Effects of Dietary Buffering Characteristics and Protected or Unprotected Acids on Piglet Growth, Digestibility and Characteristics of Gut Content[a,b]

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ABSTRACT : We conducted two experiments to evaluate the interaction among fumaric acid (FA), protected acids (PA), or no additional acid (NO) and two different levels of acid buffering capacity (BC) in diets for 14-d-old weaned pigs. BC was varied substituting mono-calcium phosphate and calcium sulfate for dicalcium phosphate and calcium carbonate. In the high BC diet plus PA, FA was also added. In Exp. 1, 48 gilts were raised for 31 days on the six diets, evaluating growth performance and fecal digestibility. In Exp. 2, 42 gilts were raised. With each diet three subjects were sacrificed after 19 days and four after 38 days. In addition, six subjects were sacrificed at weaning. Growth and carcass performance, ileal digestibility, bacterial populations and pH in the gut were assessed. The piglet performance and stomach, ileal and cecal pH, and empty body composition were not affected by the diets. Empty body composition other than ash content was affected by piglet age ($p<0.01$). The BC did not influence digestibility. The dietary inclusion of PA improved fecal digestibility of protein ($p<0.05$) compared to the addition of FA and NO. Ileal digestibility slightly increased with both acid additions ($p<0.10$), the groups receiving PA showing the higher values. Piglets fed diets with low BC had lower *Lactobacillus* and *E. coli* counts in the ileum ($p=0.07$) and higher *Lactobacillus* in the colon ($p=0.08$). Acidified diets tended to reduce *E. coli* counts in the ileum ($p=0.10$) and increased *Lactobacillus* in the colon ($p=0.09$). The addition in the diet of PA increased *Lactobacillus* in the ileum compared to the sole addition of free fumaric acid ($p=0.07$). The addition of protected acids, combined with free fumaric acid in the case of high BC diets, increased protein digestibility and *Lactobacillus* counts and reduced *E. coli* counts. Only some changes in the concentration of bacterial population can be expected with a diet of low BC. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1104-1110*)

Key Words : Piglets, Digestibility, Organic Acid, Buffering Capacity, *Lactobacillus*, *E. coli*

INTRODUCTION

It is well established that insufficient gastric acidification in early weaned piglet is a risk factor for the health of the gut (Mosenthin, 1998). This is particularly relevant when diets with high buffering capacity are fed, that bind free hydrochloric acid (Bolduan et al., 1988). The addition of organic acids sometimes successfully increased stomach acidity after weaning, but no further effect is found in the lower part of the digestive tract (Aumaitre et al., 1995). These data contrast with the growth promoting effect observed when organic acids are added to starter diets (Cho et al., 1997). This effect could be derived from the energy value of organic acids when absorbed, particularly at high levels of addition. Fumarate additions to the diet of weaned pig vary in the literature from 0.7% (Scipioni et al., 1978) to 3.0% (Cho et al., 1997). It is not clear if an acidification effect can be achieved at low (<1%) doses of acid in the diet of piglet.

An intense gastric acidification obtained by an increased acidification of the diet, can produce a reduction of the secretion of hormone gastrin and, consequently, a reduction of hydrochloric acid secretion (Kemme-Kroonsberg, 1993). This can be more important in the case of the addition of acidifiers to a diet of low binding capacity. Highly buffered diets can increase the base excess in the blood, induce a over-stimulated bicarbonate secretion from the pancreas (Kemme-Kroonsberg, 1993) and, possibly, affect digestibility. In this case the addition of acidifiers cannot be sufficient to control the growth of *E. coli* and other dangerous microbes. A valid tool to control the overgrowth of bacteria in the lower tracts of the small intestine and in the hind gut, without affecting secretions in stomach and duodenum, could be to slow the release of the acids to be added to the diet.

The present study was conducted to determine the effect of buffering capacity and protected or unprotected acids in the diet on performance, nutrient

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digestibility, carcass composition, bacterial populations and characteristics of chyme from different digestive segments in piglets.

