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A note on the effects of selected prebiotics on the performance and ileal microbiota of broiler chickens

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ABSTRACT

The objective of the experiment was to study the influence of lactose, inulin, and modified β-glucan as additives to maize-based diets on the performance of broiler chickens, as well as on the pH in the crop, gizzard, ileum, caeca and on populations of selected ileal microflora species. Over the entire experimental period (day 1 - day 42), the animals (384 male broilers, 4 treatments, 12 replicates of 8 birds per treatment) were offered maize-based diets. The diets were fed either without any supplementation (control diet), or with a supplement of either 0.025% modified β-glucan, 0.3% inulin, or 0.2% purified lactose. Body weight gain decreased ($P<0.05$) and feed conversion ratio worsened ($P<0.05$) in birds fed diets with supplements in comparison with the non-supplemented control group. The counts of lactic acid bacteria in ileal digesta were lower ($P<0.05$) in birds supplemented with lactose. No difference between the dietary treatments was observed regarding the population of intestinal coliform bacteria.

KEY WORDS: β-glucan, inulin, lactose, performance, ileal microbiota, chickens

INTRODUCTION

Questioning the use of antibiotic growth promoters (AGP) in animal production has resulted in withdrawal of these feed additives in the EU. Consequently, interest in feed additives that may act as alternatives for AGPs is growing. These additives include mixtures of organic acids, prebiotics, probiotics, symbiotics, and feed enzymes.

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Prebiotics are generally classified as indigestible feed agents that are potentially beneficial to the health of the host due to their fermentable properties that may stimulate the growth and/or activity of bacteria in the ileum and caecum (Gibson and Roberfroid, 1995). According to this definition, prebiotics might include a very variable and wide range of chemical substances. However, mostly they are carbohydrates that are not hydrolysed by endogenous host enzymes, thus being available for microbiological fermentation in the lower parts of the non-ruminant gastrointestinal tract. Non-digestible oligosaccharides, especially those containing xylose, fructose, galactose, mannose and glucose, have attracted much attention and seem to be promising (Gibson and Roberfroid, 1995). The prebiotic properties of glycooligosaccharides, trans-galactooligosaccharides, glucooligosaccharides, lactulose, maltooligosac-charides, xylo-oligosaccharides, stachyose, raffinose, and sucrose thermal oligosaccharides have also been investigated (Patterson and Burkholder, 2003). Further, some structural carbohydrate components of NSP have been used in broiler chicken diets, and have been studied as potential prebiotics. Besides its effect on microbial fermentation, β-glucan has been shown to modulate immunity by increasing the activity of mammalian macrophages *in vitro* (Kataoka et al., 2002) and protecting broilers against *Salmonella* (Lowry et al., 2005).

It has been suggested that the major effect of prebiotics includes selective growth of lactic acid-producing bacteria, resulting in an increased concentration of short-chain fatty acids (SCFA), i.e. acetate, propionate, butyrate. High fermentation activity and high concentration of the SCFA in chicken caeca is correlated with a lower pH (Józefiak et al., 2004). This may inhibit some pathogenic bacteria by dissipating the proton motive force across the bacterial cell membrane (Russell, 1992).

The objective of the experiment was to study the influence of lactose, inulin and modified β-glucan as additives to a maize-based diet on the performance of broiler chickens as well as the pH in the crop, gizzard, ileum, caeca and populations of ileal microflora.

MATERIAL AND METHODS

A total of 384 male broiler chickens (Cobb 500) were included in a feeding experiment. The study design consisted of four dietary treatments, 12 replicates of 8 birds in each. Over the entire experimental period (day 1 - day 42), the birds were offered maize-based diets, in the starter period (days 1-14) and in the grower period (days 15-42). Diets contained no antibiotic growth promoters, but an ionophore coccidiostat (Lasalocid) was used (Table 1). The concentration of dry matter, crude protein and crude fat, ash and mineral content in the raw materials was determined using the AOAC procedure (2005). The first group (C) received a non-supplemented control diet, the second group (G), a diet

Table 1. Composition and nutritional value of diets fed from 1 to 14 day and from 15 to 35 day

Item	Days 0-14	Days 15-42
Ingredients		
maize	48.55	58.3
soyabean meal, 43%	40.0	31.5
rapeseed oil	6.6	5.7
monocalcium phosphate	1.6	1.5
L-lysine, 20%	0.2	0.2
DL-methionine, 20%	0.95	0.7
NaCl	0.3	0.3
limestone	0.7	0.7
sodium carbonate	0.1	0.1
vitamin and mineral mix[1]	1.0	1.0
Calculated		
ME in MJ/kg	12.80	13.00
crude protein	22.5	19.5
lysine	1.30	1.07
methionine	0.54	0.45
Ca	0.99	0.95
P available	0.44	0.40

[1] providing per kg of diet: IU: vit. A 12 500, vit. D3 3000; mg: vit. E 30, vit. B1 2.2, vit. B2 6.5, vit. B6 3.8, pantothenic acid 12.5, choline chloride 400, folic acid 1.5, biotin 0.2, vit. B12 0.025, BHT 10, Se 0.35, Fe 60, Zn 80, Mn 80, Cu 10, I 0.75, g: lasalocid and Ca 2.45

supplemented with 0.025% modified β-glucan (Biolex Beta S), the third group (I), a diet supplemented with 0.3% inulin (Fructafit HD), and the fourth group, (L) a diet supplemented with 0.2% purified lactose. β-glucan and inulin were supplemented in accordance with the producers' recommendations, while the lactose level was chosen according to our earlier studies (unpublished). Feed intake and body weight were registered at weekly intervals, and the feed conversion ratio (FCR) and body weight gain (BWG) were calculated.

