislation (Hoszowski and Waysl, 2002).

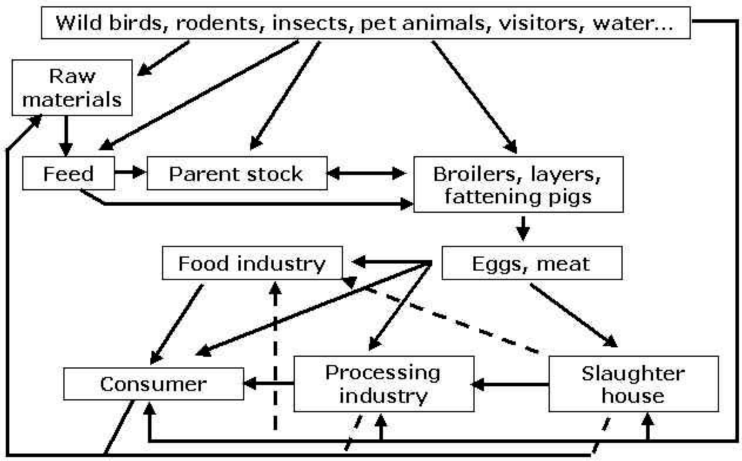

Figure 1. Infection routes of *Salmonella* in animal production (Adapted from Oostenbach, 2004)

Control of salmonella in feed

Different strategies have been suggested to control salmonella in feed (Wray, 2001). Heat treatment (above 70°C, 160°F) by pelleting reduces the risk when compared to meal feed, and salmonella prevalence in meal feeds can increase when finely ground grains are used compared with coarse ground. Although appropriate heat treatment of feed is sufficient to remove the bacteria, recontamination is likely to happen before the feed is made available to the animals. Use of fermented liquid feed reduces contamination, but this technique has many limitations and so far has not been widely implemented on commercial farms. Use of acidifiers in feed can offer an effective solution.

Mode of action of acidifiers against pathogenic bacteria

Acidifiers used in feed have two modes of action. The first one is reduction of pH in the digestive tract. Reduction of pH happens when acids dissociate in a liquid environment and liberate protons (H^+). Inorganic acids will dissociate completely and have therefore a strong effect on pH. S*almonella* spp. survive within a pH range of 4 to 9, with an optimum range for growth from 6.5 to 7.5, so either low or high pH values in the environment will inhibit the growth of the bacteria. At very low pH values (e.g. pH 3) protons (H^+) leak across the membrane faster than the homeostasis system can remove them. This causes intracellular acidification, to levels that damage or disrupt key biochemical processes.

Lowering the pH to extreme values is not practical because acids are corrosive and dangerous for both humans and animals, and can cause damage to milling and handling equipment. Moreover, it is almost impossible to significantly modify the pH value of the digestive tract of animals because homeostasis combined with buffer capacity of feed, work together to reduce variations in pH. Finally, it has been demonstrated that certain *Salmonella* spp. can develop acid resistance when exposed to low pH values for long periods (Foster, 1991; Bearson *et al*., 1998).

Organic acids (which are classified as weak acids) have a different mode of action against *Salmonella* spp. and other gram-negative bacteria. Organic acids are common products of microbial metabolism, and have a long history as food preservatives due to their antimicrobial properties. In solution, organic acids exist in pH-dependant equilibrium between uncharged, acid molecules and their respective charged anions (for example acetic acid/acetate). The key basic principle regarding their mode of action is that, in their non-dissociated (non-ionized, more lipophilic) form, they can penetrate the bacteria cell wall by traversing the membrane, and disrupt the normal processes of certain types of bacteria.

Since the proportion of dissociated acid increases as pH increases, once inside the cell, they dissociate. This exposes the acid to the near neutral intracellular pH of the bacteria, liberating an anion (A^-) and a proton (H^+) in the cytoplasm (Russell and Diez-Gonzalez, 1998). This causes internal pH to decrease, and because pH sensitive bacteria do not tolerate a big difference between the internal and the external pH, a specific mechanism (H^+ -ATPase pump) is activated, bringing the pH inside the bacteria to a normal level (figure 2). This phenomenon consumes energy, can eventually stop the growth of the bacteria, and may kill it.

The anionic (A^-) part of the acid remains trapped inside the bacteria, because it diffuses freely through the cell wall only in its non-dissociated form. This accumulation of anions becomes toxic to the bacteria (Russell, 1992) as it inhibits metabolic reactions (Krebs *et al*., 1983), reducing the synthesis of macromolecules (Cherrington *et al*., 1991), and disrupts internal membranes (Freese *et al*., 1973). Non-pH sensitive bacteria (such as lactic acid bacteria) can tolerate a larger differential between the internal and the external pH, if the internal pH becomes low enough, organic acids re-appear in a non-dissociated form and exit the bacteria by the same route as they entered. Gram-positive bacteria may also have a high concentration of intracellular potassium, which provides a counter cation for the acid anions (Russell and Diez-Gonzalez, 1998).

The antimicrobial effect of organic acids occurs at higher concentration and with acids constructed from longer carbon chains. Gram-negative bacteria are unable to tolerate long and medium-chained organic acids (Canibe *et al*., 2001).

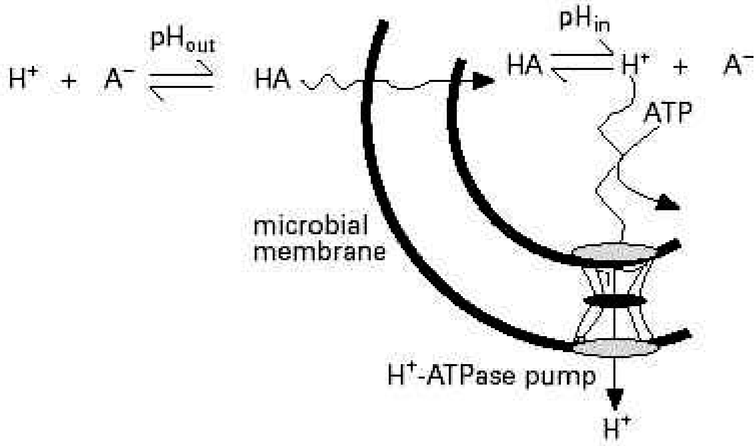

Figure 2. Control of cytoplasmic proton-level by the membrane-bound H^+-ATPase pump (Adapted from Lambert and Stratford, 1998)

Another important parameter to consider regarding the efficacy of acids is the constant dissociation of the acid (pK), which is the pH value where concentrations of dissociated and undissociated species are equal. For example, formic acid (pK = 3.75) will be 50% dissociated and 50% undissociated at a pH value of 3.75. Thus, anti-bacterial activities of organic acids are influenced by the pH of the intestinal tract. The higher the pH value, the more they will tend to dissociate.

Efficacy of different acidifiers against salmonella in animal production

A row-dilution-test (Sadler and Binder, 1996) was carried out in order to determine the anti-bacterial effect of different test substances (organic acids) against salmonella, following the same design as described in chapter "*Possibilities of E. coli control by using acidifier in livestock production*" (see page 17).

Salmonella typhimurium Strain DSM-No. 554 was used to identify minimal inhibitory concentration (table 1).

As shown by the results of the MIC-test the variety of organic acids performed differently. Especially formic acid and combinations with formic acid (e.g. formic acid + ortho-phosphoric acid) showed the strongest impact on *Salmonella typhimurium* in a laboratory trial.

Waldroup *et al.* (1995) studied the effect of supplementing citric acid at 1% inclusion in broiler feed, and observed that the number of birds contaminated with *Salmonella* spp. was increased when compared to the control group. The same researchers found that fumaric acid (0.5, 1.0 and 2.0%) in broiler diets was not

Table 1. Minimal inhibitory concentration MIC of *S. typhimurium* in different test substances

Test substance	MIC of *S. typhimurium* in %
acetic acid	0.0586
formic acid	0.0293
lactic acid	0.1172
ortho-phosphoric acid	0.1172
citric acid	0.1172
propionic acid	0.0586
fumaric acid	0.0586
ammonium acetate	>1
ammonium formate	>1
calcium formate	>1
acetic / formic acid	0.0586
formic / ortho-phosphoric acid	0.0293
formic / lactic acid	0.0293

sufficient to control caecal *Salmonella typhimurium* colonization or carcass contamination. Likewise, lactic acid (0.25, 0.5, 1.0 and 2.0%) fed to broilers, as a supplement also did not control caecal *Salmonella typhimurium* colonization or carcass contamination.

In other trials, Jorgensen *et al*. (2001) reported that a dietary inclusion of 2.8% of lactic acid reduced the number of salmonella-positive faecal samples in weaned piglets. When added to drinking water before slaughter, lactic acid at 0.45-0.5% was effective in reducing Salmonella populations in broilers (Byrd *et al*., 2001).

Izat *et al*. (1990a) studied formic acid and calcium formate in broiler feed. Adding 0.36% calcium formate and 0.25% formic acid significantly reduced levels of *Salmonella typhimurium* in poultry carcasses. Caecal salmonella counts were reduced when 0.36% calcium formate or 0.5% formic acid were added in the diet. Izat *et al*. (1990b) also examined the effects of buffered propionic acid, associated with glycol. They observed that when 0.4% of this mixture (corresponding to 0.2% of propionic acid) was added to the feed, it reduced the population of *Salmonella typhimurium* in broiler carcasses after slaughter.

Kovarik and Lojda (2000) report that formic acid at 0.5% in the diet can be successfully used on farms to reduce salmonella contamination in feed, excretion of *Salmonella* spp. and re-infection of chicken populations. Byrd *et al*. (2001) demonstrated that 0.5% of formic acid added to drinking water pre-slaughter could also control salmonella populations in broilers.

Canibe *et al*. (2001) evaluated organic acids added to stomach contents *in vitro* at pH 4, and reported that their relative efficacy against *Salmonella typhimurium* was as follows:

acetic acid < formic acid < propionic acid < lactic acid < sorbic acid < benzoic acid.

Typically, blends of organic acids, representing an array of pK optima, are more effective against *Salmonella* spp. than single acids alone. This synergism, demonstrated by Thompson and Hinton (1997), demonstrated improved efficacy from a combination of propionic and formic acids when compared to single acids.

Al Tarazi and Alshawabkeh (2003) reported that a mixture of dietary formic and propionic acids (total concentration 2% or more in the diet) for of newly hatched infected layer chicks significantly decreased the crop and caecal population of *Salmonella pullorum*; and reduced mortality. Iba and Berchieri (1995) carried out experiments on the antibacterial effects of a commercial formic acid-propionic acid mixture against different salmonella serotypes, using a dosage of 0.2% in diets. After 28 days of storage, the bactericidal effect in feed was still considerable. Chickens reared on the treated feed that had been artificially contaminated with *Salmonella enteritidis* and *Salmonella typhimurium* showed no contamination in caecal contents.

Walsh *et al*. (2003a, 2003b) observed that a combination of organic acids at 0.4% of the diet, or a mix of different organic and inorganic acids at 0.2 or 0.4%, can successfully reduce the salmonella shedding in faecal samples from piglets, whereas addition of 0.2% of inorganic acid did not have any impact.

The effect of the inclusion of an acid blend consisting of formic acid, propionic acid and their salts on a slow release medium has been determined *in vitro*. Feed samples were mixed with intestinal juice that was inoculated with 10^3 cells of *Salmonella typhimurium*, and incubated at 37°C. After 0 and 4 hours of incubation, samples were taken to determine the number of colony forming units (table 2).

Table 2. Growth of *Salmonella typhimurium* in intestinal juice under treated and non-treated conditions

CFU count	*0 hours*	*4 hours*
Control	2.9×10^3	1.4×10^5
0.3% acid blend	3.0×10^3	2.1×10^2

The combination of formic and propionic acid reduced the gram-negative bacteria *Salmonella typhimurium* under the conditions of the small intestine.

Another laboratory trial was conducted with the same acid blend against the gram-negative bacteria *Salmonella enteritidis* in order to determine the amount of the acidifier that is necessary to stop the growth of salmonella. A suitable medium was applied together with basis-bouillon Salmosyst on plates with agar according to DSMZ and incubated at 25°C±2°C. The experimental period lasted for 24 hours. Four different trial groups were applied: 0.12% acid blend, 0.25% acid blend, 0.50% acid blend and 1.00% acid blend (table 3).

Table 3. Inhibition capacity of an acid blend consisting of formic and propionic acid against *Salmonella enteritidis*

Acidifier concentration	*Inhibition at cfu per ml*
0.12% acid blend	5.00×10^5
0.25% acid blend	5.00×10^7
0.50% acid blend	$> 5.00 \times 10^8$
1.00% acid blend	$> 5.00 \times 10^8$

The blended mixture of formic and propionic acid was able to stop the growth of salmonella within the recommended dosage rate (3-5 kg/t) at a level of 10^7 to 10^8 CFU/ml in the inoculated medium. This demonstrates that the acid blend is effective in the suppression of further growth of *Salmonella enteritidis* in-vitro. It corresponds well with data obtained from Adams (2001), who used a mixture of propionic and formic acid to successfully reduce the initial *Salmonella enteritidis* contamination in wheat by 59% at 3 kg/t.

Conclusion

Pathogenic bacteria colonisation in the gastro-intestinal tract, including *Salmonella* spp., can be decreased at low pH generated by the use of acidifiers, and without inhibiting beneficial bacteria (e.g. *Lactobacilli* spp.) (Kirchgessner and Roth, 1988). Preservation of feedstuffs and complete diets with organic acids has been proven to reduce the microbial loads of salmonella in feed, thus avoiding degradation and maintaining feed safety (Eidelsburger, 1997). The efficacy of organic acids against salmonella depends upon their concentration, pK value and molecular size. Combination of organic acids (and possibly aldehydes, natural terpenes and surfactants) can bring synergistic effects. In addition, attention should also be focused on using a carrier that ensures liberation of the acids on their site of action and avoids related problems with corrosion.

References

Adams, C.A. (2001) Health promoting additives (nutricines). In: *Proceedings of the 1st World Feed Conference: Advances in Nutritional Technology*, Utrecht, The Netherlands. pp. 207–227.

Al Tarazi, Y.H. and Alshawabkeh, K. (2003) Effect of dietary formic acid and propionic acids on Salmonella pullorum shedding and mortality in layer chicks after experimental infection. *Journal of Veterinary Medicine*, Series B. **50** (3):

112-117.

Bearson, B.L., Wilson, L. and Foster, J.W. (1998) A low pH-inducible, PhoPQ-dependent acid tolerance response protects Salmonella typhimurium against inorganic acid stress. *Journal of Bacteriology* **180** (9): 2409-2417.

Byrd, J.A., Hargis, B.M., Caldwell, D.J., Bailey, R.H., Herron, K.L., McReynolds, J.L., Brewer, R.L., Anderson, R.C., Bischoff, K.M., Callaway, T.R. and Kubena, L.F. (2001) Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on salmonella and campylobacter contamination of broilers. *Poultry Science* **80**: 278-283.

Canibe, N., Engberg, R.M. and Jensen, B.B. (2001) An overview of the organic acids on gut flora and gut health. In*: Proceedings of the Workshop on alternatives to feed antibiotics and anticoccidials in the pig and poultry meat production, gut environment and feed additives*. October 13-16, 2001, Oslo, Norway.

Cherrington C.A., Hinton M., Pearson G.R. and Chopra I. (1991) Short-chain organic acids at pH 5.0 kill Escherichia coli and Salmonella spp. without causing membrane perturbation. *Journal of Applied Bacteriology* **70** (2): 161-165.

Drews, G. (1983) *Mikrobiologisches Praktikum*. Springer Verlag, Germany. pp. 176-177.

Eidelsburger, U. (1997) Organische Säuren und was sie in der Schweinefütterung bewirken. Optimierung der Futterqualität ist nur ein Teilaspekt. *Schweinewelt, Jahrgang* **22**: 18-21.

Foster, J.W. (1991) Salmonella acid shock proteins are required for the adaptative acid tolerance response. *Journal of Bacteriology* **73** (21): 6896-6902.

Freese, E., Sheu, C.W. and Galliers, E. (1973) Function of lipophilic acids as antimicrobial feed additives. *Nature* **241**: 321-325.

Hoszowski, A. and Waysl, D. (2002) Salmonella serovars found in animals and feeding stuffs in 2001 and their antimicrobial resistance. *Bulletin of the Veterinary Institute in Pulawy* **46**: 165-178.

Iba, A.M. and Berchieri, A. (1995) Studies on the use of a formic acid-propionic acid mixture (Bio-add™) to control experimental *Salmonella* infection in broiler chickens. *Avian Pathology* **24**: 303-311.

Izat, A.L., Adams, M.H., Cabel, M.C., Colberg, M., Reiber, M.A., Skinner, J.T. and Waldroup, P.W. (1990a) Effect of formic acid or calcium formate in feed on performance and microbiological characteristics of broilers. *Poultry Science* **69**: 1876-1882.

Izat, A.L., Tidwell, N.M., Thomas, R.A., Reiber, M.A., Adams, M.H., Colberg, M. and Waldroup, P.W. (1990b) Effects of a buffered propionic acid in diets on the performance of broiler chickens and on the microflora of the intestine and carcass. *Poultry Science* **69**: 818-826.

Jørgensen, L., Kjærsgaard, H.D., Wachmann, H., Jensen B.B., and Bach Knudsen, K.E. (2001) Effect of pelleting and use of lactic acid in feed on Salmonella prevalence and productivity in weaners. In: *Proceedings of the 4th International Symposium* *on the Epidemiology and Control of Salmonella and other Food Borne Pathogens in Pork*. Leipzig, Germany, September 2 to 5, 2001: 109-112.

Kirchgessner, M. and Roth, F.X. (1988) Ergotrope Effekte durch organische Säuren in der Ferkelaufzucht und Schweinemast. *Übersichten zur Tierernährung* **16**: 93-108.

Kovarik, K. and Lojda, L. (2000) WVPA news. (www.wvpa.net/newsletter/reports_czech_rebubl.html).

