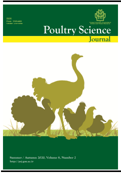

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Effects of Different Levels of Date Waste Vinegar in Diet and Water on Growth Performance, Gastrointestinal Tract Morphology, Ileal Microflora and Immune Response of Broilers

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Abstract

To investigate the effects of dietary supplementation with industrial vinegar (IV) and waste date vinegar (WDV) on growth performance, intestinal morphology, and immune response of broilers, five hundred Ross 308 chickens were randomly divided into 50 cages in a 42-day breeding period. The experiment consisted of ten treatments with five replications in each treatment including; control 1 (standard feed without WDV or IV), control 2 (control 1 + 2% water into the feed), 1, 2, and 3% of WDV and 2% industrial vinegar (IV) into the feed and 0.5, 1, and 1.5% of WDV and 1% industrial vinegar (IV) into the water. One chicken from each replicate was slaughtered on days 24 and 42 to investigate gastrointestinal tissue growth as well as intestinal morphology. The results showed that growth performance was not affected by treatments in any period. The height and width of the villus in the treatments containing 1% of WDV into the water and 2% of WDV into the feed increased with time compared to the control treatments. On day 42 of the experiment, the crypt depth was also higher in the treatment containing 1% of WDV into the feed compared to the other treatments. The ileal coliforms were also affected by WDV addition into the feed at 42 days of age compared to other groups ($P < 0.05$). The highest amount of IgM and IgG were found to levels of 2% and 1% of WDV into the diet, respectively ($P < 0.05$). However, SRBC, ND titter was not affected by treatments. Also, no difference was found between industrial vinegar in water or feed in most of the studied parameters. The results of this study showed that supplementation of the diet with WDV had a positive effect on intestinal morphology and immune system of broilers compared to industrial vinegar.

Introduction

The importance of using agricultural wastes in animal diets can be tested from different aspects, which include the growing trend of the country's livestock population. This trend indicates the need to make optimum use of available feed capacity and economical savings in livestock and poultry production using agricultural waste as cheap and useful alternative sources. Organic acids as synthetic varieties are currently used in poultry feeds. They are expensive and most farmers do not have access to these acids. Researchers are working on organic products due to the inhibition of a vast range of drugs for animal production. Organic acids, have growth stimulating properties and antimicrobial effects and have shown a good alternative to antibiotics (Paul *et al.*, 2007; Rasschaert *et al.*, 2016).

In recent years, several studies were focused on using probiotics (Awad *et al.*, 2006) and organic acids (Gunal *et al.*, 2006) in poultry diets, since they improve the intestinal tract function. It has been shown that yeast products contain beneficial nutrients such as peptides, oligosaccharides, flavorings, and aromatic substances that improve intestinal health against pathogens (Roto *et al.*, 2015). Probiotic products can also reduce the population of pathogenic bacteria such as *Escherichia coli* and reduce the pH of the small intestine of poultry, which in turn bird

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performance improves due to increased nutrient absorption in the gastrointestinal tract. Therefore, there is an increasing trend to study the effects of these products on growth performance and gut health in the poultry industry by offering antibiotic-free diets (Placha *et al*., 2014; Pirgozliev *et al*., 2015).

The fermentation of waste dates crushed in water produces vinegar, which is acidic and has viable microorganisms good for the growth and production of poultry. The use of organic acids as feed additives in animal production is one of the non-therapeutic alternatives (Adil *et al*., 2011). It has been proven that probiotics can be slow down the growth of some gastrointestinal bacteria and increase nutrient absorption that ultimately leads to improving the digestive system. According to the annual production of more than 160,000 tons of palm waste across the country and the need for its use in the agricultural industry, the use of such feed sources in livestock and poultry is strongly recommended. The use of organic acids such as citric acid to increase feed intake and consequently live weight has been reported in broilers (Waldroup *et al*., 1995). The increased feed efficiency of chickens may be partly explained by improved intestinal morphology and increased nutrient absorption capacity (Yang *et al*., 2018). It assumes that the use of date vinegar in the diet of poultry might have some positive effects due to its acidic properties and the presence of beneficial microbes in vinegar. Therefore, this study aimed to evaluate the effects of industrial and waste date vinegar on performance, ileal microflora, and immune system of broiler chickens.

