Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: Parviz Allahdo, Javad Ghodraty, Heydar Zarghi, Zohre Saadatfar, Hassan Kermanshahi & Mohammad Reza Edalatian Dovom (2018) Effect of probiotic and vinegar on growth performance, meat yields, immune responses, and small intestine morphology of broiler chickens, Italian Journal of Animal Science, 17:3, 675-685, DOI: 10.1080/1828051X.2018.1424570

To link to this article: https://doi.org/10.1080/1828051X.2018.1424570

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Published online: 12 Jan 2018.

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ITALIAN JOURNAL OF ANIMAL SCIENCE, 2018
VOL. 17, NO. 3, 675–685
https://doi.org/10.1080/1828051X.2018.1424570

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Effect of probiotic and vinegar on growth performance, meat yields, immune responses, and small intestine morphology of broiler chickens

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ABSTRACT

The present study was conducted to investigate the effect of dietary supplementation of probiotic and drinking water (DW) supplemented with different levels of vinegar on performance, meat yields, immune responses, and small intestine histology of broiler chickens. Three hundred thirty day-old Ross 308 male broiler chicks assigned to six treatments in a completely randomised design experiment with a factorial arrangement (2×3) and five replicates of 11 chicks each. The treatments included two levels of dietary probiotic supplementation (0 and 1×10^{10} CFU lactic acid/kg of diet) and three levels (0%, 1%, and 2%) of DW supplemented with vinegar (5% acetic acid concentration). The study lasted from 1 to 42 d. Growth performance, meat yields and lymphoid organs relative weight, humoral and cellular immune responses, and small intestine histomorphometry were measured. The average daily feed intake (ADFI) and feed conversion ratio (FCR) during 1–10 days of age significantly decreased in the birds that fed supplemented diet with probiotic and drunk supplemented water with vinegar than the birds fed and drunk free of any additive. Experimental treatments did not have a significant effect on performance during other growth periods, carcase yields, and lymphoid organs relative weight. DW supplemented with vinegar significantly increased villus height (VH), crypt depth (CD) and decreased small intestine muscular thickness (MT) and abdominal fat. Dietary supplementation of probiotic significantly improved immune response to sheep red blood cell (SRBC) inoculation. In conclusion, this study confirms beneficial effects of probiotic and vinegar on 1–10 d performance, immune and intestine health of broiler chickens.

ARTICLE HISTORY
Received 21 October 2017
Revised 23 December 2017
Accepted 3 January 2018

KEYWORDS
Broiler chickens; performance; immune response; intestine morphology; vinegar

Introduction

The gastrointestinal tract (GIT) of newly hatched chick is immature and sterile. It begins to develop functional and microbial flora when the chick starts to ingest feed. At this time, the chick is very susceptible to pathogenic microorganisms (Dehghani-Tafti and Jahanian 2015). Furthermore, under the modern systems of broiler chicken production, birds are exposed the considerable stress during their productive lifetime that increase birds' susceptibility to diseases. Continuous and long-term use of antibiotics for control diseases in poultry production may lead to the presence of these compounds in products (Samli et al. 2007; Baurhoo et al. 2009). Since 1997, the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) have paid much attention to the potential risks of the addition of antibiotics stimulating growth in livestock and poultry diets. Finally, use of antibiotic in Europe was banned from 2006 (Chaves et al. 2008). Therefore, it has become necessary to develop antibiotic alternatives by using either beneficial microorganisms (probiotics) or other ingredients that enhance microbial growth. This has led to the application of non-antibiotics substances (Yang et al. 2007).

A probiotic defined as a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microflora (Fuller 1989). The purpose of feeding probiotics is to stabilise beneficial microbes, to prevent the accumulation of gastrointestinal harmful bacteria and subsequently, to help maintain animal health (Patterson and Burkholder 2003). Probiotics make effective changes in the intestinal microbial population and maintain its natural microbial

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flora by stimulating the growth and proliferation of useful bacteria. Improvement in growth performance and feed efficiency of broiler chickens confirmed by feeding probiotics supplements (Cavazzoni et al. 1998; Zulkifli et al. 2000; Mountzouris et al. 2007; Samli et al. 2007; Murshed and Abudabos 2015; Abudabos et al. 2017).

The short chain fatty acids (SCFA) considered as the potential alternative to antibiotics (Van Immerseel et al. 2004). Acetic acid is one such SCFA, which has higher bactericidal activity when the acid is un-dissociated. The bacterial cell takes up un-dissociated fatty acid, which, by ionising fatty acid inside the bacterial cell, there is a change in the intracellular pH leading to the death of bacterial (Khan and Iqbal 2016). Organic acids added to feed for their various beneficial effects on gut function and microflora, feed preservation from microbial invasion, inhibition of pathogenic bacteria, enhancing mineral absorption, and improvement of nutrient digestibility (Dehghani-Tafti and Jahanian 2015).

Since the levels of SCFA are quite low in the intestine of young chicks, so they may be the best candidates for use of acidifiers (Panda et al. 2009; Abudabos et al. 2017). The addition of organic acids in diet can have a beneficial effect on the performance of poultry by decreasing pathogenic bacteria. Currently, drinking water (DW) acidification is another implementation in the broiler industry used for improving performance. Subsequent studies indicated that addition of organic acid to the DW helps to reduce the level of pathogens in the water and the crop or proventriculus, to regulate gut microflora, to increase the digestion of feed and to improve growth performance (Khan and Iqbal 2016). The use of probiotic and acidifier together are more beneficial than the use of those alone (Rodríguez-Lecompte et al. 2012; Agboola et al. 2015; Murshed and Abudabos 2015). The present study was carried out to evaluate the effect of vinegar (as a native and inexpensive acidifier with easy access) combined with probiotic on broiler chickens performance, carcase yields, immunological responses and the small intestinal morphology.

