

## THE EFFECTS OF BENZOIC ACID AND PROTEIN LEVEL ON URINE PH AND AMMONIA EMISSION OF PIGS

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### ABSTRACT

Twelve hybrids gilts (initial BW  $29.9 \pm 1.7$  kg) were used for evaluation to identify the effect of benzoic acid and dietary protein in the diets on urine pH and ammonia of the slurry by growing piglets. We used two protein concentrations - high protein 18.8 % (HP) and low protein 14.0 % (LP) and two benzoic acid levels 0.0 % and 1.0 %. The same energy level (13.3 MJ. kg) in the diets was maintained by supplementation with rapeseed oil. The piglets were housed in metabolic cages and fed with two equal doses at 7 a.m. and 5 p.m. at a daily rate of 90 g. kg<sup>0.75</sup>. Water was offered *ad libitum*. Each experimental period consisted of a 6-d adapted and was followed by a 4-d collection phase. During the collection phase feces and urine (using bladder catheters) were collected. Experimental data were subjected to ANOVA and when significant value was observed for treatment effect, the differences between means were assessed using Fisher's LSD procedure. Nitrogen and dry matter intake was significantly affected in diets. The numbers of N balance was a significant increase of N uptake only in pigs fed the diet with benzoic acid and HP. We found a significant reduction of urine pH, specifically pH (0.7 to 0.9) by both experimental groups fed with benzoic acid diets, regardless of the nitrogen content in the diet. The coefficients of excretion determination between hippuric acid and urine pH were  $R^2 = 0.57$ , the same for HP and LP diets. The higher decrease of ammonia nitrogen was observed in experimental LP groups, but it was not statistically significant.

**Key words:** ammonia excretion; benzoic acid; urine pH; piglets

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### INTRODUCTION

The excretion of minerals from pig farms on the environment is of general interest in most countries. Likewise, emissions of ammonia and odder emissions from pig production sites and manure handling are in the spotlight. The main source of ammonia emissions is a mixture of urea and excreted in the urine. Urea is converted to ammonia and carbon dioxide by urease present in faeces. The most important factors that affect the process are the urea concentration in urine and the pH and temperature of the slurry (Sommer and Husted, 1995). Other factors affecting the production of NH<sub>3</sub> is a kind of floor covering system for manure removal, climatic conditions inside the building,

diet composition and feed efficiency of animals. One of the possibilities to reduce ammonia emissions is through dietary manipulation (Gatel and Grosjean, 1992). Organic acids such as formic acid, lactic acid are added in the diet of pigs (Jongbloed *et al.*, 2000), are readily metabolized in the liver, and do not cause significant urine acidification. Benzoic acid (BA) may be one of the promising candidates to replace organic acids, nutritional use of antibiotics and affect growth performance and also reduce ammonia emissions from excrements.

The objective of the experiment described herein was to study pH changes in urine and ammonia emissions of pigs which were fed with different nitrogen level diets supplemented with benzoic acid.

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## MATERIAL AND METHODS

Eight crossbred gilts, progeny of Large White sows and Landrace boars (initial BW  $29.9 \pm 1.7$  kg) were allotted to a 2 x (4 x 4) Latin square design (n = 8). Pigs were individually housed in galvanized steel metabolism cages in an environmentally controlled room ( $20 \pm 1.3^\circ\text{C}$ ). The dietary treatments included: diet LP with lower CP content (14 %), supplemented with isoleucine, lysine, methionine, threonine, tryptophan and valine; diet LPBA was similar to diet LP with  $10 \text{ g.kg}^{-1}$  benzoic acid; diet HP contained 18.8 % CP and diet HPBP was similar to diet HP with  $10 \text{ g.kg}^{-1}$  benzoic acid. The diets were based on wheat, maize and soybean meal, diets 3 and 4 were supplemented only with lysine and threonine. Diets were formulated to an equal ME content ( $13.3 \text{ MJ.kg}$ ) by means of supplementation with rapeseed oil (Table 1). The pigs were fed in two equal feed doses of 07:00 and 17:00 at a daily rate of  $90 \text{ g.kg}^{0.75}$ .

After a 5-days preliminary period, during which the pigs were adapted to cages and the new environment, four consecutive 10-days experimental periods followed. Each experimental period consisted of a 6-days adaptation period, within which the animals were adapted to the experimental diet and followed by a 2 x 48 hour collection period (7 to 8 day and 9 to 10 day). During the collection period, samples of urine and faeces were separately collected. Urine was collected via catheters, without addition of sulphuric acid and stored in ice-cooled containers. Urine pH was measured before freezing each day. 10 % aliquot was stored at  $-20^\circ\text{C}$ . Faeces were collected, pooled, and stored at  $-20^\circ\text{C}$  until analysis.