MATERIALS AND METHODS

Female pigs weaned at 14 days of age were allotted by litter and weight to 6 dietary treatments: two different levels of buffering capacity (BC), low (L) and high (H), crossed with three levels of diet acidification no addition (NO); fumaric acid (FA) and protected organic and inorganic acids mixture (PA). PA contained 10% ortho-phosphoric acid, 20% fumaric acid, 10% citric acid, 10% malic acid and 50% triglycerides and free fatty acids.

Two experiments were conducted. In Exp. 1, 48 subjects were raised on the six diets, eight piglets per treatment. Exp. 1 ended after 31 days, and then the pigs were normally reared. In this trial only growth performance and fecal digestibility were assessd. In Exp. 2, 42 subjects were raised, seven piglets per treatment, of which three subjects were sacrificed after 19 days and four after 38 days. In addition, six subjects were sacrificed at the start for body composition assessment.

The subjects were housed in individual pens with expanded plastic coated mesh floor and solid sidewalls. The temperature in the nursery was automatically controlled to vary from 30℃ at the start to 24℃ at the end of trial. In the first three days infra-red lamps located over the piglets provided zone heating.

Ingredients and analytical characteristics of the basal diet are presented in table 1. Compared to high BC diet, low BC diet was obtained substituting mono-calcium phosphate and calcium sulfate for dicalcium phosphate and calcium carbonate. However, in the case of the high BC with PA, unprotected fumarate was added in the feed. This was to compensate in stomach the higher BC (table 1). Chromic oxide (0.3% wt/wt) was included in the diet as an indigestible marker for determination of protein and dry matter digestibilities and no anti-microbial additive was added.

In Exp. 2 at the day of sacrifice, after sedation with sodium thiopenthal (10 mg/kg live weight), the piglets were euthanised with a 0.5 ml/kg live weight intracardiac injection of Tanax (A.I.C. Hoechst Roussel Vet GmbH, Weisbaden, Germany). Two portions of

Table 1. Formulation and chemical composition of diets

Buffer capacity:	H			L		
Acid:	NO	FA	PA	NO	FA	PA
Ingredient (%):						
Basal diet[1]	97.3	97.3	97.3	97.3	97.3	97.3
Flaked corn addition	1.0	0.5		1.0	0.5	
Calcium carbonate	0.5	0.5	0.5			
Dicalcium phosphate	1.2	1.2	1.2			
Monocalcium phosphate				1	1	1
Calcium sulfate				0.7	0.7	0.7
Fumaric Acid		0.5	0.5		0.5	
Protected acid[2]			0.5			0.5
Chemical composition:						
Dry matter (%)	89.80	89.53	88.99	90.27	89.85	90.23
Crude protein (%)	18.89	18.28	18.35	18.17	18.45	18.35
Ash (%)	8.20	7.83	7.81	7.95	7.80	7.86
Lysine, calculated (%)	1.56	1.56	1.56	1.56	1.56	1.56
Ca, calculated (%)	1.07	1.07	1.07	0.88	0.88	0.88
P, calculated (%)	0.81	0.81	0.80	0.78	0.81	0.79
Buffer capacity (meq/kg, pH 3)	823	783	803	645	636	670
pH	5.62	5.06	5.09	5.32	5.26	4.86

[1] Flaked corn 29.97%, flaked dehulled barley 20%, dried skimmed milk 15%, spray-dried milk whey 10%, fish meal 5%, soybean extruded 5%, wheat bran 3%, potato concentrate 2.5%, oil 4% Lys-HCl (30%) 1.1, Met (30%) 0.55, Thr 0.16, Trp 0.05, Choline-Cl 0.34, premix 0.6% and Chromic oxide 0.3%. Vitamin and mineral mixture per kg diet: vitamin A, 26,500 IU; vitamin D_3 , 2,400 IU; vitamin E, 35 mg; vitamin B_1, 10 mg; vitamin B_2, 8 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.04 mg; niacin, 55 mg; biotin, 0.15 mg; d-pantothenic acid, 30 mg; folacin, 1 mg; Fe, 200 mg; Zn, 175 mg; Cu, 150 mg; Mn, 80 mg, I, 2.5 mg; Co, 2 mg and Se, 0.2 mg.

