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Original Research Article

Dietary organic acid and fiber sources affect performance, intestinal morphology, immune responses and gut microflora in broilers

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ABSTRACT

This experiment was designed to investigate the effects of a dietary organic acid (OA) mixture and 2 fiber sources on performance, intestinal morphology, immune responses and gut microflora in broilers. A total of 390 one-day-old broiler chicks (Ross 308) were allocated to 6 dietary treatments with 5 replicate pens and 13 chicks each based on a factorial arrangement (2 × 3) in a completely randomized design. The experiment lasted 42 d. The following experimental diets and as well as their interaction were considered: a basal diet supplemented with or without OA (0 or 1 g/kg) and 2 fiber sources (sugar beet pulp [soluble fiber] or rice hull [insoluble fiber]; 0 or 30 g/kg). Dietary supplementation of OA increased daily weight gains of broilers across the entire rearing period ($P < 0.05$). The dietary fibrous materials did not affect the performance of broilers. Antibody titer against influenza disease virus was higher in birds fed diets containing rice hull compared with other experimental groups ($P < 0.05$). The population of *Lactobacillus* bacteria was greater in birds fed OA-added diets without or with 30 g/kg rice hull supplementation compared with other experimental groups ($P < 0.05$). In conclusion, dietary supplemental OA improved performance of broilers, and dietary supplemental OA with rice hull enhanced humoral immune responses.

1. Introduction

The need of modern poultry industry to high levels of production could be achieved through application of certain feed additives, thus dietary supplementation of these compounds has been the subject of numerous studies. In general, inclusion of organic acid (OA) in the feed was reported to improve performance (Abdel-Fattah et al., 2008; Panda et al., 2009), nutrient utilization (Ao et al., 2009) and immune responses (Zhang et al., 2011) in broiler chickens. In addition, beneficial effect of OA on gut development of broiler chickens was also reported, e.g., orally administration of an OA blend (10 g/kg sorbic acid and 2 g/kg citric acid) considerably increased duodenal villus height of broiler chickens at 11 and 22 d of age (Rodríguez-Lecompte et al., 2012). And, supplementation of 2, 4 and 6 g/kg butyric acid in diets of broilers improved duodenal villus height and crypt depth (Rodríguez-Lecompte et al., 2012). Improvement in villus height might be associated with reduction in the intestinal colonization of pathogenic bacteria, as well as decreased inflammatory process at the intestinal mucosa, which eventually improves function of nutrients absorption (Iji and Tivey, 1998). However, there are trials without significant effects of OA on the performance of broiler chickens (Alp et al., 1999; Gunal et al., 2006). For example, Gunal et al. (2006) indicated that dietary inclusion of an OA mixture decreased intestinal Gram-negative bacteria of broiler chickens but failed to improve daily weight gain (DWG) and feed conversion ratio (FCR). Thereby, effect of an OA on the intestinal microflora and its relationship with performance and immune responses in broiler chickens could be the subject of further research. On the other hand, supplementation of dietary fiber has been reported to have beneficial effects on the microbial profile of gastrointestinal tract (GIT) in broiler chickens. In this

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regard, Abazari et al. (2016) demonstrated that inclusion of rice husk in diets of broiler chickens increased the population of *Lactobaccili* but reduced the number of pathogenic bacteria. The mode of action for the effect of soluble fiber (SF) on GIT microflora is through fermentation in the hindgut, production of short chain fatty acids, and their bacteriostatic effects on the pathogenic bacteria (Van der Wielen et al., 2001). Alternatively, the friction effect of dietary insoluble fiber (IF) on the mucousal layer of small intestine contributes to the removal of pathogenic bacteria (Mateos et al., 2012). There is a relationship between gut microflora and immune responses in broiler chickens. Researchers have shown better immune response of broiler chickens fed diets supplemented with OA (Emami et al., 2013) or fiber (Sadeghi et al., 2015), which might be due to the beneficial effects of them on the intestinal microflora. Antibody measurement is a proper tool to assess humoral immune responses of broilers in this trial because susceptibility of broiler chickens to disease is influenced by blood antibody level (Parmentier et al., 2004). Little experimental studies exist on the effect of fibrous materials such as sugar beet pulp (SBP) and rice hull (RH) on immune response of broiler chickens. Sadeghi et al. (2015) indicated augmented antibody titer against Newcastle disease virus (NDV) in broilers fed on dietary combination of SBP and RH. As such this subject is worthy of further investigation.

