Effect of dietary supplementation of protected organic acids on ileal microflora and protein digestibility in broiler chickens.

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This experiment was conducted to investigate the effects of 3 diets containing different levels (0, 0.2 and 0.4%) of protected organic acids (formate and propionate) on the gut microflora and ileal protein digestibility in broiler chicks. One hundred and fifty six broilers in a completely randomized design with three treatments and four replicates for each treatment were employed. The experiment lasted for 42 days. Ileal pH and gut microflora at 24 and 42 days of age and ileal protein digestibility on day 42 were determined. The result showed that different levels of protected organic acids had no significant effects on ileal protein digestibility. The acidity of ileal digesta, colony count of Lactobacillus and Coliforms were significantly affected by protected organic acids ($p<0.05$). On days 24 and 42, increasing the level of protected organic acids, significantly decreased pH of digesta flow in ileum ($p<0.05$). Organic acids significantly ($p<0.05$) increased colony count of Lactobacillus and decreased colony count of Coliforms in digesta at 24 and 42 days of age. Colony counts of Lactobacillus and Coliforms were higher on day 42 compared to 24. The results showed that dietary supplementation of 0.2% of protected organic acids can improves proliferation of useful microflora and diminishes population of harmful bacteria in poultry gut contents.

Key words: Broiler chick, protected organic acid, ileal protein digestibility, microflora

Introduction

Today, use of some antibiotics as growth promoter creates a huge problem for environmental conditions and healthy of consumers around the word. Regarding that, researchers are trying to substitute those with different natural feed additive materials, such as organic acids and probiotics. Like antibiotics, short-chain organic acids also have a specific antimicrobial activity. Unlike antibiotics, their antimicrobial activity is pH dependent. They are particularly effective against acid-intolerant species such as *E. coli, Salmonella* and *Campylobacter*. On the other hand organic acids increase population of useful bacteria like *Lactobacilli*, improve protein and energy digestibility (Runho et al., 1997) by reducing microbial competition with the host for nutrients and endogenous nitrogen losses and by reduction in ammonia and production of biogenic amins and other growth-depressing microbial metabolites (Dibner and Buttin, 2002). These actions have clear and significant benefits on growing bird performance (Bolling et al., 2000; Patten and Waldroup, 1988). Lack of consistency in demonstrating an organic acid benefit is related to form of administrated organic acid (protected or unprotected), uncontrolled variables such as buffering capacity of dietary ingredients, presence of other antimicrobial compounds, cleanliness of the production environment, and heterogeneity of gut microbiata. Chaveerach et al. (2002) demonstrated the magnitude of their antimicrobial effects varies from one acid to another and is dependent on concentration and pH. The objective of this study was to evaluate the effects of a mix of protected form of two organic acids (formic and propionic acid) on colony counts of *Lactobacilli* and *Coliforms* in ileum and on ileal protein digestibility in broiler chicks.

Materials and methods

One hundred and fifty six day-old broiler chicks (Ross 308) assigned to 12 pens of 3 treatments, randomly. The experimental design was completely random, consisting of three dietary levels (0, 0.2 and 0.4%) of a protected organic acids mixture (Salkil, combination of formic acid, propionic acid and their ammonium salts). Each treatment had four replicates of 13 birds. Chicks fed a basal of corn-soybean diet during 0-21and 22–42d of age. To determine ileal protein digestibility chromic oxide (3g/kg) as an external marker was added to the experimental diets. At 24 and 42d of age nine chicks per treatment (3chicks/pen) were killed by intravenous injection of pentobarbitone. Ileal contents (from 1 cm below Merckel's diverticulum to 4cm above the ileo-cecal junction) were gently removed. Ileal contents of three chicks from each replicate pooled and homogenized, then one gram sample diluted with 4ml deionised water and pH was measured immediately. *Lactobacilli* population were enumerated on the plate count agar following anaerobic incubation at 37 °C for 3 d using anaerobic jar techniques. *Co;iforms/Escherichia coli* population levels were estimated using lactose broth media and the most probable number (MPN) technique. On day 42 part of the pooled samples were used for determination of ileal protein digestibility. Ileal samples were dried (60°C for 24h) then analyzed for chromic oxide and N (Kjeldahl analyzer). Data for all variables were subjected to an ANOVA analysis using the gGeneral Linear Models (GLM) procedure of SAS software (SAS Institute 1992) and means were considered different at $P<0.05$ using Duncan's multiple range test.

