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Effect of Some Acidifiers on Gastrointestinal Tract Characteristics and Performance of Broiler Chickens

Research Article

S. Yakhkeshi[1], P. Shawrang[2*] and S. Rahimi[1]

[1] Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
[2] Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Karaj, Iran

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*Correspondence E-mail: pshawrang@nrcam.org

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ABSTRACT

The effects of Selko-pH (S; 1 mL/L drinking water); Termin-8 (T; 2 kg/ton feed) and Neogermicin (N; 2 L/ton feed) on gastrointestinal tract (GIT) pH, microbial population, ileal digestibility and broiler performance were investigated. The lowest feed intake (FI) was attained by chickens fed diets supplemented with S group in all periods ($P<0.05$). The highest weight gain (WG) was achieved by birds fed diet containing N set at 22-42 and 1-42 periods ($P<0.05$). The highest and lowest feed conversion ratio (FCR) were obtained by control (C) and N groups at 22-42 and 1-42 days of age, respectively ($P<0.05$). Inclusion of acidifiers increased lactic acid bacteria and reduced total coliforms at different ages. The C and N treatments induced the lowest and greatest dry matter and gross energy digestibility coefficients, respectively ($P<0.05$). Moreover, pH of different parts of GIT were significantly diminished by acidifiers' treatments compared to C group ($P<0.05$). The results of current study have shown that acidifiers improved the gut microflora conditions by pH reduction. The digestibility of nutrients and energy utilization were superior by incorporated acidifiers, but S groups played a minor role in this regard.

KEY WORDS acidifiers, broiler performance, digestibility, microflora.

INTRODUCTION

The antibiotics resistance and unreliable antibiotic therapy have lead to ban the use of antibiotics (Patterson and Burkholder, 2003). Increasing investigations were widely made regarding antibiotics alternatives (Jones and Ricke, 2003). Acidifiers (e.g. organic acids) are the candidate alternative for antibiotics, either individual or blends of several acids. The beneficial effects of organic acids in pigs and poultry have been demonstrated (Ao *et al.* 2009; Adil *et al.* 2011; Král *et al.* 2011; Lampromsuk *et al.* 2012; Khosravi *et al.* 2012). Several organic acids have been reported to improve growth performance, feed efficiency, mineral absorption and phytate-P utilization when supplemented in diets (Vogt and Matthes, 1982; Ao *et al.* 2009; Boling Frankenbach *et al.* 2001; Adil *et al.* 2011; Lampromsuk *et al.* 2012). The low acid concentrations in the intestine lead to pH rises, inability of digestive enzymes to function properly and diminish nutrient digestion and absorption (Hernandez *et al.* 2006). Acidification with various organic acids has been reported to reduce the production of bacteria toxic components and colonization of pathogens on the intestinal wall, thus prevent the damage to epithelial cells (Langhout, 2000). In addition, organic acids serve as substrates in intermediary metabolism the digestibility of proteins and mineral (calcium, phosphorus, magnesium and zinc) were improved. Commercial acidifiers, based organic acids, have potential to be used in poultry industry. Therefore, the objective of the present study was to evaluate the effects of some commercial acidifiers as antibiotic alternatives on performance, gastrointestinal tract (GIT) pH, intestinal microbial population and digestibility of nutrients in broiler chickens.

MATERIALS AND METHODS

Birds and diets

A total of 240 one-day old male broilers (Cobb 500) were randomly divided into 4 treatments and 4 replicates of 15 birds which were kept on the floor pens. Treatments were: control (C), Termin-8 (Anitox, USA) (2 kg/ton feed) (T), Neogermicin (IQF, Spain) (2 L/ton feed) (N) and Selko-pH (Selko, Netherlands) (1 mL/L drinking water) (S) which were administrated for a 42 days period. Feed and water were provided *ad libitum* throughout the study. Lighting schedule were 23L/1D while the temperature was gradually reduced by 3 ˚C from initially 32 ˚C each week. Feed was formulated for two periods of starter (1-21 days) and grower (22-42 days). The diets composition is shown in Table 1.

Digesta collection and microbial assay

On day 42 of experiment, 2 birds from each replicate were randomly selected and euthanized. Crop, ileum and cecal contents were collected for investigation of microbial population. The serial dilutions (10^{-3} to 10^{-7}) of these samples were prepared and cultured on the selective media of plate count agar, De Man Rogosa Sharpe Agar (MRS) and MacConkey agar (Merck, Germany) for enumerating total aerobics; lactic acid bacteria and coliforms, respectively. The total aerobics and coliforms population were counted after aerobic incubation at 37 ˚C for 24 hours and lactic acid bacteria after aerobic incubation at 37 ˚C for 48 hours (Witkamp, 1963).