Materials and methods

Birds, housing, and rearing conditions

The present study was performed in the Animal and Poultry Research Station of Ferdowsi University of Mashhad (Mashhad, Iran). The Animal Care Committee, Ferdowsi University of Mashhad, approved the experimental procedure. Three hundred thirty one-day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery. The chicks were individually weighed, and were randomly distributed between 30 pens. Each pen has one square metre space and, its floor covered with wood shaving. The house initially temperature was maintained at $32 \pm 2\,^{\circ}C$ and gradually decreased (2.5 °C every week) to reach a constant temperature of 20–22 °C at 24 days of age. During the experimental period, relative humidity and light-darkness programme were kept in the 50–60% and 23:1 h of light: darkness, respectively. In the course of the experiment, the chickens had continuous access to water and feed.

Additives, diet formulation, and experimental design

Prior to formulating the experimental diets, the main feed ingredients were analysed, through Evonik–Degussa office in Tehran, Iran and the data were used to formulate the experimental diets. The probiotic used in this experiment was bacterial probiotic *Lactobacillus*-Bactocell (*Pediococcus acidilactici MA 18/5M*; Lallemand Animal Nutrition, Saint-Simon, France) with the number of lactic acid-producing live bacteria 1×10^{10} CFU/g of supplement. The vinegar used in this experiment was apple vinegar with 5% acetic acid concentration. The basal diets (Table 1) were formulated by using the UFFDA software to meet the nutritional requirements of broiler chickens as provided by nutrition specifications for Ross 308 broilers, target live weight 1.70–2.40 kg (Aviagen 2014), for different growth periods; starter (1–10 days of age), grower (11–24 days of age), and finisher (25–42 days of age). The experimental design was done with a combination of probiotic and vinegar in a 2×3 factorial arrangement of a completely randomised design (CRD) experiment with six treatments and five replicates/treatment. Treatments consisted two levels (0 and 1×10^{10} CFU lactic acid/kg of diet) dietary supplementation of probiotic and three levels (0%, 1% and 2%) DW supplemented with vinegar '5% acetic acid'. The diets were provided in a way that a batch of basal diet for each age period was made and then divided into two equal portions, the definite dosage of probiotic added on top of each diet and mixed to make the two dietary treatments. DW was supplemented with three different levels (0%, 1%, and 2%) of vinegar.

Measurements

Performance traits

All birds (pen groups) were weighed at 1, 11, 24, and 42 days of age. The birds have fasted for 4 h prior to

Table 1. Feed ingredients and nutrient composition of the experimental basal diets during different growth periods[a].

Items	Starter (1–10 days of age)	Grower (11–24 days of age)	Finisher (25–42 days of age)
Ingredient, g/kg			
Corn (7.75% CP)	491.7	521.5	570.0
Soybean meal (44.25% CP)	421.5	387.0	335.5
Soybean oil	42.0	51.9	58.1
Limestone	15.6	13.4	11.7
Dicalcium phosphate	1.41	13.1	11.9
Sodium chloride	1.9	2.3	1.9
Sodium bicarbonate	1.5	0.9	1.5
Vitamin premix[b]	2.5	2.5	2.5
Mineral premix[c]	2.5	2.5	2.5
DL-Methionine	3.5	2.9	2.6
L-Threonine	1.2	0.7	0.7
L-Lysine HCL	2.0	1.3	1.2
Nutrient composition[d]			
Metabolizable energy, kcal/kg	3000	3100	3200
Crude protein, g/kg	230	215	195
Calcium, g/kg	9.6	8.7	7.8
Available phosphorus, g/kg	4.8	4.3	3.9
Sodium, g/kg	1.6	1.6	1.6
Digestible *Lys*, g/kg	12.8	11.5	10.3
Digestible *Met*, g/kg	6.5	5.8	5.3
Digestible *Met* + *Cys*, g/kg	9.5	8.7	7.9
Digestible *Thr*, g/kg	8.6	7.7	6.0

[a]A batch of basal diet for each age periods were made and then divided into two equal portions and the probiotic supplement was added in replace of equivalent weights of starch on top of each portion and mixed to make the two dietary treatments.
[b]Vitamin premix supplied the following per kilogram of diet. vitamin A (all-trans-retinol), 12,000 U; vitamin D3 (cholecalciferol), 5000 U; vitamin E (α-tocopherol), 18 U; vitamin K3 (menadione), 2.65 mg; vitamin B1 (thiamin), 2.97 mg; vitamin B2 (riboflavin), 8.0 mg; vitamin B3 (niacin), 57.42 mg; vitamin B5 (pantothenic acid), 17.86 mg; vitamin B6 (pyridoxine), 4.45 mg; vitamin B9 (folic acid), 1.9 mg; vitamin B12 (cyanocobalamin), 0.02 mg; vitamin H2 (biotin), 0.18 mg; choline chloride, 487.5 mg, and antioxidant 1.0 mg.
[c]Mineral premix supplied the following per kilogram of diet. Zn (zinc sulphate), 84.7 mg; Mn (manganese sulphate), 120.6; Fe (iron sulphate), 40.5; Cu (copper sulphate), 16.1; I (calcium iodate), 1.26; Se (sodium selenite), 0.31; choline chloride, 474.0.
[d]Determined by ingredient analysis and used result for calculated nutritional composition.

being weighed. Dead birds in each pen were recorded once observed to correct the growth performance traits. The growth performance as average daily gain (ADG) and average daily feed intake (ADFI) was recorded and calculated periodically (1–10, 11–24, 25–42, and 1–42 days of age) by pen basis. The feed conversion ratio (FCR) or the amount of feed needed to increase the live weight unit was calculated by dividing the feed intake by weight gain.

Humoral immune response

Sheep red blood cells (SRBCs), as a non-pathogenic antigen, were used for evaluating the humoral immune response of broiler chickens. Ten birds from each treatment were injected with 0.1 mL of 0.5% SRBC suspension into the brachial vein at 28 and 35 days of age, and blood samples were collected at the seventh day of post-inoculation. Subsequently, the micro hemagglutination activity (HA) of serum was estimated and the antibody concentration ($\log_2$) measured following the standard procedure (Wegmann and Smithies 1966).

