REGULAR ARTICLE

DETERMINATION OF URINE PH AND AMMONIA EMISSION AFTER ADDITION OF BENZOIC ACID AND DRIED BEET PULP IN GROWING PIGS

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ABSTRACT

Twelve crossbred gilts (initial BW 29.9 ± 1.7 kg) were used for evalution of the effect of benzoic acid and the fiber in diets on the urinary pH, ammonia of the slurry and redistribution of nitrogen between faeces and urinary. For the pigs there were randomly allotted three dietary treatments according to a replicated 3 x 3 Latin square design. The dietary treatments included: Diet control (C) was supplemented with isoleucine, lysine, methionine, threonine, tryptophan, and valine to fulfill the requirements of ideal amino acid profile; diet (BA) was similar to diet (C) with 10g.kg^{-1} benzoic acid; diet (BABP) was similar to diet (C) with 10g.kg^{-1} benzoic acid and 150 g.kg^{-1} dried beet pulp, all of them with equal ME content (13.3 MJ/kg) and supplemented with rapeseed oil. The pigs 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$^{0.75}$. Water was offered *ad libitum*. Each experimental period consisted of a 6-d adaptation phase and was followed by a 4-d collection phase. During collection phase the feces and the urine (using bladder catheters) were collected. The experimental data were subjected to ANOVA and when significant value for treatment effect (P<0.05) was observed, the differences between means were assessed with using Fisher's LSD procedure. Nitrogen and dry matter intake was not significantly affected by any of the feed additives. Nitrogen and dry matter intake was not significantly affected by any of supplemented additives. Fecal N excretion was increased (P <0.02) in pigs fed with added fiber (BABP), in the same group there was detected the reduction of urinary N excretion (P< 0.04). There was significant decrease of urine pH, concretely by three quarters of pH point in the both experimental groups fed with bonzoic acid diets, regardless of the fiber content in the diet. The coefficients of excretion determination between hippuric acid and urine pH were R^2 = 0.298. The same decrease of ammonia nitrogen was observed in both experimental groups, but significant difference (*P*< 0.03) was only in the group with beet.

Keywords: Ammonium excretion, Benzoid acid**,** Fiber, pH urine, Pigs

INTRODUCTION

Dietary manipulation has been shown as an effective method how to reduce N excretion in pigs and ammonia emissions from the slurry. An alternative methods of reducing ammonia emission are the acidification of urine by dietary means, the decrease of crude proteins with amino acid supplementation or fiber addition. Ammonia is formed predominantly from urinary urea which is relatively stable in its uncontaminated form. However, in the presence of microbial urease coming from faeces that is ubiquitous in pig facilities, it is converted to ammonia and carbon dioxide **(Aarnink *et al.* 1993).** Release of ammonia is pH-dependent and acidifying of pig slurries has shown ammonia volatilization reduction **(Freney *et al.* 1983, Stevens 1989)**. Benzoic acid might be one of the promising candidates of organic acids to substitute the nutritive use of antibiotics and to influence the growth performance and should also reduce the ammonia emissions from excrements. Addition of fiber sources to diet reduces urinary urea excretion which can be degraded enzymatically to ammonia. Fiber addition affects nitrogen excretory patterns and reduces ammonium nitrogen concentration of slurry which can lead to further reductions in ammonia emissions. Dietary fiber is resistant against digestive enzymes and potentially available for bacterial fermentation in guts of monogastric animals and leads to a shift from nitrogen in urine towards more nitrogen in faeces **(Nahm, 2003).** The diets with optimal amount of added fiber proved to be an effective way for animal health and environmental impacts on the country.

The objective of the experiment described herein was to study pH changes in urine and ammonia emissions of pigs which were fed by diets supplemented with benzoic acid and dried beet pulp.

MATERIAL AND METHODS

Twelve crossbred gilts, progeny of Large White sows and Landrace boars (initial BW 29.9 ± 1.7kg) were housed individually in metabolic cages. The dietary treatments included: Diet control (C) was supplemented with isoleucine, lysine, methionine, threonine, tryptophan, and valine to fit requirement of ideal amino acid profile; diet (BA) was similar to diet (C) with $10 g.kg^{-1}$ benzoic acid; diet (BABP) was similar to diet (C) with $10 g.kg^{-1}$ benzoic acid and $150 g.kg^{-1}$ dried beet pulp.

Diets were formulated to an equal ME content (13.3 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 $90 g.kg^{-1}$.