Although previous studies reported the individual effect of dietary OA or fibrous materials in broiler chickens, but to our knowledge, the simultaneous effect of dietary OA and fiber type has not been studied. Therefore, we expected that dietary inclusion of fiber and OA affect the gut microflora, immunity and growth performance in broiler chickens. The objective of this experiment was to study the effect of diets with or without 1 g/kg supplementation of an OA mixture and also inclusion of 0 or 30 g/kg fiber sources on production performance, morphology of small intestine, gut microflora and humoral immune responses in broiler chickens.

2. Materials and methods

All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch.

2.1. Fiber sources

Before the trial initiation, SBP and RH were purchased from a commercial supplier, ground using a hammer mill (2 mm screen), and used in the manufacturing of the feeds. Furthermore, fiber samples were analyzed for chemical composition (Table 1). Fibrous materials were measured for crude fiber (CF) by sequential extraction with diluted acid and alkali (method 978.10) as indicated by AOAC (2000), for dry matter (DM) and crude protein (CP) based on the methods 930.15 and 990.03, respectively (AOAC, 2000) and for ether extract (EE) by Soxhelt fat analysis (method 954.02) as described by AOAC (2000). Fiber was also analyzed for the neutral detergent fiber (NDF), acid detergent fiber and acid detergent lignin sequentially according to the method described by Van Soest et al. (1991) and expressed on ash free basis. Fiber moisture and ash contents were determined based on methods reported by Debon and Tester (2001).

Table 1
Chemical composition (g/kg) of rice hull (RH) and sugar beet pulp (SBP).

Item	RH	SBP
Ash	112	54
Crude protein	33	78
Crude fiber	445	150
Ether extract	5	15
Acid detergent fiber	499	155
Neutral detergent fiber	653	335
Acid detergent lignin	172	41
Moisture	74	78

2.2. Chicks, diets, and experimental procedures

A total of 390 one-day-old unsexed Ross 308 broiler chicks were randomly assigned to 6 dietary treatments with 5 replicate pens (length 120 cm × width 120 cm × height 80 cm) and 13 chicks each based on a factorial arrangement of treatments (2 × 3) in a completely randomized design. Experimental treatments were considered as a basal diet supplemented with or without an OA (0 or 1 g/kg blend of lactic acid, citric acid, acetic acid, formic acid, propionic acid, phosphoric acid and sodium butyrate (Animal Nutrition Development Group, Spain) and with or without fiber source (0 or 30 g/kg either SBP [SF] or RH [IF]) as well as their interaction. Supplementary level of OA in the feed was based on the manufacturer recommendation. The basal diet included 30 g/kg silica sand, which was replaced by the same amount of either fiber source, OA or both of them in the corresponding diets. Prior to formulating the diets, the main feed ingredients were analyzed for DM (930.15), CP (Method 990.03), CF (Method 978.10), Ca and P (methods 968.08 and 965.17) contents according to the standard procedures of AOAC (2000). Experimental diets were formulated to meet the nutritional requirements of broiler chickens as provided by Ross 308 broiler management manual (Aviagen, 2014) during starter (1 to 11 d of age), growing (12 to 28 d of age), and finisher (29 to 42 d of age) periods (Table 2). All experimental diets were fed in mash form and were formulated to be isoproteinous and isoenergetic. The birds were reared in an environmentally controlled windowless house equipped with cemented floor pens (length 100 cm × width 150 cm × height 80 cm) which covered with paper rolls as bedding material. The lighting program consisted of 23 h light and 1 h darkness. Environmental temperature was set at 33 °C for the first week and 30 °C for the second week, which was further decreased to 23 °C until the end of the study.