Materials and Methods

Preparation of date vinegar

To prepare scrap date vinegar, each kilogram of scrap date was mixed with three liters of water and nucleated for three days. The tank continuously stirred for a week and added sodium chloride as salt (15 g/kg scarp date) and vinegar (250 ml /kg scarp date) for 30 days and kept warm and tight. The total bacterial colonies in the waste date's vinegar in this project were evaluated according to the standard method (Tasharofi *et al.,* 2017).

Table 1. Dietary ingredients and chemical composition of basal diet in starter, grower, and finisher periods of broiler chickens

Ingredients (%)	Starter diet (0-10 days)	Grower diet (11-24 days)	Finisher diet (25-42 days)
Corn	51.32	53.82	54.49
Soybean meal 44%	40.09	36.52	35.82
Soybean oil	3.98	5.80	6.29
Limestone	1.09	0.86	0.80
Di-Calcium phosphate	1.87	1.64	1.50
Common salt	0.37	0.37	0.37
Vitamin and Mineral premix[1]	0.50	0.50	0.50
DL-Methionine	0.38	0.29	0.22
L-Threonine	0.11	0.05	-
L- Lysine HCL	0.29	0.15	0.01
Calculated nutrients (%)			
ME (kcal/kg)	3050	3175	3280
Crude protein	22.95	21.11	21.37
Crude fat	2.29	2.34	2.40
Crude fiber	3.95	3.77	3.68
Calcium	1.05	0.9	0.85
Available Phosphorus	0.50	0.45	0.43
K	0.96	0.90	0.87
Cl	0.32	0.29	0.26
Na	0.16	0.16	0.16
Lysine	1.43	1.24	1.09
Methionine	0.71	0.61	0.53
Methionine + Cystine	1.07	0.95	0.86
Tryptophan	0.33	0.30	0.29
Arginine	1.45	1.35	1.31
Threonine	0.94	0.83	0.76
Electrolyte balance (mEq/kg[2])	227.80	220	220

[1]One kilogram vitamin and mineral premix included: vitamin A as acetate, 8800 IU; vitamin D3, 2500 IU; vitamin E (as dl-α tocopherol) 15 IU, vitamin K3, 2.2 mg; Vitamin B12, 0.01 mg, Thiamine, 1.5 mg; Riboflavin, 4 mg; Niacin 35 mg, Folic acid 0.5 mg; Biotin, 0.15 mg; Pyridoxine 2.5 mg; Pantothenate, 8mg; Choline chloride, 50 mg; Betaine 190 mg; Zinc, 65 mg; Magnesium, 75 mg; selenium, 0.2 mg; iodide, 0.9 mg; Copper, 6 mg; Iron,75 mg.
[2] Electrolyte balance as mEq per kilogram diets calculated by $Na^+ + K^+ - Cl^-$.

Animals, diets, and experimental

The experimental procedures were approved by the Ferdowsi University of Mashhad Animal Care and Use Committee (Protocol No. 88364111). In this experiment, different amounts of vinegar were used in water and the diet. 500 day-old mixed-sex broiler chickens (Ross 308) were allocated to ten experimental treatments in a balanced completely randomized design with 50 pens (100 * 100 cm^2 each) and 10 chicks in each pen. Experimental treatments were prepared by adding different levels of waste date vinegar (WDV) to feed or industrial vinegar (IV) into drinking water and in the feed as follows: control 1 (without WDV or IV) in feed or water; control 2 (control 1 + 2% water into the feed); 1, 2, and 3% of WDV, and 2% industrial vinegar (IV) in feed, 0.5, 1, and 1.5% of WDV, and 1% industrial vinegar (IV) into the water. Dietary ingredients and chemical composition of experimental diets are shown in Table 1. All chickens had free access to feed and water. Chickens were raised under similar environmental conditions based on Ross 308 management recommendations for 42 days. At the end of each rearing period (days 10, 24, and 42) live weight and feed intake were recorded, and feed conversion ratio (FCR) was calculated.