Cellular immune response

The cell-mediated immune (CMI) response was assessed by measuring the cutaneous basophils hypersensitivity (CBH) response to intra-dermally injection phytohemagglutinin-P (PHA-P) at 42 days of age. The thickness of the web between the third and fourth inter-digital space on the left and right feet was measured with a micrometre. Ten broilers from each treatment were injected with 100 μg of PHA-P suspended in 0.1 mL of phosphate buffered saline (PBS) into the web of right foot, while in each bird the left web (control) was injected with 0.1 mL of PBS. The web swelling of both feet was measured 8, 16, and 24 h after the injection. The response was determined by subtracting the skin thickness of the first measurement from the second and the values of left foot (control) from the right foot (Corrier and Deloach 1990).

Slaughter and sampling procedures

At the end of the study (42 days of age), 10 birds from each treatment (two bird/pen close to the average pen weight) selected and after 4 h of feed withdrawal but with free access to DW, weighed, slaughtered, plucked, and visceral organs excised to determine the carcase and organs relative weight. Carcass obtained by removing the head, feathers, feet, and visceral organs. After chilling for 24 h at 4 °C, carcases were weighed and were cut according to a

standardised procedure to determine the meat yield (Tabatabaei et al. 2017). Carcass, breast, legs, and visceral organ weights were determined by weighing scale (0.001-g, model GF 400; A&D Weighing, San Jose, CA, USA), and calculated meat and organs relative weight (g/100 g of live body weight). A portion (0.5 cm in length) of the small intestine segments (duodenum, jejunum, and ileum) taken from the midpoint, flushed with 0.9% saline to remove the contents and then fixed in 10% neutral buffered formalin solution for histological study. The fixative solution of the tissue samples replaced after 24 h and then samples kept until histologically work.

Intestinal morphology

Tissue samples were taken out from formaldehyde and placed in the automatic processing machine, dehydrated through a series of graded ethanol baths to displace the water, cleared in xylene, and were infiltrated with paraffin. The infiltrated tissue samples embedded in wax blocks. Imbedded tissue samples sectioned at 5 μ thickness using an auto microtome. The slides were prepared and processed for staining with Hematoxylin and Eosin (H&E). All chemicals were purchased from Sigma-Aldrich Co. (Sigma-Aldrich Chemical Co, St. Louis, MO).

Micrographs were taken with an Olympus BX41 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera. The images were analysed using image software. Morphometric measurements were performed on nine villi chosen from each sample. Morphometric indices included were villus height (VH) from the tip of the villus to the crypt, crypt depth (CD) from the base of the villus to the submucosa, villus width (VW; average of VW at one-third and two-thirds of the villus) and muscular thickness (MT) from the submucosa to the external layer of the intestine (Ebrahimi et al. 2017). The villus surface area (VSA) was calculated according to the Formula 1 (Gangali et al. 2015).

$$\text{Formula 1}: \text{VSA} = \frac{1}{2} \times \text{VW} \times \text{VH} \times 2\,\pi$$

VSA = Villus surface area, VW = villus width, VH = Villus height and $\pi = 3.14$.

Statistical analysis

The obtained data were analysed for the main effects of different levels of vinegar added to DW and probiotic added to the diet and for interactions between them. Log_2 transformations were done on blood antibody concentration prior to statistical analysis. The results of the experiment were analysed in a completely randomised design with the factorial method, using statistical software SAS 9.1.3, general linear model (GLM) procedure (SAS 2003). Mean comparison was done by Duncan test, the results were considered statistically significant when $p < .05$. To assess the effect of vinegar level, orthogonal contrasts were used. The mathematical model of the statistical design was according to the Formula 2.

$$\text{Formula 2}: Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} = the value of the desired trait, μ = total mean, α_i = the effect of vinegar level in water, β_j = the effect of adding probiotic to the diet, $(\alpha\beta)_{ij}$ = the interaction between adding vinegar to DW and adding probiotic to the diet and ε_{ijk} = test error per observation.

Results and discussion

Performance

As shown in Table 2, the effects dietary supplementation of probiotic, DW supplemented with vinegar and the interaction between them on ADG were not significant ($p > .05$). Dietary supplementation of probiotic had a significant effect on ADFI during starter (1–10 days of age) period ($p < .02$). The interaction effects of adding probiotic to diet with adding vinegar to DW on ADFI and FCR during the starter period were significant ($p < .05$). The birds fed diet supplemented with probiotic and drank water supplemented with vinegar showed significantly lower ADFI and FCR than the birds fed diet and drunk water without any supplementation. In the birds which drunk water supplemented with vinegar ADFI and FCR significantly decreased contrast those drunk water free additives during the starter and grower period. The results showed that dietary supplementation of probiotic and DW supplemented with vinegar and the interaction between them did not have a significant effect on performance trait during the grower (11–24 days of age), finisher (25–42 days of age), and the whole (1–42 days of age) of growth periods. Total mortality percentage and production efficiency factor (PEF) were 9.08% and 282, respectively and were not significant differences among experimental groups (Table 3).

The results obtained from this research are in agreement with Yu et al. (2007) that reported, during the growing or finishing periods, the probiotic inclusion did not significantly affect the body weight gain, feed intake, and feed conversion. In contrast, some researchers have a positive assessed from dietary

Table 2. Effect of drinking water (DW) supplemented with different levels of vinegar on performance of male broiler chickens fed diets with/without probiotic (Pro.) supplemetation[c].

