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Efficacy of benzoic acid in the feeding of weanling pigs

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SUMMARY – Two trials were conducted to evaluate the effects of feeding benzoic acid on the performance of weanling pigs. Ninety-six and forty-eight piglets (4 wk of age) were used in trials 1 and 2 respectively. For each trial half of the piglets were fed a control diet and the other half the same diet with 0.5 % benzoic acid. In both trials benzoic acid improved body weight gain (P<0.01) and feed to gain ratio (P<0.1) during the pre-starter phase (0-14 days). Similarly, during the starter phase (14-28 days) benzoic acid improved weight gain in trials 1 and 2 (P<0.05 and P<0.1 respectively) and feed intake in trial 1 (P<0.1). Over the whole trial (0-28 days), benzoic acid improved weight gain in trials 1 and 2 (P<0.05) and also improved feed intake and feed to gain ratio (P<0.1) in trial 1. At the end of trial 1, twenty-four pigs were slaughtered and their urine was sampled. It was observed that benzoic acid significantly lowered (P<0.1) the pH in urine and that hippuric acid concentration in urine increased (P<0.01) as a result of the metabolism of benzoic acid. It is concluded that the inclusion of 0.5% benzoic acid in pre-starter and starter diets for piglets improves their performance and reduces the pH in urine by increasing its hippuric acid concentration.

***Key words**: Benzoic acid, Weaning piglets, Intestinal microbiota, Antibiotic alternatives*

INTRODUCTION

Under the current intensive production systems, at weaning piglets are subjected to a series of stressful conditions (ranging from nutritional to environmental and social nature) that make it one of the most critical phases in their life. Amongst the changes that the piglet must go through is a re-adaptation of the gastrointestinal microbiota. For many decades these changes in the microbiota were modulated with the use of antimicrobial growth promoters that kept pathogens under control and favoured the establishment of a "healthy" microbiota. However, the recent ban on the use of antimicrobial growth promoters for the risk that they may cause antibiotic resistance that may be transferred to human microorganisms, has encouraged the research for new alternatives. Amongst these, organic acids have been of interest as reviewed by Partanen and Mroz (1999) [1]. More recently, in vitro studies report that benzoic acid has the strongest antimicrobial effect amongst several organic acids tested (Knarreborg et al. 2002) [2]. The objectives of present trial were to test benzoic acid as a supplement for weaning pig diets and to study its effects on the microbial ecology of their gastrointestinal tract.

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

[2] 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.

MATERIAL AND METHODS

Animals and housing

Two trials were conducted in two different experimental farms of IRTA's Nutrition Department Centre de Mas Bové and Estació Experimental de El Prat, respectively. Ninety-six and forty-eight piglets (*Landrace*; mixed sexes) of four weeks of age with 9.1 kg (SD 1.56) and 7.9 kg (SD 1.21) initial body weight were used, respectively. Piglets were distributed by initial body weight in pens of four animals according to a randomised block design.

Diet, treatments and experimental procedures

A single dietary composition (13.5 MJ ME/kg; 1.2% Lysine; Tables 1,2) was offered to the animals for the whole experimental period. Feed was presented in pelleted form and offered *ad libitum*.

Two experimental treatments were tested; consisting of a Negative control (T-1; basal diet) and a diet with Benzoic acid (VevoVitall®, DSM Nutritional Products) added at 0.5% replacing the same amount of maize starch.

The piglets were housed in 24 and 12 pens of four animals for trials 1 and 2, respectively. The animals were randomly distributed by initial weight into blocks of two pens and within each block the two treatments were randomly assigned.

Measurements

Feed and pigs were weighed at the start, at 14 days and at the end of the experiment (day 28). Initial and final body weight, daily weight gain, feed intake and feed conversion rate were calculated.

At the end of trial 1, twenty-four piglets were killed by an intravenous overdose of sodium pentobarbital. Urine samples were obtained from the urinary bladder and their pH and hippuric acid concentration in urine were measured.

Samples of ileal and caecal digesta from these animals were also obtained to study the microbial ecology by restriction fragment length polymorphism (RFLP) according to the procedures decribed by (Badiola et al. 2001)[3]. From the RFLP profiles, the biodiversity degree and the frequency of detection of certain bacterial genus were determined, and dendrograms with the degree of similarity between RFLP profiles were constructed.