Krebs, H.A., Wiggins, D., Stubs, M., Sols, A. and Bedoya, F. (1983) Studies on the mechanism of the antifungal action of benzoate. *Biochemical Journal* **214**: 657-663.

Lambert, R.J. and Stratford, M. (1998) Weak-acid preservatives: modeling microbial
inhibition and response. *Journal of Applied Bacteriology* **86**: 157-164.

Oostenbach, P. (2004) Combining strategies for salmonella control. In: *Livestock Focus Asia*, 23-25 February 2004, Bangkok, Thailand.

Russel, J.B. (1992) Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. *Journal of Applied Bacteriology* **73**: 363-370.

Russell J.B. and Diez-Gonzalez, F. (1998) The effects of fermentation acids on bacterial growth. *Advances in Microbial Physiology* **39**: 205-234.

Sadler, M. and Binder, J. (1996) Determination of the minimal inhibiting concentration of different substances and substance mixtures with the aid of a row-dilution test, IFA Tulln, In-house report: 1-5.

Thompson, J.L. and Hinton, M. (1997) Antibacterial activity of formic and propionic acids in the diet of hens on salmonellas in the crop. *British Poultry Science*. **38** (1): 59.

Waldroup, A., Kaniawato, S. and Mauromoustakos, A. (1995) Performance characteristics and microbiological aspects of broiler fed diets supplemented with organic acids. *Journal of Food Protection* **58**: 482-489.

Walsh, M., Sholly, D., Kelly, D., Cobb, M., Trapp, S., Hinson, R., Hill, B., Sutton, A., Radcliffe, S., Harmon, B., Smith, J. and Richert, B. (2003a) The effects of supplementing weanling pigs diets with organic and inorganic acids on growth performance and microbial shedding. *2003 Swine Research Report*. Purdue University. pp. 89-98.

Walsh, M., Sholly, D., Kelly, D., Cobb, M., Trapp, S., Hinson, R., Sutton, A., Radcliffe, S., Harmon, B., Smith, J. and Richert, B. (2003b) Evaluation of organic and inorganic acids in various feeding programs as alternatives to antibiotic growth promoters for nursery pigs. *2003 Swine Research Report*. Purdue University. pp. 99-107.

Wray, C. (2001) Review of research into salmonella infections in pigs. *Review commisioned by the Meat and Livestock Commission*, UK.

4

THE USE OF DIFFERENT DOSAGES OF ACIDIFIER BASED ON INORGANIC ACIDS IN POST-WEANING PIGLETS

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Introduction

It is well known that the swine industry has been interested in reducing piglet weaning age in order to maximize the annual sow productivity, saving costs and improving the economics of pig production on farm. However, weaning at an earlier age exposes the piglet to a wide variety of problems, including nutritional and environmental stresses, which can result in depressed growth, diarrhoea and high mortalities (Ravindran and Kornegay, 1993). During the last few decades, diets for weaning piglets have been supplemented with various antibiotics in prophylactic doses, to prevent gastrointestinal disorders and improve growth rates (4 to 15%) and feed efficiency (2 to 6%; Mroz, 2003), thereby maximising the economics of production. However, in more recent years, public concern has increased regarding the use of antibiotics in animal agriculture and the risk of developing cross-resistance of pathogens to antibiotics used in human therapy, especially in European countries. This has prompted the pig industry to look for alternatives to antibiotic growth promoters, which will maintain pig performance and control gastric disorders.

Acidifiers in animal feed were initially used in piglets to compliment their limited capacity to maintain a low gastric pH, which is linked to problems with digestion (Easter, 1988). Antibiotics inhibit all microbial growth (Cromwell, 1990), whereas acidifiers are more selective in their activity – they can reduce harmful micro organisms and promote beneficial microflora colonisation of the gastrointestinal tract (Mathew *et al*., 1991). The most widespread benefit from acidification of weaner pig diets has been seen with organic forms of acids (Kim *et al*. 2005). Research to date has been primarily focussed on types and levels of applied organic acids (Cole *et al*., 1968; Giesting and Easter, 1991; Eckel *et al*., 1992).

The use of acidifiers containing inorganic acids in-feed has become popular due to their relatively cheaper costs compared to organic forms, and weaner diets including these acids are considered a low cost option. To examine the relative efficacy of inorganic acids, studies were carried out using hydrochloric acid, sulphuric acid and phosphoric acid (Giesting, 1986; Roth and Kirchgessner, 1989; Oh, 2004). These trials were based on experience gained from the use of organic acids in feed, such as fumaric acid, and included high amounts (e.g. 3%) of these pure and corrosive liquids.

Supplementation of feed with concentrated hydrochloric acid or sulphuric acid resulted in severe growth depression in weaning piglets. The addition of phosphoric acid did not reduce growth performance, but neither was there any indication of improvement in performance (Schoenherr, 1994). Roth and Kirchgessner (1989) concurred that, despite the lowering of the pH value in the diet with phosphoric acid, no additional growth performance was seen. These results demonstrate that the use of inorganic acids at such high doses has no, or even a negative, effect on growth performance, and it was postulated that this may be due to alteration of electrolyte balance or to feed palatability.

More recent investigations have lead to more promising data. Schoenherr (1994) reported comparable growth benefits in piglets for supplementation with fumaric acid and a phosphoric acid-based product immediately post-weaning. These results are based on 5 nursery tests, using piglets with an average initial weight of around 6.3 kg and a weaning age of 19.1 days (table 1).

Table 1. Phosphoric acid-based acidifier for weaned piglets (0-13 days post weaning) (Schoenherr, 1994)

	Negative Control	*Fumaric acid*	*Phosphoric acid*
Daily gain (g)	167.8	181.4	186.0
Daily feed (g)	217.7	222.3	231.3
FCR	1.30	1.23	1.24

Trials continued until day 33 post weaning, but the response to the addition of both the phosphoric acid-based product and the fumaric acid in the diet in the older nursery pigs was less pronounced than during the first two weeks post weaning.

Mahan *et al*. (1996) investigated the use of inorganic acids in weaning pigs as a potential source of minerals. They reported that an addition of hydrochloric acid (as a source of chloride) to the starter diet resulted in improved performance. In a later study (Mahan *et al*., 1999) they also showed that the addition of HCl increased daily weight gain and improved nitrogen retention and apparent digestibility (table 2).

In recent studies, Oh (2004) and Kil (2004) have experimented with the salts of hydrochloric acid, which are less corrosive – one of the major drawbacks of using inorganic acids in animal nutrition. Acid was bound to a carrier (scoria), which is a modified form of volcanic ash. They observed that the inorganic acidifier (scoria-hydrochloric acid complex) was less corrosive or volatile and improved the growth performance of weaning pigs during the 3 week post-weaning period. Furthermore, the level of blood IgA was lowered in pigs fed on the inorganic acid complex (Oh, 2004). Seven days following weaning, IgA levels in the negative control group were around 250 ng/ml, while the treated groups (1-3 kg scoria-hydrochloric acid complex) had IgA levels well below 200 ng/ml. This effect became more pronounced on day 16 post weaning, when the negative control group reached levels of more than 300 ng/ml IgA, while the treatments reached only around 200 ng/ml IgA. This suggests that supplementation with hydrochloric acid has a bactericidal effect, resulting in fewer pathogens and lower IgA production.

Table 2. Effect of added dietary hydrochloric acid on performance and N utilization of weaning pigs (Mahan *et al*., 1999)

Piglet performance	*Dietary chloride level (%) from HCl*					
	0.20	*0.26*	*0.32*	*0.38*	*0.42*	*SEM*
Daily gain (g)						
0-7 d	120	123	156	147	138	11[a]
7-14 d	294	316	342	351	310	10[b]
14-21 d	372	422	447	418	427	12[b]
N retention (g/d)	6.09	6.58	6.66	6.80	6.36	0.11[b]
Apparent digestibility (%)[d]	88.6	90.7	91.4	93.2	94.9	0.54[c]

[a]Quadratic response (P<0.10), [b]Quadratic response (P<0.01), [c]Linear response (P<0.01)
[d]Apparent digestibility (%) = [(N intake – faecal N) / N intake] x 100

Promising results reported with phosphoric acid, compared to organic acids, lead to several attempts at using silica-phosphoric acid complexes in early weaned piglets. The objective of these studies was to evaluate the potential of phosphoric acids as alternatives to AGPs in nursery pig diets in terms of weight gain, diarrhoea and resistance to illness of piglets under field conditions.

The first trial was conducted at a pig farm at Cai Be, in Tien Giang province. Sixty post-weaning piglets (Yorkshire x Landrace x Duroc), aged 26 days were assigned to two treatment groups, based on sex and weight. The treatments included a basal diet as negative control and a diet supplemented with 3 kg/t inorganic acid blend on the other hand. Each pen held 10 piglets, with 3 replicate pens per treatment. The experiment lasted for 28 days. The pens measured 4m x 3m x 0.6m, and faced in the same direction to avoid climate factors that could influence piglet performance. All pigs had *ad libitum* access to feed and water in each pen.

The second trial was run at Tan Trung farm, belonging to the Saigon Agriculture Corporation, and examined the effects of the inorganic acidifier fed at a lower inclusion rate of 2 kg/t, against a commercial piglet diet. One hundred and twenty piglets (Yorkshire x Landrace x Duroc hybrid) aged 25 days were randomly selected and divided into 2 treatment groups as per experiment 1, and were fed and watered *ad libitum*. Piglets were housed in individual weaner cages (4 m x 3 m x 0.6 m) over the 28-day trial period. Performance data were recorded on a weekly basis.

The compound feed for both trials was obtained from My Tuong feed mill of Tien Giang province, Vietnam (table 3).

Table 3. Feed formula for inorganic acidifier trial with piglets

Raw material	*Inclusion (kg/t)*
Corn	351.2
Broken rice	100.0
Wheat bran	62.9
Organic Fe 60	1.5
Milk powder K10	40.0
Dabomb –P	40.0
Lactose	50.0
Expelled soybean meal	120.0
Soybean oil	10.0
Soybean meal 44	155.6
Fish meal 60 (low salt)	30.0
Lysine	1.3
Methionine	0.094
Oyster shell	4.1
DCP (18%P, 24%Ca)	19.8
Salt (NaCl)	4.2
Premix CM 2325	3.0
Antioxidant	1.0
Mould Inhibitor	0.5
Milk flavour	1.0
Total	**1000.0**

Table 4. Feed analysis

Nutrients	*Percentage*	*Nutritive value at 87% dry matter*
Dry matter (%)	90.62	87.00
ME (Kcal/kg)	3200.00	3072.25
Crude protein (%)	21.00	20.16
Crude fat (%)	5.50	5.28
Crude fibre (%)	3.38	3.25
Lysine (%)	1.30	1.25
Methionine (%)	0.37	0.35
Met +Cysteine (%)	0.69	0.66
Threonine (%)	0.85	0.82
Isoleucine (%)	1.02	0.97
Tryptophan (%)	0.27	0.26
Calcium (%)	0.90	0.86
Total phosphorus (%)	0.85	0.82
Phosphorus available (%)	0.59	0.57
Salt (%)	0.50	0.48

Monitored parameters included weekly individual body weight, and feed consumption was estimated by monitoring the daily feed intake. Health status was established every day, and included checks for fever, poor feed intake, arthritis and diarrhoea. These figures were used to calculate the daily diarrhoea and live piglet percentages. Data was analysed using Excel and Minitab software for statistics, with significance levels set at P<0.05.

Table 5. Weight gain of piglets fed 3 kg/t inorganic acidifier versus a negative control (trial 1)

Parameter	*Control*	*Treatment*	*P value*
Average weight, kg			
P0 weight initial	8.00 ± 0.76	8.03 ± 0.76	> 0.05
P1 weight week 1	9.23 ± 0.70	9.25 ± 0.78	> 0.05
P2 weight week 2	10.41 ± 1.05	10.95 ± 1.18	< 0.05
P3 weight week 3	11.69 ± 1.48	12.56 ± 1.57	< 0.05
P4 weight week 4	13.94 ± 1.91	15.25 ± 1.99	< 0.01
Weekly weight gain, kg			
P1 – P0	1.23 ± 0.55	1.49 ± 0.58	> 0.05
P2 – P1	1.18 ± 0.77	1.43 ± 0.78	> 0.05
P3 – P2	1.28 ± 0.55	1.61 ± 0.73	< 0.05
P4 – P3	2.25 ± 0.68	2.69 ± 0.76	< 0.05
P4 – P0	5.94 ± 0.63	7.22 ± 0.71	< 0.01

The trial results showed significant improvements in weight gain for piglets fed the acidified diet. Body weight differences of more than 1 kg were observed for piglets fed the acidified diet after 4 weeks (P<0.01).

Table 6. Feed consumption and FCR of piglets fed 3 kg/t inorganic acidifier versus a negative control (trial 1)

Parameter	*Control*	*Treatment*	*P value*
Total feed consumed/group, kg	315.20	317.90	-
Feed consumed /piglet, kg	10.50	10.60	> 0.05
Average weight gain, kg	5.94	7.22	< 0.01
FCR	1.77	1.47	< 0.01

There were no significant differences between treatments for feed intake, however FCR was lower (1.47 vs. 1.77) by nearly 17% (P<0.01).

Table 7. Illness and diarrhoea incidence for piglets fed 3 kg/t inorganic acidifier versus a negative control (trial 1)

Parameter	*Control*	*Treatment*	*P value*
Total days of piglets in the trial			
30 piglets x 28 days	840	840	-
No. of days of illness	11	8	-
No. of days of diarrhoea	52	26	-
Illness ratio (%)	1.31	0.95	> 0.05
Diarrhoea ratio (%)	6.19	3.09	< 0.05

Illness and diarrhoea incidences in the acidified feed group were around 50% lower than those piglets fed the control diet, but this was only statistically significant for diarrhoea scores (P<0.05). No mortality occurred during the trial in both groups.

Data from the second trial are shown in table 8.

Table 8. Animal performance for piglets fed 2 kg/t inorganic acidifier versus a negative control (trial 2)

	Control	*Acidifier treatment*
Number of pigs	60	60
Initial weight (kg)	6.72	6.68
Final weight (kg)	14.15^{A}	15.63^{B}
Daily weight gain (g)	248^{A}	298^{B}
FCR	1.54^{a}	1.45^{b}
Daily diarrhoea ratio (%)	2.83^{A}	1.11^{B}
Daily illness ratio (%)	1.56	0.89

Means not sharing a superscript differ significantly at P<0.05 and P<0.001 (capitalised) respectively

Body weight data from piglets in trial 2 showed significant improvements in those groups fed the acidified diets, even with a lower inclusion rate of 2 kg/t. The body weight difference of nearly 1.5 kg for the acidified group after 4 weeks was statistically significant (P<0.001). FCR was lower (1.45 vs. 1.54) by nearly 6% in piglets supplemented with acidifier (P<0.05). Daily diarrhoea ratio was statistically lower for the acidifier treatment compared to the control diet (P<0.001). No mortality occurred in either group.

Conclusions

Dietary acidifiers have been accepted as potential alternatives to antibiotics in order to improve performance and health status of livestock. In post weaning piglets particularly, this is relevant for the application of acidifiers based on inorganic acids, especially phosphoric acid. Using phosphoric acid offers the advantage of reducing the cost of acidifying starter pig diets, and requires less space in formulation compared to organic acid supplementation. Similar or even better growth performance of piglets can be achieved in the first couple of weeks post-weaning, in comparison with fumaric acid.

References:

Cole, D.J.A., Beal, R.M. and Luscombe, J.R. (1968) *Veterinary Record* **83**: 459-464.

Cromwell, G.L. (1990) Antimicrobial agents. In: *Swine Nutrition*. Edited by E.R. Miller, D.E. Ullrey and A.J. Lewis. Butterworth-Heiemann, Boston, MA, USA. pp. 297-313.

Easter, R.A. (1988) Acidification of diets for pigs. In: *Recent Advances in Animal Nutrition*. Edited by W. Haresign and D.J.A. Cole. Butterworths, London. UK. pp. 61-72.

Eckel, B., Kirchgessner, M., and Roth, F.X. (1992) Influence of formic acid on daily weight gain, feed intake, feed conversion rate and digestibility: 1. Nutritive value of organic acids in the rearing of piglets. *Journal of Animal Physiology and Animal Nutrition* **67**: 93-100.

Giesting, D.W. (1986) Utilization of soy protein by the young pig. Ph. D. Thesis. University of Illinois, Urbana, Champaign.

Giesting, D.W. and Easter, R.A. (1991) Effect of protein source and fumaric acid supplementation on apparent digestibility of nutrients by young pigs. *Journal of Animal Science* **69**(6): 2497-2503.

Kil, D.Y. (2004) Comparison of growth performance, nutrient digestibility and white blood cell counts by organic or inorganic acid supplementation in weaned pigs. MSc Thesis. Seoul National University, Korea.

Kim, Y.Y., Kil, D.Y., Oh, H.K. and Han, I.K. (2005) Acidifier as an Alternative Material to Antibiotics in Animal Feed. *Asian-Australasian Journal of Animal Science*s **18**(7): 1048-1060.

Mahan, D.C., Newton, E.A. and Cera, K.R. (1996) Effect of supplemental sodium chloride, sodium phosphate, or hydrochloric acid in starter pig diets containing dried whey. *Journal of Animal Science* **74**: 1217-1222.

Mahan, D.C., Wiseman, T.D., Weaver, E. and Russell, L. (1999) Effect of supplemental sodium chloride and hydrochloric acid added to initial diets containing sprayed-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3-week-old weaned pigs. *Journal of Animal Science* **77**: 3016-3021.

Mathew, A.G., Sutton, A.L., Scheidt, A.B., Forsyth, D.M., Patterson J.A. and Kelly D.T. (1991) Effects of a propionic acid containing feed additive on performance and intestinal microbial fermentation of the weanling pigs. In: *Proceedings*

Sixth International Symposium on the Digestive Physiology in Pigs. PUDOC. Wageningen, The Netherlands. pp. 464-469.

