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# Ileal digestibility of amino acids in pigs fed diets of different buffering capacity and with supplementary organic acids

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ABSTRACT

A 2 x 4 factorial experiment was carried out with eight SICV-cannulated pigs of 30 kg initial BW. Dietary buffering capacity (632 vs 578 meq/kg in Diets H and L, respectively) in combination with supplementary organic acids (formic acid, fumaric acid or n-butyric acid in acid-equivalent doses of 300 mmol/kg) were evaluated for their effects on the apparent ileal digestibility (AID) of protein and amino acids. In the presence of these organic acids in Diet H ("practical"), the AID of crude protein and several amino acids increased by 2.9 to 5.9%-units ($P<0.05$). This increase was linear and positively correlated with increasing dietary acidity. Feeding Diet L without organic acids resulted in an increase of urinary acidity by 1.6 pH-units, and thereby, ammonia emission from manure was 44% lower.

KEY WORDS: amino acids, ileal digestibility, pigs

## INTRODUCTION

Numerous authors have reported positive effects of dietary acidification with supplementary organic acids (formic, propionic, citric, fumaric) on the performance of weaned or growing pigs (Kirchgessner and Roth, 1978, 1980; Easter, 1988; Ravidran and Kornegay, 1993). The acidity of diets can be also elevated by reducing buffering capacity (BC) with Ca-benzoate $(C_6H_5COO)_2Ca \cdot 3H_2O$. This is an acidogenic, antifungal salt of benzoic acid (Ka x 105 = 6.3) used in preserving foods. In contrast to organic acids, this salt is reported to lower urinary pH values via biotransformation of its anionic part into hippuric acid (Bridges et al., 1970). A lowered urinary pH is preferable, from the environmental point of view, since an indoor volatilization of $NH_3$ and its irritative effect on pigs and stockmen can be diminished (Mroz et al., 1996). So far, little is known about the influence of Ca-benzoate in combination with organic acids on the ileal digestibility (AID) of amino acids. Therefore, the objective of this study was to evaluate the effects of BC (High vs Low) and organic acid supplementation (formic, fumaric and n-butyric acid) on the AID of N and amino acids.

## MATERIAL AND METHODS

### *Animals and experimental design*

Eight barrows (Y x [FL x DL]) of approximately 30 kg initial BW were fitted with steered ileo-caecal valve (SICV) cannulae under inhalation anaesthesia as described by Mroz et al. (1996a). Pigs were kept in specially designed metabolic pens (1.15 m x 1.35 m) throughout the whole experimental phase (75 d), at an average ambient temperature of 18°C. Quantitative collections of faeces and urine were carried out from unrestrained (freely moving) pigs. The following experimental factors were implemented: (a) BC (Low vs High), and (b) supplementation of organic acids (none [-], formic acid [For], fumaric acid [Fum] or n-butyric acid [But]), arranged in a 2 x 4 factorial trial.

### *Feeding, measurements and statistical analysis*

Basal diets H (BC=632 meq/kg) and L (BC=578 meq/kg) consisted of tapioca (27.7%), wheat middlings (15.0%), maize (11.0%), extracted soyabean meal (19.8%) and maize gluten feed (10.0%) as major ingredients. The lower buffering capacity in Diet L was obtained by adding Ca-benzoate (24 g/kg) into Diet H at the expense of $CaCO_3$ and maize starch. Diets H and L were fed either alone or with acid-equivalent doses of formic acid (300 mmol/kg), fumaric acid (150 mmol/kg) or n-butyric acid (300 mmol/kg). These organic acids were added to the diets at the expense of maize starch. The pigs were fed twice daily at 2.8 x their maintenance requirement, i.e. at 418 kJ ME/$BW^{0.75}$. Chromic oxide (0.25 g/kg) was used as a marker. The feed was given to the pigs in a dry form and water was available *ad libitum*.

Each experimental period lasted 14 d. After 5 d of adaptation, faeces and urine were collected quantitatively for 5 d, and thereafter ileal digesta were collected twice for 12 h, with a 3-day interval in-between.

The data were subjected to analysis of variance using the ANOVA procedure of Genstat 5, according to the following model: $Yijkl = \mu + Ai + Pj + Bk + Ol + (B \times O)kl + \varepsilon ijkl$, where $\mu$ = the overall mean, A = the effect of the animal (i = 1...8), P = the effect of the period (j = 1...5), B = the effect of the dietary buffering capacity (k = 1,2), O = the effect of the organic acid supplementation (l = 1...4), and ( = error contribution with average 0 and variance). The differences between the treatments were tested with the Student t-test at $P<0.05$ and 0.01.