Digestibility determination

To determine nutrients digestibility, titanium oxide (TiO_2) as an indigestible marker was included in experimental diets at 0.2%. After a 4-day adaptation period to experimental diets, 2 birds from each replicate were euthanized at 42 days of age. Contents of ileum were gently and immediately removed (Gong *et al.* 2002). Samples of oven-dried feed and digesta were grounded to a fine texture. Feed and digesta were analyzed for chemical composition. Dry mater (DM), organic matter (OM), crude protein (CP) and ether extract (EE) of diets and digesta were determined according to AOAC (1990) methods. Apparent digestibility coefficients of DM, OM, CP and EE of feed ingredients were calculated based on standard formulas for the total collection and marker methods (Maynard and Loosli, 1969). Following equations were used for digestibility estimations:

$$AD = 100 - [NE \times (TiO_2 \text{ feed}/TiO_2 \text{ fecal}) \times NF] \times 100$$

Where:
AD: is apparent digestibility.
NF: is nutrient in feed.
NE: is nutrient in excreta.

Table 1 The composition and nutrient values of diets

Ingredients	Starter (day 1-21)	Finisher (day 22-42)
Maize (g/kg)	498.0	527.8
Soybean meal (480 g P/kg)	408.7	361.3
Wheat (g/kg)	45.3	66.0
Soybean oil (g/kg)	10.0	10.0
Dicalcium phosphate (g/kg)	24.6	22.0
DL-methionine (980 g/kg)	3.4	2.7
L-lysine (980 g/kg)	2.3	1.9
Vitamin permix[1] (g/kg)	2.5	2.5
Mineral permix[2] (g/kg)	2.5	2.5
Limestone (g/kg)	-	0.5
Salt (g/kg)	2.7	2.8
Calculated nutrients		
ME (kcal/kg)	2820	2950
Crude protein (g/kg)	215.3	188.5
Ether extract (g/kg)	40.4	50.5
Calcium (g/kg)	9.3	8.3
Available P (g/kg)	4.7	4.1
Lysine (g/kg)	11.8	11.2
Methionine (g/kg)	3.8	3.7
Methionine + cystine (g/kg)	9.0	8. 2

[1] Supplied the following per kg of diet: vitamin A (retinyl acetate): 8000 IU; vitamin D_3 (cholecalciferol): 3000 IU; vitamin E (DL-alpha-tocopheryl acetate): 25 IU; menadione: 1.5 mg; vitamin B_{12} (cyanocobalamin): 0.02 mg; Biotin: 0.1 mg; Folacin (folic acid): 1 mg; Niacin (nicotinic acid): 50 mg; Pantothenic acid: 15 mg; Pyridoxine (pyridoxine_HCl): 4 mg; Riboflavin: 10 mg and Thiamin: 3 mg (thiamin mononitrate).

[2] Supplied the following per kilogram of diet: Copper (CuSO4): 10 mg; Iodine Ca $(IO3)_2$: 1.0 mg; Iron (FeSO4_H2O): 80 mg; Manganese (MnSO4_H2O): 100 mg; Selenium (NaSeO3): 0.15 mg; Zinc (ZnSO4_H2O): 80 mg and Cobalt (CoSO4): 0.5 mg.

pH determination

On 21 and 42 days of age, 2 birds from each replicate were euthanized by cervical dislocation. The pH of various segments of GIT (crop, gizzard, different parts of small intestine and cecum) were assayed.

One g sample from contents of each segment were homogenized by distilled water (2 mL) and pH was determined by digital pH-meter (Metrohm, Germany) according to Chaveerach *et al.* 2004.

Statistical analysis

A completely randomized design was employed. One-way analysis of variance was performed using the GLM procedure of SAS software (SAS, 2004). Duncan's multiple range tests were used for means comparison. Statistical significance was considered at P < 0.05.

RESULTS AND DISCUSSION

Performance

The effects of dietary treatments on broiler performance are presented in Table 2. The lowest feed intake (FI) was observed in S group (P<0.05), which was similar to C one at 22-42 days of age (P>0.05).

The highest body weight (BW) was gained in N treatment (P<0.05), which was similar to T set at 22-42 and 1-42 periods (P>0.05). The highest and lowest feed conversion ratio (FCR) were in C and N groups at 22-42 and 1-42 days of age, respectively (P<0.05).