Mroz, Z. (2003) Organic acids of various origin and physicochemical forms as potential growth promoters for pigs. In: *Digestive Physiology in Pigs. Proceedings 9th Symposium*. pp. 267-293.

Oh, H.K. (2004). Effect of dietary supplements on growth, nutrient digestion and intestinal morphology in monogastric animals. Ph. D. Thesis. Seoul National University, Korea.

Ravindran, V. and Kornegay E.T. (1993) Acidification of weaner pig diets: A review. *Journal of the Science of Food and Agriculture* **62**: 313-322.

Roth, F.X. and Kirchgessner, M. (1989) Bedeutung von pH-Wert und Pufferkapazität des Futters für die Ferkelfütterung. 2. Miteilung: Effekt einer pH-Absenkung im Futter durch Orthophosphorsäure-Zusatz auf Wachstum und Futterverwertung. *Landwirtschaftliche Forschung* **42**: 168-175.

Schoenherr, W.D. (1994) Phosphoric acid-based acidifiers explored for starter diets. *Feedstuffs* **66**: 40.

5

EFFECTS OF ORGANIC ACIDS ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITIES IN PIGS

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Introduction

As in-feed antibiotics have been completely banned in the EU, the beginning of 2006 saw intensified research to find suitable replacements. Organic acids and their salts have received much attention as potential alternatives in order to improve the performance and health of weaning and fattening pigs. It is generally accepted that organic acids and their salts lower gastric pH, resulting in increased activity of proteolysis and consequent improved amino acid and protein digestion. Additionally, organic acids selectively inhibit the growth of potential harmful bacteria, like *Escherichia coli*. However, growth-promoting and microbial effects depend on type and inclusion level of the acid used, the buffering capacity of the diet and age of animals. In general, the response to supplementation with organic acids is more pronounced in the young pig, especially around weaning when the digestive system is still immature.

At weaning, pigs are exposed to physiological and environmental stress, which often results in reduced feed intake and little or no weight gain. In some instances diarrhoea, morbidity and even fatalities may occur. The transition from liquid to solid feed and abrupt changes in feed intake (Aumaître *et al.*, 1995) have been identified as major stressors after weaning. At this time, piglets have a limited digestive and absorptive capacity due to an insufficient secretion of hydrochloric acid (HCl) required maintaining a low pH of approximately 3.5 in the stomach (Cranwell and Titchen, 1974). They also have inadequate secretion of pancreatic and brush border enzymes. It takes 3–4 weeks after weaning before the acid secretion in the stomach of piglets is sufficient to reduce gastric pH to a suitable level. In particular, diets with a high buffering capacity exert a negative effect on pepsin activity in the stomach, which, in turn, may have adverse effects on performance (Eidelsburger *et al.*, 1992a). Additionally, during the fattening period, digestive disorders associated with poor performance may also occur, particularly when pigs from different rearing compartments or farms are transferred to other production units and feeding regimes

(Partanen and Mroz, 1999). In the past, in-feed antibiotics have proven efficient tools to compensate for post-weaning or post-rearing stress. Public concerns about the potential development of resistant pathogen strains and general food safety as well as animal health matters resulted in the complete ban of in-feed antibiotics within the EU at the beginning of 2006. Consequently, both nutritionists and feed manufacturers have been challenged to search for new, “safe” alternative growth promoters.

Particular attention has been paid to organic acids, which are widely distributed in nature as constituents of plants, animal tissue or common metabolites of microbial fermentation in the digestive tract (Partanen and Mroz, 1999; Mroz, 2003). They are considered to be weak acids containing up to seven carbon atoms, such as formic, sorbic, citric, propionic, fumaric and lactic acid (table 1). Due to their antimicrobial activities and their beneficial effects on pig performance, organic acids have proven to be an efficient alternative to in-feed antibiotics in modern pig production systems. It has to be emphasized that this effect is more pronounced in weaning rather than fattening pigs (Gabert and Sauer, 1994; Roth and Kirchgessner, 1998).

Table 1. Formulas, physical and chemical characteristics of organic acids used as dietary acidifiers for pigs (Foegeding and Busta, 1991)

Acid	*Formula*	*MM (g/mol)*	*Density (g/ml)*	*Form*	*pK*	*Solubility in water*
Formic	$HCOOH$	46.03	1.22	liquid	3.75	∞
Acetic	CH_3COOH	60.05	1.049	liquid	4.76	∞
Propionic	CH_3CH_2COOH	74.08	0.993	liquid	4.88	∞
Butyric	$CH_3CH_2CH_2COOH$	88.12	0.958	liquid	4.82	∞
Lactic	$CH_3CH(OH)COOH$	90.08	1.206	liquid	3.83	v
Sorbic	$CH_3CH:CHCH:CHCOOH$	112.14	1.204	liquid	4.76	s
Fumaric	$COOHCH:CHCOOH$	116.07	1.635	liquid	3.02 4.38	s
Malic	$COOHCH_2CH(OH)COOH$	134.09		liquid	3.4 5.1	∞
Tartaric	$COOHCH(OH)CH(OH)COOH$	150.09	1.76	liquid	2.93 4.23	v
Citric	$COOHCH_2C(OH)(COOH)CH_2COOH$	192.14	1.665	solid	3.13 4.76 6.4	v

MM: molecular mass; ∞: soluble in all proportions; v: very soluble; s: sparingly soluble

The most likely mechanisms, by which organic acids improve feed conversion ratio and animal health, include acidification, either of the feed or of the stomach, and/or selective inhibition of pathogenic intestinal micro-organisms. They may also be absorbed along the digestive tract, providing an additional energy source for the animal (Roth *et al.*, 1993; table 2). In this review, special attention will be paid to the mode of action of organic acids in the gastrointestinal tract (GIT) of pigs, and to their impact on nutrient digestibility and growth performance.

Table 2. Mechanisms of organic acids and their salts (Kirchgessner and Roth, 1988)

Area targetted	*Acid mechanism*	
Feed		pH decrease
		Antimicrobial effect (bacteria, yeast, fungi)
		Reduced buffering capacity
GIT	Proton	Decreases pH in the stomach
		Increase in efficiency of pepsin (pH optimum 2.5 and 3.5)
		Antimicrobial effect
	Anion	Antimicrobial effect
		Complexing agent (Ca^{2+}, Mg^{2+}, Fe^{2+} etc.)
Intermediary metabolism		Energy source

GIT: Gastrointestinal tract

Mode of action of organic acids in the gastrointestinal tract of pigs

Supplemental organic acids and their salts mainly act in the proximal part of the GIT (stomach and small intestine). The beneficial effects of organic acids cannot be simply attributed to the acidification of the intestinal content, because neither HCl nor phosphoric acid (which are known as ‘strong acids’), improve growth rate or feed conversion in piglet trials. It is likely that the anion is responsible for the beneficial effects of these acids, as it controls bacterial populations in the upper intestinal tract (Roth and Kirchgessner, 1998). The antimicrobial activity of organic acids *in vivo* may be a resultant of various factors including (a) the carbon chain length and inclusion level; (b) the proportion of dissociated to undissociated forms; (c) intra-luminal digesta acidity and acid-binding capacity; (d) the time of retention/exposure of digesta in particular gut segments; (e) specific potency of pathogens for colonization and enterotoxin production (Mroz *et al.*, 2006). Recently, the potential modes of action of organic acids and their salts in the gastrointestinal tract have been reviewed by Mroz (2003) and Mroz *et al.* (2006).

Supplementation with organic acids induces a more rapid reduction of the pH value in the stomach, with optimal values between pH 3 and 4. Lower gastric pH compensates for inadequate endogenous secretion of HCl in weaning pigs, and facilitates the activation of pepsinogen to pepsin. As a result, protein digestibility may be improved and gastric retention time may be increased. Due to the lower rate of gastric emptying, large protein molecules may be more rapidly hydrolysed, improving protein digestion (Ravindran and Kornegay, 1993; Gabert and Sauer, 1994). Organic acids can also stimulate the exocrine pancreatic secretion of enzymes and bicarbonate, assisting fat and protein digestion. Additionally, at lower gastric pH conditions the proliferation and/or colonization of undesirable microbes in the gastro-ileal region are inhibited. This effect has been referred to as the ‘bacteriocidal’ or ‘bacteriostatic’ effect of organic acids (Partanen and Mroz, 1999). Such activity is specific towards certain bacteria only, as the development of beneficial bacteria, such as *lactobacilli* spp. is improved under these conditions.

Protons (liberated during dissociation of organic acids) and anions may serve as a pH barrier against pathogen colonization at the brush border. In the undissociated form, organic acids are lipid soluble, enabling them to penetrate the microbial cell, destroying the cytoplasm or inhibiting growth by deactivating bacterial decarboxylases and catalases (Mroz *et al.*, 2006). An alternative hypothesis for the mode of action of organic acids has been proposed by Russell (1992), who attributed the toxic effects of organic acids to an accumulation of polar anions in the cell wall, which is dependent on the pH gradient across the membrane of the bacterial cell. Bacteria that try to resist internal pH changes are more sensitive to organic acids than those that do not respond to the changing environment, allowing pH to decline (Russell and Diez-Gonzales, 1998). Organic acids also influence mucosal morphology, and serve as substrates in intermediary metabolism. This multifunctional role may lead to improved digestion, absorption and retention of dietary nutrients (Partanen and Mroz, 1999).

Effects of organic acids on growth performance and nutrient digestibilities

Numerous studies have been carried out to specifically investigate the effects of organic acids and their salts on nutrient digestibility and growth performance in growing pigs (e.g. Mroz *et al.*, 2000, 2002; Øverland *et al.*, 2000; Partanen *et al.*, 2001; Canibe *et al.*, 2005). Improvements in growth performance, following dietary supplementation with organic acids, have frequently been attributed to increased apparent ileal digestibility of protein and amino acids (Mosenthin *et al.*, 1992; Blank *et al.*, 1999; Mroz *et al*., 2000).

GROWTH PERFORMANCE

Generally, performance response to organic acid supplementation is more pronounced in piglets than in fattening pigs due to the immature digestive physiology of the younger animal. A recent meta-analysis showed that fumaric acid inclusion in feed usually results in better performance criteria than formic or citric acid, while formic acid was more effective in fattening pigs (Partanen and Mroz, 1999). As a result, most growth performance studies with weaning pigs have been conducted to evaluate the efficacy of formic acid and its salts as well as of fumaric and citric acid as well. (table 3; Partanen and Mroz, 1999).

Table 3. Effect of organic acids or their salts on average daily weight gain (ADG) and feed conversion ratio in piglets and fattening pigs

Growth period	*Acid*	*Dose (%)*	*ADG (g)*	*Feed/gain ratio*	*Reference*
Pre-starter period	Control	0	372	1.32	Roth *et al.*, 1996
	Formic acid	0.85	432	1.20*	
Starter period	Control	0	580	1.82	
	Formic acid	0.85	613	1.83	
Pre-starter period	Control	0	123	2.49	Giesting *et al.*, 1991
	Fumaric acid	3	174*	1.85*	
Pre-starter period	Control	0	336	1,10	Eidelsburger *et al.*, 1992b
	Fumaric acid	1.8	323	1,12	
Starter period	Control	0	452	1,78	
	Fumaric acid	1.8	469	1,69	
Starter period	Control	0	431	1.70	Kirchgessner *et al.*, 1995
	Sorbic acid	1.2	490*	1.63*	
	Sorbic acid	1.8	523*	1.60*	
	Sorbic acid	2.4	546*	1.59*	
Starter period	Control	0	344	2.24	Boling *et al.*, 2000
	Citric acid	1	371	1.98	
	Citric acid	2	398*	1.9*	
Growing period	Control	0	752	2.25	Øverland *et al.*, 2000
	Ca/Na-formate	0.85	758	2.24	
	K-formate	0.8	797*	2.17*	
Finishing period	Control	0	1118	2.65	
	Ca/Na-formate	0.85	1099	2.71	
	K-formate	0.8	1130	2.68	
Finishing period	Control	0	860	3.57	Krause *et al.*, 1994
	Fumaric acid	2.5	880	3.45	
Growing period	Control	0	586	3.74	Grela and Lipiec, 1991
	Citric acid	2.5	622*	3.52*	
Finishing period	Control	0	693	4.49	
	Citric acid	2.5	714	4.39	

*Significantly different from the control diet ($P<0.05$)

Other organic acids, such as acetic, lactic and sorbic acid, have shown potential growth-promoting effects, but studies on these treatments are much less numerous (e.g. Roth and Kirchgessner, 1988; Roth *et al*., 1993).

Formic (or formate), citric and fumaric acids significantly improved ($P<0.05$) weaning piglet average daily gain and feed conversion ratio in comparison to the non-acidified diet. Eckel *et al.* (1992) reported that piglet diets acidified with 0.6-1.2% formic acid resulted in improved daily weight gain (up to 22%) and feed conversion ratio (7%), although responses varied considerably between the different acids. Potential reasons for these varying results may be related to differences in the type and inclusion level of the acid used, composition and buffering capacity of the diet, age of animals and existing levels of performance (Ravindran and Kornegay, 1993).

Certain acids, such as tartaric and formic acid, have a strong odour and flavour which may cause feed refusal, and are therefore associated with lower daily gain (due to reduced feed intake) when the recommended threshold levels of these acids in the diet were reached or exceeded (Eckel *et al.*, 1992). Consequently, the growth promoting effects of organic acids in weaning pigs depend considerably on their influence on palatability (Partanen and Mroz, 1999). The minimal effective level of each individual acid should be evaluated (Best, 2000) to avoid such problems. Using the salts of organic acids instead can be a way around these issues, as they are tasteless and do not influence feed intake.

The most effective acidification can be attained in feeds that are formulated with cereals and plant proteins, as, in comparison, growth promoting effects are smaller in diets based on milk products (e.g. Giesting *et al.*, 1991; Weeden *et al.*, 1991). This is due to the conversion of lactose to lactic acid by lactobacilli in the stomach, which reduces gastric pH and reduces the benefits of supplemental organic acids (Easter, 1988). Differences in performance response may also be influenced by the buffering capacity of the diet, which is lowest in cereals and their by-products. Addition of protein and certain minerals to the diet increases the buffering capacity, and consequently weakens the pH-lowering effect of organic acids (Roth and Kirchgessner, 1989; Blank *et al.*, 1999, 2001).

Research data shows that benefits with organic acids for improved average daily gain in fattening pigs were maximised when formic acid and formates, followed by fumaric acid (Partanen and Mroz, 1999) were used, whereas propionic acid did not show any effect (Thacker *et al.*, 1992). From all the studies conducted in post-weaning pigs, responses were more pronounced in the grower compared to the finisher phase (Kirchgessner *et al.*, 1997). For example, Øverland *et al.* (2000) and Grela and Lipiec (1991) observed a significantly higher daily weight gain and better feed conversion ratio in grower pigs versus finishing pigs (table 3).

NUTRIENT DIGESTIBILITY

In table 4, studies on the influence of organic acids on the apparent total tract digestibility of crude protein and energy in weaning pigs are summarized.

Table 4. Influence of organic acids or theirs salts on the apparent total tract digestibility of crude protein and energy in weaning pigs

Organic acid	*Level*	*Diet*	*Crude protein*		*Gross energy*	
	(%)		*D (%)*	*ΔD*	*D (%)*	*ΔD*
Formic acid						
Eckel *et al*. 1992	0.6	Pre-starter	86.2	+2.2*	88.4	+0.9
	1.2	Pre-starter	86.2	+2.9*	88.4	+1.4
	1.8	Pre-starter	86.2	+3.8*	88.4	+1.9*
	2.4	Pre-starter	86.2	+3.8*	88.4	+2.2*
	0.6	Starter	87	+1.1	89.3	+0.7
	1.2	Starter	87	+1.4	89.3	-0.6
	1.8	Starter	87	+1.2	89.3	+0.4
	2.4	Starter	87	+2.6*	89.3	+0.5
Sodium formate						
Eidelsburger *et al*.. 1992b	1.8	Pre-starter	87.1	3.1*	88.4	1.5*
		Starter	86.4	0	87.7	0
Fumaric acid						
Gabert and Sauer. 1995	1.5	Starter	89.2	-0.2	86.6	-0.2
	3	Starter	89.2	-0.8	86.6	-0.9
Eidelsburger *et al*.. 1992b	1.8	Pre-starter	87.1	+0.4	88.4	+0.6
	1.8	Starter	86.4	+1.0	87.7	-0.2
Sodium fumarate						
Gabert and Sauer, 1995	1.5	Starter	89.2	-1.4	86.6	-0.7
Propionic acid						
Kirchgessner and Roth, 1982	1	Pre-starter	84.5	+1.5	81.9	+1.4
	2	Pre-starter	84.5	+1.6	81.9	+2.2*
	1	Starter	83.0	+0.2	81.5	-0.1
	2	Starter	83.0	+0.5	81.5	+0.7
Calcium propionate						
Roth and Kirchgessner, 1982	1.8	Pre-starter	83.8	+1.6**	81.2	0.3
	1.8	Starter	81.3	+1.6**	81.3	0.2

D: Digestibility of the non-acidified control diet; ΔD: Percentage unit change in the digestibility in relation to the non-acidified control diet
*Significantly different from the control diet ($P<0.05$), **$P<0.1$

Corresponding digestibility data and the values for N retention in fattening pigs are shown in table 5.

Table 5. Influence of organic acids or theirs salts on the apparent total tract digestibility of crude protein and energy and nitrogen (N) retention in growing pigs

	Level	*Crude protein*		*Gross energy*		*N retention*	
Organic acid	*(%)*	*D (%)*	*ΔD*	*D (%)*	*ΔD*	*R (%)*	*ΔR*
Formic acid							
Mroz *et al*. 2000	1.4	80.6	+1.4	82.2	+0.7	48.3	+4.9*
Butyric acid							
Mroz *et al*.. 2000	2.7	80.6	+1.4	82.2	+1.6*	48.3	+4*
Fumaric acid							
Mroz *et al*.. 2000	1.8	80.6	-1.0	82.2	+0.7	48.3	+2.9*
Propionic acid							
Mosenthin *et al*.. 1992	2	80.2	+2.3	77.9	+1.4	-	-

D: Digestibility of non-acidified control diet; ΔD: Percentage unit change in the digestibility relative to the non-acidified control diet; R: N retention of non-acidified control diet as % of intake; ΔR: Percentage unit change in the N retention relative to the non-acidified control diet
*Significantly different from the control diet (P<0.05)

Apparent ileal amino acid and protein digestibilities determined in weaned and fattening pigs are presented in table 6.