Table 1 Composition of diets and analyzed content of nutrients

Ingredient *($g.kg^{-1}$ diet)*	C	BA	BABP
Maize	571.80	552.00	388.00
Wheat	300.00	300.00	300.00
Soybean meal	83.00	86.90	88.80
Beet pulp	-	-	150.00
Rapeseed oil	2.00	8.30	22.60
L-isoleucine	0.87	0.90	1.00
L-lysine.HCl	5.85	5.80	5.70
DL-methionine	0.85	0.90	1.20
L-threonine	2.20	2.20	2.40
L-tryptofan	0.44	0.40	0.50
L-valine	0.86	0.90	1.10
Monocalcium phosphate	14.10	14.10	14.10
Limestone	11.00	11.00	7.90
Salt	3.90	3.90	3.50
Vit.-min. premix[1]	3.00	3.00	3.00
Benzoic acid	-	10.00	10.00
Analyzed nutrient contents	*($g.kg^{-1}$ air-dry)*		
Dry matter	884.4	883.5	887.5
Crude protein	137.1	139.2	140.8
Crude fibre	30.6	30.4	45.6

C- control 0% benzoic acid, BA- 1% benzoic acid, BABP- 1% benzoic acid and 15% beat pulp.

[1] Supplied per kg of diet: vit. A 9 000 IU, vit. D3 1 500 IU, α– tocopherol 35.0 mg, vit. B1 1.7 mg, vit. B2 6.0 mg, vit. B6 2.5 mg, Ca-panthothenate 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.

After a 5-day preliminary period, during which the pigs were adapted to cages and the new environment, four consecutive 10-d experimental periods followed. Each experimental period consisted of a 6-d 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°C. Faeces were collected, pooled, and stored at -20°C until analyse.

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 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_2SO_4. The air left the system after passing through a water trap, a flow controller at the rate of 2.3 $L.min^{-1}$, 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 0.2N 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 (Nx6.25) was determined by Kjeldahl method using a Kjell-Foss 16200 auto analyser (method 978.02).The crude fiber contents were determined using The Fibertec™ 2010 fibre analyzer Tecator, Hoganas, Sweden (method 2002.04). 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 (C) diet was slightly lower than that expected from the formulation Table 1. However treatments with 1% of benzoic acid and 15% beet pulp in the diet did not significantly influence nitrogen intake or dry matter intake (1.22, 1.23 and 1.14 $kg.d^{-1}$). Nitrogen balance (Table 2) showed a slightly higher N intake for diet

BA, due to the higher feed intake. No significant difference was observed between treatments, in accordance with **Nørgaand *et al.* (2010)**. But nitrogen excretion affected on both experimental diets. Switch from urinary to faecal excretion was detected in all groups, the largest and significant in the group BABP (*P* = 0.02). Faecal N excretion increased by +7.35% (C vs. BA) and +20.4% (C vs. BABP). The reduction for urinary nitrogen by -16 % was in the group BABP(*P* = 0.04). Our result with 15 % (CF 4.56%) beet pulp diets is similar to the findings of **Galassi *et al.* (2007)**. On the traditional Italian heavy pig (CP 14.0 % and CF 4.1 %) was N faecal excretion 12.3 % as compared to control group (CP 13.5 and CF 3.2%). In the diet with higher protein content and increasing fibre (CF 6.7%) found the reduction of faecal N to19.3%.

Table 2 Nitrogen balance

Items	C	± SEM	BA	± SEM	BABP	± SEM
N intake *[g.d^{-1}]*	30.16	± 0.34 ^{a}	31.07	± 0.27 ^{a}	28.96	± 0.32 ^{a}
Faecal N *[g.d^{-1}]*	4.31	± 0.14 ^{a}	4.63	± 0.19 ^{ab}	5.19	± 0.15 ^{b}
Urinary N *[g.d^{-1}]*	7.92	± 0.21 ^{a}	8.02	± 0.18 ^{a}	6.65	± 0.19 ^{b}
Faecal N excretion *[%]*	14.79	± 0.30 ^{a}	15.26	± 0.41 ^{ab}	18.34	± 0.28 ^{b}
Urinary N excretion *[%]*	27.39	± 0.51 ^{a}	25.73	± 0.14 ^{a}	22.94	± 0.21 ^{b}

C- control 0% benzoic acid, BA- 1% benzoic acid, BABP- 1% benzoic acid and 15% beat pulp.

^{a,b} means not sharing the same superscript letter are significantly different (P<0.05).

Total N retention in absolute terms, was detected by+0.49 g.d^{-1} (BA vs. C) and -0.82 g.d^{-1} (BABP vs. C), however N intake was +0.91 g.d^{-1} (BA) and -1.21 g.d^{-1} (BABP). Difference in the results is not significant, but corresponds to the value of N retention for the diet group BA 59.01 % and 58.72 % for diets BABP. The numerically higher N retention is consistent with the trend for a better growth performance observed by **Bühler *et al.* (2006)** using diets additioned with 1% benzoic acid in the grower and the finisher phase.