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 and caeca were quantitatively collected and randomly pooled into seven replicates, each containing digesta from three birds. The ileum was defined as the small intestinal segment caudal to Meckel's diverticulum. The pH in the contents of all gastrointestinal segments was measured with a combined glass/reference electrode (CP-40 Elmetron, Poland). Diluted ileal digesta for counting microflora was further homogenized for 2 min in CO_2-flushed plastic bags using a stomacher homogenizer (Interscience, France). Subsequently, the samples were serially diluted in 10 steps using pre-reduced salt medium according to the technique of Miller and Wolin (1974). Lactic acid bacteria were counted on de Man Rogosa and Sharp agar (Merck 1.10660, Darmstad, Germany) after anaerobic incubation at 39°C for two days. Coliform bacteria were enumerated on MacConkey agar (Merck 1.05465) after aerobic incubation at 39°C for one day.

The obtained results were analysed using the statistical software package SAS (1996), SAS®/STAT. The results were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. Statistical significance was established at $P<0.05$.

RESULTS AND DISCUSSION

In general, the selected prebiotics did not improve the final performance of broiler chickens (Table 2). Terada et al. (1994) also reported that the application of oligosaccharides did not improve the performance of broilers. In the present experiment, however, in the starter period, all groups fed diets supplemented with prebiotics had a higher BWG in comparison with the control group. Irrespective of the diet, there were no changes in pH value in the crop and ileal chyme. However, in gizzard chyme, the pH was lower when birds were fed diets supplemented with prebiotics as compared with the control and was significantly lower ($P\leq0.05$) in groups I and L (Table 2). Only the supplementation with inulin and lactose

Table 2. Performance of broiler chickens , pH and bacterial counts in ileal digesta

Item	Dietary treatments				
	Control (C)	β-glucan (G)	inuline (I)	lactose (L)	SEM
Body weight gain, g					
0 - 14 day	281^{b}	287^{a}	287^{a}	300^{a}	0.004
15 - 42 day	1948^{a}	1858^{b}	1836^{b}	1838^{b}	0.037
0 - 42 day	2229^{a}	2146^{b}	2122^{b}	2133^{b}	0.031
Feed conversion ratio, kg feed/kg BWG					
0 - 14 day	1.66^{b}	1.64^{b}	1.76^{a}	1.60^{b}	0.012
15 - 42 day	1.98^{b}	2.00^{b}	2.29^{a}	2.04^{b}	0.018
0 - 42 day	1.94^{b}	1.95^{b}	2.22^{a}	1.98^{b}	0.016
pH of digesta					
crop	4.82	4.90	4.87	4.82	0.063
gizzard	4.10^{a}	3.87^{ab}	3.65^{b}	3.69^{b}	0.072
ileum	5.80	5.93	5.79	5.89	0.051
caeca	6.58^{a}	6.58^{a}	6.12^{b}	6.31^{b}	0.083
Lactic acid bacteria log cfu × g $^{-1}$ wet digesta					
ileum	8.5^{a}	8.7^{a}	9.0^{a}	8.2^{b}	0.178
Coliform bacteria log cfu × g $^{-1}$ wet digesta					
ileum	4.9	4.0	4.9	5.0	0.159

^{ab} - means in the rows with different letters are significantly different at $P\leq0.05$

SEM - pooled standard error

lowered the pH in the caeca of broiler chickens, which is in agreement with the definition of a prebiotic as suggested by Gibson and Roberfroid (1995).

Patterson and Burkholder (2003) reported that the positive effect of prebiotics becomes most apparent under poor environmental conditions where the birds are exposed to pathogenic microflora. It was reported that bacteria which inhabit the poultry GIT, *Lactobacillus* spp. (Grill et al., 1995) or *Bifidobacterium* spp. (Grill et al., 2000), deconjugate bile salts. It is well documented that deconjugated bile salts lose their emulsifying properties, resulting in decreased fat digestibility. In addition to potential energy loses, increased competition between the host and the microflora for nutrients may appear. In the present trial, feeding prebiotics, particularly inulin, impaired feed utilization and this could be explained by lower fat digestibility and/or other nutrient availability caused by activity of native microflora.

The caecum in broiler chickens is the main fermentative chamber and, in comparison with other gastrointestinal tract (GIT) segments, contains the largest number of bacteria (Józefiak et al., 2004). Thus, the ability of microbiota to ferment carbohydrates there is very high. In the present study, the pH of caecal digesta was lower in birds provided diets with inulin and lactose. Also Corrier et al. (1991), who provided 2.5% lactose in drinking water, and Tellez et al. (1993), who administrated 10% of lactose in feed, reported a lowered pH in the caecal contents of broilers.

The intestinal colonization of different bacteria populations interacting with each other, as well as with the host, is correlated with the growth performance of the bird. In the present experiment, the number of lactic acid bacteria in the ileal content tended to be higher in groups fed diets supplemented with β-glucan and inulin as compared with the control group, whereas in the group fed the diet with lactose, the LAB counts were lower ($P<0.05$; Table 2). The used feed additives had no influence on the population of coliform bacteria. More distinct effects on the intestinal microbiota would be expected in the caecum, which is indicated by the marked changes in pH observed in caecal content (Table 2). However, the growth-depressing and potentially pathogenic bacteria can influence absorption processes more strongly in the ileum. Even though this region of the GIT is less colonized than the caecum, the competition of bacteria with the host can exert the most important effects on performance. It is therefore suggested that potential alternatives, similar to AGPs, should also affect this part of the GIT.

The results of the present experiment strongly question the effectiveness of the tested additives as potential growth-promoting agents in broiler chicken production.

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