Blank *et al.* (1999) reported an increase in the apparent ileal protein and amino acid digestibilities in early-weaned pigs (5.5 to 11.6 kg BW) following dietary supplementation with 1, 2 or 3% fumaric acid. The highest responses were found with 2% fumaric acid. Apparent ileal protein digestibility was significantly improved by 7%, and the amino acid digestibilities from 4.9 to 12.8%, except for methionine, tyrosine and cysteine. Supplementation with fumaric acid in a diet with high buffering capacity caused smaller, numerical increases in the digestibilities of protein and amino acids. In growing pigs, Mosenthin *et al.* (1992) observed no effect on the apparent ileal digestibilities of dry matter, organic matter, crude protein or ash when 2% propionic acid was added to a barley-soybean meal based diet. Only the ileal digestibilities of certain essential amino acids (arginine, histidine, leucine, phenylalanine and valine) were improved.

Mroz *et al.* (2000) reported an increase (P<0.05) of up to 6% in the apparent ileal digestibilities of protein and several essential and non-essential amino acids in growing pigs fed 1.4% of formic acid, 1.8% of fumaric acid or 2.7% of n-butyric acid in a diet with high buffering capacity. For feed with low buffering capacity, only the apparent ileal digestibilities of arginine, isoleucine and leucine were improved by acidification (P<0.05).

Organic acids may improve the absorption of minerals, such as Ca, P, Mg and Zn, in the small intestine (Kirchgessner and Roth, 1988). The presence of anions appears to facilitate cation mineral absorption, and formic, fumaric and n-butyric acid have all been shown to improve apparent total tract digestibility of Ca and total P, as well

Table 6. Influence of organic acids on the ileal digestibility of crude protein and amino acids in weaned and growing pigs

Acid	*Weaned piglets*[1]		*Growing pigs*[2]			
	Control	*2% Fumaric*	*Control*	*1.4% Formic*	*1.8% Fumaric*	*2.7% n-Butyric*
	D (%)	*ΔD*	*D (%)*	*ΔD*	*ΔD*	*ΔD*
Crude protein	73.6	+7.3	69	+4.2*	+3.3	+5.2*
			Essential amino acid			
Arginine	82.7	+5.8*	83.4	+2.0	+1.2	+2.2
Histidine	81.2	+7.9*	76.4	+4.5*	+4.2*	+4.8*
Isoleucine	78.7	+6.7*	75.4	+3.9*	+3.3*	+3.7*
Leucine	78.9	+6.8*	77.6	+3.2*	+2.9*	+3.5*
Lysine	83.4	+5.6*	77.5	+4.7*	+4.0*	+3.9*
Methionine	86.2	+2.5	83.7	+2.3	+0.8	+2.0
Phenylalanine	80.1	+6.1*	77	+3.4*	+2.8	+3.7*
Threonine	68.8	+8.3*	70.4	+4.7*	+4.3*	+4.8*
Tryptophan	-	-	70.2	+2.6	+2.2	+3.4*
Valine	76	+7.1*	71.3	+4.5*	+4.2*	+5.3*
			Nonessential amino acid			
Alanine	67.1	+12.8*	68.3	+5.3*	+5.0*	+6.2*
Aspartate	74.6	+7.6*	71.2	+4.6*	+4.3*	+4.7*
Cysteine	80.1	+2.0	70.6	+5.4*	+2.6	+6.5*
Glutamate	86.2	+4.9*	77.5	+4.3*	+4.8*	+5.1*
Glycine	65.6	+8.6	57.7	+8.4*	+6.7*	+9.8*
Proline	-	-	66.7	+2.6*	-7.3	+4.7*
Serine	75.2	+7.4*	72.6	+3.9*	+4.6*	+4.3*
Tyrosine	83.4	+2.2	77.6	+3.1*	+3.0*	+3.4*

[1]Blank *et al*., 1999; [2]Mroz *et al*., 2000
*Significantly different from the control diet (P<0.05)

as the retention of Ca in growing pigs (Mroz *et al.*, 2000). In piglets, Kirchgessner and Roth (1980) demonstrated that 2% of fumaric acid supplementation improved the balance and retention of Ca, P, Mg and Zn.

Antimicrobial effects of organic acids

Besides growth promoting effects, interest has focussed on the effects of organic acids on the activity and composition of the microflora in the gut of weaned and fattening pigs. Variation in form of organic acids appears to affect populations of micro-organisms differently. Research by Naughton and Jensen (2001) and Knarreborg *et al.*

(2002), showed a clear selective removal of target species by acids, such as coliform bacteria but not lactobacilli, an effect which seems to be pH dependent.

Killing potency of acids for coliform bacteria was in the following order: benzoic > fumaric > lactic > butyric > formic > propionic acid. Canibe *et al.* (2001), highlighted that K-diformate and formic acid both reduce the growth of yeasts in the GIT, while lactic acid may promote yeast. Roth and Kirchgessner (1998) reported that formic acid did not change the composition of the microflora in the ileum, but decreased the numbers of lactobacilli, *E. coli*, bacteroidaceae and enterococci in the duodenum and jejunum. After adding 1.8% of fumaric acid to the diet, Gedek *et al*. (1992) observed significantly reduced counts of lactobacillus, bifidobacterium and eubacterium, and the total count of the main flora was lower in the duodenum, jejunum and ileum. Lactobacillus and bifidobacterium counts in the caecum and colon were significantly lower, although supplemental fumaric acid reduced *E. coli* counts in the jejunum rather than in other segments of the GIT. It must be remembered that inducible tolerance (adaptation) to acidic environments is recognised as an important survival strategy for many micro-organisms. This should be monitored when acids are added to feed over a longer period of time (Piva, 1998).

FERMENTATION PRODUCTS

Supplementation with 2% propionic acid did not affect the concentrations of NH_3, cadaverine or putrescine in ileal digesta, but it did decrease concentrations of cadaverine in caecal digesta of growing pigs (Mosenthin *et al.*, 1992). Gabert *et al.* (1995) reported no effects on volatile fatty acid concentrations when 1 % of formic acid was supplemented to the diet of piglets. Trials with fumaric acid decreased the concentrations of lactic, acetic and propionic acid as well as of ammonia and spermidine in ileal digesta of weaning pigs, the response being dependent on the supplementation level (Blank *et al.*, 2001). When dietary buffering capacity was increased, these responses were lost.

Lipopolysaccharides (used as a marker for gram-negative bacteria) were significantly ($P<0.05$) lower in the presence of fumaric acid at 2 or 3% in the diet compared to the control treatment, regardless of the buffering capacity of the diet. Risley *et al.* (1992) found no effect of fumaric acid on volatile fatty acid (VFA) concentrations in the different segments of the GIT of weaning pigs when 1.5% of fumaric or citric acid was added to the diet. Although not significant, the concentration of succinic acid was considerably increased in the stomach of piglets receiving fumaric acid, indicating changes in microbial activity. Pigs receiving a diet containing 1.8% K-diformate showed decreased lactic acid levels in the stomach and small intestine after 29 days, while VFA concentrations (acetic, propionic and butyric acids) increased in the distal part of the small intestine, caecum and proximal colon (Canibe *et al.*, 2001).

Conclusions

Literature available to date reveal that organic acids represent a useful alternative to in-feed antibiotics in order to enhance and secure growth performance, and reduce pathogen loading in weaning and fattening pigs. This effect is more pronounced in the younger animal. Despite increasing information about the possible modes of action of organic acids in the different sections of the GIT, several aspects still require elucidation. The extent to which organic acids affect growth performance and digestibility depends on the type and the inclusion level of the acid used, as well as on diet and animal factors. Although research results vary, organic acids may enhance the digestibilities of protein and amino acids in weaned and fattening pigs. Regarding their antimicrobial activity, organic acids selectively inhibit the proliferation of harmful bacteria in the proximal part of the digestive tract (stomach and small intestine).

References

Aumaître, A., Peiniau, J. and Madec, F. (1995) Digestive adaptation after weaning and nutritional consequences in the piglet. *Pig News and Information* **16**: 73N-79N.

Best, P. (2000) Adding acids to swine diets. *Feed management* **51**: 19-22.

Blank, R., Mosenthin, R., Sauer, W.C. 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.

Blank, R., Sauer, W.C., Mosenthin, R., Zentek, J., Huang, S. and Roth, S. (2001) Effect of fumaric acid supplementation and dietary buffering capacity on the concentration of microbial metabolites in ileal digesta of young pigs. *Canadian Journal of Animal Science* **81**: 345-353.

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M. and Baker, D.H. (2000) The effect of citric acid on phytate-phosphorus utilization in young chicks and pigs. *Journal of Animal Sciences* **78**: 682-689.

Canibe, N., Steien, S.H., Øverland, M. and Jensen, B.B. (2001) 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.

Canibe, N., Højberg, O., Højsgaard, S. and Jensen, B.B. (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.

Cranwell, P.D. and Titchen, D.A. (1974) Gastric secretion in newly born pigs. *Research in Veterinary Science* **16**: 105-107.

Easter, R.A. (1988) Acidification of diets for pigs. In: *Recent Advances in Animal Nutrition*. pp. 61-71. Edited by W. Haresign and D.J.A. Cole. Butterworth, London, UK.

Eckel, B., Kirchgessner, M. and Roth, F.X. (1992) Zum Einfluss von Ameisensäure auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 1. Mitteilung. Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Influence of formic acid on daily weight gain, feed intake, feed conversion rate and digestibility. 1. The nutritive value of organic acids in the rearing of piglets] *Journal of Animal Physiology and Animal Nutrition* **67**: 93-100.

Eidelsburger, U., Kirchgessner, M. and Roth, F.X. (1992a) Zum Einfluss von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 7. Mitteilung. Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Influence of formic acid, calcium formate and sodium hydrogen carbonate on dry matter content, pH value, concentration of carbonic acids and ammonia in different segments of the gastrointestinal tract. 7. Communication. Investigations about the nutritive efficacy of organic acids in the rearing of piglets] *Journal of Animal Physiology and Animal Nutrition* **68**: 258-267.

Eidelsburger, U., Kirchgessner, M. and Roth, F.X. (1992b) Zum Einfluss von Fumarsäure, Salzsäure, Natriumformiat, Tylosin und Toyocerin auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 11. Mitteilung. Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Influence of fumaric acid, hydrochlorid acid, sodium formate, tylosin and toyoerin 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 pigs] *Journal of Animal Physiology and Animal Nutrition* **68**: 82-92.

Foegeding, P.M. and Busta, F.F. (1991) Chemical food preservatives. In: *Disinfection, Sterilization and Preservation*. pp. 802-832. Edited by S.S. Block. Lea and Febiger, Philadelphia, USA.

Gabert, V.M. and Sauer, W.C. (1994) The effects of supplementing diets for weanling pigs with organic acids. A review. *Journal of Animal and Feed Sciences* **3**: 73-87.

Gabert, V.M., Sauer, W.C., 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 Sciences* **75**: 615-623.

Gedek, B., Roth, F.X., Kirchgessner, M., Wiehler, S., Bott, A. and Eidelsburger, U. (1992) Zum Einfluss von Fumarsäure, Salzsäure, Natriumformiat, Tylosin und Toyocerin auf die Keimzahlen der Mikroflora und deren Zusammensetzung in verschiedenen Segmenten des Gastrointestinaltraktes. 14. Mitteilung.

Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Influence of fumaric acid, hydrochlorid acid, sodium formate, Tylosin and Toyocerin on the microflora in different segments of the gastrointestinal tract. 14. Communication. Investigations avout the nutritive efficacy of organic acids in the rearing of piglets] *Journal of Animal Physiology and Animal Nutrition* **68**: 209-217.

Giesting, D.W., Roos, M.A. and Easter, R.A. (1991) Evaluation of the effect of fumaric acid and sodium bicarbonate addition on performance of starter pigs fed diets of different types. *Journal of Animal Science* **69**: 2497-2503.

Grela, E. and Lipiec, A. (1991) Über den Einsatz von verschiedenen Getreidearten und Zitronensäure im Alleinfutter für Mastsschweine. [Use of various cereals and of citric acid in pig fattening on an all-mash diet] *Züchtungskunde* **63**: 399-407.

Kirchgessner, M. and Roth, F.X. (1980) Verdaulichkeit und Bilanz von Protein, Energie und einigen Mineralstoffen bei Fumarsäurezulagen an Ferkel. [Digestibility and balance of protein, energy and some minerals in piglets given supplements of fumaric acid] *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **44**: 239-246.

Kirchgessner, M. and Roth, F.X. (1982) Propionsäure als Futteradditiv in der Ferkelaufzucht und Schweinemast. [Propionic acid as a feed supplement in rearing piglets and in fattening pigs] *Das wirtschaftseigene Futter* **28**: 225-234.

Kirchgessner, M. and Roth, F.X. (1988) Ergotrope Effekte durch organische Säuren in der Ferkelaufzucht und Schweinemast. [Energy value of organic acids in the rearing of piglets and the fattening of pigs] *Übersichten zur Tierernährung* **16**: 93-108.

Kirchgessner, M., Gedek, B., Wiehler, S., Bott, A., Eidelsburger, U. and Roth, F.X. (1992) Zum Einfluss von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf die Keimzahlen der Mikroflora und deren Zusammensetzung in verschiedenen Segmenten des Gastrointetinaltraktes. 10. Mitteilung. Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. [Influence of formic acid, calcium formate and sodium hydrogen carbonate on the microbial count and composition in different parts of the gastrointestinal tract. 10. Communication. Investigations about the nutritional efficacy of organic acids during rearing of piglets] *Journal of Animal Physiology and Animal Nutrition* **68**: 73-81.

Kirchgessner, M., Roth, F.X. and Paulicks, B.R. (1995) Zur nutritiven Wirkung von Sorbinsäure in der Ferkelaufzucht. [Nutritive efficacy of sorbic acid in the rearing of piglets] *Journal of Animal Physiology and Animal Nutrition* **74**: 235-242.

Kirchgessner, M., Paulicks, B.R. and Roth, F.X. (1997) Effects of supplementation of diformate complexes (Formi™ LHS) on growth and carcass performance of piglets and fattening pigs in response to application time. *Agribiological Research* **50**: 1-7.

Knarreborg, A., Miquel, N., Granli, T. and Jensen, B.B. (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.

Krause, D.O., Harrison, P.C. and Easter, R.A. (1994) Characterization of the nutritional interactions between organic acids and inorganic bases in the pig and chick. *Journal of Animal Sciences* **72**: 1257-1262.

Mosenthin, R., Sauer, W.C., Ahrens, F., de Lange, C.F.M. 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., Jonbloed, A.W., Partanen, K.H., Vreman, K., Kemme, P.A. 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.

Mroz, Z., Reese, D.E., Øverland, M., Van Diepen, J.T.M. and Kogut, J. (2002) The effects of potassium diformate and its molecular constituents on the apparent ileal and fecal digestibility and retention of nutrients in growing-finishing pigs. *Journal of Animal Science* **80**: 681-690.

Mroz, Z. (2003) Organic acids of various origin and physico-chemical forms as potential alternatives to antibiotic growth promoters for pigs. In: *Proceedings of the 9th International Symposium on Digestive Physiology in Pigs*. Vol. 1. pp. 267-293. Banff, Canada.

Mroz, Z., Koopmans, S.J., Bannink, A., Partanen, K., Krasucki, W., Øverland, M. and Radcliffe, S. (2006) Carboxylic acids as bioregulators and gut growth promoters in nonruminants. In: *Biology of Nutrition in Growing Animals*. pp. 81-133. Edited by R. Mosenthin, J. Zentek and T. Zebrowska. Elsevier, Edinburgh, UK.

Naughton, P.J. and Jensen, B.B. (2001) A porcine intestinal organ culture model to study the adhesion of *Salmonella* and *E. coli in vitro*. In: *Proceedings of the 8th Symposium on Digestive Physiology in Pigs*. pp. 272-274. CABI Publishing, Wallingford, UK.

Øverland, M., Granli, T., Kjos, N.P., Fjetland, O., Steien, H. 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.

Partanen, K.H. and Mroz, Z. (1999) Organic acid for performance enhancement in pig diets. *Nutrition Research Review* **12**: 117-145.

Partanen, K., Jalava, T., Valaja, J., Perttilä, S., Siljander-Rasi, H. and Lindberg, 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.

Piva, A. (1998) Non-conventional feed additives. *Journal of Animal Feed Science* **7**: 143-154.

Radcliffe, J.S., Zhang, Z. and Kornegay, E.T. (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.

Ravindran, V. and Kornegay, E.T. (1993) Acidification of weaner pig diets: a review.
Journal of the Science of Food and Agriculture **62**: 313-322.

Risley, C.R., Kornegay, E.T., Lindemann, M.D., Wood, C.M. and Eigel, W.N. (1992) Effect of feeding organic acids on selected intestinal content measurements at varying times postweaning in pigs. *Journal of Animal Sciences* **70**: 196-206.

Roth, F.X. and Kirchgessner, M. (1982) Zum nutritiven Einsatz von Ca-Propionat in der Ferkelaufzucht und Schweinemast. [Ca-Propionate as nutritive supplement for rearing piglets and fattening pigs] *Das wirtschaftseigene Futter* **28**: 235-242.

Roth, F.X. and Kirchgessner, M. (1988) Zum Einsatz von Essigsäure in der Ferkelfütterung. [Use of acetic acid in piglet feeding] *Landwirtschaftliche Forschung* **41**: 253-258.