Microbial population

The results of treatments on microflora population are shown in Table 3. The lowest total of aerobic bacteria in crop was attained to S group at 21 days of age (P<0.05). A significant decrease in coliforms was found in ileum contents in all experimental treatments as compared to C group at 21 day of age (P<0.05). Termin-8 and N treatments caused a significant increase in the lactic acid bacteria in crop at 42 days of age (P<0.05). Inversely acidifiers' treatments caused a significant decrease in total aerobic bacteria and coliforms in crop at day 42 (P<0.05). All acidifiers' treatments caused a significant increase in lactic acid bacteria in ileum at 42 days of age (P<0.05). The lowest and highest total aerobic counts and total coliforms in ileum were obtained in N and C groups at day 42 of experiment, respectively (P<0.05). Moreover, the lowest and highest lactic acid bacteria counts in cecum were obtained in control and N groups at 42 days of age, correspondingly (P<0.05).

Digestibility

The effects of dietary treatments on nutrient digestibility are shown in Table 4. The lowest and greatest DM and GE digestibility coefficients were attained in C and N groups, respectively (P<0.05). No significant differences were observed in OM and CP digestibility (P>0.05). The more EE digestibility was obtained in N group (P<0.05) which was similar with T group.

Gastrointestinal tract pH

The treatment effects on pH of different GIT sites are shown in Table 5. All experimental treatments caused a significant decrease in crop pH at 21 and 42 days of age in comparison with C group (P<0.05). Selko-pH treatment caused a significant decrease in gizzard pH at 21 and 42 day of age (P<0.05). A significant decrease in pH of duodenum, jejunum and ileum were seen in all experimental treatments as compared to C group at 21 and 42 days of age (P<0.05). Also, organic acids treatments caused a significant decrease in cecum pH at 42 days of age (P<0.05).

The use of organic acids in feed and water has a significant effect on FI. In this experiment, using N and T acidifiers in broiler diets improved FI, but the use of S acidifier in broiler drinking water decreased FI.

This highlighted importance of acidifier administration. Skinner *et al.* (1991) reported that FI increased by adding fumaric acid to cockerels feed. They suggested that feed consumption was increased because of reduction in the bacteria load and improvement in the palatability of diet. Denli *et al.* (2003) showed that adding propionic acid in water decreased FI in turkeys.

It is reported that the use of organic acid affect FI (Cave, 1984; Adil *et al.* 2011; Khosravi *et al.* 2012). These data are in agreement with results of the present study. It is proposed that organic acids control FI by affecting feed regulation center (Cave, 1984).

Moreover, the presence of some elements (such as Cu) in the used mixtures, variations in feed ingredients, nutrient levels and acidifier administration way might also have a multiple effects on feed palpability and FI.

The N treatment had better WG compared to the T group. Roy *et al.* (2002) showed that addition of a blend of organic acids significantly decreased the WG of male and female turkeys at 21 day of age. Vale *et al.* (2004) showed that a 0.5% blend of organic acids in feed led to improvement in WG at 42 day of age.

Furthermore, some studies observed that different levels of formic acid (0 to 7.5%) had a significant effect on WG of birds.

The results of present study are in opposite with studies conducted by Izat *et al.* (1990), Vale *et al.* (2004) and Adil *et al* (2011), but are in agreement with results of other researches (Ranho *et al.* 1997; Roy *et al.* 2002). The observed improvement in WG and FCR might be due to direct antimicrobial effect of the used acidifier administration that may affect the integrity of microbial metabolism.

Moreover, pH reducing properties of acidifiers might have resulted in intestinal bacteria inhibition, reducing bacterial competition with host and lessened bacterial fermentation resulting in the improvement of protein and energy digestibility, thereby ameliorating the WG, FCR and performance of broiler chicken (Adil *et al.* 2011). Vogt and Matthes (1982) reported that diet supplementation with some organic acids improved FCR in broiler and laying hens.

It has been suggested that organic acids by producing an appropriate pH in the gut would improve the microflora, reduce gut harmful bacteria, increase utilization of nutrients, and improve performance of chickens (Roser, 2006).