Briefly, 2 kg of fresh slurries prepared maintaining the respective proportion of urine and faeces in the excreta, and placed in a 10 L bowl, 280 mm high and 230 mm in diameter, covered by a lid connected to a tube system. Air entered the bowl

**Table 1: Composition of diets and analysed content of nutrients**

Ingredients ( $\text{g.kg}^{-1}$ diet)	Diet <sup>1</sup>			
	LP	LPBA	HP	HPBA
Maize	571.80	552.00	445.60	425.50
Wheat	300.00	300.00	300.00	300.00
Soybean meal	83.00	86.90	218.8	222.7
Beet pulp	-	-	-	-
Rapeseed oil	2.00	8.30	3.30	9.60
L-isoleucine	0.87	0.90	-	-
L-lysine.HCl	5.85	5.80	1.74	1.66
DL-methionine	0.85	0.90	-	-
L-threonine	2.20	2.20	0.33	0.32
L-tryptophan	0.44	0.40	-	-
L-valine	0.86	0.90	-	-
Monocalcium phosphate	14.10	14.10	12.70	12.70
Limestone	11.00	11.00	10.70	10.70
Salt	3.90	3.90	3.80	3.80
Vit.-min. premix <sup>2</sup>	3.00	3.00	3.00	3.00
Benzoic acid	-	10.00	-	10.00
Analysed nutrient contents	<i>(g.kg<sup>-1</sup> air-dried)</i>			
Dry matter	884.4	883.5	889.6	886.3
Crude protein	137.1	139.2	182.6	183.8
Crude fibre	30.6	30.4	31.5	34.6

<sup>1</sup>LP- control low protein diet + 0 % benzoic acid; LPBA- low protein + 1 % benzoic acid; HP- control high protein diet + 0 % benzoic acid; HPBA - high protein + 1 % benzoic acid.

<sup>2</sup>Supplied per kg of diet: vit. A 9 000 IU. vit. D3 1 500 IU.  $\alpha$ -tocopherol 35.0 mg. vit. B1 1.7 mg. vit. B2 6.0 mg. vit. B6 2.5 mg. Ca-pantothenate 15.0 mg. niacin 38.0 mg. vit. K3 2.0 mg. biotin 0.12 mg. cyanocobalamin 0.03 mg. choline 156 mg. Fe 103.0 mg. Zn 116.5 mg. Mn 49.0 mg. Cu 40.0 mg. I 1.2 mg. Co 0.4 mg. Se 0.3 mg.

through a small hole at the edge of the lid and left the bowl from the centre. Ammonia was removed from the air by passing through 2 flasks, each containing 100 mL 1N H<sub>2</sub>SO<sub>4</sub>. The air left the system after passing through a water trap, a flow controller at the rate of 2.3 L.min<sup>-1</sup>, and a vacuum pump. The first flask was replaced daily whereas the second was replaced after 7 days. The concentration of ammonia in the liquid was determined using titration with 0.2 N NaOH. Nitrogen emission was determined daily for 7 consecutive days.

Analyses of diet, urine and faeces samples for dry matter, total N and fibre were performed in accordance with standard methods of AOAC (1998). Feeds and faeces sample were analysed for dry matter (DM) after drying at 105 °C for 8 hours. Crude protein (N x 6.25) was determined by Kjeldahl method using a Kjell-Foss 16200 auto analyser (method 978.02). Chemical analyses were conducted in duplicate.

The data were subjected to one-way ANOVA using Statgraphic Plus package (version 3.1., Statistical Graphics Corp., Rockville, MD, USA). In statistical significance testing, the differences between means were assessed using Fisher's LSD procedure.

## RESULTS AND DISCUSSION

The analysed amount of crude protein in the diet was slightly lower than would be expected from the proposed formulation components in Table 1. Treatments with 1 % benzoic acid in the diet did not significantly influence nitrogen intake or dry matter intake (1.22 vs. 1.23, and 1.16 vs. 1.12 kg.d<sup>-1</sup>; *P* = 0.34).

Reduction in dry matter intake of 4 % and 9 %

was found in a diet with higher nitrogen vs. diets with low nitrogen content. Nitrogen balance (Table 2) showed adoption amount of nitrogen intake, which correspond to the dry matter intake and is higher by 17 to 27 %, despite the fact that the nitrogen content on the HP diet was higher by 32-33 %.

