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Effects of acidified drinking water on performance, carcass, immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine

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ABSTRACT

A 2 × 4 factorial arrangement of treatments was used to investigate the effects of 2 levels of an acidifier supplement (0 or 350 mL/1000 L) in drinking water on growth performance, carcass, immune response, intestinal microbial flora, and jejunum morphology of broiler chickens fed diets based on 4 levels of dietary Thr (100, 110, 120, and 130% of the requirements). A total of 320 broiler chickens were assigned to 8 treatments with 4 replicate pens of 10 broiler chickens per pen. Growth performance traits, including daily weight gain, feed intake, and feed conversion ratio (FCR), were recorded. The broiler chickens immunized against Newcastle disease virus (NDV) at 8 d of age. Blood samples were drawn from the wing vein 7 and 14 d after vaccination for the determination of primary and secondary antibody responses. In addition, blood samples were collected in tubes containing anticoagulant to determine the number of heterophil (H) and lymphocyte (L). At the end of the experiment, 4 broiler chickens per treatment were selected and killed, and, then, the relative weights of carcass parts, jejunum morphology, and intestinal microbial population were determined. The results showed that broiler chickens received acidified drinking water (ADW) had greater ($P < 0.05$) feed intake (28.51 vs. 27.30 g/chicken/d) and weight gain (20.01 vs. 19.26 g/chicken/d) than those received without water additive during the starter period. Neither Thr nor ADW had any effect on any carcass traits of broiler chickens. Heterophil count and H to L ratio were increased (35.87 and 0.63%, respectively) and lymphocyte count was decreased (56.50%) in broiler chickens fed diets containing 110% threonine ($P < 0.05$). However, antibody titer against NDV was not influenced by experimental treatments. In the jejunum, the villus width (136.8 μm) and crypt depth (188.9 μm) were greater and the ratio of VH to CD was lower (6.50) in broiler chickens received ADW ($P < 0.05$). Moreover, the population of *Escherichia coli* decreased (5.79 vs. 6.23 $\log_{10}$ cfu/g) in broiler chickens received ADW, while *lactobacilli* population increased (7.45 vs. 6.90 $\log_{10}$ cfu/g; $P < 0.05$). However, dietary Thr had no effect on jejunum morphology and intestinal microbial population of broiler chickens. The present findings indicate that ADW improves growth performance (0–10 d), jejunum morphology, and intestinal *lactobacilli* population of broiler chickens. In addition, the use of 110% Thr increased H to L ratio in broiler chickens. However, different graded concentrations of Thr did not alter growth performance, intestinal morphology, and microbiota activity.

1. Introduction

Acidifiers (organic acids) are important additives which can be included in the diets of poultry as a suitable alternative for antibiotic growth promoters. Several studies have demonstrated that supplemental dietary organic acids have a significant effect on growth performance (Ogunwole et al., 2011; Rafacz-Livingston et al., 2005), nutrient utilization (Ao et al., 2009), intestinal morphology (Cengiz et al., 2012), and microbial population (Chaveerach et al., 2002) of broiler chickens. However, in recent years, addition of organic acids in drinking water is another implementation in the broiler farms for improving growth performance (Açıkgöz et al., 2011; Alzawqari et al., 2013; Chaveerach et al., 2004). Acidifier added to the diet promotes machine corrosion, moisture absorption and acid volatilization during the process of granulating or storing (Zhu et al., 2014). Therefore, it is hypothesized that addition of organic acids via drinking water can avoid these problems. It is well known that drinking water is the most important factor for the spread of bacterial infection on the farm. Most studies have concentrated mainly on the effects of

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water acidification on *Campylobacter* and *Salmonella* contaminations in broilers (Byrd et al., 2003; Chaveerach et al., 2004; van Bunnik et al., 2012). However, data on the effects of acidified water on other species of intestinal bacteria in broiler chickens are limited. Therefore, it seems that addition of organic acids to drinking water may decrease *Escherichia coli* populations in the digestive tract of broiler chickens.

Threonine is the third most limiting amino acid, especially in a low crude protein diet (Rezaeipour et al., 2012). Threonine has been added to poultry and swine diets to meet dietary amino acid requirements. Threonine is involved in important metabolic processes, such as uric acid formation and protein synthesis. Also, poultry are not capable of synthesizing Thr de novo, which makes it a nutritionally indispensable amino acid. It has been reported that Thr is an important component of mucus (40% of protein in mucus glycoproteins) in the digestive tract (Carlstedt et al., 1993; Corzo et al., 2007). Mucins are not highly digestible and the associated Thr cannot be recovered (Fuller, 1994). Therefore, it is necessary to meet Thr in broiler diets by adding L-threonine or use soybean meal and meat meal as most important ingredients, which supply Thr in the poultry diets. There are many reports on the Thr requirements of broiler chickens (Corzo et al., 2007; Kidd et al., 1997, 2005; Rosa et al., 2001), but no information is available on the effects of different levels of dietary Thr in combination with an acidifier supplement on immune response, intestinal morphology, and microbial population.

Therefore, the objective of this research was to investigate the effects of ADW and four levels of dietary Thr in broiler chicken diets on growth performance, immune response, carcass characteristics, intestinal morphology, and microflora population.