		ADFI, g/d per chicken				ADG, g/d per chicken				FCR, g feed/g gain			
Pro[d]	Vinegar[e]	1–10 d	11–24 d	25–42 d	1–42 d	1–10 d	11–24 d	25–42 d	1–42 d	1–10 d	11–24 d	25–42 d	1–42 d
None	0%	27.21[a]	78.10	123.40	85.40	18.57	53.17	66.84	50.79	1.47[a]	1.55	1.84	1.70
	1%	24.94[a,b]	72.74	125.93	84.16	18.23	53.23	67.72	51.10	1.39[ab]	1.41	1.84	1.65
	2%	25.06[a,b]	77.34	131.95	88.30	17.89	54.11	67.90	51.40	1.42[ab]	1.49	1.94	1.73
Sup.	0%	24.79[a,b]	79.33	133.53	89.57	17.75	54.08	67.55	51.20	1.40[ab]	1.56	1.93	1.75
	1%	24.11[b]	74.74	125.35	84.38	18.15	53.80	66.80	50.88	1.38[b]	1.44	1.84	1.65
	2%	23.58[b]	71.00	130.99	85.42	17.86	50.62	71.24	51.66	1.34[b]	1.45	1.84	1.67
SEM		0.81	2.46	6.31	3.30	0.66	1.50	1.87	1.03	0.04	0.05	0.09	0.06
None		25.74[a]	76.06	127.10	85.95	18.23	53.50	67.49	51.10	1.43	1.49	1.87	1.69
Sup.		24.16[b]	75.03	129.96	86.46	17.92	52.83	68.53	51.25	1.37	1.48	1.87	1.69
SEM		0.46	1.42	3.65	1.91	0.38	0.86	1.08	0.59	0.02	0.03	0.05	0.04
	0%	26.00	78.72	128.46	87.49	18.16	53.62	67.20	51.00	1.43	1.55[a]	1.88	1.73
	1%	24.53	73.74	125.64	84.27	18.19	53.52	67.26	50.99	1.39	1.43[b]	1.84	1.65
	2%	24.32	74.17	131.47	86.86	17.87	52.37	69.57	51.53	1.38	1.47[a,b]	1.89	1.70
SEM		0.57	1.73	4.46	2.33	0.47	1.06	1.32	0.72	0.03	0.04	0.06	0.04
Source of variation		*p* value											
Pro.		.02	.61	.58	.84	.57	.59	.50	.85	.29	.98	.97	.97
Vinegar		.09	.10	.65	.60	.87	.65	.36	.83	.66	.05	.82	.52
1% and 2% vs. 0%		.03	.03	.98	.50	.82	.60	.45	.76	.37	.02	.82	.38
1% vs. 2%		.80	.86	.36	.44	.64	.45	.22	.60	.94	.35	.56	.44
Pro. × OA		.04	.19	.61	.56	.80	.28	.53	.94	.04	.80	.58	.62

ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio; SEM: standard error of the mean.
[a,b]Mean values within the same variable for each effect with unlike superscript letters were significantly different ($p < .05$).
[c]Every mean for the probiotic, vinegar and interaction effects is the average of 15, 10 and 5 pens of 11 chicks each, respectively.
[d]Probiotic (Pro.) supplemented at the levels of 0 and 1×10^{10} CFU lactic acid producing live bacteria/kg of diet.
[e]Drinking water supplemented at the levels of 0%, 1% and 2% vinegar with 5% acetic acid concentration/litre of drinking water.

Table 3. Effect of drinking water (DW) supplemented with different levels of vinegar on meat yields, abdominal fat, mortality and the production efficiency factor (PEF) in male broiler chickens fed diets with/without probiotic (Pro.) supplementation.

		Meat yields (% of live body weight)[c]					
Pro[d]	Vinegar[e]	Carcass	Breast	Legs	Abdominal fat[c]	Mortality (%)	PEF[f]
None	0%	72.33	26.70	20.48	1.67	8.79	280
	1%	72.85	26.50	20.08	1.15	9.27	288
	2%	72.80	26.00	19.70	1.20	8.94	277
Sup.	0%	72.50	25.50	19.40	1.50	9.27	273
	1%	73.10	26.60	19.30	0.90	8.94	287
	2%	73.20	26.20	20.30	0.80	9.27	290
SEM[g]		0.91	0.81	0.59	0.25	0.17	13.36
None		72.66	26.40	20.09	1.34	9.00	282
Sup.		72.93	26.10	19.67	1.07	9.16	283
SEM[g]		0.53	1.04	0.34	0.14	0.10	7.72
	0%	72.42	26.10	19.94	1.59[a]	9.03	277
	1%	72.98	26.55	19.69	1.03[b]	9.11	288
	2%	73.00	26.10	20.00	1.00[b]	9.11	284
SEM[g]		0.64	1.28	0.42	0.18	0.12	9.45
Source of variation		*p* value					
Pro.		.17	.38	.37	.20	.26	.89
Vinegar		.77	.67	.87	.03	.88	.72
1% and 2% vs. 0%		.55	.65	.60	.01	.61	.46
1% vs. 2%		.25	.35	.45	.45	.95	.75
Pro. × OA		.50	.70	.34	.50	.06	.74

[a,b]Mean values within the same variable for each effect with unlike superscript letters were significantly different ($p < .05$).
[c]Every mean for the probiotic, vinegar and interaction effects is the average of 30, 20 and 10 chicks, respectively.
[d]Probiotic (Pro.) supplemented at the levels of 0 and 1×10^{10} CFU lactic acid producing live bacteria/kg of diet.
[e]Drinking water supplemented at the levels of 0%, 1% and 2% vinegar with 5% acetic acid concentration/litre of drinking water.
[f]PEF: [liveability (%) × live weight (kg)] / [age (d) × FCR] × 100.
[g]SEM: standard error of the mean.

supplementation of probiotic (Samli et al. 2007; Baurhoo et al. 2009). These positive effects may influenced by the general characteristics of probiotics, such as the production of lactic acid, the competitive elimination of pathogenic bacteria, and the improvement of the condition of the intestine (Patterson and Burkholder 2003). The reason for not seeing the positive effects of adding organic acid to a diet or DW on functional parameters in some experiments can be due to the health conditions of rearing and proper feeding of broiler chickens (Hernández et al. 2006).