On days 24 and 42 of breeding periods, one chicken from each cage was selected and killed for immunity and digestive system assays, ileal microbial population, the weight of different parts of the small intestine, and jejunal morphology (including villus height (VH), villus width (VW) and crypt depth (CD). To ascertain the sheep red blood cell (SRBC) test after washed sheep red blood cell injection to broiler chickens at 36 days of age, the samples of blood via wing veins were taken. Using the hemagglutination inhibition test (HI) according to the method explained by Cheema *et al.* (2003), Newcastle disease (ND) and SRBC antigen, geometric mean titers were assessed. Immunoglobulin titer was also conducted by a single radial immunodiffusion (SRID) technique (Sarker *et al.*, 1999).

Jejunal morphology and analysis

For the morphology of the intestinal tissue, the jejunum of the slaughtered chickens was separated and about 2 cm from the middle part of them removed and their contents were completely drained. The specimens were placed in different solutions of alcohol, xylene, and paraffin for one hour, and then the height and depth of the crypts of the specimens after the molding, cutting, sliding, paraffin removal, dehydration, slide staining and finally using optical microscopy was measured (Kermanshahi *et al.*, 2017).

To count the coliforms in the ileal samples, one gram of the sample in 9 ml of Ringer serum with the dilution of 10^{-6} was used. Eventually, one ml of the dilutions provided with a 20 ml mix of VRB-A medium incubated at 37°C for 24 to 48 h and evaluated for coliforms colonization (VRB-A culture medium is a selective medium for isolation and enumeration of *Escherichia coli*) (Guban *et al.*, 2006).

Statistical analysis

The data were tested for normality using the Shapiro-Wilk test. All data were analyzed by the GLM procedure of SAS software and if needed, the appropriate transformation was performed. Statistical differences between the treatments were conducted using Tukey's test when the P-value was less than 0.05.

Results

Growth Performance and small intestine weight

Data related to the growth performance are presented in table 2. The results showed that daily gain and FCR were not affected by treatments in any period. However, a trend ($P = 0.069$) was observed for feed intake with the dietary WDV and IV at 25-42 of age. Similarly, a trend ($P = 0.067$) was detected for feed intake between treatments throughout the rearing period of 1-42 days of age. So, the highest feed intake was seen in 2% of IV and 2% of WDV when compared to those of other treatments. The results (Table 3) indicated that different dietary IV and WDV sources had no significant effect on the relative weight of duodenum, jejunum, and ileum at 24 days and 42 days ($P > 0.05$)

Jejunal morphology and ileal microflora

The effect of different levels of IV and WDV on jejunal morphology and ileal microflora of broilers chicken are shown in Table 4. The results indicated that levels of used IV and WDV in feed or water significantly affected VH, VW, CD, and coliform count. The highest VH at 24 days of age, was owned to 1% of IV into the water, 1% of WDV into the water, and 2% of WDV into the feed treatments, while the lowest VH was observed in control 1 treatment when no vinegar was added. Also, at 24 days of age, the results showed that the highest VW was seen at the level of 3% of WDV into the feed and the lowest VW was in Control 1 treatment. There was no significant difference between the control and the treatments with vinegar when added into the water. At 24 days of age, the highest CD was owned to levels of 2 and 3% of WDV and 2% IV into the feed, while the least CD was seen at control 1 and 0.5% of WDV into the water treatments. The highest VH to CD ratio (VH/CD) at 21 days of age was seen at the level of 2% of IV and 2% of WDV into the feed while at 42 days of age, the addition of 3% WDV into the feed was seen when compared to those of other treatments. At 42 days of the chickens, the highest VH, VW, and CD belonged to 2% WDV into the feed and 1% of WDV into the water. The lowest CD was seen in the control 2 group and the lowest VH were seen at 1% IV into the water and control 2 treatments. The lowest VW was observed in the group with 1% IV into the feed as well as the control 1 group.