Roth, F.X. and Kirchgessner, M. (1989) Bedeutung von pH-Wert und Pufferkapazität des Futters für die Ferkelfütterung. 1. pH-Wert und Pufferkapazität von Futtermischungen bei Einsatz von organischen Säuren. [Significance of dietary pH and buffering capacity in piglet nutrition. 1. pH and buffering capacity in diets supplemented with organic acids] *Landwirtschaftliche Forschung* **42**: 157-167.

Roth, F.X. 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.

Roth, F.X., Kirchgessner, M. and Eidelsburger, U. (1993) Nutritive value of lactic acid in piglet rearing. *Agribiological Research* **46**: 229-239.

Roth, F.X., Kirchgessner, M and Paulicks, B.R. (1996) Nutritive use of feed additives based on diformates in the rearing and fattening of pigs and their effects on performance. *Agribiological Research* **49**: 307-317.

Russell, J.B. (1992) Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling (A review). *Journal of Applied Bacteriology* **73**: 363-370.

Russell, J.B. and Diez-Gonzales, F. (1998) The effect of fermentation acids on bacterial growth. *Advances in Microbial Physiology* **39**: 205-234.

Siljander-Rasi, H., Alaviukola, T. and Suomi, K. (1998) Carbadox, formic acid and potato fibre as feed additives for growing pigs. *Journal of Animal Feed Science* **7** (Suppl. 1): 205-209.

Thacker, P.A., Campbell, G.L. 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.

Weeden, T.L., Nelssen, J.L., Hansen, J.A. and Richardson, K.L. (1991) The effect of diet acidification on starter performance and nutrient digestibility. *Journal of Animal Science* **69**: 105-106.

6

IMPROVING THE PERFORMANCE OF WEANED PIGS WITH NATURAL PRODUCTS

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Introduction

Mortality, morbidity and depressed pig performance associated with disease during the early post-weaning period continue to be major problems facing the swine industry (Cutler *et al.*, 1992). Although there are many managerial and environmental factors that can contribute to these losses, increased susceptibility of young pigs to disease is a direct reflection of their relatively poor immunological competence (Kelly *et al.*, 1993). For the newly weaned pig, poor immune status, in combination with other stresses associated with high-intensity pig production systems, and the concomitant suppression of feed intake and immune responsiveness are major contributors to enhanced disease susceptibility (Westley and Kelly, 1984; Hennessy and Jackson, 1987; Brown-Borg *et al.*, 1993). These issues are exacerbated in nurseries, where pigs of multiple origins provide an increased chance of pathogen exposure and consequent disease (Boeckman, 1996).

Currently, the majority of the U.S. swine industry relies on the inclusion of sub-therapeutic concentrations of antibiotics in the diets of young pigs to promote growth and mitigate disease problems. Increasing concerns regarding the development of antibiotic-resistant bacteria, and its potential implications for human health, have created public concern worldwide regarding such management practices (Liem, 2004). The development of alternatives to the use of antibiotics is therefore a priority for the industry.

The research discussed in this chapter was conducted to assess the use of natural products, designed to enhance feed intake and reduce disease susceptibility, versus antibiotics used at sub-therapeutic growth promoting doses (AGP's) on the performance of pigs from weaning to the grower phase. At present, acidifiers consisting of organic and/or inorganic acids are considered a promising option for replacing AGPs in livestock production (Steiner, 2006).

Successful application of organic acids in the diets for pigs requires an understanding of their modes of action. It is generally considered that dietary organic acids or their salts lower gastric pH, which results in increased activity of proteolytic enzymes and gastric retention time, thus improving protein digestion (Partanen and Mroz, 1999).

This may lead to a better growth of the animals. Furthermore, there is a direct antimicrobial effect of organic acids, as, in their undissociated form, the acid molecules freely diffuse through the semi-permeable membrane of micro-organisms into their cell cytoplasm. Once inside the cell, where the pH is maintained near 7, the acid dissociates and suppresses cell enzymes (decarboxylases and catalases) and nutrient transport systems (Lueck, 1980). Supplementation of diets for weaning pigs with organic acids has been shown in many cases an increased daily weight gain in the range from 4% to 27% (Gabert and Sauer, 1994).

Not only organic acids have proved to be beneficial for pigs. Jones (2001) outlined the efficacy of phytogenic or phytobiotic feed additives, which may improve hygiene and safety of feed, increase palatability and hence promote feed intakes. These potential benefits were examined in a number of trials (Jones, 2002), and were demonstrated in the resulting 10% increase in production. Steiner (2006) mentioned nutritional strategies to maintain gut health in livestock by combining different feed additives. Following on from this research, the main aim of the following study was to evaluate the effects of a combination of acidifiers and phytobiotics on weaned piglets.

Determining the efficacy of acids and phytobiotics in feed on pig performance

Pig trials were conducted at the Kingsville Swine Unit of Texas A&M University in the USA. The unit is a farrow-to-finish operation and has been in production for approximately 25 years. This facility has experienced episodic problems with post-weaning scours, which on occasion has resulted in relatively high morbidity and mortality rates.

For the feeding trials, crossbred pigs (Yorkshire x Landrace, n=144), from three separate farrowing groups, were selected at weaning and assigned by litter, gender and initial body weight to one of two treatments. The treatments included the unit’s standard nursery diets as a positive control (1% Mecadox antibiotic, n=73), or the same diet supplemented with 3 kg/t acidifier and 2 kg/t phytobiotic (Biotronic® and Biomin® P.E.P. from Biomin GmbH, Austria, n=71). Within each farrowing group, pigs were assigned by weight and treatment to one of six pens, to give 3 pens per treatment containing pigs of High, Moderate, and Low initial body weight class. Group averages for initial body weight were not statistically different by design (6.7±0.3 and 6.6±0.2 kg for Control and Supplemented pigs, respectively).

Two phases of standard nursery diet formulation were used during the experiment, each phase lasting 28 days following weaning. Daily feed intake and weekly weight gain were recorded for all the pigs used in the trial, and treatment groups were maintained using the same groups of piglets for both diet phases. The number of pigs that died or needed to be removed to sick pens was recorded.

Results for both phases of the trial are shown in table 1. Replacement of the AGP with acidifier and phytobiotic in the diet resulted in comparable weight gains during both dietary phases ($P>0.05$). Supplementation with the acid-phytobiotic blend increased ($P<0.05$) feed intake during both phases, although in the supplemented pigs, this did not affect (P>0.05) gain to feed ratio. It was, however, associated with a significant reduction (P<0.05) in mortality during phase 1 feeding (16.4% vs. 8.5% for Control vs. Supplemented pigs, respectively). This resulted in an increased net return per pig ($2.94 vs. $5.80 for Control vs. Supplemented pigs, respectively).

Table 1. Effect of antibiotic supplemented diets versus those containing Biomin® P.E.P. and Biotronic® on pig performance post weaning

	Phase 1 (Days 1 to 28)		*Phase 2 (Days 28-56)*	
Measure	*Control*	*Supplement*	*Control*	*Supplement*
Number of pigs	73	71	56	62
Initial Weight (kg)	6.5 ± 0.3	6.6 ± 0.3	14.8 ± 0.6	14.4 ± 0.5
Final Weight (kg)	14.8 ± 0.6	14.4 ± 0.5	30.5 ± 1.0	30.4 ± 0.8
Mortality (%)	16.4[a]	8.5[b]	1.8	1.6
ADFI (kg/d)	0.50 ± 0.02[a]	0.55 ± 0.02[b]	1.28 ± 0.03[a]	1.34 ± 0.02[b]
ADG (g/d)	277 ± 16	279 ± 15	563 ± 18	572 ± 12
Gain:Feed Ratio	0.59 ± 0.09	0.54± 0.03	0.45 ± 0.02	0.45 ± 0.02
Net Return/Pig[c]			$2.94	$5.80

[a, b] Values within rows with different superscripts differ P<0.05.
[c] Net return per pig was calculated at the end of the Nursery II period based on estimates of Revenue/pig and Cost/pig. Cost per pig was calculated as the (sum of the feed costs during the Nursery I and II periods + estimated sow cost) / the total number of pigs at the end of the Nursery II period). Revenue per pig was based on the market value of 60 lb feeder pigs as of 09/04/03; http://www.ams.usda.gov/mnreports).

Regardless of initial piglet body weight, fed intake immediately after weaning was low, but increased (P<0.01) rapidly from approximately 3 to 4 days into the trial period through the end of the first feeding phase (figure 1).

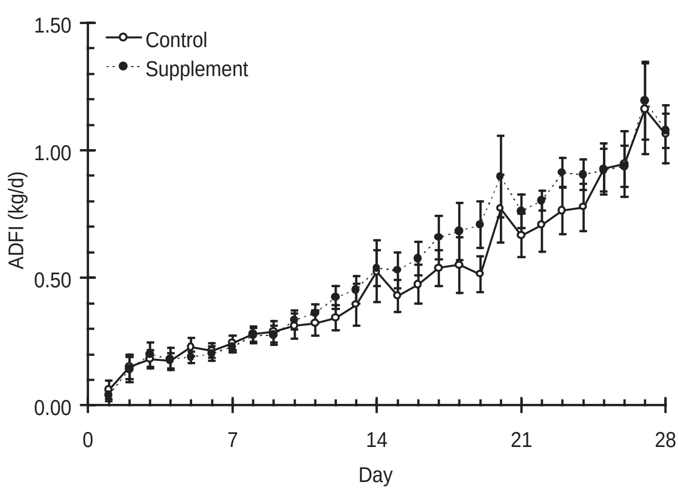
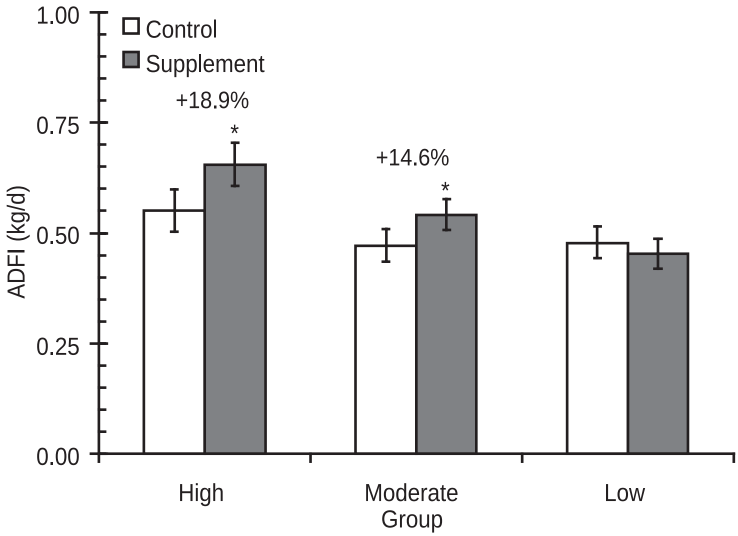


Figure 1. Average daily feed intake by day during the first feeding phase (1-28 days post-weaning)

Feed intake improved more rapidly in pigs receiving the acidifier and phytobiotic treatment compared to those fed the control AGP diets.



*Differs significantly from control (P<0.05)

Figure 2. Average daily feed intake by initial body weight class during the first feeding phase (1-28 days post-weaning)

The effect of the combined natural products was more pronounced in heavier pigs, with pigs from the high and moderate weight groups having 18.9% and 14.5% respectively greater average intake during the first feeding phase ($P<0.05$) (figure 2). In contrast, feed intake was similar ($P>0.05$) for the AGP groups and the pigs receiving the acid/phytobiotic diet from the low weight group. This is probably more reflective of the differences in mortality rate between the treatments, as the mortality rate in control pigs was high (16.4%) during the first feeding phase, and the majority of these mortalities occurred in the low weight group in the early part of that period. This illustrates the potential ability of natural products to substantially reduce ($P<0.05$) the mortality rate by 8.5% during the first phase, and saw an increased number of pigs, experiencing the lowest feed intakes, surviving throughout this initial trial period.

Similar to the first phase, feed intake significantly increased ($P<0.01$) for all weight classes during the second feeding phase (figure 3).

The magnitude of the intake response was greater for the pigs fed the acidifier/phytobiotic diet, resulting in the average intake during the second phase period being higher (1.28 ± 0.03 vs. 1.34 ± 0.02 for AGP vs. acidifier/phytobiotic respectively) ($P<0.05$). At this age, the effect of the natural products was more apparent in pigs from the moderate and low weight class groups, with acidifier/phytobiotic pigs having increases of 5.5% for the moderate and 6.7% for the low groups ($P<0.05$) (figure 4). In contrast, average intake was similar ($P>0.05$) in both treatment groups for the high weight classification pigs.

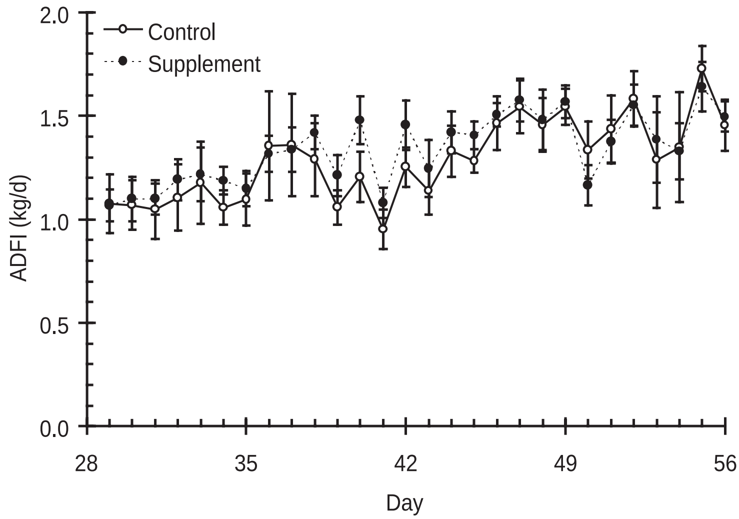


Figure 3. Average daily feed intake by day during the second feeding phase (29-56 days post-weaning)

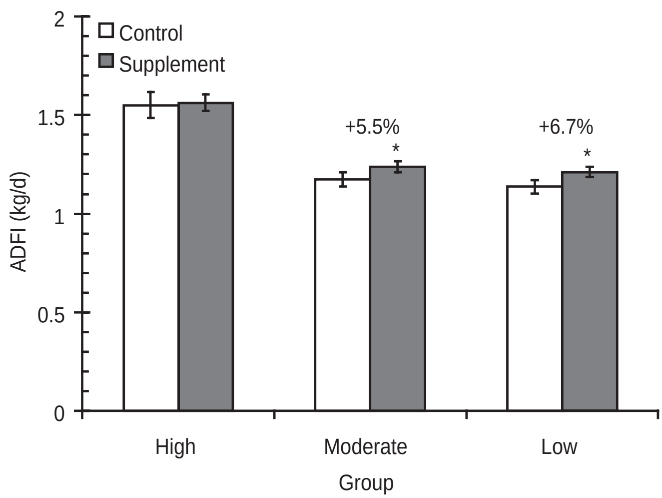


Figure 4. Average daily feed intake by initial body weight classification during the second feeding phase (29-56 days post-weaning)

Collectively, these results suggest that the use of natural products is most beneficial for improving ADFI during the Nursery I period, and with lighter weight pigs. This is particularly significant, as these represent the most vulnerable pigs to disease and those that are most apt to be fed antibiotics.

Initial pig weights significantly differed (P<0.01) between the allocated weight classes. As expected, the final weights of pigs at the end of both phases (figures 5 and 6) were influenced by the weight of the pig at weaning, with pigs from the high weight group being the heaviest (P<0.05) and pigs from the low weight group be the lightest (P<0.05).

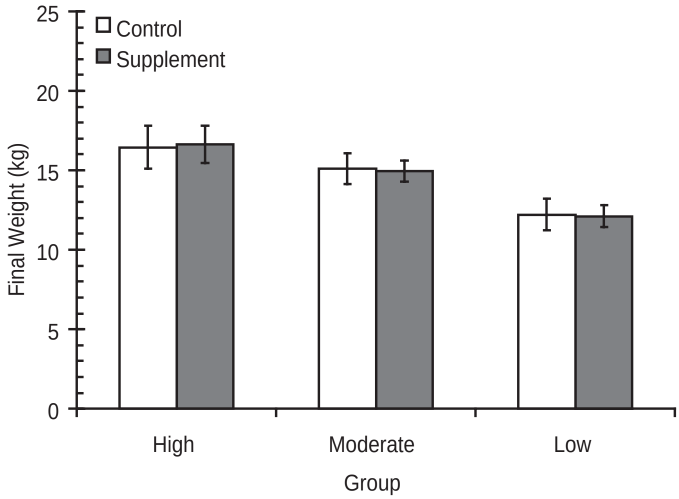


Figure 5. Final weight of pigs by initial body weight classification at 28 days post-weaning

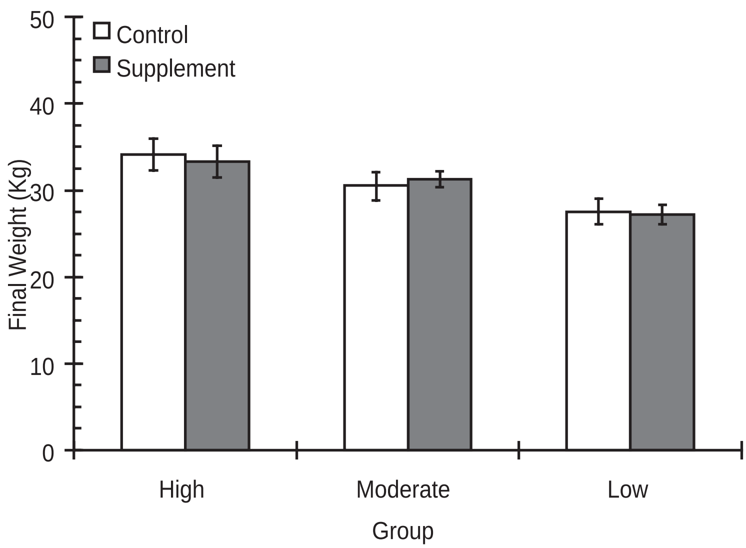


Figure 6. Final weight of pigs by initial body weight classification at 56 days post-weaning

Regardless of weight class, replacement of antibiotics with the acidifier and phytobiotic products resulted in similar levels (P>0.05) of growth performance as indicated by final pig weights and daily gain (figure 7) during the two feeding phases.