The results of this experiment showed that ADFI reduced by the addition of probiotic to diet during the starter period. Adding probiotics to diet will improve the bioavailability of nutrients and minerals by better solubilising and absorbing (Khaksefidi 2005), thereby it will be reducing feed intake. Brenes et al. (2003) reported, adding different levels of citric acid from 1.5% to 3.1% to diet decrease the passage velocity of GIT contents and reduce the bird's feed intake (Brenes et al. 2003). Our results showed that ADFI and FCR reduced by DW supplemented with vinegar, it might be due to the strong taste associated with the organic acids, which would have decreased the bird's appetite, thereby reducing feed intake. The birds that drunk water supplemented with vinegar, FCR significantly decreased than those drunk non-supplemented water. The improvement in feed efficiency could be possibly due to better utilisation of nutrients with organic acids supplementation (Khan and Iqbal 2016). Although, against to the results of this experiment and the above reports, Yu et al. (2007) reported adding probiotic lactobacillus to broiler diets did not effect on FCR.

Carcass yield

The addition of probiotic to diet and vinegar to DW and the interaction between them did not have a significant effect on carcase yield (Table 3). Only, DW supplementation of vinegar significantly effect on the abdominal fat percentage ($p < .03$). Abdominal fat as a percentage of live body weight at 42 days of age in the birds that drunk 1% and 2% vinegar supplemented water was lower than those drunk non-supplemented water (1% and 1.03% vs. 1.59%).

Similar with our results, reported that organic acid has no effect on the carcase yield (Skinner et al. 1991; Leeson et al. 2005; Garcia et al. 2007). The result of our experiment show adding vinegar to DW can reduce the accumulation of abdominal fat, similarly, Agboola et al. (2015) reported that dietary supplementation of organic acids, probiotics, and prebiotics can reduce serum cholesterol and abdominal fat of broiler chickens. It is accepted that inhibiting the absorption of dietary fat and fatty acid synthesis, and/or promoting fatty acid β-oxidation reduces abdominal fat deposition by decreasing the size and/or the number

Table 4. Effect of drinking water (DW) supplemented with different levels of vinegar on immunity response of broiler chickens fed diets with/without probiotic (Pro.) supplementation.[c]

		Lymphoid organs relative weight (%)[f]		Variation webbed toes thickness hours after PHA-P injection (mm)			Antibody titres (log_2) response to SRBC					
							Seven days post first inoculation			14 days post first inoculation		
Pro.[d]	Vinegar[e]	Bursa of Fabricius	Spleen	8	16	24	Ig T	Ig Y	Ig M	Ig T	Ig Y	Ig M
None	0%	0.20	0.10	0.38	0.24[b]	0.10[b]	4.33	1.67	2.67	5.33	2.67	2.67
	1%	0.15	0.12	0.45	0.52[a,b]	0.59[a,b]	3.17	1.67	1.50	4.50	2.00	2.50
	2%	0.13	0.11	0.46	0.57[a,b]	0.61[a,b]	3.83	1.83	2.00	3.83	2.33	1.50
Sup.	0%	0.16	0.09	0.62	0.71[a]	0.78[a]	4.67	1.50	3.17	4.50	2.17	2.33
	1%	0.14	0.11	0.52	0.32[a,b]	0.76[a]	3.83	1.50	2.33	4.50	2.83	1.67
	2%	0.15	0.11	0.37	0.26[b]	0.53[a,b]	5.33	2.17	3.17	4.83	3.00	1.83
SEM[g]		0.02	0.02	0.15	0.16	0.18	0.55	0.22	0.55	0.52	0.53	0.42
None		0.16	0.11	0.43	0.44	0.44	3.78[b]	1.70	2.06[b]	4.56	2.33	2.22
Sup.		0.15	0.10	0.50	0.43	0.69	4.61[a]	1.72	2.89[a]	4.61	2.67	1.94
SEM[g]		0.01	0.01	0.08	0.09	0.10	0.32	0.13	0.32	0.30	0.31	0.24
	0%	0.18	0.09	0.50	0.47	0.44	4.50	1.58	2.92	4.92	2.42	2.50
	1%	0.15	0.11	0.48	0.42	0.67	3.50	1.58	1.92	4.50	2.42	2.08
	2%	0.14	0.12	0.41	0.42	0.57	4.58	2.00	2.58	4.33	2.67	1.67
SEM[g]		0.02	0.02	0.10	0.11	0.13	0.38	0.16	0.39	0.37	0.38	0.29
Source of variation		*p* value										
Pro.		.52	.60	.55	.92	.10	.07	.95	.07	.89	.45	.42
Vinegar		.25	.14	.82	.91	.46	.10	.12	.20	.52	.86	.16
1% and 2% vs. 0%		.65	.58	.68	.68	.27	.34	.29	.17	.27	.79	.09
1% vs. 2%		.61	.77	.64	.97	.58	.06	.08	.24	.75	.64	.32
Pro. × OA		.37	.86	.57	.05	.03	.55	.45	.83	.23	.41	.39

[a,b]Mean values within the same variable for each effect with unlike superscript letters were significantly different ($p < .05$).
[c]Every mean for the probiotic, vinegar and interaction effects is the average of 30, 20 and 10 samples, respectively.
[d]Probiotic (Pro.) supplemented at the levels of 0 and 1×10^{10} CFU lactic acid producing live bacteria/kg of diet.
[e]Drinking water supplemented at the levels of 0%, 1% and 2% vinegar with 5% acetic acid concentration/litre of drinking water.
[f]Lymphoid organs relative weight (g/100 g of live body weight) at 42 days of age.
[g]SEM: standard error of the mean.

of abdominal adipose cells. The regulation of lipid metabolism to reduce the abdominal fat content based on dietary composition and feeding strategy, as well as elucidating their effects on the key enzymes associated with lipid metabolism, could facilitate the production of lean meat and help to understand the fat-lowering effects of diet and different feeding strategies (Fouad and El-Senousey 2014).

Immunological response

As presented in Table 4, dietary supplementation of probiotic and watery supplemented with vinegar had no significant effect on the relative weight of lymphoid organs ($p > .05$). Dietary supplementation of probiotic significantly ($p < .07$) increased the primary immune response to SRBC injection, serum IgT and IgM concentration significantly increase in response to the SRBC inoculation but in the second stages it is not significant ($p > .05$). The interaction effect of dietary supplemented of probiotic and DW supplemented with vinegar on the cellular immune response to PHA-P injection was significant ($p < .05$).