Table 3 Effects of crude fiber and benzoic acid intake on pH urine and emission from slurry

Items	C	± SEM		BA	± SEM		BABP	± SEM	
Crude fibre intake *[g.d⁻¹]*	42.17	± 0.40	a	42.40	± 0.32	a	58.62	± 0.45	b
Benzoic acid intake *[g.d⁻¹]*	0.00	± 0.00	a	13.96	± 0.20	b	12.86	± 0.22	b
Hippuric acid excretion *[g.d⁻¹]*	0.83	± 0.06	a	16.34	± 0.39	b	18.53	± 0.35	b
Initial urine pH	5.73	± 0.04	a	5.06	± 0.04	b	5.05	± 0.06	b
NH_3-N emission *[g.d⁻¹]* *	0.94	± 0.09	a	0.82	± 0.06	ab	0.73	± 0.05	b

C- control 0% benzoic acid, BA- 1% benzoic acid, BABP- 1% benzoic acid and 15% beat pulp.

[a,b] means not sharing the same superscript letter are significantly different (P<0.05).

* average per day of total emission from 2 kg fresh slurries during 7 days determination

The pH of urea and the ammonia release from the slurries are reported in Table 3. The addition of benzoic acid to the diet determined a numerically 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)** lowering urinary pH and it is well known that ammonia emission is affected by urinary pH.

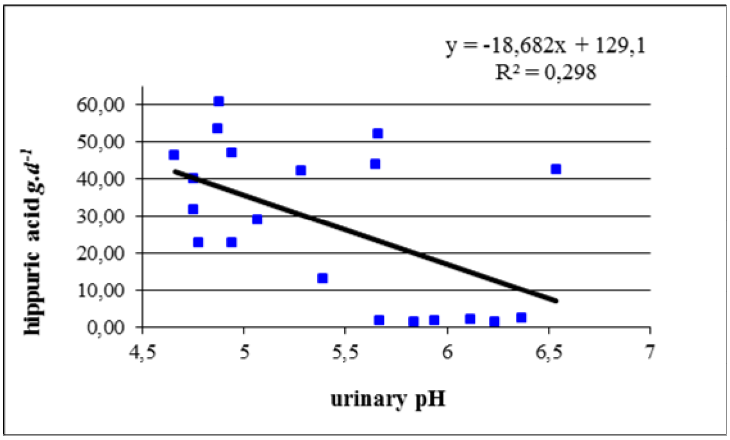

Figure 1 Relationship hippuric acid ratio and pH in the urine of pigs

The concentration of hippuric acid in urine of pigs treated with benzoic acid was significantly higher (*P*<0.01) as compared to the control animals. Regression analysis showed that urinary pH (y) was a linear function content of hippuric acid (x) Figure 1. There was a significant negative relationship relationship was described with an equation: $y = -18{,}682x + 129.1$; $R^2 = 0{,}298$. The slope of the regression equation showed that each point of pH increased 18,682 g.d^{-1} hippuric acid in urine. The data obtained indicate lower pH of urea with the diets containing benzoic acid (BA = 5.06) and beet pulp (BABP = 5.05) compared to the C diet (5.73). The decrease in urine pH (three quarters points of pH) is much lower than that (two points of pH decrease) obtained by **Kristensen *et al.* (2009)** on pigs of 50 kg body weight. Diets containing 1% benzoic acid pH was decreased by 11% in both diets. However, increasing fiber intake by 39% (16 g.d^{-1}) did not affect the pH value (P = 0.4).

The trend was lower for a NH_3-N emission from the diets C diet 0.94 g.d^{-1} to the diet with containing benzoic acid was 0.82 g. d^{-1}, however because of the high residual variation of the values detected no significant difference was observed among treatments. Reducing value of ammonia emission from slurry determined for the diet with beet pulp (BABP) of 0.73 g. d^{-1} was significant (P = 0.03). In the experiment **Galassi *et al.* (2010)**, determined high fiber diets, in comparison control diet, there was similar decrease of ammonia emission from slurry. In the diet with benzoic acid (BA) the value of ammonia emission was observed between the results of the control (C) diet with fiber (BABP). This fact shows that nitrogen created by after microbial fermentation is more stable than ammonia nitrogen

CONCLUSION

Nitrogen excreted in the feces in the form of microbial protein is advantageous because its degradation is slower. Dietary fiber is used as energy source for microbial populations in the digestive tract and thereby reduces ammonia emissions, but has no effect on the pH of urine. Benzoic acid as a dietary supplement has also led to a decrease in pH of urine, accompanied by reduction of ammonia emissions in growing pigs.

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