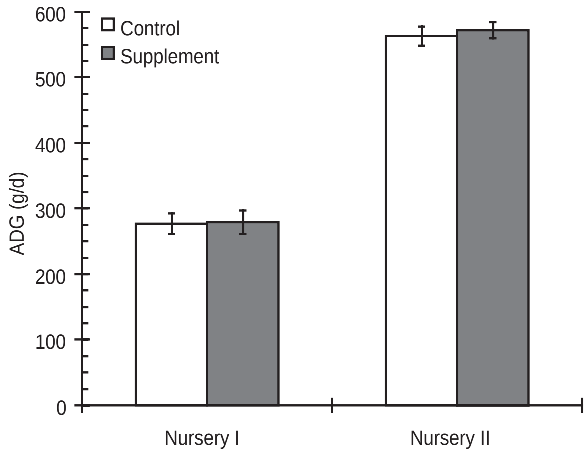


Figure 7. Average daily gain of pigs in two feeding phases post-weaning, 1-28 days (Nursery I) and 29-56 days (Nursery II)

Replacement of antibiotics with the natural products also resulted in similar (P>0.05) gain to feed ratios during both feeding phases (figure 8).

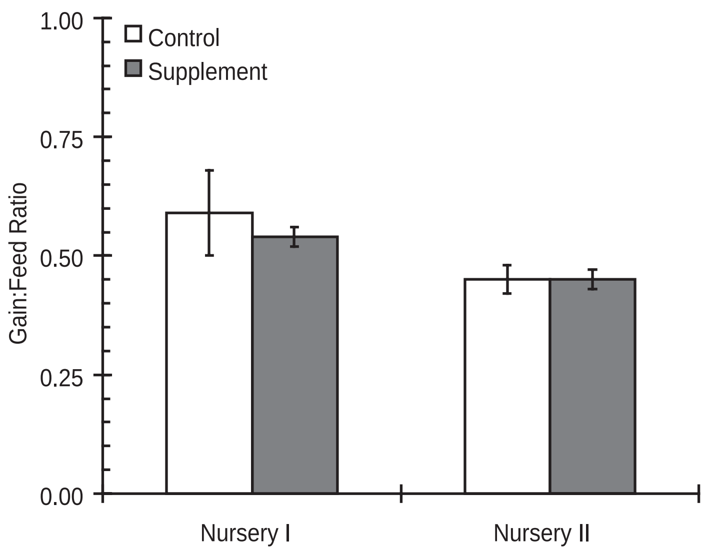


Figure 8. Gain to feed ratio of pigs in two feeding phases post-weaning, 1-28 days (Nursery I) and 29-56 days (Nursery II)

Conclusions

Results from pigs used in this study indicated that replacement of antibiotics with an acid/phytobiotic blend of natural products maintains performance in weaned pigs. In addition, these products appear to be more effective than the antibiotic in reducing mortality due to post-weaning scours, which improves economic return.

References

Boeckman, S. (1996) SEW: Panacea or Pandora's box. *Swine Practitioner* **September**:18.

Brown-Borg, H.M., Klemcke, H.G. and Blecha, F. (1993) Lymphocyte proliferative responses in neonatal pigs with high or low plasma cortisol concentration after stress induced by restraint. *American Journal of Veterinary Research* **54**: 2015.

Cutler, R.S., Fahy, V.A. and Spicer, E.M. (1992) Preweaning Mortality. In: *Diseases of Swine* (7th Edition). Edited by A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire and D.J. Taylor. pp. 847-860. Iowa State Univ. Press, Ames, USA.

Gabert, V.M. and Sauer, W.C. (1994) The effects of supplementing diets for weaning pigs with organic acids. A review. *Journal of Animal and Feed Sciences* **3**: 73-87.

Hennessy, D.P. and Jackson, P.N. (1987) Relationship between adrenal responsiveness and growth rate. In: *Manipulating Pig Production. Proceedings of the Australian Pig Science Conference*. Albury, NSW, November 23, 1987.

Jones, G. (2001) High-performing livestock and consumer protection are not contradictory. *Feed Magazine* **12**: 468-472.

Jones, G. (2002) Phytobiotic solutions. *Pig Progress* **18**: 2-3.

Kelly, K.S., Kent, S. and Dantzer, R. (1993) Why sick animals don't grow: an immunologic explanation. In: *Growth of the Pig*. Edited by G.R. Hollis. pp.119-132. CAB Intl., Wallingford, UK.

Liem, D.T. (2004) E.coli resistant to most antibiotics in Vietnam. *Asia Pork Magazine* **8/9**: 22-24.

Lueck, E. (1980) Antimicrobial Food Additives: Characteristics, Uses, Effects. Springer Verlag, Berlin, Germany.

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

Steiner, T. (2006) The potential benefits of Natural Growth Promoters. *Far Eastern Agriculture* **January/February**:18-20.

Westley, H.J. and Kelly K.W. (1984) Physiologic concentrations of cortisol suppresses cell-mediated immune events in the domestic pig. *Proceedings of the Society for Experimental Biology and Medicine* **177**: 156.

7

ACIDIFIERS IN POULTRY DIETS AND POULTRY PRODUCTION

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Introduction

Both the feed industry and the food production sector still suffer from losses due to the contamination of feed with pathogenic bacteria and their related impacts in the animal, such as lower weight gains or increased mortality. The ban on the use of in-feed antibiotics (AGPs) in livestock in the EU puts more pressure on animal producers, and also poses a challenge to innovative animal nutrition. Addressing these problems in a suitable manner can regain the trust of people concerned about the safety of food. Alternative feed ingredients are being adopted in order to fill the gap from the AGPs. From this point of view, acidifiers can be part of the feeding concept to replace AGPs and it is expected that the market segment for this feed additive will continue to rise.

Activity of acidifiers

The potential of single organic acids in feed preservation lies in their ability to protect feed from microbial and fungal destruction, and its effect on stomach pH and gut flora, and has been known for decades and proven in many laboratory and field trials (Eidelsburger *et al*., 1992; Eidelsburger and Kirchgessner, 1994; Freitag *et al.*, 1999). Acidifiers act as performance promoters by lowering the pH of gut (mainly upper intestinal tract), reducing potential proliferation of unfavourable microorganisms. Acidification of gut stimulates enzyme activity and optimises digestion and the absorption of nutrients and minerals. Un-dissociated forms of organic acids penetrate the lipid membrane of bacterial cells and dissociate into anions and protons. After entering the neutral pH of the cell’s cytoplasm, organic acids inhibit bacterial growth by interrupting oxidative phosphorylation and inhibiting adenosine triphosphate-inorganic phosphate interactions.

Acidifiers for poultry

Improved broiler performance by supplementation with single acids was noticed for formic acid (Vogt *et al.*, 1981) and fumaric acid (Patten and Waldroup, 1988; Kirchgessner *et al.*, 1991), and Izat *et al.* (1990a) found significantly reduced levels of *Salmonella* spp. in carcass and caecal samples after including calcium formate to broiler diets. In another trial from Izat *et al.* (1990b) buffered propionic acid was used to counteract pathogenic microflora in the intestine and carcass of broiler chickens, and resulted in a significant reduction in *E. coli* and *Salmonella* spp..

The use of pure formic acid in breeder feed reduced the contamination of tray liners and hatchery waste with *S. enteritidis* drastically (Humphrey and Lanning, 1988). Kirchgessner *et al.* (1992) found significantly better feed utilization in laying hens after adding fumaric acid, but only when the feed was low in protein and methionine and cysteine. Performance enhancement was influenced by both quantity and quality of the protein, although these trials were performed either with single organic acids or with the corresponding salt of a single acid.

Hinton and Linton (1988) examined controlling salmonella infections in broiler chickens by using a mixture of formic and propionic acid (table 1). They demonstrated that under experimental conditions 6 kg/t of that organic acid blend was effective in preventing intestinal colonization with *Salmonella* spp. from naturally or artificially contaminated feed.

Table 1. Isolation rate of salmonella after feeding naturally contaminated feed in chicken faeces (Hinton and Linton, 1988)

Acidifier in kg/t	*No. of positive samples*	*% positive in flock*
0	42	60
3	55	79
6	0	0
9	0	0

A trial with 240 one-day-old (Ross) chicken was carried out at the Trakya University, Turkey (Lückstädt *et al.*, 2004) where an acid blend was compared against a negative control. Each of the two applications was repeated 12 times, with 10 chickens per replicate in a randomly selected design. The trial period was from 1-35 days of age. The acidifier treatment (a combination of formic and propionic acid and their salts, based on an inorganic sequential release medium) was added at a dosage rate of 3 kg/t feed. The trial feed (based on corn and full fat soybean) was applied as starter (0-14 days), grower (15-28 days) and finisher phases (29-35 days). Feed and water was available *ad libitum*.

Measured parameters (taken weekly) included feed intake, live weight, average weight gains and mortality. FCR and European Broiler Index (Average Daily Weight Gain (g) x Survival (%) / 10 x FCR) were calculated from these results.

The effect of the acidifier on weight performance is shown in table 2. The acidifier affected the body weight of the chicken from week 1 until the termination of the experiment.

Table 2. Body weight of broiler chickens fed a combined acidified diet in g (Lückstädt *et al.*, 2004)

	Control	*Acidifier (3 kg / t)*+	*P value*
1. week	142±7.05	147±4.22	0.01
2. week	368±21.77	375±11.32	0.01
3. week	731±46.47	773±27.15	0.01
4. week	1194±82.27	1263±62.08	0.05
5. week	1662±115.24	1759±97.67	0.06

+ acidifier: Biotronic® (acid blend of formic and propionic acid and corresponding salts on sequential release medium)

The same effect was observed in weight gain (table 3). However, this time only at week 3 a significantly higher daily growth in the acidifier treated group was observed.

Table 3. Daily weight gain of broiler chickens fed a combined acidified diet in g (Lückstädt *et al.*, 2004)

	Control	*Acidifier*	*P value*
1. week	14±1.01	15±0.60	n.s.+
2. week	26±2.84	26±1.86	n.s.
3. week	52±4.84	57±2.62	0.01
4. week	66±11.66	70±6.78	n.s.
5. week	67±6.08	71±5.79	n.s.

+ n.s.: not significant

Comparing the feed intakes (table 4), it was observed that the group receiving the acidifier consumed more feed than the control group throughout the experimental duration. However, in week 5, feed intake fell below expected levels, probably because of increasing ambient temperature.

FCR data showed no significant differences between the applications. The data appeared similar between treatments until week 5, after which the acidifier treated group had an FCR of 1.52 compared to the negative control group, which reached 1.53. Mortality did not differ significantly between the applications and was only observed during the first week of the trial, which included one chick from the control group and two from the acidifier treatment. The European Broiler Index (EBI) was highest in the acidifier treatment (310.5) compared to the negative control (291.7).

Table 4. Average daily feed intake of broiler chickens fed a combined acidified diet in g (Lückstädt *et al.*, 2004)

	Control	*Acidifier*	*P value*
1. week	12±0.52	19±0.52	0.01
2. week	42±1.64	43±1.25	0.01
3. week	76±5.38	81±3.63	0.05
4. week	107±7.15	114±6.81	n.s.
5. week	121±10.69	126±12.46	n.s.

In a recent trial at the Bombay Veterinary College (BVC), the same combined acidifier (a blend of formic and propionic acid and their salts on sequential release medium) was investigated as an alternative for AGPs. Feed was based on a corn-soy mash formulation. 300 one-day-old male and female chicks were divided into 3 groups of 100 birds each. The experimental period lasted again for 35 days. Three treatments were as following:

- Trial group A: AGP 0.5 kg/t of feed
- Trial group B: AGP 0.5 kg/t of feed and 1 kg acidifier/t of feed
- Trial group C: 1.5 kg acidifier/t of feed

Performance results for the broilers are shown in table 5.

Table 5. Growth parameter and production efficiency in broiler production (data from In-house report, BVC 2006)

	AGP	*AGP with acidifier*	*Trial group with acidifier*
Average weight (g)	1532	1515	1568
Average daily weight gain (g)	42.5	42.0	43.5
Feed intake (g/bird)	2609.0	2586.2	2608.6
FCR	1.75	1.75	1.71
Mortality (%)	2	1	4
European Broiler Index $^{+}$	237.8	237.7	244.2
European Production Efficiency Factor †	2.45	2.45	2.52

$^{+}$ EBI = ADWG (g) x Survival (%) / 10 x FCR
† EPEF (European Production Efficiency Factor= Average weight (kg) x Survival (%) / FCR x Age (d)

Trial data showed that the inclusion of an acidifier in broiler feed can successfully replace an antibiotic growth promoter in commercial Indian broiler production, and can support increased production efficiency.

Published papers describe the effect of acidifiers for significantly reducing the number of *E. coli* or *Salmonella* spp. in the gastro-intestinal tract of chickens. These pathogenic gram-negative bacteria often lead to diarrhoea in poultry. A trial was carried out at the China Agricultural University, Beijing, with the aim being to test the previously trialled acidifier (used at BVC) against an unsupplemented commercial broiler diet (basal corn-soybean diet). Four hundred and twenty birds (one-day-old Arbor Acres broiler) were divided into 2 groups of 210 birds each. The trial lasted for 42 days. Acidifier was included in the trial diet at a rate of 2 kg/t of feed (table 6).

Table 6. Health status and production efficiency in broiler treated with acidifier (data from In-house report, China Agricultural University, 2005)

	Control group	*Acidified feed*
Diarrhoea incidence (%) at day 6	34.0	25.7*
Average diarrhoea intensity ADI† at day 6	0.47	0.31*
Mortality (%)	3.81	1.43
Feed conversion rate FCR	1.85	1.84
European Broiler Index EBI+	200.9	203.2

† ADI = sum of scores in a replicate / number of chicks per replicate (1 = weak-, 2 = medium-, 3 = heavy diarrhoea)
+ EBI = ADWG (g) x Survival (%) / 10 x FCR
(* $P<0.05$)

The data showed that diarrhoea cases in the trial group with the acidifier were significantly lower ($P<0.05$).

Finally, an experiment was conducted by the BVC regarding use and efficiency of a liquid acidifier, in which broilers were divided in four groups of 50 birds. Birds were reared on deep litter system of housing under ideal and identical management and environmental conditions. The acidifier (a blend containing equal amounts of propionic acid and formic acid) was used at a rate of 0.5 litres per 1000 litres drinking water (table 7).

Table 7. The use of a liquid acidifier in drinking water for broiler chickens (data from In-house report, BVC 2006)

	Control	*Acidifier*
Initial weight (g)	38.2	37.6
Final weight (g)	1347.1	1478.2
Total weight gain (g)	1308.9	1440.6
Total feed consumption (g)	2315.4	2389.2
Feed conversion ratio	1.77	1.66
Mortality (%)	2.0	4.0

Birds from the group supplemented with acidifier through drinking water had the highest weight gain, which was thought to be due to higher nitrogen retention.

Conclusions

The inclusion of single organic acids in broilers has often showed growth performance increases (Vogt *et al.*, 1981; Skinner *et al.* 1991). For formic acid, it has been concluded that inclusion rates lower than 0.5% can increase the animal performance (Eidelsburger and Kirchgessner, 1994), and dosage rates for commercially produced acidifiers are recommend between 0.2% and 1.0%. Results from commercially managed poultry farms with this type of acidifiers were promising. Iba and Berchieri (1995) tested a mixture of formic and propionic acid at a dosage of 2 kg/t against different salmonella serotypes and demonstrated that *Salmonella enteritidis* and *Salmonella typhimurium* could not be isolated from caecal samples.

The mode of action of acidifier in poultry is mainly due to its anti microbial action, unlike in pigs where a key activity is the reduction of the stomach-pH. The effect of acids on gram-negative bacteria is increased if the organic acid is not dissociated. Because of this mode of action, effective acidifiers need to contain organic acids that are undissociated at different pH-values, so that the antimicrobial action is prolonged over a wider pH range.

In the trials discussed, final body weight of the broiler chickens fed acidified diets was significantly increased. Average daily weight gain was higher in the acidifier group, and FCR was slightly reduced, even though this reduction was not significant. Additionally, the health status of the chickens was better, with significantly lower diarrhoea incidence.

It can be concluded that the addition of a balanced acid blend, such as combinations of formic and propionic acid based on a sequential release medium, increases the performance of broiler chicken and is an option for maintaining or improving broiler growth and efficiency results without resorting to supplementation with an AGP.

Reference

Eidelsburger, U., Roth, F.X. and Kirchgessner, M. (1992) Zum Einfluß von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 7.Mitteilung. Untersuchungen zu nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. *Journal of Animal Physiology and Animal Nutrition* **67**: 258-267.

Eidelsburger, U. and Kirchgessner, M. (1994) Zum Einfluß organischer Säuren und Salze im Futter auf die Mastleistung von Broilern. *Archiv für Geflügelkunde*

58: 268-277.

Freitag, M., Hensche, H.U., Schulte-Sienbeck, H. und Reichelt, B. (1999) Biologische Effekte konventioneller und alternativer Leistungsförderer. *Feed Magazine*: 49-57.

Hinton, M. and Linton, A.H. (1988) Control of Salmonella infections in broiler chickens by the acid treatment of their feed. *Veterinary Record* **123**: 416-421.

Humphrey, T.J. and Lanning, D.G. (1988) The vertical transmission of salmonellas and formic acid treatment of chicken feed. A possible strategy for control. *Epidemiology and Infection* **100**: 43-49.