## RESULTS AND DISCUSSION

The SICV-cannulated pigs had no health problems and grew well (827 g/d). In our study, the organic acids in Diet H ("practical") improved significantly the AID of N and several essential and nonessential amino acids by 2.9 to 5.9%-units (Table 1). This higher AID can most probably be attributed to a stimulatory role of organic acids on endocrine and exocrine pancreatic secretion in pigs, as indicated by Harada et al. (1986) and Sano et al. (1995). These authors found that the pancreatic exocrine responses induced by monocarboxylic acid solutions (250 mM) of pH 2.0 were in the following order: formic acid>lactic acid>pyruvic acid>>acetic acid>butyric acid>propionic acid. Similarly, an intravenous injection of sodium acetate, propionate or butyrate (625 M/kg BW) stimulated pancreatic juice secretion and output of protein and amylase (Kato et al., 1989) in 2-week-old calves, which had been given only whole milk and milk replacement and which were considered as nonruminant animals. The responses increased with increasing chain length of the fatty acids, and butyrate was the most effective stimulant.

Acidification of duodenal contents appears to affect pancreatic exocrine secretion via release of secretin, as elevated serum secretin content has been reported in pigs after intestinal acidification with hydrochloric acid or monocarboxylic acids (Moazam et al., 1982). Biliary secretion is also stimulated via released secretin, regulating glucagon and/or insulin levels (Harada et al., 1986). Gálfi and Bokori (1990) reported elevated plasma insulin levels with Na-butyrate supplemented diets. However, Sano et al. (1995) failed to observe any effect of acetate, propionate or butyrate injections on plasma insulin concentrations. Improved apparent digestibility of protein may also be due to an increased absorptive capacity of the intestine. Short-chain fatty acids (SCFAs), such as acetic, propionic and n-butyric acid, produced by microbial fermentation of dietary fibre in the hindgut appear to increase proliferation of epithelial cells in the gastrointestinal mucosa (Sakata et al., 1995). The strength of the effect was found to be in the order of n-butyric>propionic>acetic acid. Increased epithelial cell proliferation has also been observed when SCFAs have been given orally or provided by intravenous or gastrointestinal infusions. Gálfi and Bokori (1990) observed that 0.17% of Na-butyrate increased the length of ileal microvilli and the depth of caecal crypts in growing pigs. Using short-term culture, Sakata et al. (1995) reported that n-butyric acid increased cryptal cell production rate of pig distal colon. The SCFAs may also have local trophic effects on ileal or colonic mucosa, stimulating gut epithel-

TABLE 1

Apparent ileal digestibility (AID) of DM, N and amino acids in pigs as influenced by dietary buffering capacity (BC) and supplementation of organic acids at an equivalent acid dose of 300 mmol/kg diet

| Treat. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BC | H | H | H | H | L | L | L | L | | | basal | acid x |
| Org.ac. | [-] | For | Fum | But | [-] | For | Fum | But | s.e. | acid | diet | basal |
| DM | 65.6$^{a}$ | 67.3$^{a}$ | 67.2$^{a}$ | 66.6$^{a}$ | 66.8$^{a}$ | 66.9$^{a}$ | 71.3$^{b}$ | 68.3$^{ab}$ | 1.66 | ns | ns | ns |
| N | 68.7$^{a}$ | 73.4$^{b}$ | 72.1$^{ab}$ | 74.0$^{b}$ | 72.5$^{ab}$ | 73.4$^{b}$ | 75.6$^{b}$ | 74.3$^{b}$ | 2.02 | ns | ns | ns |
| Arg | 83.2$^{a}$ | 85.5$^{abc}$ | 84.4$^{ab}$ | 85.5$^{abc}$ | 85.1$^{abc}$ | 85.4$^{abc}$ | 87.2$^{c}$ | 86.6$^{bc}$ | 1.11 | ns | * | ns |
| His | 76.2$^{a}$ | 81.0$^{bc}$ | 80.4$^{bc}$ | 81.1$^{bc}$ | 79.6$^{ab}$ | 79.9$^{bc}$ | 83.3$^{c}$ | 81.2$^{bc}$ | 1.62 | * | ns | ns |
| Ileu | 75.3$^{a}$ | 79.4$^{b}$ | 78.5$^{b}$ | 78.9$^{b}$ | 78.4$^{ab}$ | 79.8$^{b}$ | 81.6$^{b}$ | 80.7$^{b}$ | 1.62 | * | * | ns |
| Leu | 77.5$^{a}$ | 80.9$^{bc}$ | 80.4$^{bc}$ | 81.0$^{bc}$ | 79.7$^{ab}$ | 81.2$^{bc}$ | 83.4$^{c}$ | 82.2$^{bc}$ | 1.46 | * | * | ns |
| Lys | 77.4$^{a}$ | 82.3$^{b}$ | 81.3$^{b}$ | 81.2$^{b}$ | 80.3$^{ab}$ | 81.5$^{b}$ | 83.6$^{b}$ | 81.9$^{b}$ | 1.59 | * | ns | ns |
| Meth | 83.6$^{a}$ | 86.0$^{ab}$ | 84.4$^{ab}$ | 85.6$^{ab}$ | 83.8$^{ab}$ | 85.1$^{ab}$ | 86.5$^{b}$ | 85.4$^{ab}$ | 1.28 | ns | ns | ns |
| Phen | 76.8$^{a}$ | 80.5$^{bc}$ | 79.7$^{abc}$ | 80.6$^{bc}$ | 79.3$^{ab}$ | 80.7$^{bc}$ | 82.9$^{c}$ | 81.4$^{bc}$ | 1.50 | * | ns | ns |
| Thr | 70.2$^{a}$ | 75.3$^{bc}$ | 74.5$^{bc}$ | 75.1$^{bc}$ | 73.4$^{ab}$ | 74.5$^{bc}$ | 78.3$^{c}$ | 75.4$^{bc}$ | 1.28 | ns | ns | ns |
| Val | 71.1$^{a}$ | 75.9$^{bc}$ | 75.3$^{bc}$ | 76.4$^{bc}$ | 74.4$^{ab}$ | 75.8$^{bc}$ | 78.7$^{c}$ | 76.5$^{bc}$ | 1.84 | * | ns | ns |
| Ala | 68.1$^{a}$ | 73.8$^{b}$ | 73.1$^{b}$ | 74.4$^{b}$ | 72.8$^{b}$ | 74.2$^{b}$ | 76.8$^{b}$ | 75.2$^{b}$ | 2.01 | * | * | ns |
| Asp | 71.0$^{a}$ | 75.9$^{bc}$ | 75.3$^{c}$ | 75.8$^{bc}$ | 74.6$^{ab}$ | 76.0$^{bc}$ | 79.1$^{c}$ | 76.8$^{bc}$ | 1.85 | * | ns | ns |
| Glu | 77.3$^{a}$ | 82.0$^{bc}$ | 82.2$^{bc}$ | 82.5$^{bc}$ | 80.5$^{ab}$ | 81.6$^{bc}$ | 84.6$^{c}$ | 82.4$^{bc}$ | 1.81 | * | ns | ns |