2.3. Data collection and sampling

Daily feed intake (DFI) and daily weight gain (DWG) were recorded in different periods of experiment (1 to 11, 12 to 28, and 29 to 42 d of age) by pen basis after 3 h of feed withdrawal. The feed conversion ratio (feed intake/weight gain) was calculated. On d 42 of experiment, 2 birds close to the mean body weight (BW) of pen were individually weighed and slaughtered. Carcass traits containing carcass, liver, abdominal fat and heart were collected, weighed and expressed as a percentage of live BW. Proportional weights of digestive organs including pancreas, gizzard, segments of small intestine and cecum were also calculated. The length of small intestine was also measured and recorded.

2.4. Morphology of small intestine

At 28 d of age, 2 birds from each pen were slaughtered and small intestinal segments were sampled from duodenum, jejunum and ileum. Samples were evaluated for the villus height, crypt depth and villus height to crypt depth ratio (VH:CD). Intestinal segments were gently flushed twice with physiological saline solution (1% NaCl) to remove intestinal contents and placed in 10% formalin in 0.1 mol/L phosphate buffer saline (PBS) (pH = 7.0) for fixation. The samples were processed for 24 h in a tissue processor with ethanol as dehydrant and were embedded in paraffin. Sections (5 μm) were

Table 2
Dietary composition and nutrients during different periods.

Item	Starter (1 – 11 d)	Grower (12 – 28 d)	Finisher (29 – 42 d)
Ingredients, g/kg			
Corn	533	555	567
Soybean meal	375	350.6	335.8
Soybean oil	15	25	30
Dicalcium phosphate	19.1	16.1	15.1
Calcium carbonate	11.7	9.6	9.3
DL-methionine	3.5	2.9	2.5
L-lysine	2.1	1.2	0.8
L-threonine	1.1	0.6	0.5
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
Sodium chloride	2.5	2	2
Sodium carbonate	2	2	2
Silica sand	30	30	30
Total	1,000	1,000	1,000
Calculated nutrient level, g/kg, as fed basis			
ME, MJ/kg	11.57	12.03	12.27
Crude protein	207.6	198.6	193.1
Lysine	13.1	11.8	11.1
Methionine + Cystine	10	9.2	8.6
Threonine	8.9	8.1	7.8
Calcium	9.8	8.3	7.9
Available phosphorous	4.7	4.1	3.9
Analyzed values, g/kg, as fed basis			
Crude protein	208.5	199.6	194.2
Crude fiber	4.7	4.67	3.91
Calcium	9.6	8.1	7.6
Total phosphorous	6.9	6.1	6.1
Dry matter	908.1	905.9	903.4

[1] Vitamin premix provided per kilogram of diets: vitamin A (retinol), 2.7 mg; vitamin D_3 (cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K_3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg.

[2] Mineral premix provided per kilogram of diets: Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; I (KI, 58% I), 1 mg; Se ($NaSeO_3$, 45.56% Se), 0.2 mg.

made by the use of a microtome (Rotary Microtome, Model MK1120, Pooyanmedical Co., Mashhad, Iran) and stained with hematoxylin-eosin. Morphological examination of samples was applied through an optical microscope (Olympus CX31, Tokyo, Japan). A total of 10 intact well-oriented villus–crypt units were selected for each intestinal cross section (3 cross sections/sample and 30 cross sections/treatment for a total of 300 measurements/treatment). Villus height in micrometre was measured from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between 2 villi. Villus height to crypt depth ratio was then calculated. The average of values for each cross section was used for further analysis.