Iba, A.M. and Berchieri, A. (1995) Studies on the use of a formic acid-propionic acid mixture (Bio-Add™) to control experimental *Salmonella* infection in broiler chickens. *Avian Pathology* **24**: 303-311.

Izat, A.L., Adams, M.H., Cabel, M.C., Colberg, M., Reiber, M.A., Skinner, J.T. and Waldroup, P.W. (1990a) Effect of formic acid or calcium formate in feed on performance and microbiological characteristics of broilers. *Poultry Science* **69**: 1876-1882.

Izat, A.L., Tidwell, N.M., Thomas, R.A., Reiber, M.A., Adams, M.H., Colberg, M. and Waldroup, P.W. (1990b) Effects of a buffered propionic acid in diets on the performance of broiler chickens and on the microflora of the intestine and carcass. *Poultry Science* **69**: 818-826.

Kirchgessner, M., Roth, F.X. und Steinruck, U. (1991) Nutritive Wirkung von Fumarsäure bei Änderung der Proteinqualität und des Proteingehaltes im Futter auf die Mastleistung von Broilern. *Archiv für Geflügelkunde* **55**: 224-232.

Kirchgessner, M., Roth, F.X. und Steinruck, U. (1992) Ergotroper Effekt von Fumarsäure bei suboptimaler Proteinmenge und –qualität im Futter auf die Produktionsleistung von Legehennen. *Archiv für Geflügelkunde* **56**: 27-36.

Lückstädt, C., Senköylü, N., Akyürek, H. and Ağma, A. (2004) Acidifier – a modern alternative for anti-biotic free feeding in livestock production, with special focus on broiler production. *Veterinarija ir Zootechnika* **27**: 91–93.

Patten, J.D. and Waldroup, P.W. (1988) Use of organic acids in broiler diets. *Poultry Science* **67**: 1178-1182.

Skinner, J.T., Izat, A.L. and Waldroup, P.W. (1991) Research note: Fumaric acid enhances performance of broiler chickens. *Poultry Science* **70**: 1444-1447.

Vogt, H., Matthes, S. und Harnisch, S. (1981) Der Einfluß organischer Säuren auf die Leistungen von Broilern und Legehennen. *Archiv für Geflügelkunde* **45**: 221-232.

8

EFFECT OF ORGANIC ACID CONTAINING ADDITIVES IN WORLDWIDE AQUACULTURE - SUSTAINABLE PRODUCTION THE NON-ANTIBIOTIC WAY

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Introduction

The current situation in world food supplies calls for supreme efforts to ensure the increasing requirements of the growing world population for staple diets and high-quality food. Additionally, bridging the widening gap between food demand and supply is required, especially in developing areas. Setbacks in any food production sector places greater pressure on other areas for supplying the increasing urban and rural populations, particularly in less developed countries.

Around one billion people are dependent on fish as their main protein source, and this number is likely to increase further (Becker and Focken, 1998), as the world population is increasing at an estimated annual rate of 2%. Aquaculture now provides more than 22% of all consumable aquatic products (Guillaume *et al*., 2001). Between 1987 and 1996, aquaculture production of food fish increased by 148% (Tomasso and New, 1999). In comparison, livestock meat and fisheries have grown yearly only by 3% and 1.6% respectively. Aquaculture is, at present, the only enlarging sector within the fishing industry and is also reputed to be the fastest growing food production sector in the world.

Since the early 1980s, yearly growth rates of around 10% have been reported for aquaculture business. Because of this situation, global production of farmed fish and shellfish has more than doubled in both volume and value in the past 15 years (Naylor *et al*., 2000). If products from aquaculture that are not directly used for human consumption are included (e.g. seaweed), then the world's aquaculture production more than tripled by weight and value between 1984 and 1996 (Dagoon, 2000). The contribution of aquaculture to total fish production directly consumed by humans is currently more than 25%.

Aquaculture production differs greatly between countries due to different retail opportunities, climatic zones and local conditions as well as the types of farmed animals, leading to diverse production practices and a variety of impacts on the ecosystem. Williams *et al*. (2000) described certain targets required for the aquaculture industry if sustainability is to be achieved, in particular the promotion of environmentally sound practices in all fields of fish and shrimp production.

Increased awareness from consumers and producers has resulted in calls for more responsible and sustainable aquaculture. Public opinion and regulation authorities in most export countries focus now on the misuse of antibiotics in aquaculture, and public attention has focussed on alternative production methods (Verbeeke, 2001; Feedinfo, 2005). Furthermore, the EU has banned all antibiotic growth promoters (AGPs) from livestock production since January 2006, as the use of low level AGPs in animal feeds carries the possibility of resistance transfer from the bacterial community to species that are pathogenic in humans (Liem, 2004).

Antibiotic use in aquaculture

In the field of aquaculture, it is well established that the inclusion of antibiotics into the diets of fish (Ahmad and Matty, 1989) can promote growth and feed conversion. However, it was acid preservation in the use of fish silage from preserved fish and fish viscera (Gildbert and Raa, 1977; Åsgård and Austreng, 1981) that attracted scientific attention to this group of additives, as it is interesting to investigate the effects of these short-chain acids in the fish directly. Research was initially conducted with carnivore species, like rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and Arctic charr *Salvelinus alpinus*, and has now included herbivorous filter feeders, like tilapia, and shrimp as well (Lückstädt, 2006).

Benefits of acidified diets in Arctic charr

Ringø (1991) fed Arctic charr on commercial diets with or without supplementation with sodium salts of lactic and propionic acid. Fish were held in brackish water at 8°C, and the inclusion rate of the sodium salts was 10 kg/t of feed. Fish fed the Na-lactate diet increased in weight from around 310 g to about 630 g within an 84-day trial period, which was significantly higher than the negative control group (final weight of fish: 520 g) ($P<0.05$). The gut content from Arctic charr fed the sodium-lactate supplemented diet contained significantly ($P<0.05$) lower amounts of water, energy, lipid, protein and free amino acids. It has been observed that charr feeding on high doses of commercial feeds, as it often appears under aquaculture conditions, have a tendency for diarrhoea. When charr received Na-lactate, no nutritive diarrhoea appeared, probably because of much lower amounts of remaining nutrients and water in the gut. Furthermore, it was proposed that the growth promoting effect of dietary lactate in Arctic charr is caused by the relatively slow gastric emptying rate (Gislason *et al.*, 1996). An increased holding time in the stomach augments the antibacterial potential of the lactic acid salt and can have a larger inhibitory effect against possible pathogenic bacteria (Sissons, 1989). The improved growth of the Arctic charr did not affect the chemical composition of the fish (Ringø *et al*., 1994).

Trials in Salmon

Feeding Na-lactate to Atlantic salmon juveniles (15 kg/t) did not show such a prolonged effect (Ringø, 1994; Gislason *et al*., 1996) compared to the reported results from trials using charr. Ringø (1994) found slightly increased survival rates in salmon feeding on lactate (84.8% compared to 80.1%), while Gislason *et al.* (1996) determined a higher specific growth rate (SGR), which is calculated as:

SGR = ln final weight – ln initial weight) x 100 / days of culture period

However, none of these differences were statistically significant. These findings suggest that the influence of lactate is a result of certain differences in digestive physiology between the two fish species, for instance a longer retention time of lactate in the stomach in charr. Lower bacterial challenge, due to the use of the organic acid salt, may contribute to the tendency of higher survival rates.

Studies with acids for rainbow trout

Recently, a trial with organic acid salts was also carried out with rainbow trout *Oncorhynchus mykiss* (de Wet, 2005). This study evaluated an organic acid blend (5–15 kg/t), mainly consisting of formate and sorbate, for use in trout nutrition to improve performance parameters, in comparison with some commonly used antibiotic growth promoters (40 ppm Flavomycin). Rainbow trout fingerlings (initial weight ~ 40 g) were kept in flow-through ponds and fed three times daily to apparent satiety. The experiment lasted for three months (table 1).

Table 1. Influence of dietary treatment with an organic acid blend on rainbow trout Oncorhynchus mykiss performance compared to an antibiotic growth promoter (AGP) (de Wet, 2005)

Parameter	*Control*	*AGP*	*5 kg/t acidifier*	*10 kg/t acidifier*	*15 kg/t acidifier*
Initial weight (g)	40.3	42.3	40.0	37.3	37.2
Final weight (g)	184.8[a]	235.4[b]	205.6[ab]	231.2[b]	231.4[b]
FCR	1.22	1.10	1.09	1.08	1.04
SGR (%)	1.23[a]	1.37[b]	1.23[a]	1.29[ab]	1.37[b]
Survival (%)	82.7	88.8	85.0	85.8	89.6

[ab]within rows, means without common superscripts are significantly different (P<0.05)

Fish receiving 10 or 15 kg acidifier per ton of feed had significantly higher final weights compared to the negative control group, while there was no difference in comparison with the group treated with AGP. Feed conversion ratio tended to be lower with increasing dosages of the acid blend, even if compared to the AGP group. The results of this study showed that the application of acidifier at 15 kg/t feed improved weight gain and feed conversion ratio in trout compared to a negative control by 20% and 15% respectively.

This data demonstrated that organic acid inclusion is suitable for use in rainbow trout grower feeds at, and above, levels of 10 kg acidifier per ton of finished feed and that this level can be an effective alternative compared to the use of AGPs in trout aquaculture.

Acidifer research in warm-water species

The use of organic acids however was not only tested in Salmoniformes, but also in tropical warm-water species, like tilapia or catfish. Ramli *et al.* (2005) tested the use of potassium-diformate as a non-antibiotic growth promoter in tilapia grow-out in Indonesia. In this study fish were fed 6 times a day over a period of 85 days with different concentrations of potassium-diformate (0, 2, 3 and 5 kg/t feed). Fish were challenged orally, starting at day 10 of the trial period, with *Vibrio anguillarum* at 10^5 CFU per day over a period of 20 days.

Table 2. Effects of potassium-diformate on growth performance in tilapia challenged with *V. anguillarum* (Ramli et al. (2005)

Parameter	*Control*	*2 kg/t acidifier*	*3 kg/t acidifier*	*5 kg/t acidifier*
Initial weight (g)	16.7	16.7	16.7	16.7
Final weight (g)	218^{a}	258^{c}	246^{b}	252^{bc}
FCR	1.34^{a}	1.23^{b}	1.25^{b}	1.22^{b}
Mortality (%), day 10-85	33.0^{a}	20.8^{b}	18.4^{b}	11.0^{c}

^{abc}within rows, means without common superscripts are significantly different (P<0.05)

Over the whole feeding period, from day 1 to day 85, potassium-diformate supplementation significantly increased the weight gain and feed efficiency in tilapia. Survival rates of fish after the challenge with *V. anguillarum* on day 10 were also significantly higher compared to the negative control, and this effect was dose dependent. The 2 kg/t inclusion of the potassium salt of the formic acid lead to an improvement in weight gain and feed conversion ratio in tilapia by 19% and 8% respectively and indicated that the acidifier was able to counteract bacterial infections in tilapia.

Owen *et al.* (2006) tested the sodium salt of butyric acid as a feed additive in the omnivorous tropical catfish *Clarias gariepinus* at 2 kg/t using a fishmeal and a defatted soya concentrate diet. No significant differences were found while supplying sodium butyrate if compared with the negative control. However, in the catfish fed on fishmeal diet, the SGR was slightly higher in the supplemented fish (% body weight gain 131% for control and 141% for the Na-butyrate group), with a concomitant reduction in the FCR for the supplemented fish. Subjectively, sodium butyrate supplementation appeared to increase the proportion of gram-positive bacteria in the hindgut of *C. gariepinus*, even though this increase was not statistically significant.

The beneficial application of organic acid salts for shrimps was reported by Tung *et al.* (2006), who used 5 kg/t Na-citrate and inactivated Lactobacilli to boost the growth of the Kuruma shrimp *Masurpenaeus japonicus*.

Finally, a recent report from Taiwan (Lückstädt, unpublished data) suggests that a dosage of 2.5 kg/t Ca-formate can enhance the survival rates in brackish water shrimp grow-out. However, those achieved results need to be evaluated in more than just one grow-out season.

Conclusions

From research into the effectiveness of acidifiers in aquaculture, it can be concluded that the use of organic acid salts or blends provides an interesting option for promoting the performance of a wide variety of aquaculture species worldwide. Data suggests that the impact of bacterial infections may be reduced in fish receiving acidified feed, which could lead to higher survival rates. The use of acidifier in aquaculture can be therefore an efficient tool to achieve more sustainable and economic fish and shrimp production.

References

Ahmad, T.S. and Matty, A.J. (1989) The effect of feeding antibiotics on growth and body composition of carp (*Cyprinus carpio*). *Aquaculture* **77**: 211-229.

Åsgård, T. and Austreng, E. (1981) Fish silage for salmonids: a cheap way of utilizing waste as feed. *Feedstuffs* **53**: 22-24.

Becker, K. and Focken, U. (1998) Resource utilisation by aquaculture in the Tropics: Requirements of different production systems on the operational resources of fish farmers. *Animal Research and Development* **48**: 84-91.

Dagoon, N.J. (2000) Whither aquaculture? *SEAFDEC Asian Aquaculture* **22**: 32-33.

de Wet, L. (2005) Can organic acid effectively replace antibiotic growth promotants in diets for rainbow trout *Oncorhynchus mykiss* raised under sub-optimal water temperatures? *Abstract CD-Rom*, WAS Conference, May 9-13, 2005, Bali, Indonesia.

Feedinfo. (2005) Hard-hit Thai Shrimpers Re-Think Quality Control, Some Go Organic. *Feedinfo News Service*. www.feedinfo.com (accessed: 26.04.2005).

Gilbert, A. and Raa, J. (1977) Properties of a propionic acid / formic acid preserved silage of cod viscera. *Journal of the Science of Food and Agriculture* **28**: 647-653.

Gislason, G., Olsen, R.E. and Ringø, E. (1996) Comparative effects of dietary Na-lactate on Arctic charr, *Salvelinus alpinus* L., and Atlantic salmon, *Salmo salar* L. *Aquaculture Research* **27**: 429-435.

Guillaume, J., Kaushik, S., Bergot, P. and Métailler, R. (2001) Nutrition and Feeding of Fish and Crustaceans. Springer Verlag, Praxis publishing, Chichester, UK.

Liem, D.T. (2004) E. coli resistant to most antibiotics in Vietnam. *Asian Pork Magazine* **August / September**: 22-24.

Lückstädt, C. (2006) Use of organic acids as feed additives – sustainable aquaculture production the non-antibiotic way. *International Aquafeed* **9** (2): 21-26.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. (2000) Effect of aquaculture on world fish supplies. *Nature* **405**: 1017-1024.

Owen, M.A.G., Waines, P., Bradley, G. and Davies, S. (2006) The effect of dietary supplementation of sodium butyrate on the growth and microflora of *Clarias gariepinus* (Burchell 1822). *Proceedings of the XII International Symposium Fish Nutrition & Feeding*, May 28 – June 1, 2006: 149.

Ramli, N., Heindl, U. and Sunanto, S. (2005) Effect of potassium-diformate on growth performance of tilapia challenged with *Vibrio anguillarum*. *Abstract CD-Rom*, WAS Conference, May 9-13, 2005, Bali, Indonesia.

Ringø, E. (1991) Effects of dietary lactate and propionate on growth, and digesta in Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* **96**: 321-333.

Ringø, E., Olsen, R.E. and Castell, J.D. (1994) Effect of dietary lactate on growth and chemical composition of Arctic charr *Salvelinus alpinus*. *Journal of the World Aquaculture Sociecty* **25** (3): 483-486.

Sissons, J.W. (1989) Potential of probiotic organisms to prevent diarrhoea and promote digestion in farm animals. A review. *Journal of the Science of Food and Agriculture* **49**: 1-13.

Tomasso, J. and New, M. (1999) Aquaculture in Global Fisheries Production. **Fisheries** 24: 32.

Tung, H.T., Koshio, S., Teshima, S., Ishikawa, M., Ren, T. and Phuong, N.D.T. (2006) Effects of heat-killed Lactobacillus plantarum on Kuruma shrimp *Masurpenaeus japonicus* juveniles. *Proceedings of the XII International Symposium Fish Nutrition & Feeding*, May 28 – June 1, 2006: 46.

Verbeeke, W. (2001) The influence of consumerism on livestock production and eventually the feed industry. In: Recent Developments in animal feeds and feeding. *Proceedings of the Animal Feed Manufactures Association (AFMA) Forum 2001*, 21st – 23rd of February 2001, Sun City, Northwest Province, South Africa, 6: 1-18.

Williams, M.J., Bell, J.D., Gupta, M.V., Dey, M., Ahmed, M., Prein, M., Child, S., Gardiner, P.R., Brummet, R. and Jamu, D. (2000) Responsible aquaculture can aid food problems. *Nature* **406**: 673.

9

THE USE OF ACIDS TO PRESERVE FEEDSTUFFS

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Introduction

To secure health and a good growth performance, animals need a constant supply of high quality nutrients throughout the year. A primary objective in any profitable farming operation should be the use and production of good quality feedstuffs. Preservation of forage feedstuffs is of key importance for maintaining nutritive value and avoiding the losses caused by undesirable microorganisms and the contamination with toxins such as fungal mycotoxins.

According to the presence or absence of oxygen, feedstuffs can be stored under aerobic or anaerobic conditions respectively. Anaerobic procedures (without oxygen) include the age-old practice known as ensiling. The practice of ensiling was originally a management tool to fulfill feed demand for ruminants in seasons where forage was scarcer, by storing and preserving the excess forage resources during periods of overproduction or abundance, e.g. spring grass 'flush'. In more recent times its importance has extended, especially for high input systems utilizing so called "zero-grazing" strategies, with the accompanying benefits derived from increased productivity per animal and per area unit (Ogle, 1990; Muller and Botha, 1997; Klein and Ledgard, 2001). Ensiling is also less dependent on weather and can be used to preserve a great variety of forage crops and regionally available byproducts (Schroeder, 2004).