[2] Containing 10% ortho-phosphoric acid, 20% fumaric acid, 10% citric acid, 10% malic acid and 50% triglycerides and free fatty acids.

the central jejunum and of the ascending colon of each subject were isolated, resected, placed in a sterile basin and opened longitudinally, for microbial determination. The mucosa of jejunum was gently scraped and the two samples carefully mixed. From the colon about 5 g of content was collected. The digesta contents of stomach, ileum and cecum were sampled for pH measurement. The empty digestive tract was then weighted for empty body determination and along with the carcass was milled and homogenized. The individual samples of the empty body and of the ileal chyme were freeze-dried and analyzed for dry matter, ash and nitrogen content.

The analysis for chemical composition of the diet was carried out according to the Association of Official Analytical Chemists Methods (1995). Dietary pH was measured with a pH meter (HI 8418, Hanna Instruments, Padova, Italy) three times by adding 100 ml of distilled, deionized water to a 20 g sample, according to Risley et al. (1992). Buffering capacities of the experimental diets were determined according to Gabert et al. (1995) with some modifications. Five grams of each diet, in 50 ml of 0.1 N hydrochloric acid, were titrated with 0.1 N sodium hydroxide until pH 3. The BC was calculated according to following equation:

$$BC\ (meq/kg)=((50-X)\times 0.1/\text{weight sample})\times 1000,$$

where X=milliliters of 0.1 N NaOH

For microbial counts, 1 g of each sample was diluted in 4 ml of Ringer's solution (1:4 v/v) and tenfold serial dilutions were made up to 10-10 dilution. Aliquots of 0.1 ml were inoculated onto a Violet Red Bile Agar medium (with added 4-methyl-umbelliferyl-glucoronide, 0.1 g/L) for enumeration of *E. coli* and on to a deMan-Rogosa-Sharpe (MRS) agar for enumeration of *Lactobacillus*. Violet Red Bile Agar was incubated at 43℃ for 24 hours and the MRS plates were incubated in 5% (v/v) CO_2 at 37℃ for 72 hours. The specificity of colonies was confirmed by Wood's lamp fluorescence, growth on Kligler Iron Agar media and API 20E test for *E. coli*, and by Gram coloration and catalase test for *Lactobacillus*. The counts were effected at the first dilution that permitted the enumeration of colonies.

In Exp. 1, feces were individually collected on days 17 to 19 and 29 to 31. The total tract digestibility was measured with the indirect method. The apparent ileal digestibility (AID) was measured by the slaughter method (Donkoh et al., 1994). The chromic oxide contents of diets, feces and ileal digesta were determined by the Fenton and Fenton's method (1979).

The performance data of Exp. 1 were analyzed by means of a linear model considering the effects of dietary treatments, their interaction and litter. For the analysis of the data from Exp. 2 the effects of time period and of its interaction with dietary treatments, were added to the factors considered for Exp. 1. In the case of fecal digestibility (Exp. 1), data were analyzed considering also the period of measurement and its interactions, as fixed effects, and the subject within treatment as random effect. The analyses were performed by SAS (1996), with dietary treatment least squares means being adjusted for the other effects. Three orthogonal contrasts were evaluated: Buffering Capacity, H vs. L; levels of acid addition, NO vs. FA, NO vs. PA.

RESULTS AND DISCUSSION

For all the statistically processed data no significant first level interaction was found between BC and acid addition or with time period. Therefore, the least squares means of the main effects are presented here.

Piglet performance in Exp. 1 is presented in table 2. No effect of buffering capacity was found for growth and feed efficiency. Feed intake was higher for the PA group ($p<0.01$), but no statistically significant difference was found for average daily gain and feed to gain ratio, in relation to diet acidification. For Exp. 2, piglet performance and carcass composition are presented in table 3 and table 4, respectively. No effect of dietary treatments was found for the

Table 2. Effect of buffering capacity and acid dietary addition on piglet performance (Exp. 1)