The performance parameters from the two trials were compared together using the GLM procedure of the statistical package SAS. For statistical analysis, a randomised block design was used. The average measurements of performance for each pen were used to calculate the mean values for each treatment, and they were compared taking into account the block effect

[3] Badiola, J.I., Pérez de Rozas, A.M., Francesch, M., Esteve, E., Brufau, J. (2001). Evaluación del efecto de los componentes de la ración sobre los componentes de la microbiota intestinal. XXXVIII Symposium Científico de Avicultura. Córdoba. Pag.: 109-116.

(initial weight and trial site). Urine pH and hippuric acid concentration for each treatment were analysed using the individual values by one-way ANOVA. The biodiversity degree of the digestive microbiota was analysed by ANOVA according to a factorial distribution of treatments with two treatments (control and benzoic acid) and two GIT segments (ileum and caecum). Differences in the frequencies of detection of certain bacterial genus due to GIT segment, and due to the treatments (globally or within each segment) were analysed by chi square analysis.

RESULTS AND DISCUSSION

The addition of benzoic acid to the diet resulted in an improved performance of the animals (Table 3). Particularly, it increased ($P<0.05$) average weight gain in all the experimental periods considered, feed intake was also increased ($P<0.05$) during the second half of the trial (14-28 days) and over the whole experimental period, and finally, it improved ($P<0.05$) feed to gain ratio during the first half of the trial (0-14 days) and over the whole trial.

Benzoic acid tended ($P<0.1$) to reduce the pH of the urine (Table 4), and this was due to the higher ($P<0.001$) concentration of hippuric acid in the urine of these pigs resulting from the metabolism of benzoic acid.

The biodiversity of the gastrointestinal microbiota was increased ($P<0.05$) in the ileum of the pigs fed benzoic acid (Table 5). It is generally accepted that a higher degree of biodiversity is associated with a healthier microbiota, and this would be in good agreement with the productive results described above.

A small impact of benzoic acid on the frequencies of detection of certain bacterial genera was found (Figure 1). Only a tendency ($P<0.1$) for a higher frequency of detection of Vibrio in ileal digesta and a lower ($P<0.05$) frequency of Ruminococcus in the caecum were observed.

The dendrogram with the degree of similarity between RFLP profiles (Figure 2) suggests a higher similarity between ileal and caecal microbiotas in the animals on benzoic acid than in those from the control group. Although in order to be able to draw conclusion from the relevance of this similarity further work is required, the better productive results suggest that it may be advantageous.

CONCLUSIONS

It is concluded that the addition of benzoic acid at 0.5% in the diet of weanling pigs improves their performance and acidifies their urine by increasing their hippuric acid concentration. Additionally benzoic acid increases the biodiversity of the microbiota at ileal level and results in a higher similarity between the ileal and caecal microbiotas.

Table 1 *Composition of the experimental diet (%, as fed)*

Ingredients	Basal diet
Maize	30.0
Barley	36.0
Extruded soybeans	6.2
Soybean meal (48% CP)	8.0
Potato protein concentrate	5.0
Sweet milk whey	10.0
Lard	1.2
DL-Methionine	0.07
L-Lysine-HCl	0.27
L-Threonine	0.03
Calcium carbonate	0.69
Dicalcium phosphate	1.38
Salt	0.19
Vit-Min complex (*)	0.40
Maize starch or Benzoic acid	0.50

(*) Providing per kg of diet: vitamin A: 10000 IU; vitamin D3: 2000 IU; vitamin E: 15 mg; thiamin: 1,3 mg; riboflavin: 3,5 mg; vitamin B12: 0.025 mg; vitamin B6: 1,5 mg; calcium pantothenate: 10 mg; nicotinic acid: 15 mg; biotin: 0.1 mg; folic acid: 0.6 mg; vitamin K3: 2 mg; Fe: 80 mg as iron sulfate; Cu: 6 mg as copper sulfate; Co: 0.75 mg as cobalt sulfate; Zn: 185 mg as zinc oxide; Mn: 60 mg as manganese sulfate; I: 0.75 mg as potassium iodate; Se: 0.10 mg as sodium selenite; ethoxiquin: 0.15 mg.