At 42 day of age, coliform microbial counts showed that the addition of 3% WDV into the feed decreased the number of ileal coliforms when compared to those of control 1, control 2, birds fed 1% IV into the water, and 0.5% WDV into the water groups (*P < 0.05*).

Table 2. Effect of industrial vinegar (IV) and different levels of waste date's vinegar (WDV) on growth performance of broiler chickens

Treatments	daily gain (g/bird/d)				feed intake (g/bird/d)				feed conversion ratio (g feed/g gain)			
	d 1 to 10	d 11 to 24	d 25 to 42	d 1 to 42	d 1 to 10	d 11 to 24	d 25 to 42	d 1 to 42	d 1 to 10	d 11 to 24	d 25 to 42	d 1 to 42
Control 1 (without vinegar)	13.56	33.65	67.66	42.95	23.77	62.04	128.41	80.02	1.75	1.84	1.89	1.70
Control 2 (control 1 + 2% water in feed)	13.63	33.61	67.43	42.90	23.85	62.04	127.91	80.12	1.75	1.85	1.90	1.85
1% IV in water	13.71	33.75	65.90	43.05	24.12	62.04	125.78	80.17	1.76	1.85	1.91	1.87
2% IV in feed	13.59	33.52	66.98	43.12	23.88	62.04	127.40	81.53	1.76	1.82	1.90	1.89
0.5% WDV in water	13.46	33.25	67.36	44.43	23.91	62.04	126.42	79.30	1.78	1.83	1.88	1.87
1% WDV in water	13.55	33.29	67.53	43.26	23.67	62.04	127.90	80.53	1.75	1.83	1.89	1.86
1.5% WDV in water	13.64	33.85	65.10	42.76	23.84	62.04	124.67	80.43	1.75	1.84	1.91	1.87
1% WDV in feed	13.52	32.05	67.00	42.51	23.78	62.04	125.75	80.20	1.76	1.83	1.88	1.88
2% WDV in feed	13.40	32.88	66.97	42.85	23.82	62.04	127.61	81.71	1.78	1.85	1.91	1.90
3% of WDV in feed	13.67	33.07	68.67	43.71	24.10	62.04	131.01	81.25	1.76	1.83	1.91	1.86
SEM	0.109	0.631	0.800	0.334	0.156	1.109	1.353	0.601	0.011	0.007	0.007	0.004
P-Values	0.289	0.33	0.186	0.454	0.595	0.210	0.069	0.067	0.604	0.100	0.225	0.141

SEM: Standard error of mean.

Table 3. Effect of industrial vinegar (IV) and different levels of waste date's vinegar (WDV) on relative weight (relative to live body weight, w/w) of different parts of the small intestine of broiler chickens.

Treatments	day 21			day 42		
	Relative weight of duodenum	Relative weight of jejunum	Relative weight of ileum	Relative weight of duodenum	Relative weight of jejunum	Relative weight of ileum
Control 1 (without vinegar)	1.12	2.15	2.01	0.56	1.03	0.99
Control 2 (control 1 + 2% water in feed)	1.24	2.21	2.37	0.71	1.33	1.24
1% IV in water	1.15	2.21	2.11	0.74	1.36	1.19
2% IV in feed	1.18	2.27	1.95	0.56	1.31	1.01
0.5% WDV in water	1.16	2.26	2.09	0.73	1.24	1.20
1% WDV in water	1.02	2.01	2.10	0.66	1.09	1.13
1.5% WDV in water	1.24	2.19	2.13	0.68	1.21	1.02
1% WDV in feed	1.13	2.02	2.08	0.75	1.26	1.16
2% WDV in feed	1.12	2.21	2.04	0.63	1.16	1.13
3% WDV in feed	1.05	1.95	1.97	0.62	1.26	1.13
SEM	0.0631	0.0846	0.1147	0.0588	0.809	0.0896
P-Values	0.2613	0.1054	0.4154	0.1938	0.1083	0.5799

SEM: Standard error of mean.