2.5. Immune responses and intestinal microbial populations

Subcutaneous injection of Newcastle and influenza antigens (0.2 mL per chick) was done on 9 d of age, with dual vaccine of Newcastle-influenza (H9N2 subtype). On d 19 of age, chicks were orally vaccinated against Newcastle Disease (Lasota). Two chicks per pen were randomly selected for intraperitoneal injection with 1.0 mL of sheep red blood cells (SRBC) suspension diluted with PBS on d 23. Five days post SRBC injection, birds were bled from the wing vein to determine antibody titers against SRBC, influenza disease virus (IDV) and NDV. Serum was collected after centrifugation (1,500×*g* for 15 min) at room temperature. The hemaglutination assay method was used to measure the antibody titer against SRBC. Antibody titers against IDV and NDV were separately measured by hemaglutination inhibition (HI) method. The HI antibodies were then converted to log2. Antibody titer against SRBC was measured by the microtiter procedure described by Wegmann and Smithies (1966). Spleen and bursa of Fabricius were evaluated after slaughter at the end of experiment. Furthermore, to assess the intestinal microbial populations, the carcasses were opened and the whole GIT was removed aseptically. Intestinal samples (from Meckel's diverticulum to the ileal cecal–colon junction) were collected directly into 80-mL sampling cups under CO_2, sealed, and put on ice until they were transported to the laboratory for enumeration of bacterial populations. Immediately, the contents of ileum were cultured on specific culture media to enumerate the populations of *Lactobacilli* bacteria and coliforms. Digesta samples were serially diluted in 0.85% sterile saline solution for enumeration of *Lactobacilli* bacteria and coliforms by conventional microbiological techniques using selective agar media. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. The *Lactobacilli* bacteria were anaerobically assayed using MRS agar (Fluka 80961). Colonies from each agar media were counted, log transformed and expressed per gram of digesta.

2.6. Statistical analysis

Data were analyzed as a 2 × 3 factorial arrangement based on a completely randomized design using the GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of OA (0 and 1 g/kg) and fiber source (0, 30 g/kg SBP and 30 g/kg RH) and their interactions. Data were analyzed considering all birds in a cage as an experimental unit. When a significant F-test was detected ($P < 0.05$), corresponding means were separated by Tukey's test, and the interaction between treatments were analyzed using an least squares (LS) means test adjusted for Tukey's test. For all statistical analyses, significance was declared at $P \leq 0.05$, unless otherwise stated.

3. Results

3.1. Growth performance, carcass traits and digestive organs

The effects of treatments on the performance of broilers are presented in Table 3. Interaction effect of fiber source and OA showed that fiber had no significant effect on growth performance of broilers. Irrespective of fiber, dietary supplementation of 1 g/kg OA remarkably increased DWG of broiler chickens compared with those did not receive OA across the entire rearing period ($P < 0.05$). Similarly, OA inclusion in the feed improved DWG of broilers during 1 – 11 d and 1 – 42 d of trial as suggested by the main effect of treatments ($P < 0.05$). Feed consumption of broiler chickens was not influenced by experimental treatments whereas FCR improved with the inclusion of 1 g/kg OA in the feed compared with those did not receive OA during the whole production period ($P < 0.05$).

There was an interaction effect of fiber source × OA for abdominal fat in which broilers fed diets supplemented with SF without inclusion of OA deposited more fat in the abdomen area than those fed diets supplemented with either SF or IF substances and 1 g/kg OA ($P < 0.05$). Dietary treatments failed to affect carcass yield, liver and heart proportional weights ($P < 0.05$; Table 4).

3.2. Morphology of small intestine

Duodenal morphometric features were not influenced by the fiber source × OA effect. Villus height of duodenum increased in birds receiving diets added with IF compared with those given diets without fiber inclusion ($P < 0.05$). In jejunum, diets containing IF

with OA supplementation increased villus height of boilers, relative to diets included with fibrous materials without OA supplementation ($P < 0.05$). Furthermore, birds given diets containing 1 g/kg OA had higher villus height than those received diets without OA supplementation ($P < 0.05$). Crypt Dietary supplementation of IF increased crypt depth of broilers compared with diets with SF or without exogenous fiber ($P < 0.05$; Table 5).