2. Materials and methods

2.1. Broiler chickens and dietary treatments

All animal care and use procedures were approved by the Department of Animal Science, Islamic Azad University (Qaemshar Branch, Qaemshar, Iran). The study was conducted at a commercial broiler chicken farm (Sari, Mazandaran, Iran).

Three hundred and twenty 1-d-old broiler chickens (Ross 308) were obtained from a local hatchery (Zarbal Company, Amol, Iran) and randomly allocated into 8 treatments with 4replicate pens of 10 broiler chickens per pen. The broiler chickens were kept in floor pens (1.0×1.7 m^2) for the experimental period of 42 d. Each pen was equipped with a separate feeder and a manual drinker. The house temperature was maintained at 35 °C during the first week, and it was reduced 2 °C per week until reaching the temperature of 23 °C. The broiler chickens were provided access to feed and water ad libitum.

All experimental diets were formulated to meet or exceed the energy and nutrient requirements (Aviagen, 2009). The composition of the experimental diets is presented in Table 1. The experiment used a completely randomized design with a 2×4 factorial arrangement of treatments, including supplementation of drinking water with organic acids [0 or 350 mL Agrocid Solution (AGROCID, Belgium)/1000 L] and 4 dietary Thr (100, 110, 120 or 130% of the requirements). The acidifier product contained lactic acid, formic acid, propionic acid, sorbic acid, and citric acid. Fresh water was provided every for all the treatment groups.

2.2. Measurements

2.2.1. Growth performance and carcass characteristics

The broiler chickens were fed the 8 experimental diets until 42 d of age. Feed intake and body weight gain of each pen was measured at the end of each week. Feed conversion ratio for each pen was calculated by dividing feed intake by body weight gain. Mortality was recorded and weight gain feed consumption data were corrected accordingly.

Table 1
Composition of basal diets (as-fed basis).

Item	Starter d 0 to 10	Grower d 10 to 24	Finisher d 24 to 42
Ingredient (g/kg)			
Maize	589.0	606.6	634.4
Soybean meal (440 g CP/kg)	349.5	327.8	290.5
Soybean oil	12.0	25.0	37.20
Oyster shell	13.9	11.2	11.30
Dicalcium phosphate	18.4	16.3	14.90
Common salt	4.60	4.10	3.60
Vitamin premix[a]	2.50	2.50	2.50
Mineral premix[b]	2.50	2.50	2.50
DL-Met	3.70	2.60	2.20
L-Lys-HCl	2.90	1.10	0.80
L-Thr	1.00	0.20	0.10
Chemical composition			
ME (MJ/kg)	12.35	12.77	13.19
CP (g/kg)	224.4	213.1	196.8
Ca (%)	10.3	8.70	8.40
Available P (%)	4.90	4.40	4.10
Na (%)	2.00	1.80	1.60
Lys (%)	14.0	12.1	10.8
Met+Cys (%)	10.6	9.30	8.50
Thr (%)	9.20	8.10	7.40

[a] Provides per kilogram of diet: 9000 IU vitamin A; 2000 IU vitamin D3; 18 IU vitamin E; 2 mg menadion; 1.8 mg thiamine; 6.6 mg riboflavin; 30 mg niacin; 3 mg pyridoxine; 15 μg vitamin B12; 100 mg D-pantothenic acid; 1 mg folic acid; 0.1 mg biotin; 500 mg choline chloride; and 100 mg antioxidant.
[b] Provides per kilogram of diet: 100 mg Mn; 84.7 mg Zn; 50 mg Fe; 10 mg Cu; 1 mg I; and 0.2 mg Se.

At the end of the study (42 d of age), 1 broiler chicken from each pen, which was close to the mean weight of the pen, was selected and killed by cervical dislocation for the assessment of carcass characteristics, intestinal morphology, and microflora population. After removing viscera manually, carcass characteristics, including the weight of the breast, thigh, liver (without gallbladder), pancreas, heart, gizzard, intestine and proventriculus, was recorded. The weight of bursa and spleen, as lymphoid organs, was also recorded. All carcass data are presented based on percent of live weight of each broiler chicken.

2.2.2. Jejunum morphology

A segment of the jejunum (2 cm) was excised for morphological evaluation. The jejunum was defined as the midway between the end of the duodenum and Meckel's diverticulum. The jejunum segments were flushed clean with phosphate buffered saline to avoid damage to the tissues. Samples were fixed in Clark solution for 1 h. Samples were then transferred in 50% ethanol solution. A 0.5-cm section was processed, embedded in paraffin, stained with eosin blue, and examined with a light microscope. The 15 longest and straightest villi and associated crypts were measured in each segment.

2.2.3. Microbial enumeration

At 42 d of age, 4 broiler chickens (1/pen) per treatment were selected, weighed, and killed by cervical dislocation. The intestinal tract of each broiler chicken was removed and samples of fresh digesta (1–2 g) from the ileum (Meckel's diverticulum to 1 cm proximal to the ileocecal junction) and ceca were collected and gently placed in sterile sampling tubes. Samples were put on ice untill they were transported to the laboratory for enumeration of microbial populations. The populations of *E. coli* and *Lactobacilli*