	Buffering capacity		SEM	Acid			SEM
	H	L		NO	FA	PA	
Initial weight (kg)	4.22	4.17	0.11	4.16	4.21	4.21	0.13
Final weight (kg)	11.24	11.16	0.26	11.02	11.02	11.55	0.32
Feed intake (g/day)	298	297	3.6	291^{A}	287^{A}	315^{B}	4.4
Average daily gain (g/day)	226	226	9.8	221	220	237	12
Feed:gain	1.37	1.36	0.06	1.32	1.30	1.33	0.07

^{A,B} Means, within each main effect, with different superscripts are significantly different at $p<0.01$.

parameters shown. Similar results on growth performance were reported by Henry et al. (1985) and Giesting and Easter (1985) with FA (10 g/kg fed), but most research suggested that organic acid improved daily live weight gain when added to diets for post-weaning piglets (Cho et al., 1997). Compared to control group, the daily protein gain increased in the current study by 12% with FA and PA addition, but the difference did not reach statistical significance. Radecki et al. (1988) observed that protein accretion of piglets was not affected by organic acid supplementation, while other data indicated an improvement in nitrogen balance with a high dose of organic acids (Kirchgessner and Roth, 1980). The lack of effect on performance could be due to an excellent state of health of the animals in the present study, to optimal environmental conditions and to the low dose of acidifiers. Moreover, a long trial, as in our case, could have masked any effect that diet acidification had on nutrient retention (Ravindran and Kornegay, 1993). However in our trial no interaction was found between time period and diets.

The rate of protein deposition of the early weaned piglets fell within the ranges cited by Close and Stanier (1984). Growth and carcass composition other than ash content were affected by piglet age ($p<0.01$). Fenton et al. (1985) observed a similar change in body chemical composition with the age of the early weaned pig.

The buffering capacity did not influence the apparent fecal (table 5) and apparent ileal digestibility (table 6). The lack of response in the present study may have been due to the high buffering capacity of milk products that were included in both diets (Bolduan et al., 1988). It is also likely that the lactose contained in these diets was available for the formation of lactic acid, converted by the *Lactobacillus* bacteria normally resident in the stomach, which could have reduced the need for diet acidification (Easter, 1988).

The dietary inclusion of a mixture of protected organic and inorganic acids improved fecal digestibility of protein ($p<0.05$) compared to the addition of unprotected acid and to control, while the dry matter

Table 3. Effect of buffering capacity and acid dietary addition on piglet performance (Exp. 2)

	Buffering capacity		SEM	Acid			SEM	Days in trial[a]	
	H	L		NO	FA	PA		19	38
Initial weight (kg)	4.96	4.96	0.11	4.93	5.00	4.94	0.13	4.98±0.13	4.93±0.12
Final weight (kg)	11.46	11.63	0.41	11.67	11.53	11.45	0.50	7.13±0.49^{A}	15.96±0.42^{B}
Feed intake (g/day)	267	276	8.5	266	265	282	10	176±10^{A}	366±8.8^{B}
Average daily gain (g/day)	202	206	8.7	209	200	201	11	113±10^{A}	292±8.9^{B}
Feed:gain	1.32	1.34	0.08	1.27	1.32	1.40	0.09	1.56±0.09^{A}	1.25±0.08^{B}

[a] Values reported as means±SE.
[A,B] Means, within each main effect, with different superscripts are significantly different at $p<0.01$.

Table 4. Effect of buffering capacity and acid dietary addition on body composition and nutrients growth

	Buffering capacity		SEM	Acid			SEM	Days in trial[a]	
	H	L		NO	FA	PA		19	38
Chemical composition									
Dry matter (%)	26.90	28.20	0.56	27.72	27.67	27.24	0.69	25.56±0.60^{A}	29.54±0.52^{B}
Proteins (N×6.25, %)	14.85	14.91	0.15	14.76	14.96	14.91	0.19	14.50±0.17^{A}	15.27±0.15^{B}
Lipids (%)	8.29	8.80	0.38	8.35	8.60	8.69	0.47	7.19±0.41^{A}	9.91±0.35^{B}
Ash (%)	2.97	3.14	0.08	3.20	3.04	2.93	0.09	3.05±0.08	3.06±0.07
Daily nutrient gain									
Proteins (N×6.25, g)	28.88	27.56	1.81	25.81	29.37	29.44	2.19	14.56±1.94^{A}	41.88±1.69^{B}
Lipids (g)	12.93	15.18	1.55	13.25	14.13	14.78	1.90	0.58±1.68^{A}	27.53±1.38^{B}
Ash (g)	5.17	5.46	0.37	5.34	5.48	5.13	0.45	2.77±0.40^{A}	7.86±0.34^{B}