3.3. Immune responses and intestinal microbial populations

The effect of dietary treatments on lymphoid organs and humoral immunity in broiler chickens are presented in Table 6. Antibody titer against SRBC and NDV were unaffected by interaction effect of fibrous materials and OA. However, 1 g/kg supplementation of OA in the diet increased antibody titer against IDV in broilers fed on IF-containing diets ($P < 0.05$). Dietary OA supplementation increased antibody titer against IDV in broilers compared with those did not receive OA ($P < 0.05$). Furthermore, fibrous materials increased antibody titer against IDV compared with diets lacking added fiber, and IF resulted in significantly greater antibody titer than SF ($P < 0.05$). Lymphoid organs including spleen and bursa of Fabricius proportional weights were not influenced by experimental treatments.

The population of *Lactobacillus* bacteria was affected by the interaction of fiber source and OA, and dietary supplementation of IF and 1 g/kg OA caused significantly higher population than other dietary treatments except for dietary supplementation of OA without fiber inclusion ($P < 0.05$). Generally, birds fed diets containing OA had higher intestinal population of *Lactobacillus* bacteria than those did not receive OA ($P < 0.05$). Furthermore, supplemental IF caused greater population of *Lactobacillus* bacteria than supplemental SF ($P < 0.05$). Population of coliform bacteria remained unaffected after dietary inclusion of fiber and OA (Table 7).

4. Discussion

Dietary supplementation of 1 g/kg OA improved DWG of broilers across the entire rearing period, particularly in diets without fiber supplementation. Beneficial effects of OA on growth performance of broiler chickens have been largely investigated (Abdel-Fattah et al., 2008; Panda et al., 2009; Dehghani-Tafti and Jahanian, 2016). On the contrary, Biggs and Parsons (2008) suggested inefficiency of dietary OA to promote the performance of chickens. These researchers believed that differences in dietary phosphorous content and conducting experiment under the ideal condition were possible reasons for lack of growth-promoting action of applied OA. Dietary fiber supplementation had no remarkable effect on the performance of broiler chickens. Although there are studies reporting positive effect of SF- and IF-containing diets on the

Table 4
Effects of dietary treatments on carcass measurements (% BW).

Item		Carcass yield	Abdominal fat	Heart	Liver
Organic acid, g/kg	Fiber source[1]				
0	No fiber	71.60	0.98^{ab}	0.41	2.00
	SF	72.39	1.45^{a}	0.46	1.97
	IF	72.98	1.21^{ab}	0.42	1.99
1	No fiber	70.60	1.00^{ab}	0.41	2.16
	SF	73.54	0.74^{b}	0.41	2.03
	IF	74.53	0.80^{b}	0.41	2.15
Organic acid, g/kg					
0		72.324	1.21^{a}	0.42	1.98
1		72.891	0.84^{b}	0.41	2.11
Fiber source					
No fiber		71.099	0.988	0.410	2.084
SF		72.968	1.092	0.435	2.000
IF		73.755	1.007	0.416	2.069
Pooled SEM		1.53	0.15	0.03	0.04
P-value					
Organic acid		0.591	0.001	0.219	0.091
Fiber source		0.123	0.657	0.291	0.606
Organic acid × fiber source		0.569	0.020	0.321	0.806

SF = soluble fiber; IF = insoluble fiber.
[a, b] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).
[1] Rice hull as IF or sugar beet pulp as SF was supplemented at 30 g/kg to replace silica sand in the basal diet.

Table 3
Effects of dietary treatments on performance of broiler chickens at different ages.