The different nitrogen content of diet caused variability in the content of nitrogen in the faces (- 3 to + 3 %), but the differences were not statistically significant. The diets with the same nitrogen content, with different contents of benzoic acid was found to increase nitrogen in the faces of + 1 to + 6 %, but without being significant statistically. However, the nitrogen content of the urine was about 73-86 % higher than for lower nitrogen diet (33 % different of the nitrogen in the diet HP). In all groups there was a switch from urinary to faecal excretion, the largest but not significant in the group LPBA (*P* = 0.19), although not statistically significant. Faecal N excretion increased by + 7.35 % (LP vs. LPBA). The reduction for urinary nitrogen by - 5.5 % was in the HPBA group (*P* = 0.28). Retention of nitrogen was affected in both experimental groups. The retention calculated as a percentage of nitrogen intake was to improve the impact of benzoic acid 3.2 % (*P* = 0.06), and retention calculated as a percentage of the absorbed nitrogen was up to 4.6 % (*P* = 0.04). The numerically higher N retention is consistent with the trend for a better growth performance observed by Bühler *et al.* (2006) using diets supplemented with 1 % benzoic acid in the grower and the finisher phases.

The value of benzoic-, hippuric acids, pH of urea and the ammonia release from the slurries are reported in Table 3. Intake of benzoic acid was methodically conducted and was not statistically evaluated.

**Table 2: Effects of dietary protein and benzoic acid level on nitrogen (N) balance**

Items	Diet <sup>1</sup>				pooled SEM	P-value <sup>a</sup>	
	LP	LPBA	HP	HPBA		1	2
DM intake, [kg.d <sup>-1</sup> ]	1.22	1.23	1.16	1.12	0.22	0.34	0.34
N intake [g.d <sup>-1</sup> ]	30.16	31.07	38.21	36.58	6.52	0.27	0.33
Faecal N [g.d <sup>-1</sup> ]	4.31	4.63	4.46	4.52	1.10	0.19	0.47
Urinary N [g.d <sup>-1</sup> ]	7.92	8.02	14.76	13.94	2.19	0.40	0.28
Absorbed N of intake [%]	85.21	84.74	88.17	87.27	3.76	0.36	0.26
Retained N of intake [%]	57.81	59.01	49.43	52.66	6.52	0.36	0.06
Retained N of absorbed [%]	67.76	69.41	55.98	60.56	6.79	0.29	0.04

<sup>1</sup>LP- control low protein diet + 0 % benzoic acid; LPBA- low protein + 1 % benzoic acid; HP- control high protein diet + 0 % benzoic acid; HPBA - high protein + 1 % benzoic acid.

<sup>a</sup>Contrast: 1 = LP vs. LPBA; 2 = HP vs. HPBA.

**Table 3: Effects of crude protein and benzoic acid intake on urine pH and emission from slurry**

Items	Diet <sup>1</sup>				pooled SEM	P-value <sup>a</sup>			
	LP	LPBA	HP	HPBA		1	2	3	4
Benzoic acid intake [g.d <sup>-1</sup> ]	0.00	13.96	0.00	13.23	1.96	0.00	0.00	-	0.25
Hippuric acid excretion [g.d <sup>-1</sup> ]	0.83	16.34	0.90	17.54	3.75	0.00	0.00	0.28	0.31
Initial urine pH	5.73	5.06	6.82	5.94	0.35	0.00	0.00	0.00	0.00
NH <sub>3</sub> -N emission [g.d <sup>-1</sup> ]*	0.94	0.82	1.21	1.15	0.22	0.12	0.36	0.06	0.01

<sup>1</sup>LP- control low protein diet + 0 % benzoic acid; LPBA- low protein + 1 % benzoic acid; HP- control high protein diet + 0 % benzoic acid; HPBA - high protein + 1 % benzoic acid.

<sup>a</sup>Contrast: 1 = LP vs. LPBA; 2 = HP vs. HPBA; 3 = LP vs. HP; 4 = LPBA vs. HPBA

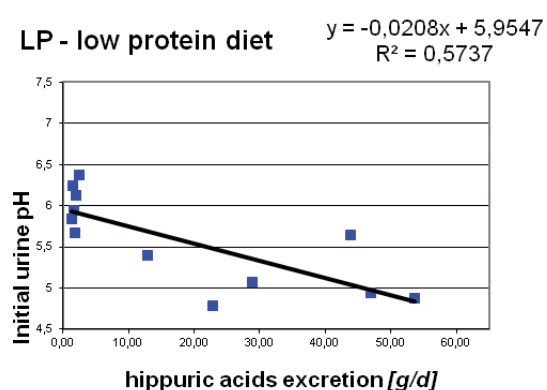
\*average per day of total emission from 2 kg fresh slurry during 7 days' determination

Hippuric acid is a direct metabolite of benzoic acid. The addition of benzoic acid to the diet determined a numerical decrease of the urinary pH due to the conversion of benzoic acid into hippuric acid in the liver. Hippuric acid is then excreted with urine (Bridges *et al.*, 1970). The concentration of hippuric acid in urine of pigs treated with benzoic acid was significantly higher ( $P < 0.001$ ) as compared to the control animals.