Result and Discussion

The pH in the ileum of the chicks fed diet supplemented with 0.4% organic acids mixture was significantly ($p<0.05$) lower than in the chicks fed control diet at both 24 and 42 days of age (Table 1). These results clearly show that use of protected form of organic acids allowed slow release of the acid, so that a sufficient amount of product reached the lower intestinal tract (Van Immerseel et al. 2002). Thus, dietary inclusion of the organic acids reduced pH and produced a suitable environment for lactic acid bacteria proliferation in ileum (Table 2). The magnitude of the digesta pH responses to dietary organic acids in chicks is similar to that observed by number of other researchers (Canibe and Jensen, 2003; Waldroup et al., 1995; Rafacz-Livingston, et al., 2005; Bolling et al., 2000). However, Izat et al. (1990) reported that dietary organic acid (formic acid or calcium formate) didn't have any significant effect on intestinal pH. It seems that unprotected organic acids used in feed can be neutralized easily by bile and pancreatic secretions. In addition, effect of buffering capacity of the diets on the organic acids effectiveness in changing the pH along the gastrointestinal tract not to be ignored. Waldroup et al. (1995) reported that formic acid/propionic acid 1% in the feed reduces the caecal pH. Regardless of treatment, ileal pH of the digesta in all chicks on day 24 were clearly lowest compared with that of chicks on day 42 (Table 1). Ileal protein digestibility also not affected by different levels of protected organic acids supplemented to the diets (Table 1).

Table 1. Effect of different levels of dietary organic acids on Ileal pH and protein digestibility

	Ileal pH		Ileal protein digestibility
Treatments	**24d**	**42d**	**(%)**
0% organic Acid	5.92 [a]	7.27 [a]	67.7
0.2 % organic Acid	5.56 [a b]	6.74 [a b]	67
0.4 % organic Acid	5.15 [b]	6.36 [b]	68
Standard Error (SE)	0.14	0.16	2.1

[a,b] Means within a column followed by different superscripts differ significantly ($P<0.05$).

Ileum contents of the birds fed diet containing 0.2% organic acids mixture had significantly higher counts of *lactiobacilli* grow at 37°C. However feeding diets containing 0.4% organic acid resulted in lower ileum counts of lactic acid bacteria than when feeding 0.2% organic acids. It seems that using high level of organic acid caused sever reduction in ileal pH (Table 1) and produced an unconventional atmosphere for a normal proliferation of *Lacobaciili* bacteria.

The *coliforms* counts of the ileal digesta grow on lactose broth and counted by MPN method showed that diets supplemented with 0.2 and particularly 0.4% organic acids mixture dramatically (not significant) diminished population of the bacteria which are potentially pathogenic for animals or humans. Short chain fatty acids (SCFA) are bacteriostatic or bactericidial *in vitro* for gram-negative bacteria, provided that there are sufficient undissociated acid molecules present and that they are in contact with the bacteria for sufficiently long time (Thompson and Hinton, 1997).Within practical limits, lowering the pH increases the concentration of undissociated molecules. Therefore the products will be more efficient at low pH. This was proved in *in vitro* experiments by a decreased growth rate of *Salmonella Typhimorium* at decreasing pH levels in the present of VFA (Durant et al., 2000). Concentrations of VFA sufficient to cause growth inhibition of *E. cloli in vitro* immediately slowed the rates of RNA, DNA, protein, lipid and cell wall synthesis (Cherington et al., 1991). Ozturk et al. (2004) also reported decreasing gram-negative bacteria counts in the intestine of broiler chicks fed organic acid supplementation diet. However, it seems that we needed to high sample number for investigation on gut microflora using the conventional method. On the other hand use of new methods such as molecular methods are recommended to permit studying on colony counts and particularly determination of diversity of intestinal bacterial community in chickens (Ricke and Pillai, 1999).

Table 2. Effect of dietary levels of organic acids and age on microbial counts (cfu/g sample) in Ileal digesta

Treatments	*Lactobacilli*	*Coliforms*
Organic acid concentration(%)		
0	$23.2 \times 10^{6\,b}$	14.56×10^{4}
0.2	$90.9 \times 10^{6\,a}$	10.03×10^{4}
0.4	$45.7\times 10^{6\,a\,b}$	8.88×10^{4}
SE	0.55	0.695
Age (day)		
24	$21\times10^{6\,b}$	10.26×10^{4}
42	81.58×10^{6a}	12.08×10^{4}
SE	0.42	0.555

[a,b] Means within a column followed by different superscripts differ significantly (P<0.05).

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