Table 4. Effect of industrial vinegar (IV) and different levels of waste date's vinegar (WDV) on jejunum morphology and ileum microflora of broiler chickens.

Treatments	day 24				day 42				
	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/ Crypt depth	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus Height/ Crypt depth	Coliforms (Log 10 cfu g^{-1})
Control 1 (without vinegar)	1047.60^{d}	141.20^{c}	138.40^{d}	6.96^{ab}	1353.20^{ef}	170.60^{b}	166.80^{abc}	7.30^{e}	4.96^{a}
Control 2 (control 1 + 2% water in feed)	1082.80^{cd}	161.20^{ab}	149.60^{abc}	6.50^{b}	1344.00^{f}	178.40^{ab}	160.80^{d}	7.54^{cde}	4.81^{a}
1% IV in water	1160.40^{a}	166.40^{ab}	150.00^{ab}	6.97^{ab}	1345.60^{f}	180.40^{ab}	166.40^{bc}	7.46^{de}	4.50^{ab}
2% IV in feed	1046.80^{d}	155.60^{ab}	156.40^{a}	7.29^{a}	1526.40^{bc}	179.80^{ab}	164.40^{cd}	8.49^{ab}	3.83^{bc}
0.5% WDV in water	1109.00^{bc}	158.80^{ab}	138.40^{d}	7.00^{ab}	1416.80^{def}	177.60^{ab}	166.40^{bc}	7.98^{bcde}	4.23^{ab}
1% WDV in water	1179.20^{a}	157.60^{ab}	139.60^{cd}	7.49^{a}	1507.20^{bcd}	184.00^{ab}	171.20^{ab}	8.12^{bcd}	4.04^{bc}
1.5% WDV in water	1154.80^{ab}	159.20^{ab}	142.00^{bcd}	7.08^{ab}	1573.60^{ab}	183.60^{ab}	165.20^{cd}	8.57^{ab}	3.98^{bc}
1% WDV in feed	1082.60^{cd}	153.60^{bc}	150.80^{ab}	7.08^{ab}	1453.20^{cde}	176.00^{b}	172.00^{a}	8.26^{bc}	3.79^{bc}
2% WDV in feed	1190.80^{a}	162.20^{ab}	156.40^{a}	7.42^{a}	1635.20^{a}	185.60^{a}	166.80^{abc}	8.57^{ab}	3.79^{bc}
3% WDV in feed	1146.80^{ab}	168.00^{a}	153.60^{a}	7.09^{ab}	1575.60^{ab}	181.20^{ab}	168.80^{abc}	9.03^{a}	3.42^{c}
SEM	10.7764	3.030	2.1392	0.1463	21.4807	1.8612	1.9266	0.1553	0.308
P-Values	<0.0001	<0.0001	<0.0001	0.0023	<0.0001	0.0043	0.0110	<0.0001	0.040

[a-f] Means within a column with different superscripts differ significantly ($P < 0.05$). *SEM*: Standard error of mean.

Immune response

The results of dietary treatments on the immune response of broilers are displayed in table 5. The levels of IgM and IgG were affected by treatments so that at 42 days of age the highest amount was found to levels of 2% and 1% of WDV into the feed for IgM and IgG, respectively (*P < 0.05*). However, anti SRBC, ND titter, and total Ig were not affected by treatments (*P > 0.05*).