Table 2 The effects of dietary treatments on performance of broiler chicken

Measurement	Treatments					
	C	S	T	N	SEM	P-value
Feed intake (g/day/bird)						
Days 1-21	59.61[a]	55.08[b]	59.56[a]	59.26[a]	0.769	0.007
Days 22-42	158.07[bc]	154.08[c]	165.52[a]	162.15[ab]	1.45	0.004
Days 1-42	108.51[a]	102.23[b]	111.34[a]	109.83[a]	1.21	0.009
Weight gain (g/day/bird)						
Days 1-21	38.53	38.97	39.36	40.00	0.330	0.497
Days 22-42	71.09[b]	70.49[b]	78.07[ab]	82.35[a]	1.90	0.046
Days 1-42	52.84[b]	53.28[b]	56.83[ab]	59.95[a]	1.00	0.008
Feed conversion ratio (g/g)						
Days 1-21	1.54	1.41	1.51	1.48	0.022	0.192
Days 22-42	2.23[a]	2.18[ab]	2.12[ab]	1.97[b]	0.041	0.043
Days 1-42	2.05[a]	1.91[ab]	1.95[ab]	1.83[b]	0.030	0.032
Mortality (%)						
Days 1-21	4.77	4.55	4.44	4.22	0.201	0.864
Days 22-42	0.68	0.00	0.00	0.00	0.290	0.0601
Days 1-42	6.66	6.44	6.22	5.73	0.085	0.811

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
C: control; S: selko-pH; T: termin-8 and N: neogermycin.
SEM: standard error of means.

Table 3 The effects of dietary treatments on microbial population ($\log_{10}$ cfu/g)

Measurement	Dietary treatment					
	C	S	T	N	SEM	P-value
Crop day 21						
Total aerobic	8.85[a]	8.11[c]	8.24[c]	8.43[b]	0.074	0.0001
Lactic acid bacteria	7.88[b]	8.14[a]	8.04[ab]	8.09[a]	0.072	0.036
Total coliforms	3.98[a]	3.85[c]	3.88[bc]	3.92[ab]	0.020	0.006
Crop day 42						
Total aerobic	9.83[a]	9.46[b]	9.58[b]	9.56[b]	0.042	0.003
Lactic acid bacteria	8.61[b]	8.67[ab]	8.85[a]	8.89[a]	0.026	0.006
Total coliforms	4.93[a]	4.73[b]	4.79[b]	4.75[b]	0.024	0.002
Ileum day 21						
Total aerobic	8.87[a]	8.77[b]	8.64[c]	8.60[c]	0.029	0.0001
Lactic acid bacteria	8.05[b]	8.37[a]	8.38[a]	8.41[a]	0.039	0.0001
Total coliforms	6.33[a]	5.99[b]	6.05[b]	6.01[b]	0.038	0.0001
Ileum day 42						
Total aerobic	9.87[a]	9.62[b]	9.69[b]	9.44[c]	0.045	0.0003
Lactic acid bacteria	9.09[b]	9.36[a]	9.38[a]	9.44[a]	0.039	0.0003
Total coliforms	7.12[a]	6.73[b]	6.77[b]	6.05[c]	0.101	0.0001
Cecum day 21						
Total aerobic	8.42[a]	8.41[a]	8.47[a]	8.31[b]	0.020	0.007
Lactic acid bacteria	9.87[c]	9.99[b]	10.09[a]	10.11[a]	0.019	0.0001
Total coliforms	8.68[a]	8.50[b]	8.48[b]	8.11[c]	0.057	0.0001
Cecum day 42						
Total aerobic	9.40	9.31	9.39	9.36	0.027	0.701
Lactic acid bacteria	10.73[b]	10.82[b]	10.84[ab]	10.98[a]	0.031	0.019
Total coliforms	9.01	8.89	8.94	8.92	0.030	0.563

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
C: control; S: selko-pH; T: termin-8 and N: neogermycin.
SEM: standard error of means.

Microbial population of GIT significantly influences intestinal functions. The effects of treatments on GIT microbial population were significantly different in this study. Overall, increase trend was observed in the formation of all colonies from ileum to cecum and with birds ageing in all treatments. The organic acid treatments reduced coliforms and total aerobic bacteria counts at 21 and 42 days of age. It is reported that the addition of propionic acid buffer significantly decreased the total number of coliforms in comparison to the control (Izat *et al.* 1990).

However, buffering capacity of feed and diet ingredients are effective in organic acid effectiveness. It is noted that

Table 4 Effects of dietary treatments on nutrient digestibility at 42 day of age

Digestibility %	Dietary treatment					
	C	S	T	N	SEM	P-value
DM	72.65[b]	74.56[ab]	76.71[ab]	78.91[a]	0.868	0.040
OM	70.44	72.29	73.87	75.01	0.812	0.220
CP	73.91	72.51	74.88	75.88	1.01	0.721
EE	62.39[b]	62.78[b]	64.19[ab]	68.86[a]	0.968	0.047
GE	80.73[b]	83.82[ab]	84.03[ab]	87.65[a]	0.827	0.011

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
C: control; S: selko-pH; T: termin-8 and N: neogermycin.
DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extracts and GE: gross energy.
SEM: standard error of means.