The ineffectiveness of adding vinegar to DW on lymphoid organs relative weight that observed in our study is consistent with Brisbin et al. (2008). Against, reported use of organic acids leads to an increase in the number of contributing cells in immune and then increases the bursa of Fabricius weight (Haque et al. 2010). The adding vinegar to DW and or probiotic to diet significantly increased the thickness of the web between the third and fourth inter-digital space on the left foot that measured within 24 h after PHA-P injection ($p < .05$). The response to the CBH test by subcutaneous injection of PHA-P is a thymus-dependent response that occurs through T lymphocytes. In other words, this assay used to measure T-cell activity in cellular immunity. The swollen skin caused by the presence of leukocytes and fluid filtration after PHA-P injection. PHA-P binds to glycoproteins and attaches to the surface of T cells. PHA-P stimulates lymphocyte T and produces lymphokine, resulting in increased vascular permeability and leukocytes invading the injection site (Grasman 2010).

Intestinal morphology

Drinking water supplemented with vinegar lead to an increase in the VH and CD and a decrease in the MT thorough the small intestine, although the values were

Table 5. Effect of drinking water (DW) supplemented with different levels of vinegar on histomorphological measurements of small intestine of broiler chickens fed diets with/without probiotic (Pro.) supplementation at 42 days of age[c].

		Duodenum[f]						Jejunum[f]						Ileum[f]					
Pro[d]	Vinegar[e]	VH	VW	CD	MT	AVSA	VH/CD	VH	VW	CD	MT	AVSA	VH/CD	VH	VW	CD	MT	AVSA	VH/CD
None	0%	1526	177	221	201[a]	855	7.00	1777	189	191	194	1050[a,b]	9.37	1281[b]	153	165	206	614	7.76
	1%	1766	167	231	195[a]	924	7.79	1599	172	216	195	864[c]	7.43	1288[b]	179	153	206	719	8.42
	2%	1897	222	251	206[a]	1310	7.63	1629	150	216	164	754[c]	7.55	1176[b]	162	185	168	603	6.36
Sup.	0%	1752	185	251	213[a]	1022	7.02	1465	196	233	180	899bc	6.36	1278[b]	172	179	196	689	7.14
	1%	1814	150	208	180[a,b]	853	9.22	1850	181	219	183	1051[a,b]	8.45	1127[b]	186	185	198	651	6.09
	2%	1929	184	296	155[b]	1109	6.72	1747	219	245	146	1206[a]	7.30	1532[a]	129	180	170	619	8.51
SEM4		98.41	11.56	16.08	6.72	84.39	0.77	99.00	15.78	9.59	6.24	92.08	0.65	41.79	12.40	4.43	5.32	43.66	0.55
None		1730	188	234	201[a]	1030	7.47	1669	170[b]	208	183	886[b]	8.12	1248	165	167[b]	193	654	7.51
Sup.		1831	173	251	183[b]	995	7.65	1687	199[a]	232	170	1041[a]	7.06	1312	162	181[a]	188	653	7.25
SEM4		63.17	6.72	9.28	3.88	48.72	0.442	58.31	9.11	5.53	3.60	49.96	0.38	24.12	7.16	2.56	3.07	25.20	0.32
	0%	1639[b]	181[a,b]	236[b]	207[a]	939[b]	7.01	1621	192	212	186[a]	975	7.87	1279[a,b]	163[a,b]	172[b]	201[a]	652	7.45
	1%	1790[a,b]	158[b]	219[b]	188[b]	888[b]	8.51	1725	176	218	189[a]	953	7.93	1207[b]	183[a]	169[b]	202[a]	685	7.26
	2%	1913[a]	203[a]	273[a]	181[b]	1210[a]	7.18	1688	184	231	155[b]	980	7.43	1354[a]	145[b]	183[a]	169[b]	611	7.43
SEM4		77.36	8.17	11.37	4.75	59.67	0.54	71.42	11.16	6.78	4.41	61.19	0.46	29.55	8.77	3.13	3.76	30.87	0.39
Source of variation		*p* value																	
Pro.		.26	.12	.20	.02	.61	.77	.82	.03	.20	.55	.03	.50	.07	.80	.01	.85	.82	.45
Vinegar		.06	.02	.01	.03	.01	.12	.58	.60	.19	.03	.94	.25	.01	.02	.01	.04	.25	.64
1% and 2% vs. 0%		.03	.97	.45	.01	.14	.22	.33	.38	.43	.22	.96	.85	.97	.90	.32	.24	.91	.56
1% vs. 2%		.27	.01	.02	.95	.01	.09	.72	.61	.25	.01	.75	.10	.01	.01	.01	.02	.10	.46
Pro. × OA		.62	.16	.10	.03	.11	.32	.06	.10	.45	.85	.01	.25	.01	.10	.09	.25	.27	.11

[a,b]Mean values within the same variable for each effect with unlike superscript letters were significantly different ($p < .05$).
[c]Every mean for the probiotic, vinegar and interaction effects is the average of 30, 20 and 10 samples, respectively.
[d]Probiotic (Pro.) supplemented at the levels of 0 and 1×10^{10} CFU lactic acid producing live bacteria/kg of diet.
[e]Drinking water supplemented at the levels of 0%, 1% and 2% vinegar with 5% acetic acid concentration/litre of drinking water.
[f]VH: Villus Height; VW: Villus Width; CD: Crypt Depth; MT: Muscular thickness; AVSA: Apparent villus surface area; and VH/CD: Villus height/Crypt depth; SEM: standard error of the mean.