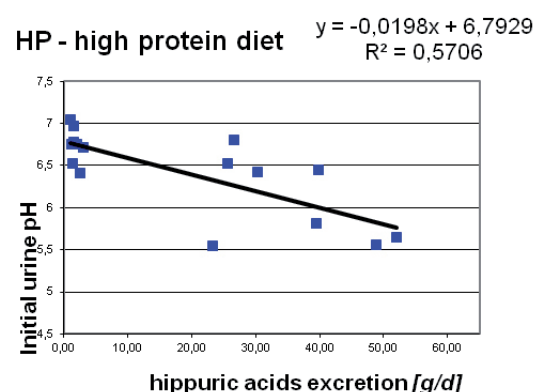
Regression analysis showed that content of hippuric acid ( $y$ ) was a linear function of urinary pH ( $x$ ) (Figure 1 and 2). Their negative relationship was almost identical as described with an equation for both levels of nitrogen. The constant in the equation is calculated as pH value for zero contents of hippuric acid by 5.95 and 6.79 for LP, HP respectively. The slope of the regression equation showed that each g.day<sup>-1</sup> hippuric acid in urine reduces the pH of 0.018 to 0.02 point.

Significantly reduced pH in urine was detected in both groups ( $P < 0.01$ ): in the group with lower nitrogen content by 0.67 (LP vs. LPBA 12 %) and in the group with higher nitrogen content by 0.88 (HP vs. HPBA 13 %). The difference between the groups with the addition of benzoic acid and different nitrogen content was as point of pH 0.88, which means up to 17 % (LPBA vs. HPBA). The decrease in urine pH (0.67 - 0.88 points of pH) is much lower than that (two points of pH decrease) obtained by Kristensen *et al.* (2009) in pigs of  $63 \pm 1$  kg body weight. But Guingand *et al.* (2005) noted a decrease of 0.9 points as a result of adding 1 % benzoic acid to the feed.

The trend for a NH<sub>3</sub>-N emission was from the diets LP diet 0.94 g.d<sup>-1</sup> to the diet with containing benzoic acid LPBA was 0.82 g.d<sup>-1</sup> (- 13 %), however because of the high residual variation of the values no significant differences were observed among



**Fig. 1: Relationship between hippuric acid and urine pH of pig with low protein diets**



**Fig. 2: Relationship between hippuric acid and urine pH of pig with high protein diets**

treatments ( $P < 0.12$ ). Slightly insignificant decrease of  $0.7 \text{ g.d}^{-1}$  (- 5 %) ammonia emissions from the manure intended for diets with a higher nitrogen content ( $P = 0.36$ ) was noted. Emission reduction was more pronounced with reduction of the nitrogen content in the diet. The diet free benzoic acid was  $0.27 \text{ g.d}^{-1}$  (27 %;  $P = 0.06$ ) which corresponds approximately to the amount of reduced nitrogen in the feed. However, the addition of benzoic acid to the diet resulted in loss of emission of  $0.33 \text{ g.d}^{-1}$  (40 %;  $P < 0.01$ ). Hansen *et al.* (2007) measured concentrations of  $\text{NH}_3$  in the exhaust air found in the reduction of dietary crude protein (from 14 % to 16 %), and with the addition of 3 % benzoic ammonia emission reduced by 30 % and 57 %, respectively. In our experiment, we observed a greater effect of the nitrogen content as benzoic acid content. Philippe *et al.* (2011) concluded that diets with reduced crude protein content are highly effective in reducing the emissions with almost a 10 % reduction for every  $10 \text{ g.kg}^{-1}$  reduction in dietary crude protein. That argument is confirmed by our experiment, the nitrogen content where reduced by  $10 \text{ g.kg}^{-1}$  and reduction of emissions decreased by 40 %.

## CONCLUSION

In conclusion, it may be stated that a strong interaction between the total quantity of nitrogen in the diet and its emissions provides the benefit to the environment. It's a close match between dietary intake and requirement of pigs under physiological and growth stages when there is limited manipulation. A more favourable impact in the experiment was that the addition of benzoic acid in the diet of the pigs resulted in a numerical decrease of the urinary pH and is beneficial to the environment without effects on N balances.

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