a, b, c – means within a row lacking a common superscript letter differ significantly ($P<0.05$)
dietary buffering capacity: High = 632 meq/kg diet; Low = 578 meq/kg

ial cell proliferation via the (afferent) autonomic nervous system, as caecal infusion of SCFAs increased ($P<0.05$) jejunal DNA, villous height, surface area, crypt depth, and gastrin in innervated rats (Sakata et al., 1995). The increased epithelial cell proliferation results in longer microvilli, and, therefore, it may increase absorptive surface and capacity in the small and large intestine.

Positive effects of organic acids on the AID of amino acids of a similar range as in our "practical" diet (high buffering capacity) were also reported by Mosenthin et al. (1992) and Blank et al. (1998). They reported that 2% of propionic or fumaric acid improved ($P<0.05$) the AID of Lys, Arg, His, Leu, Phen and Val. Also, Kemme et al. (1995) observed that lactic acid supplementation (30 g/kg) improved ($P<0.05$) the AID of Arg, Ileu, Lys, Meth, Phen, Tre and Val in diets for fattening pigs. Giesting and Easter (1991) reported a slight, but nonsignificant increase in AID of N when 2% of fumaric acid was added to a maize-soyabean meal diet. Bolduan et al. (1988) and Gabert et al. (1995) reported no effect of formic acid on the AID of crude protein and/or amino acids. In contrast, Gabert and Sauer (1995) reported that the AID of CP, Arg, Gly and Tyr decreased linearly ($P<0.05$) with increasing level of fumaric acid in the diet. These controversial results may be due to differences in the composition of the diets.

In our study, Ca-benzoate reduced the urinary pH on average by 1.6 pH-units, i.e., from 7.25 to 5.66. This is in agreement with the previous results of Mroz et al. (1996 b). As a result, ammonia emission was reduced approximately by 44 %. The lowered urinary pH is a result of increased urinary excretion of hippuric acid, an end product of benzoate metabolism. In the liver, benzoates are conjugated with glycine to form hippuric acid or benzoylglucuronic acid, which are rapidly excreted in the urine (Bridges et al., 1970).

## CONCLUSIONS

Formic, fumaric and n-butyric acids (300 mmol/kg) improved the apparent ileal digestibility of crude protein and amino acids in a pig "practical diet" (buffering capacity = 632 meq/kg) by 2.9 to 5.9%-units ($P<0.05$). In a diet of low buffering capacity (578 meq/kg) containing Ca-benzoate (24 g/kg), all three organic acids also exerted a positive effect on the apparent ileal digestibility of these nutrients, but to a lower degree than with the "practical" diet. In general, this increased digestibiliy was linearly and positively correlated with increasing dietary acidity. Feeding the diet of low buffering capacity (with Ca-benzoate and without organic acids) resulted in an increase of urinary acidity by 1.6 pH-units, and thereby, ammonia emission from manure was approximately 44% lower.

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