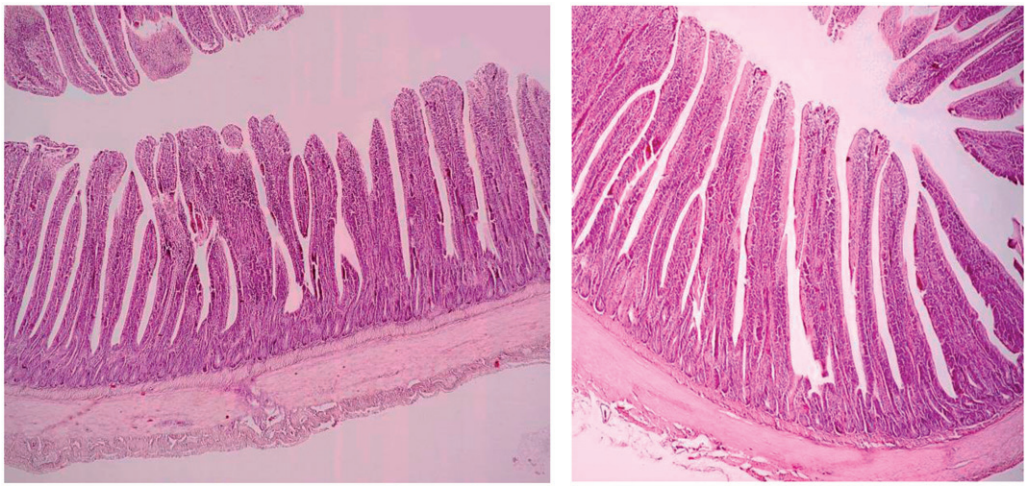

Figure 1. Effects of drinking water supplemented with vinegar on the histology of the duodenum in broiler chickens 42 days of age. Optical micrographs are shown of the duodenum stained with Hematoxylin and Eosin (H&E) of a chickens drunk (left) none supplemented water and (right) supplemented drinking water with vinegar.

significant in the duodenum and ileum and in the jejunum segment only MT was significant ($p < .03$). Chickens that drunk water contained 2% vinegar had the longer villi, deeper CD and thinner MT than those drunk none supplemented water. Dietary supplementation of probiotic increased CD and decreased MT, but the values were significant for MT in the duodenum and for CD in the ileum segment of small intestine (Table 5). Figure 1 shows cross-sections of the duodenum in a chicken at 42 days of age that drunk supplemented water with 2% vinegar compared a bird fed and drunk non-supplemented diet and water.

The results obtained from this study showed dietary supplementation of probiotic and DW supplemented with vinegar significantly improved intestinal growth and development. The results of another experiment showed that intestinal morphometric characteristics such as increase the VH and CD following the incorporation of organic acid in the diet have improved (Garcia et al. 2007). The length and diameter of the intestinal villi are morphological indexes, each increment leading to an increase in its absorption ability (Marković et al. 2009). Therefore, this kind of morphological changes induced in the intestine can indicate the effect of growth promoters on changes in the level of intestinal absorption and hence the change in the performance of broiler chickens (Oliveira et al. 2008). The histological observation of intestinal parts revealed that DW supplemented with vinegar increased the VH of different segments of the small intestine than the birds' drunk non-supplemented water (Table 5). Increasing the VH suggests an increased surface area capable of greater absorption of available nutrients (Caspary 1992). Consequently, used of organic acid reduced intestinal colonisation and infectious process, thereby, decreased inflammatory process at the intestinal mucosa, this improved VH and function of secretion, digestion and absorption of nutrients (Khan and Iqbal 2016).

Results obtained from this experiment indicate by either dietary supplementation of probiotic or DW supplemented with vinegar significantly increase intestinal CD; this reveals an increasing in the epithelial cell turnover. Similarly, Rodríguez-Lecompte et al. (2012) reported supplementation of a combined probiotics and organic acids to broiler diets resulted in inconsistent gut morphology associated responses where their effects were more apparent in the duodenum and ileum when the gut is fully developed. Increases in the VH and CD correlated with increased epithelial cell turnover (Fan et al. 1997). The villus crypt considered as the villus factory, deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue (Yason et al. 1987). Increases in the VH and VH to CD ratio directly correlated with increased epithelial cell turnover, and longer villi are associated with activated cell mitosis (Awad et al. 2009).

Conclusions

The current study revealed that broiler chickens DW supplemented with vinegar and dietary supplementation of probiotic improves feed efficiency during 1–10 days of age. No effects of dietary supplementation of probiotic and or DW supplemented with vinegar on relative weights of lymphoid organs, and meat yields

found. DW supplemented with vinegar significantly increased VH, CD and decreased small intestine muscular thickness measured at 42 days of age. Another important finding of the present study was the reduction in carcase abdominal fat content by DW supplementation of vinegar. Dietary supplementation of probiotic improved primary immune response to SRBC inoculation. Finally, probiotic, and organic acid might be promising alternatives for to eliminate antibiotic in broiler chicken production.

Disclosure statement

No potential conflict of interest was reported by the authors. The authors alone are responsible for the content and writing of this article.

Funding

We greatly appreciate the financial support of this research from vice president of research at the Ferdowsi University of Mashhad, Iran (Project code: 3/30644).

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References

Abudabos AM, Alyemni AH, Dafalla YM, Al-Owaimer AN. 2017. Effect of organic acid blend and bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to clostridium perfringens challenge during the finisher phase. J Anim Plant Sci. 27:1101–1107.

Abudabos AM, Alyemni AH, Dafalla YM, Khan RU. 2017. Effect of Organic acid blend and bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant status in broilers exposed to salmonella typhimurium challenge during the starter phase. J Appl Anim Res. 45:538–542.

Agboola AF, Omidiwura BRO, Odu O, Popoola IO, Iyayi EA. 2015. Effects of organic acid and probiotic on performance and gut morphology in broiler chickens. S Afr J Aim Sci. 45:494–501.

Aviagen. 2014. Ross 308: broiler nutrition specification.

Awad WA, Ghareeb K, Abdel-Raheem S, Böhm J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci. 88:49–56.

Baurhoo B, Ferket PR, Zhao X. 2009. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poult Sci. 88:2262–2272.

Brenes A, Viveros A, Arija I, Centeno C, Pizarro M, Bravo C. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim Feed Sci Tech. 110:201–219.