[a] Values reported as means±SE.
[A,B] Means, within each main effect, with different superscripts are significantly different at $p<0.01$.

digestibility was not affected. Moreover ileal digestibility of crude protein slightly increased with both acid additions ($p<0.10$), the group with PA showing the higher values. The current study agrees with the results of Kirchgessener and Roth (1982) and Scipioni et al. (1978) who reported a tendency toward increased protein digestible values with acidifiers. In contrast, Giesting and Easter (1991) found no effect for supplementation with 2% FA, and Mosenthin et al. (1992) also reported that supplementation with 2% propionic acid did not affect the ileal digestibility coefficients of crude protein. Finally, Mroz et al. (1997) found that the positive effect of organic acids on the ileal digestibility of nitrogen and amino acids was greater with a high BC than with a low BC diet. The improvement of the protein digestibility could be associated with a reduction of *Enterobacteriaceae* population in the small intestine determined by organic acid addition (figure 1). This can bring about a decrease of endogenous protein losses (Caine, 1997). The digestibility of dry matter and protein increased with age, in accord with Falkowski and Aherne (1984).

Buffering capacity, organic acid supplement into the diet and post-weaning age did not affect the intestinal pH (table 7). As expected, the pH of the stomach content was lowest, followed by that of colon and ileum contents. The absence of an effect of the BC contrasts with the results from Gabert et al. (1995) and Decuypere et al. (1997), but agrees with Mroz et al. (1997). In the present study the range of BC was not as large as in the trial of Decuypere et al. (1997), but in our formulation we tried to limit the difference in the Ca to P ratio between high and low BC diets.

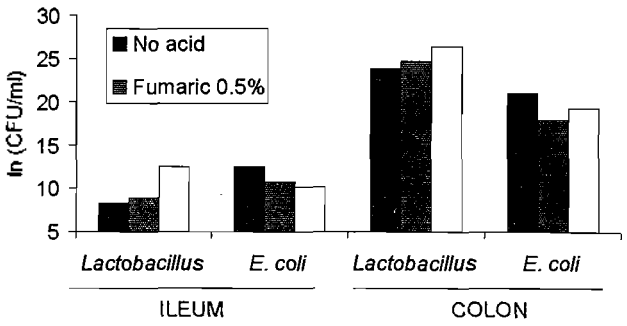

Figure 1. Effect of acid dietary addition on the *Lactobacillus* and *E. coli* content in ileum and cecum of early weaned pigs

Concerning the organic acid addition, a reduction of gastric pH in piglets has not been observed with diets containing 1.5% fumaric acid (Risley et al., 1992) or citric acid (Scipioni et al., 1978). The absence of effect could be due to the presence of dried whey in the complex diet, which lowers the pH of the feed mixture (Risley et al., 1993). Furthermore Ravindran and Kornegay (1993) conclude that reducing gastric pH does not seem to be the primary effect of acidifiers. In addition, due to the buffering capacity of milk products, higher levels of fumaric acid or protected organic acid may be required to decrease gut pH (Bolduan et al., 1988).

Piglets fed diets with low BC (figure 2) showed lower *Lactobacillus* and *E. coli* counts in the ileum ($p=0.07$) and higher *Lactobacileae* in the colon ($p=0.08$). Acidified diets (figure 1) showed a tendency toward reduced *E. coli* counts in the ileum ($p=0.10$) and increased *Lactobacillus* in the colon ($p=0.09$). The PA addition to the diet increased *Lactobacillus* in the

Table 5. Effect of buffering capacity and acid dietary addition on apparent fecal digestibility (%) (Exp. 1)

	Buffering capacity		SEM	Acid			SEM	Days in trial		SEM
	H	L		NO	FA	PA		17-19	29-31	
Dry matter	83.29	83.58	0.54	82.87	83.36	84.07	0.66	82.55^{A}	84.31^{B}	0.44
Crude protein	87.02	87.18	0.18	86.89^{b}	86.83^{b}	87.59^{c}	0.22	87.07	87.14	0.18

^{b,c} Means, within each main effect, with different superscripts are significantly different at $p<0.05$.
^{A,B} Means, within each main effect, with different superscripts are significantly different at $p<0.01$.