Table 5. Effect of industrial vinegar (IV) and different levels of waste date's vinegar (WDV) on immune response ($\log_2$) of broiler chickens at 42 days of age.

Treatments	Items*				
	Anti-SRBC	ND titter	Total Ig	IgM	IgG
Control 1 (without vinegar)	5.0	5.55	4.6^{d}	1.8^{d}	2.8^{c}
Control 2 (control 1 + 2% water in feed)	5.2	6.0	6.2^{c}	2.4^{cd}	3.8^{ab}
1% of IV in water	4.6	5.8	6.3^{c}	2.2^{cd}	4.1^{ab}
2% of IV in food	5.2	6.5	6.2^{c}	2.6^{c}	3.6^{abc}
0.5% of WDV in water	5.6	5.5	5.6^{c}	2.2^{cd}	3.4^{bc}
1% of WDV in water	5.0	5.7	6.1^{c}	2.5^{c}	3.6^{abc}
1.5% of WDV in water	4.8	5.9	7.0^{bc}	2.8^{c}	4.2^{ab}
1% of WDV in feed	4.9	6.0	7.2^{bc}	2.8^{c}	4.4^{a}
2% of WDV in feed	4.4	6.7	9.8^{a}	5.6^{a}	4.2^{ab}
3% of WDV in feed	5.8	6.3	8.6^{b}	4.4^{b}	4.2^{ab}
SEM	0.60	0.55	0.74	0.35	0.47
P-Value	0.50	0.18	0.004	<0.0001	0.02

$^{a-d}$Means within a column with different superscripts differ significantly (*P < 0.05*). *SEM*: Standard error of mean. * Sheep red blood cell (SRBC), Newcastle disease titer (ND titer), Total Ig=IgM +IgG in any replicates..

Discussion

The current research aimed to investigate the use of different dietary sources of IV and WDV at different levels in the feed or water of broilers and their effects on growth performance, gastrointestinal tract morphology, and immune response. In the current study, treatments had no significant improvement in the growth performance of broilers. Youssef *et al.* (2017) reported that probiotics and organic acids did not affect body weight or body weight gain of broilers that is possibly due to several variables including the type of used organic acid and doses. The positive effect of organic acids in the lime juice could be due to improved digestibility of nutrients (Ndelekwute *et al.*, 2015). On the other hand, Probiotic supplements are maintained through beneficial microbial populations with competitive deprivation (Fuller, 1989; Katoch *et al.*, 2017), feed assimilation and digestion enhancement (Ghafari *et al.*, 2017), and bacterial metabolism modification (Jin *et al.*, 1997; Pourakbari *et al.*, 2016). It is reported that the use of organic acids and probiotics protect deprived chickens (La Ragione and Woodward, 2003; Hassan *et al.*, 2010) and thus improve the performance of the chickens (Denli *et al.*, 2003; Adil *et al.*, 2010; Khan and Iqbal, 2016).

Intestinal health will lead to a better growth rate and feed efficiency in poultry (Montagne *et al.*, 2003). The combination of acetic acid and probiotic in WDV might be the main reason which controls the balance of intestinal microflora and similarly affects intestinal functions and metabolism. The improvement in the FCR might be due to better utilization of nutrients and also because of increased feed intake. In this study, the relative weight of different parts of the small intestine in broilers was not affected by the treatments, which is consistent with the study done by Tasharofi *et al.* (2017) but oppose with other studies including Seifi *et al.* (2013) and Mahdavi and Torki (2009). They reported that the use of acetic and butyric acids in the diet of chickens increased the ileum and jejunum weight of broilers. In the Study done by Mohiti-Asil and Ghanaatparast-Rashti (2017), they reported that broilers fed with a commercial blend of phytogenic compounds had a higher relative duodenal length than those fed 500 ppm of oregano essential oil (OEO). Sarica *et al.* (2014) showed that OEO (250 or 500 ppm) did not affect the relative length of jejunum in 3-day-old chickens. However, the relative length of ileum with 500 ppm OEO was higher at day 14 of the chickens when compared to that of control. The results showed that at the middle and the end of the broiler breeding periods, the WDV increased the VH, VW, and CD in the small intestine, but these indices decreased at the end of the rearing period when chickens fed IV. Previous studies indicated that the organic acids increased the height of the villus and width of the crypts in the ileum of the chickens (Pelicano *et al.*, 2005; Paul *et al.*, 2007; Mohamed *et al.*, 2014).