Brisbin JT, Zhou H, Gong J, Sabour P, Akbari MR, Haghighi HR, Sharif S. 2008. Gene expression profiling of chicken lymphoid cells after treatment with *Lactobacillus acidophilus* cellular components. Dev Comp Immunol. 32:563–574.

Caspary WF. 1992. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr. 55:299S–308S.

Cavazzoni V, Adami A, Cstrivilli C. 1998. Performance of broiler chickens supplemented with Bacillus coagulans as probiotic. Br Poult Sci. 39:526–529.

Chaves AV, Stanford K, Dugan MER, Gibson LL, McAllister TA, Van Herk F, Benchaar C. 2008. Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. Livest Sci. 117:215–224.

Corrier DE, Deloach JR. 1990. Evaluation of cell mediated cutaneous basophil hypersensitivity in young chickens by an inter-digital skin test. Poult Sci. 69:403–408.

Dehghani-Tafti N, Jahanian R. 2015. Effect of supplemental organic acids on performance, carcass characteristics, and serum biochemical metabolites in broilers fed diets containing different crude protein levels. Anim Feed Sci Tech. 211:109–116.

Ebrahimi E, Sobhani RS, Zarghi H. 2017. Effect of triticale level and exogenous enzyme in the grower diet on performance, gastrointestinal tract relative weight, jejunal morphology and blood lipids of Japanese Quail (Coturnix coturnix Japonica). J Agr Sci Tech. 19:569–580.

Fan YK, Croom J, Christensen VL, Black BL, Bird AR, Daniel LR, McBride BW, Eisen EJ. 1997. Jejunal glucose uptake and oxygen consumption in turkey poults selected for rapid growth. Poult Sci. 76:1738–1745.

Fouad AM, El-Senousey HK. 2014. Nutritional factors affecting abdominal fat deposition in poultry: a review. Asian-Aust J Anim Sci. 27:1057–1068. eng.

Fuller R. 1989. Probiotics in man and animals. J Appl Bacteriol. 66:365–378.

Gangali H, Raji AR, Zarghi H. 2015. Effect of post hatch delayed access to feed on performance, GIT physical and histological development and yolk absorption in young broiler chicks. Biomed Pharmacol J. 8:945–955.

Garcia V, Catala Gregori P, Hernandez F, Megias MD, Madrid J. 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. J Appl Poult Res. 16:555–562.

Grasman KA. 2010. In vivo functional tests for assessing immunotoxicity in birds. Methods Mol Biol. 598:387–398.

Haque MN, Islam KM, Akbar MA, Chowdhury R, Khatun M, Karim MR, Kemppainen BW. 2010. Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. Can J Anim Sci. 90:57–63.

Hernández F, García V, Madrid J, Orengo J, Catalá P, Megias MD. 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. Br Poult Sci. 47:50–56.

Khaksefidi ASR. 2005. Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. Asian-Aust J Anim Sci. 18:1153–1156.

Khan SH, Iqbal J. 2016. Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res. 44:359–369.

Leeson S, Namkung H, Antongiovanni M, Lee EH. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult Sci. 84:1418–1422.

Marković R, Šefer D, Krstić M, Petrujkić B. 2009. Effect of different growth promoters on broiler performance and gut morphology. Arch Med Vet. 41:163–169.

Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K. 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci. 86:309–317.

Murshed MA, Abudabos AM. 2015. Effects of dietary inclusion of probiotic, prebiotic and symbiotic on growth performance of broiler chickens. Braz J Poultry Sci. 117:99–104.

Oliveira MC, Rodrigues EA, Marques RH, Gravena RA, Guandolini GC, Moraes VMB. 2008. Performance and morphology of intestinal mucosa of broilers fed mannan-oligosaccharides and enzymes. Arq Bras Med Vet Zootec. 60:442–448.

Panda AK, Rao SVR, Raju MVLN, Sunder GS. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Aust J Anim Sci. 22:1026–1031.

Patterson JA, Burkholder KM. 2003. Application of prebiotics and probiotics in poultry production. Poult Sci. 82: 627–631.

Rodríguez-Lecompte JC, Yitbarek A, Brady J, Sharif S, Cavanagh MD, Crow G, Guenter W, House JD, Camelo-Jaimes G. 2012. The effect of microbial-nutrient interaction on the immune system of young chicks after early probiotic and organic acid administration. J Anim Sci. 90:2246–2254.

Samli HE, Senkoylu N, Koc F, Kanter M, Agma A. 2007. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch Anim Nutr. 61:42–49.

SAS. 2003. User's guide: Statistics Version 9.1. Cary, NC: S.A.S Institute.

Skinner JT, Izat AL, Waldroup PW. 1991. Research note: fumaric acid enhances performance of broiler chickens. Poult Sci. 70:1444–1447.

Tabatabaei YF, Golian A, Zarghi H, Varidi M. 2017. Effect of wheat-soy diet nutrient density and guanidine acetic acid supplementation on performance and energy metabolism in broiler chickens. Ital J Anim Sci. 16:593–600.

Van Immerseel F, Fievez V, de Buck J, Pasmans F, Martel A, Haesebrouck F, Ducatelle R. 2004. Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with *Salmonella enteritidis* in young chickens. Poult Sci. 83:69–74.

Wegmann TG, Smithies O. 1966. A simple hemagglutination system requiring small amounts of red cells and antibodies. Transfusion. 6:67–73.

Yang P, Iji PA, Choct M. 2007. Effects of different dietary levels of mannan oligosaccharide on growth performance and gut development of broiler chickens. Asian-Aust J Anim Sci. 20:1084–1091.

Yason CV, Summers BA, Schat KA. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am J Vet Res. 48:927–938.

Yu B, Liu JR, Chiou MY, Hsu YR, Chiou PWS. 2007. The effects of probiotic *Lactobacillus reuteri* Pg4 strain on intestinal characteristics and performance in broilers. Asian-Aust J Anim Sci. 20:1243–1251.

Zulkifli I, Abdullah N, Azrin NM, Ho YW. 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus cultures* and oxytetracycline under heat stress conditions. Br Poult Sci. 41:593–597.