Table 2 *Estimated nutrient composition of the experimental diet (%)*

Nutrients	Basal diet
Moisture	9.69
Crude Protein	17.68
Crude Fibre	3.18
Fat	4.45
Ash	5.23
Energy (MJ ME/kg)	13.5
Calcium	0.75
Total phosphorous	0.61
Inorganic phosphorous	0.36
Lysine	1.20
Tryptophan	0.22
Threonine	0.78
Methionine	0.38
Methionine+Cystine	0.72

Table 3 *Effect of benzoic acid supplementation on the productive parameters of weanling pigs.*

	Initial BW (kg)	Final BW (kg)	Weight gain (g/day)	Feed Intake (g/day)	Feed to gain ratio
0-14 days postweaning					
Control	**8.9**	**11.9 a**	**209 a**	**324**	**1.62 a**
Benzoic acid (0.5 %)	**8.9**	**12.6 b**	**261 b**	**356**	**1.40 b**
Pooled Standard Error	*0.03*	*0.21*	*14.2*	*14.1*	*0.065*
14-28 days postweaning					
Control	**11.9 a**	**18.9 a**	**506 a**	**764 a**	**1.52**
Benzoic acid (0.5 %)	**12.6 b**	**20.6 b**	**574 b**	**838 b**	**1.47**
Pooled Standard Error	*0.21*	*0.35*	*13.2*	*20.2*	*0.031*
0-28 days postweaning					
Control	**8.92**	**18.9 a**	**358 a**	**544 a**	**1.54 a**
Benzoic acid (0.5 %)	**8.91**	**20.6 b**	**417 b**	**597 b**	**1.44 b**
Pooled Standard Error	*0.033*	*0.35*	*12.4*	*15.6*	*0.035*

Table 4 *Effect of benzoic acid supplementation on pH and hippuric acid concentration of urine of weanling pigs at day 28 postweaning.*

	Urine pH	Hippuric acid (mg/100ml)
Control	**5.9**	**455 a**
Benzoic acid (0.5 %)	**5.4**	**741 b**
Pooled Standard Error	*0.18*	*67.3*

Table 5 *Effect of benzoic acid supplementation on the biodiversity degree of ileal and caecal digesta of weanling pigs at day 28 postweaning.*

		Biodiversity degree
Control	**Ileum**	**637 a**
	Caecum	**757 ab**
Benzoic acid (0.5 %)	**Ileum**	**1277 b**
	Caecum	**631 a**
Pooled Standard Error		*184.0*

Figure 1 *Benzoic acid supplementation and frequency of detection by RFLP profiles of certain bacterial genera in ileal and caecal digesta of piglets at day 28 postweaning (%)*

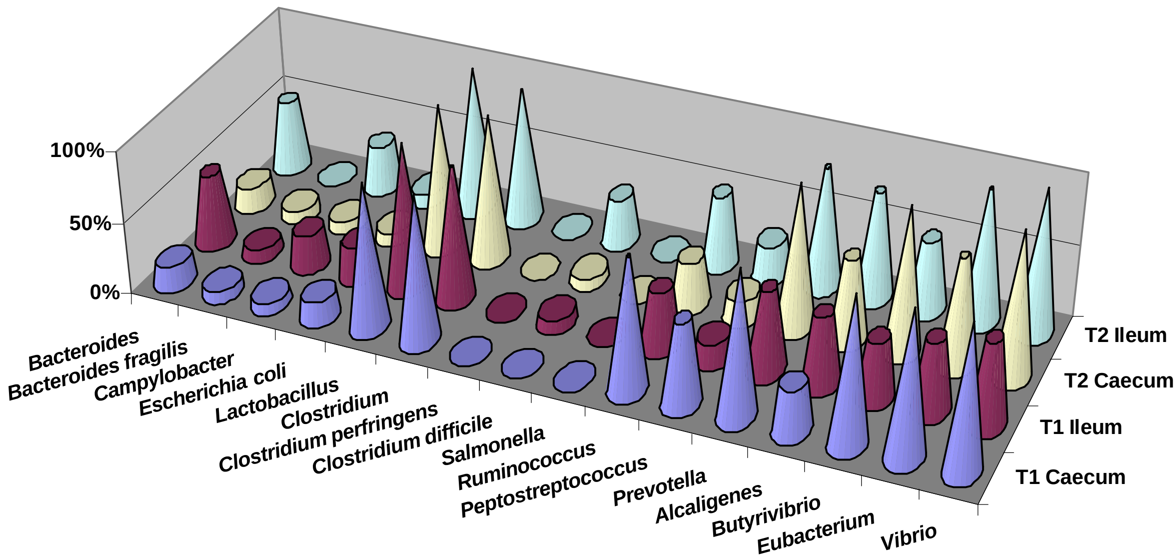

Figure 2 *Dendrogram of the degree of similarity between RFLP profiles of ileal and caecal microbiota of pigs at day 28 postweaning fed diets with or without Benzoic acid supplementation.*