Table 5 The pH of different parts of gastrointestinal tract in response to dietary treatment (21 and 42 days of age)

Measurement	Dietary treatment					
	C	S	T	N	SEM	P-value
			Day 21			
Crop	4.65[a]	3.62[c]	3.90[b]	3.87[b]	0.119	0.0001
Gizzard	3.19[a]	2.79[c]	2.86[cb]	2.88[b]	0.047	0.0001
Duodenum	6.35[a]	5.95[b]	6.03[b]	5.98[b]	0.052	0.0041
Jejunum	6.31[a]	6.11[b]	6.05[b]	5.97[b]	0.045	0.016
Ileum	6.37[a]	6.14[b]	6.11[b]	6.02[b]	0.043	0.005
Cecum	6.42[a]	6.26[b]	6.22[b]	6.15[c]	0.031	0.0001
			Day 42			
Crop	4.93[a]	3.80[b]	4.13[b]	4.10[b]	0.142	0.005
Gizzard	3.41[a]	2.98[c]	3.06[cb]	3.08[b]	0.050	0.0001
Duodenum	6.46[a]	6.14[b]	6.19[b]	6.17[b]	0.044	0.006
Jejunum	6.43[a]	6.22[b]	6.18[b]	6.16[b]	0.040	0.020
Ileum	6.49[a]	6.32[b]	6.30[b]	6.23[b]	0.036	0.031
Cecum	6.54[a]	6.42[b]	6.38[b]	6.35[b]	0.025	0.021

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
C: control; S: selko-pH; T: termin-8 and N: neogermycin.
SEM: standard error of means.

some elements in commercial organic acid combinations could make agglutination of cytoplasm proteins of pathogenic bacteria and increase the favorable microflora (Roser, 2006). The study has shown that addition of organic acids in broilers diets decreases bacteria in the small intestine and cecum (Adams, 2004). In present experiment using organic acid based acidifiers reduced harmful bacteria and increased the population of lactic acid in GIT as compared to C group. It is documented that organic acids presence in broiler chicken diets has pH reducing properties (Canibe *et al.* 2001; Abdel Fattah *et al.* 2008). The lowered pH is conducive for the growth of favorable bacteria simultaneously hampering the growth of pathogenic bacteria which grow at relatively higher pH (Langhout, 2000; Canibe *et al.* 2001). These findings are in agreement with results of current study and could be good explanation for obtained results.

The beneficial effect of organic acids on performance is probably related to a more efficient use of nutrients, which in turn results in improvement of FCR. This effect was seen almost in all birds in present study which were fed the acidifiers based organic acids. Improved retention of DM, OM, CP, ash and NDF by using organic acid was reported in numerous studies in swine and broilers (Boling *et al.* 2001; Hernandez *et al.* 2006; Ao *et al.* 2009).

Increased GIT enzymatic secretions and increased favorable microflora induced by acidifier based diets could have beneficial effects on the utilization and nutrients digestibility.

Partanen and Mroz (1999) reported that addition of organic acids in pig diets did not affect the digestibility of protein. On the other hand, Biggs and Parsons (2008) observed that digestibility of protein in the broiler diets was improved by addition of organic acids, which is in disagreement with current study.

The lower microbial proliferation produced by organic acids in the GIT reduces the competition of the microflora with the host for nutrients, improving nutrient digestion, absorption and reducing endogenous losses. The source and level of organic acids are critical in relation to obtained digestibility results. In addition, the extent and proportion of the improved digestibility effect of non antibiotic additives depends on the sanitary conditions of the place where tests are developed.

Organic acids exert their antimicrobial action in feed and GIT of the animal. The antibacterial activity increases with a decreased pH value because organic acids can maintain their un dissociated form, in which they are able to enter the microbial cell, a characteristic that depends on the pKa value of the respective acid (Ravindran and Kornegay, 1993). The antibacterial effect of dietary organic acids in broiler chickens is believed to occur mainly in the upper part of the digestive tract, such as crop and gizzard (Canibe *et al.* 2001), where pH is more appropriate for the action of these acids.

The capacity to change the pH of digesta in other sections of digestive tract of broiler chickens is open to debate. Whereas, the results of current study indicated that all organic acids treatments, in comparison with control diet, caused a decrease in pH of all measured parts. These results are inconsistent with finding of Král *et al.* (2011). Type of based acidifier might be involved in obtained results.

CONCLUSION

According to results of current study it could be concluded that acidifiers based organic acids improve the microflora of GIT by reducing harmful bacteria and producing appropriate pH. Utilization of nutrients was better and led to better performance. Further studies are required to clear the effects of tested commercial acidifiers based organic acids in industrial poultry farms.

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