Table 6. Effect of buffering capacity and acid dietary addition on apparent ileal digestibility (%) (Exp. 2)

	Buffering capacity		SEM	Acid			SEM	Days in trial[a]	
	H	L		NO	FA	PA		19	38
Dry matter	75.87	75.91	2.57	73.32	74.98	78.32	3.16	74.10±3.10	76.98±2.68
Crude protein[c]	78.03	79.17	1.88	75.40	79.35	81.05	2.31	76.73 ± 2.26^{A}	80.47 ± 1.96^{B}

[a] Values reported as means±SE.
^{A,B} Means, within each main effect, with different superscripts are significantly different at $p<0.01$.
[c] NO vs FA and PA ($p<0.10$).

Table 7. Effect of buffering capacity and acid dietary addition on pH of intestinal contents of piglets

	Buffering capacity		SEM	Acid			SEM	Days in trial[a]	
	H	L		NO	FA	PA		19	38
Stomach	3.65	3.49	0.24	3.88	3.34	3.50	0.30	3.58±0.28	3.56±0.24
Ileum	7.13	7.14	0.09	7.19	7.10	7.11	0.11	7.18±0.09	7.10±0.08
Cecum	5.76	5.79	0.07	5.90	5.69	5.73	0.09	5.96±0.08[A]	5.58±0.07[B]

[a] Values reported as means±SE.

[A,B] Means, within each main effect, with different superscripts are significantly different at $p<0.01$.

ileum compared to the sole free fumaric acid addition ($p=0.07$). The effect of the BC and organic acid on bacterial populations contrasts with the results of Gabert et al. (1995).

The reduction of *E. coli* counts in the small intestine with the addition of acidifiers agrees with the results of Roth and Kirchgessner (1997) and Sutton and Patterson (1996), but contrasts with the findings from Gabert et al. (1995) and Risley et al. (1992), who also did not find any effect on *Lactobacillus* count. In the present study the positive response of acidifiers on microbial counts in the ileum was in part determined by the presence of the protected acids that on the whole performed slightly better than the free fumaric acid.

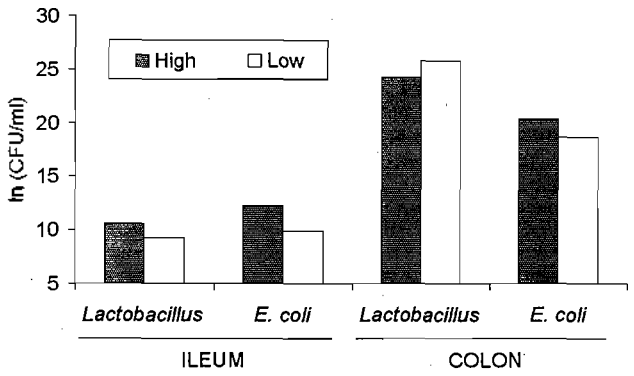

Figure 2. Effect of acid buffering capacity of diet on the *Lactobacillus* and *E. coli* content in ileum and colon of early weaned pigs

CONCLUSION

The variation range of the buffering capacity of diet was not sufficient to show any improvement of the piglet performance, notwithstanding a moderate variation of the contents of *Lactobacillus* and *E. coli* in some tracts of the gut.

Performance, carcass composition and pH in the gut were not affected by the addition of acidifiers. But this study suggests that the addition of organic acid in diet for early weaned pigs can improve protein digestibility, increase the *Lactobacillus* counts and reduce the *E. coli* counts.

Though performance was not positively affected, the use of protected acidifiers in general was the determinant of these effects.

The research could not clarify the reason for the contrast between the growth promoting effect and the response of physiological parameters to the dietary addition of the acidifiers.

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