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A note on effect of benzoic acid supplementation on the performance and microbiota population of broiler chickens

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ABSTRACT

In order to determine the efficiency of benzoic acid, a feeding experiment was carried out on 400 one-day-old Cobb 500 cockerels. The birds were divided into four dietary treatments: control without additives, or with 2.5, 5 and 7.5 g of benzoic acid per kg diet, respectively. Performance was similar in birds fed the control diet and that with 2.5 g benzoic acid. The dietary inclusion of 5 and 7.5 benzoic acid per kg depressed growth and increased the feed conversion ratio ($P<0.05$). The pH of the caecal contents tended to decrease following increased benzoic acid supplementation. No differences were found in the pH of ileal and gizzard digesta. Lactic acid bacteria populations in the caecal contents were most numerous in the group fed the diet with 7.5 g benzoic acid ($P<0.05$). Coliform bacteria tended to decrease in the crop and ileal contents following increased benzoic acid supplementation, while there was no effect on the caecal contents.

KEY WORDS: broiler chickens, feed supplementation, benzoic acid, intestinal microflora

INTRODUCTION

The impact of gastrointestinal microbiota on animal health and growth performance has been demonstrated in animals fed antibiotic growth promoters (AGP). Apart from many doubts concerning the usage of antibiotic growth promoters, it is also well documented that their presence in poultry diets enhances broiler growth and improves feed conversion ratio. In recent years, we have seen growing interest in feed additives that may be alternatives for AGPs. These include organic acids as well as their mixtures (Ricke, 2003). The modes of their

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action differ, but they are all considered to be bacteriostatic agents. Benzoic acid and its salts are common food preservatives but are also used as feed additives in fur animals (Polonen et al., 2000) and pigs (Mroz et al., 2000). In the available literature there is no information about effects of benzoic acid in poultry.

The objective of the experiment was to study the influence of benzoic acid supplementation on the performance of broiler chickens as well as the pH of digesta and selected populations of bacteria inhabiting the gastrointestinal tract.

MATERIAL AND METHODS

The total of 400 one-day-old male broiler chickens (Cobb 500) was used in the experiment. The study design consisted of four dietary treatments, 10 replicates each. The birds were kept in floor pens on straw bedding. Chickens were offered the starter type experimental diets *ad libitum* between 1-14 d and grower type between 15-42 days of life (Table 1). The diets were prepared without additives-control (C) or with, g/kg: 2.5, 5 or 7.5 pure benzoic acid. Benzoic acid replaced the respective amount of maize in the basic diet. The diets were fed in mash form. Feed intake and body weight were registered at weekly intervals, and the body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

Table 1. Composition (g/kg) and nutritional value of control diets in the starter (0-14 d of life) and grower (15-42 d of life) period

Item	Diets	
	starter type (0-14 d)	grower type (15-42 d)
Ingredients		
maize	277.0	313.5
wheat	250.0	300.0
soyabean meal	376.5	291.0
rape seed oil	49.0	50.0
monocalcium phosphate	14.0	13.0
limestone	4.0	4.0
Dl-methionine 20%	10.0	8.0
L-lysine 20%	5.0	6.0
$NaHCO_3$	1.0	1.0
NaCl	3.5	3.5
mineral and vitamin premix[1]	10.0	10.0
Calculated		
ME MJ/kg	12.5	13.0
crude protein	210.0	190.0

[1] providing per kg diet, IU: vit. A 12 500, vit. D_3 4000; mg: vit. E 50, vit. K 3, vit. B_1 2.2, vit. B_2 6.5, vit. B_6 3.8, pantothenic acid 12.5, choline chloride 400, folic acid 1.5, biotin 0.2, vit. B_{12} 0.025, BHT 10, Se 0.35, Fe 60, Zn 80, Mn 80, Cu 10, I 0.75; g: Ca 2.45