Over time, VH/CD decreased in the birds fed with Control 1 (without vinegar) or Control 2 (Control 1 + 2% water in feed) compared to other experimental treatments. Organic acids in vinegar improved the digestibility of the protein and thereby improved the absorption of nutrients and ultimately improved the appearance and function of the intestine (Pelicano *et al*., 2005). Some reports indicated that dietary addition of oregano and thyme increased VH (100 ppm of phytogenic blends), VW (200, 300, and 500 ppm of phytogenic blends), and villus surface area (300 and 500 ppm of phytogenic blends) of broilers (Khattak *et al*., 2014). It is shown that yeast product supplementation to broiler diets could improve villi attributes and enhance gut histo-morphometry (Rahbar *et al*., 2011).

Samanta *et al*. (2010) showed that adding probiotics to broilers feed for 7 days increases VH compared to the control group. Improvement in the condition of the villi may be due to the function of organic acids used in the diet that reduce the intestinal pH and reduce intestinal colonization by harmful microorganisms (Iji *et al*., 2001). The highest levels of acidified yeast product and acidified whey powder with yeast products significantly increased the VH of treated birds. Similarly, 0.45% acidified yeast and acidified whey powder with yeast products increased the CD and mucosal layer thickness. Additional levels of dietary supplements had no extra effects on the aforementioned indices (Kermanshahi *et al*., 2017).

In this study, the body weight of healthy chickens was not affected by treatments. The results of this study regarding weight gain are already confirmed by others (Owens *et al*., 2008; Adil *et al*., 2011; Ghazalah *et al*., 2011) who reported that supplementation of organic acids in broilers did not increase body weight. The use of an organic acid mixture significantly reduces the total number of bacteria as well as the number of gram-negative bacteria in broilers (Gunal *et al*., 2006). The reduction of intestinal bacteria may enhance the ability of the epithelial cells to proliferate and thus improve the absorption capacity of the intestine (Zeng *et al*., 2015). In the current study, no pathogen challenge was applied and organic acids along with beneficial bacteria used in this study might act better when some challenges with pathogens in the gut are presented.

Yeast products supplementation in broiler diets increased the number of beneficial bacteria such as lactobacillus (Roto *et al*., 2015), and reduced the number of pathogenic microbes such as Clostridium (Yitbarek *et al*., 2012) and *E Coli* (Huff *et al*., 2007). In this study, treatments containing different levels of WDV in water and diet reduced the intestinal coliforms, which probably exerted their antibacterial activity (Ricke, 2003). The decrease in bacterial load in the intestine of broiler chickens might be due to the presence of organic acids (citrus and ascorbic acid) and their antibacterial properties (Waldroup *et al*., 1995; Lawal *et al*., 2012; Tomar *et al*., 2013). Researchers have shown that diets containing organic acid and useful bacteria enhance the immune response by T and B lymphocytes development, and improve the immune system (Kabir *et al*., 2004). The stimulation of the immune system by probiotics might increase T cells and phagocytic cells (Fuller, 1989). Kabir *et al*. (2004) showed that antibiotics stimulate gram-positive bacteria which in turn stimulate the immune system.

Overall, in the present study, there is no difference between IV and WDV (in feed and water), however, it was concluded that WDV was capable to improve intestinal morphology and immune system of broilers and in terms of economic benefits, can be recommended to poultry producers. More research is needed to clarify if this product is useful for poultry when they are challenged with different pathogenic bacteria.

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