Over the last few years, silage additives have been utilised more and more by silage producers (Knický, 2005). Their main purpose for inclusion in silage is to increase its nutritional value, improve fermentation (so that storage losses are reduced) and increase aerobic stability of the finished silage after the opening of the silo (Jones *et al*., 2004). Responses to additives depend not only on what type of forage is used, but also dry matter (DM) content, for example (Burns *et al*., 2005).

Acidifying silage

Acidifying silage has been particularly practiced on high-moisture silage produced in regions where the wilting is difficult (Campbell, 2002). The main aim is to rapidly acidify the forage material, rather than waiting for the accumulation of acid from fermentation, because high moisture conditions provide an optimal environment for the growth of undesirable bacteria and mould (Jones *et al.*, 2004). The addition of organic acids and their salts influences the ensiling process via H^+ ions and undissociated acids, and both these effects contribute to antimicrobial activity.

Immediate acidification of forage also inactivates plant proteases, reducing protein losses from the silage mass (Khorasani, 2001). This results in a better yield of nutrients to the animals and has a positive effect on the preservation in the clamp. Typical amounts of acid produced may range between 5 to 10 kg/t.

Adding acidifiers to silage does incur additional costs to the producer, in comparison to biological ensiling products (€2.50-7.70 versus €0.80-2.00 per tone silage mass treated respectively; Thaysen, 2002), and issues regarding handling and the corrosive nature of acids and potential lower forage palatability must be taken into consideration. However, the use of acid salts provides a viable alternative that does not have these drawbacks.

The most commonly used acids, e.g. organic formic and propionic acids or the mineral sulfuric and hydrochloric acids, have been shown to improve the preservation of forage. Weak acids play a major role in reducing spoilage after it has been opened. Weak acids are added to the forage at the time of ensiling, in the same manner as inoculants and other additives. An appropriately dosed acid will rapidly reduce the pH value in the silage and limit fermentation losses of protein and carbohydrates.

The most common organic forms used under practical conditions are formic and propionic acids, which are often used in combination (Chamber of Agriculture Schleswig-Holstein, 2002). Formic acid (HCOOH) is a transparent liquid, soluble in water and an irritant to eyes, skin and mucous membranes and is produced by heating carbon monoxide and sodium hydroxide together under pressure, and treating the resulting sodium formate with sulfuric acid. It diminishes the pH values very quickly, allowing detrimental growth of certain undesirable bacteria. As stated previously, the antimicrobial effect of formic acid is not only caused by the pH decreasing but also by undissociated molecules under acidic conditions (figure 1).

Undissociated molecules penetrate the cell wall, dissociate in the cell cytoplasm and disrupt cell functions, inhibiting both yeast and bacterial growth. There is a long tradition of using this acid to produce suitable silage for ruminants, but more recently it has been used to acidify the young piglets diets, where it can act as a growth promoter (Partanen and Mroz, 1999).

Propionic acid is weaker than formic (and mineral acids), but is still an important additive in silages because it has an antifungal activity against moulds and yeasts, which are responsible for aerobic deterioration and spoilage in silages. Using propionic acid increases the aerobic stability of silages.

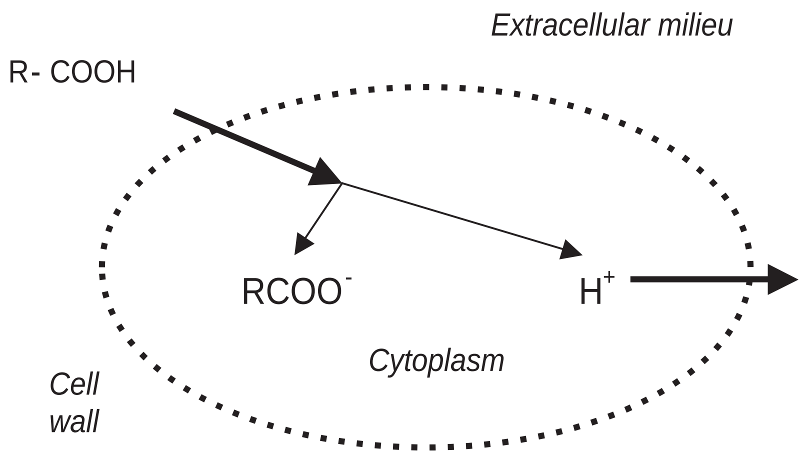

Figure 1. Schematic representation of the mode of action of preservative acids and their salts

Propionic acid is added at the rate of 0.5 to 1% of the wet forage weight (Schroeder, 2004). It is relatively expensive, and use is often limited to the last few loads of silage at the top of conventional or bunker silos, where it is most beneficial in reducing surface spoilage (Kunkle and Chambliss, 2002). The amount of propionic acid required increases with decreasing DM silage content, and because the cost of adding sufficient propionic acid is relatively high, this strategy is seldom cost effective. The efficacy of propionic acid and its salts is closely related to their solubility in water (Kung, 2006). The stronger the bond between the acid-base, the less soluble the product is resulting in less effective inhibition of fungal growth.

Scientific studies have shown improvements in the aerobic stability of the silage produced with propionic acid. Kung *et al.* (2000) found an increase in stability after the opening treated silos compared with untreated control silages, but without statistical significance. Other authors, including Britt and Huber (1975), Brit *et al.* (1975) and Leaver (1975), used propionic acid to demonstrate enhanced aerobic stability. Kung *et al*. (2004) tested buffered propionic acid-based additives alone or in combination with a microbial inoculant (containing lactic acid bacteria) on ground, high moisture corn or whole-crop barley, to investigate its effect on silage fermentation and aerobic stability. Corn treated with only 0.1 and 0.2% propionic acid tended to be more stable (161 and 218 h respectively) when exposed to air than untreated control (122 h). With whole-crop barley, the propionic acid alone, and in combination with lactic acid bacteria, prevented the production of butyric acid that was found in the untreated silage (0.48%).

For corn silage, propionic acid included at 0.2 to 0.5% has been shown to be effective (Beck, 1975). Nevertheless Kleinschmit *et al.* (2005), when investigating the effect of a buffered propionic acid-based additive on fermentation, DM recovery, or aerobic stability, found no effects on these parameters. The activity of a buffered propionic acid-based product (included at 0.1% of fresh forage weight) on the fermentation and aerobic stability of corn silage was compared with other commercial inoculants by Ranjit and Kung (2000). The silage treated with the chemical preservative took longer to generate heat than the untreated silage when exposed to air, but improvements were numerically small (6.3 to 10.5 h).

Other acids with preservative action include benzoic acid (C_6H_5COOH), the use of which has increased in recent years in animal feed. In its chemically pure form, this acid is a white granular or crystalline powder, however the main form used is sodium benzoate, due to its higher solubility in water. Benzoate inhibits yeasts more than moulds or bacteria. The undissociated form of benzoic acid, as well as sodium benzoate, is essential to its antimicrobial activity, as both are soluble in cell walls and facilitate proton leakage across membranes. This elevates energy requirements to maintain the internal cell pH.

The corrosive action of the acids can be avoided by using their salts. Their principal barrier to their use on farms is cost. Using salts can selectively prevent the growth of certain microorganisms, typically via the decomposition products of acid salts. Nitrite acts against *Clostridia* and *Listeria* spp., while the lactic acid bacteria will not be markedly affected. A further aspect to take into account is the length of time required for ensiling using acid salts. A waiting period of at least four weeks must be allowed between the ensiling process and feeding out the finished silage. Depending on the type of salts used, fermentation and aerobic stability can be improved.

A mixture of acetic acid and its sodium salt, usually known as sodium diacetate, provides another acidic preservative, which has similar results to propionic acid inclusion in its effectiveness to control yeasts and moulds in the top layers of silage. Following the application of this mixture, the silage must be covered immediately. The following table (table 1) gives an overview of the dosage of acids and their salts required to successfully treat corn silage.

Table 1. Recommended dosage of acids and salts for the treatment of the top of corn silage (Thaysen, 2002)

DM- content in the silage mass (%)	*Dosage*			
	Acid (l/ dt) in the treatment of 1 m layer*	*Acid + water (litre/ m² top area)*		*Salt (kg/ m²)*
		Acid	*Water*	
< 25	0.5	1.5	5.0	1.75
25 to 35	0.6	1.75	7.0	2.00
> 35	When DM content over 35%, recommended full treatment, not only the top layer			2.25

* 1% acid means 1 litre acid per 100kg silage mass

Commercial usage of acids in silage

In a survey taking a sample of the main 35 chemical products used for ensiling in the German market (Chamber of Agriculture Schleswig- Holstein, 2002), most farmers use propionic or formic acid or its salts (ammonium or calcium; or calcium, ammonium and sodium respectively), benzoic acid, but above all its salts (sodium benzoate) and common salt. Where propionic acid is used, the ammonium propionate salt is most soluble in water (90%), followed by sodium propionate (25%) and calcium propionate (5%) (Kung, 2006). Other chemical substances included in these products include hexamethylenetetramine (a form of formaldehyde) and sodium nitrite (well known as the “Clostridia killer”). A large number of these products contain mixtures of acids and/or salts, taking advantage of the synergistic effects of the combinations.

Feedstuffs can also be stored under aerobic conditions. Under these circumstances, the use of acids has been shown to significantly reduce the extent of microbial contamination in treated grain or finished feed. This decontamination secures the nutritional value of the stored grain, grain crush and feed mixtures and can improve the health of the animals consuming the final feedstuffs, promoting performance, and ensuring more efficient and economic animal production.

Practical storage and ensiling solutions

Despite the availability of these storage techniques, the agricultural industry faces increasing losses each year due to spoiled grain, which was not properly stored and preserved after harvest. Unlimited mould growth leads to poor nutritional value; mycotoxins formed from fungal growth can cause acute health risks in animal husbandry. Signs of spoiled grain ingestion in animals include disturbed metabolic activity, reduced performance and fertility problems.

Therefore it is of great importance to ensure that any preservation method under consideration should be carefully examined to avoid inadequate dosages, aerobe spoilage of the grain and the incorrect use of the necessary dosage equipment. Successful preservative products should guarantee the preservation and stabilisation of the grain combined with easy and user-friendly handling for the customer.

Several practical studies have demonstrated the efficiency of acidic preservation products in both grain and finished feed, compared to technical drying methods, which have become more expensive due to increasing fuel prices. A wide range of preserving agents are available commercially, many of them based on propionic acid, which is a very effective mould inhibitor. Propionic acid has been used for the preservation of grain for more than a decade, and results in excellent preservation; however it remains a corrosive and hazardous ingredient. Such corrosive characteristics can damage dosing equipment and the storing facility and can be inconvenient for staff that has to handle it.

A trial to illustrate the benefits available from using acids to preserve feedstuffs was carried out under Chinese field conditions near Shanghai, with wet corn, using a non-corrosive, skin friendly preservative containing buffered propionic acid and sodium benzoate (Lückstädt *et al.*, 2006). The trial lasted for 90 days, and examined corn storage as a two by two factorial design, including moisture levels (24% and 29%) and presence or absence of acid product (11 litres/ t and 16.5 litres/t of corn product use in the treated groups respectively), giving four experimental groups. Initial mean fungal and yeast counts in the corn were 3.8 x 10^6 and 8.3 x 10^4 CFU/g, respectively (table 2).

All stored corn samples were checked regularly for temperature profile and microbial development. Final data showed germination in both negative control groups, with maximal temperatures reaching between 48°C and 54°C. The two treated groups remained at lower, more stable temperatures (between 14°C and 15°C). At the end of the experiment the microbial load for the four treatments differed dramatically, with the treated corn yielding fungal and yeast counts ranging only from 0 to 30 CFU/g and 0 to 130 CFU/g, respectively. The untreated corn showed a higher degree of spoilage, with fungal counts ranging between 2.6 x 10^6 and 2.7 x 10^6 CFU/g in the low and high moisture content respectively and yeast counts varying from 1.6 x 10^6 to 4.3 x 10^7 CFU/g for both moisture levels. Furthermore, untreated corn showed elevated mycotoxin levels, with Aflatoxin AFB1 and Zearalenone reaching more than 80 µg/kg and 11,000 µg/kg respectively.

Table 2. Initial and final results of fungi and yeast count and mycotoxin analysis after 90 days of storage – with and without preservative

Parameter	*Unit*	*Initial*	*Treatment* 1	2	3	4
Moisture	%	24 / 29	24		29	
Dosage	litres/t	-	0	11.0	0	16.5
Avg. temperature	°C	15.6	29.3	15.2	41.2	13.9
Fungi	CFU/g	3.8 x 10^6	2.6 x 10^6	33	2.7 x 10^6	0
Yeast	CFU/g	8.3 x 10^4	1.6 x 10^6	133	4.3 x 10^7	0
Aflatoxin B1	µg/kg	n.d.	30	bdl*	83	bdl*

*bdl: below detection limit
n.d. – not determined

It was concluded that the use of buffered propionic acid combined with sodium benzoate as a preservative for wet corn storage, even without prior drying, provides an interesting option for long term storage and secures the quality of the stored raw material.

References

Beck, T. (1975) The effect of ensiling agents on aerobic deterioration. *Das Wirtschaftseigene Futter* **4**: 8-12.

Britt, D.G. and Huber, J.T. (1975) Preservation and animal performance of high moisture corn treated with ammonia or propionic acid. *Journal of Dairy Science* **59**: 668-674.

Britt, D.G., Huber, J.T. and Rogers, A.L. (1975) Fungal growth and acid production during fermentation and re-fermentation of organic acid treated corn silages. *Journal of Dairy Science* **58**: 532-539.

Burns, H., Piltz, J., Kaiser, A., Blackwood, I. and Griffiths, N. (2005) Making high quality silage. Research Update for Growers- Southern Region (High Rainfall) - August 2005. In: http://www.grdc.com.au/growers/res_upd/hirain/h05/burns.htm.

Campbell, J. (2002) Wet weather takes it toll. *Irish Farmer Journal*- Interactive. In: http://www.farmersjournal.ie/2002/0601/home/home2.html.

Chamber of Agriculture Schleswig- Holstein (2002) Futterkonservierung. Siliermittel, Dosiergeräte, Silofolien. 6. Auflage, 2002. 207.

Jones, C.M., Heinrichs, A.J., Roth, G.W. and Ishler, V.A. (2004) From harvest to feed: Understanding silage management. In: http://www.das.psu.edu/dairynutrition/documents/silage2004.pdf.

Khorasani, R. (2001) Manipulation of silage fermentation and aerobic instability. Forage Inoculants and Additives. Dairy Research and Technology Resources- University of Alberta.

Klein, C.A.M. de and Ledgard, S.F. (2001) An analysis of environmental and economical implications of nil and restricted grazing systems designed to reduce nitrate leaching from New Zealand dairy farms. I. Nitrogen losses. *New Zealand Journal of Agricultural Research* **22**: 201-215.

Kleinschmit, D.H., Schmidt, R.J. and Kung, L.Jr. (2005) The Effects of Various Antifungal Additives on the Fermentation and Aerobic Stability of Corn Silage. *Journal of Dairy Science* **88**: 2130-2139**.**

Knický, M. (2005) Possibilities to improve silage conservation. Effects of crop, ensiling technology and additives. PhD thesis at the Swedish University of Agricultural Science Uppsala, Sweden.

Kung, L.Jr. (2006) A review on silage additives and enzymes. In: http://ag.udel.edu/anfs/ faculty/kung/articles/a_review_on_silage_additives_and.htm.

Kung, L.Jr., Robinson, J.R., Ranjit, N.K., Chen, J.H., Golt, C.M. and Pesek, J.D. (2000) Microbial Populations, Fermentation End-Products, and Aerobic Stability of Corn Silage Treated with Ammonia or a Propionic Acid-Based Preservative. *Journal of Dairy Science* **83**: 1479-1486.

Kung, L.Jr., Myers, C.L., Neylon, J.M., Taylor, C.C., Lazartic, J., Mills, J.A. and

Whiter, A.G. (2004) The effects of buffered propionic acid-based additives alone or combined with microbial inoculation on the fermentation of high moisture corn and whole-crop barley. *Journal of Dairy Science* **87**: 1310-1316.

Kunkle, W.E. and Chambliss, C.G. (2002) Silage Harvesting, Storing and Feeding. In: http://edis.ifas.ufl.edu/AG180.

Leaver, J.D. (1975) The use of propionic acid as an additive for maize silage. *Journal of the British Grassland Society* **30**: 17-21.

Lückstädt, C., Zhang, C. and Wu, J. (2006) The use of a non-corrosive preservative for wet corn storage under Chinese field conditions. In: *Proceedings of the International Conference on Grain Storage and Drying (ICGSD*). May 18- 20, 2006. Chengdu, China.

Muller, C.J.C. and Botha, J.A. (1997) Production responses of lactating Jersey cows on two intensive grazing systems *versus* a zero-grazing system. *32nd Congress of the Grassland Society of Southern Africa*. 20 - 23 Jan: 90.

Ogle, B. (1990) Suggestions for intensive livestock-based smallholder systems in semi-arid areas of Tanzania. *Livestock Research for Rural Development* **2**: 1. In: http://www.cipav.org.co/lrrd/lrrd2/1/ogle.htm.

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

Ranjit, N.K. and Kung, L.Jr. (2000) The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science* **83**: 526-535.

Schroeder, J.W. (2004) Haylage and Other Fermented Forages. *NDSU Extension Service*. North Dakota State University, USA. In: http://www.ext.nodak.edu/extpubs/ansci/dairy/as1252w.htm.

Schroeder, J.W. (2004) Silage fermentation and preservation. *NDSU Extension Service*. North Dakota State University. In: http://www.ext.nodak.edu/extpubs/ansci/dairy/as1254w.htm.

Thaysen, J. (2002) Siliermittelprüfung nach den Prüfrichtlinien. Futterkonservierung- Siliermittel, Dosiergeräte, Silofolien. 6.Auflage: 39-44.

Thaysen, J. (2002) Dosierung von Silierzusätzen. Futterkonservierung- Siliermittel, Dosiergeräte, Silofolien. 6.Auflage, 57-60.

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