During two days in the sixth week of the experiment, 21 chickens from each group were sacrificed by cervical dislocation. The contents of the crop, gizzard, ileum, caeca and rectum and cloaca were collected. The ileum was defined as the small intestinal segment caudal to Meckel`s diverticulum. The rectum and cloaca were defined as the segment from the ileo-caecale junction to the end of the GIT. The pH in the contents of all gastrointestinal segments was measured with a combined glass/reference electrode (CP-40 Elmetron, Poland). The dietary concentration of dry matter, crude protein, crude fat and mineral content in the raw materials was determined using AOAC procedure (2005). Diluted caecal digesta were suspended in pre-reduced salt medium and homogenized for 2 min in CO_2-flushed plastic bags using a stomacher homogenizer (Interscience, France). Subsequently, serial decimal dilutions were made, avoiding aeration, using the same medium according to the technique of Miller and Wolin (1974). Samples incubated under anaerobic conditions at 37ºC for 48 h on MRS agar medium (Merck 1.10660, Darmstad, Germany) were used for determination of the total number of lactic acid bacteria whereas samples incubated under aerobic conditions at 37ºC for 24 h on MacConkey agar (Merck 1.05465), were used for determination of the total number of coliform bacteria.

The results were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test using the statistical computer program package SAS 9.1.3 (1996). Statistical significance was established at $P \leq 0.05$.

RESULTS

The performance of birds fed the diet without supplemented benzoic acid and of birds fed the diet with 2.5 g benzoic acid per kg were comparable. Supplementation of 5 and 7.5 g/kg benzoic acid, however, decreased BWG and increased FCR ($P<0.05$) (Table 2).

In comparison with other treatments, the pH of colon contents in group B 7.5 was statistically lower (6.65 vs 7.67). The pH of the caeca tended to decrease following benzoic acid supplementation, but reached statistical significance only in the case of B 2.5 group ($P<0.05$). No differences were found in pH of ileum and gizzard contents. The pH of the crop content was highest in control group C in comparison with groups B 2.5 and B 7.5 ($P<0.05$).

Irrespective of the benzoic acid supplementation, no differences were found in lactic acid bacteria populations in the crop and ileum, while more lactic acid bacteria were in the caeca of group B7.5 ($P<0.05$). Coliform bacteria tended to decrease in the crop and ileal contents following increased benzoic acid supplementation but only in group B 7.5 was the difference statistically significant in comparison with the other treatments ($P<0.05$). No differences were found in the coliform bacteria populations in the caeca of broiler chickens (Table 2).

Table 2. Performance results of broiler chickens, pH values and microbiota population

Item	Dietary treatments				
	C	B 2.5	B 5	B 7.5	SEM
Body weight gain, kg					
0 - 14 d	0.32[a]	0.33[a]	0.30[b]	0.25[c]	0.005
15 - 42 d	2.06[a]	2.06[a]	1.86[b]	1.62[c]	0.027
0 - 42 d	2.38[a]	2.39[a]	2.16[b]	1.87[c]	0.031
Feed conversion ratio, kg feed/kg BWG					
0 - 14 d	1.36[c]	1.39[bc]	1.44[ab]	1.50[a]	0.012
15 - 42 d	1.8[b]	1.85[b]	2.00[a]	1.97[a]	0.018
0 - 42 d	1.74[b]	1.79[b]	1.92[a]	1.90[a]	0.016
Digesta pH					
crop	5.56[a]	5.11[b]	5.27[ab]	5.01[b]	0.073
gizzard	4.23[a]	4.25[a]	4.26[a]	3.94[a]	0.077
ileum	6.33[a]	6.63[a]	6.37[a]	6.48[a]	0.055
caeca	7.04[a]	6.72[b]	6.9[ab]	6.83[ab]	0.483
rectum and cloaca	7.67[a]	7.42[a]	7.65[a]	6.65[b]	0.996
Lactic acid bacteria, log cfu × g-1 digesta					
crop	9.60[a]	8.94[a]	9.57[a]	9.58[a]	0.181
ileum	8.10[a]	8.80[a]	7.47[a]	8.75[a]	0.297
caeca	9.39[b]	9.38[b]	9.27[b]	9.87[a]	0.849
Coliforms, log cfu × g-1 digesta					
crop	6.53[a]	6.38[a]	5.37[ab]	4.13[b]	0.318
ileum	5.92[a]	5.22[a]	5.33[a]	1.50[b]	0.500

B - benzoic acid

[a,b,c] means in a row with a different letter differ significantly at $P \leq 0.05$

SEM - pooled standard error

DISCUSSION

Mammals eliminate ingested benzoic acid mainly by conjugation with glycine in the liver and kidneys. The conjugation product - benzoyl glycine (i.e. hippuric acid) - is excreted in urine, lowering its acidity. Thus, beside bacteriostatic features, benzoic acid is considered as ammonia reducing agent, which indirectly stimulates the growth of pigs (Mroz et al., 2000). Due to differences in basic physiology of mammalian and bird species, it is hard to expect the same results in broiler chickens.

Knarreborg et al. (2002) demonstrated in the batch culture conditions that benzoic acid and, to a lesser extent, fumaric acid exerted strong bacteriocidal properties towards lactic acid bacteria. These authors suggested pH-dependent effects on coliform bacteria and lactic acid bacteria in stomach content, and on coliform bacteria in the small-intestine content. The pH values used in the batch culture system by Knarreborg et al. (2002) were supposed to mimic physiology of the pig and so were lower than those observed in the chicken. The results of present experiment show the antibacterial effects of benzoic acid in broiler chickens, although lower bacterial counts in gastrointestinal digesta were not reflected in better performance of the birds.

Bridges et al. (1970) reported that the metabolic pathways of supplemented benzoic acid differ among mammalian species. Polonen et al. (2000) studying the effect of benzoic acid in mink, blue fox and racoon have found species-specific differences in excreting of this compound among its effects on poultry is scarce. In broilers fed vegetable diets based on soyabean meal, glycine is limiting amino acid in the early period of life (Corzo et al., 2004). Dietary benzoic acid may thus depress glycine availability and may reduce growth rate in chickens..

CONCLUSIONS

It my be concluded that dietary supplementation of pure benzoic acid at a rate exceeding 2.5 g/kg may have negative effects on feed conversion as well as body weight gain, by broiler chickens, even though its bacteriostatic effects are dose-dependent.

REFERENCES

AOAC, 2005. Association of Official Analytical Chemists, Official Methods of Analysis. 18th Edition. Arlington, VA

Bridges J.W., French M.R., Smith R.L., Williams R.T., 1970. The fate of benzoic acid in various species. Biochem. J. 118, 47-51

Corzo A., Kidd M.T., Burnham D.J., Kerr B.J., 2004. Dietary glycine needs of broiler chicks. Poultry Sci. 83, 1382-1384

Knarreborg A., Miquel N., Granli T., 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. Anim. Feed Sci. Tech. 99, 131-140

Miller T.L., Wolin M.J., 1974. A serum bottle modification of Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. 27, 985-987

Mroz Z., Jongbloed A.W., Partanen K.H., Vreman K., Kemme P.A., Kogut J., 2000. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients and manure characteristics in swine. J. Anim. Sci. 78, 2622-2632

Polonen I.J., Partanen K.H., Jalava T.K., Toivonen V.F., 2000. Effect of dietary glycine and benzoate level on benzoate metabolism in mink (*Mustela vison*), blue fox (*Alopex lagopus*), and raccoon dog (*Nyctereutes procyonoides*). J. Anim. Sci. 78, 976-986

Ricke S.C., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Sci. 82, 632-699

SAS, 1996. SAS®/STAT User`s Guide: Statistics Institute Inc. Cary, NC