Item		Daily weight gain, g				Daily feed intake, g				Feed conversion ratio			
		1 – 11 d	12 – 28 d	29 – 42 d	1 – 42 d	1 – 11 d	12 – 28 d	29 – 42 d	1 – 42 d	1 – 11 d	12 – 28 d	29 – 42 d	1 – 42 d
Organic acid, g/kg	Fiber source[1]												
0	No fiber	25.0	57.0	70.1	53.5^{b}	31.3	93.9	130.7	85.4	1.25	1.65	1.86	1.60
	SF	25.7	57.5	71.6	56.7^{ab}	32.7	92.9	134.5	90.0	1.27	1.62	1.87	1.59
	IF	26.2	53.5	71.5	55.0^{ab}	33.8	93.6	130.0	88.5	1.29	1.75	1.84	1.61
1	No fiber	26.5	55.6	75.9	57.7^{a}	32.6	93.8	134.3	89.7	1.23	1.69	1.78	1.55
	SF	26.6	58.4	70.6	56.9^{ab}	32.6	94.6	129.3	88.6	1.23	1.63	1.85	1.56
	IF	26.3	55.3	72.4	56.2^{ab}	32.1	93.3	127.7	87.6	1.22	1.69	1.77	1.56
Organic acid, g/kg													
0		25.6^{b}	56.0	71.0	55.1^{b}	32.6	93.5	130.7	88.6	1.27	1.67	1.91	1.61^{a}
1		26.5^{a}	56.4	73.0	56.9^{a}	32.5	93.9	130.4	88.6	1.23	1.67	1.80	1.56^{b}
Fiber source													
No fiber		25.7	56.3	73.0	55.6	31.9	93.9	132.5	88.6	1.24	1.67	1.88	1.57
SF		26.2	57.9	71.1	56.8	32.7	93.8	132.0	89.3	1.25	1.63	1.88	1.57
IF		26.3	54.4	71.9	55.6	33.0	93.5	128.8	88.0	1.26	1.72	1.81	1.58
Pooled SEM		0.42	0.61	1.36	0.42	0.37	0.39	1.16	0.35	0.01	0.02	0.03	0.01
P-value													
Organic acid		0.024	0.710	0.145	0.016	0.851	0.599	0.888	0.987	0.131	0.841	0.116	0.008
Fiber source		0.395	0.065	0.831	0.284	0.509	0.918	0.408	0.344	0.900	0.082	0.561	0.646
Organic acid × fiber source		0.306	0.530	0.120	0.049	0.287	0.539	0.440	0.065	0.824	0.411	0.591	0.553

SF = soluble fiber; IF = insoluble fiber.
[a, b] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).
[1] Rice hull as IF or sugar beet pulp as SF was supplemented at 30 g/kg to replace silica sand in the basal diet.

Table 5
Effects of dietary treatments on intestinal morphology.

Item		Duodenum			Jejunum			Ileum		
		VH, μm	CD, μm	VH:CD	VH, μm	CD, μm	VH:CD	VH, μm	CD, μm	VH:CD
Organic acid, g/kg	Fiber source[1]									
0	No fiber	1,384	233	6.1	1,060^{abc}	188	5.8	536	130	4.0
	SF	1,240	215	6.3	843^{bc}	192	4.4	569	130	4.3
	IF	1,472	207	7.3	780^{c}	188	4.3	603	171	3.9
1	No fiber	1,228	229	5.3	1,009^{abc}	191	5.5	664	127	5.2
	SF	1,512	230	6.8	1,088^{ab}	169	5.0	465	119	4.1
	IF	1,485	246	6.3	1,190^{a}	230	4.7	582	162	3.5
Organic acid, g/kg										
0		1,365	219	6.6	894^{b}	189	4.8	569	136	4.0
1		1,408	235	6.1	1,096^{a}	196	5.0	570	144	4.3
Fiber source										
No fiber		1,306^{b}	231	5.7	1,034	189	5.6	600	129^{b}	4.6
SF		1,376^{ab}	223	6.5	966	180	4.7	517	124^{b}	4.2
IF		1,479^{a}	227	6.8	985	209	4.5	592	166^{a}	3.7
Pooled SEM		142.57	20.02	0.22	95.50	31.51	0.23	100.01	6.11	0.22
P-value										
Organic acid		0.433	0.207	0.295	0.001	0.669	0.645	0.984	0.446	0.604
Fiber source		0.047	0.859	0.094	0.602	0.380	0.119	0.460	0.007	0.227
Organic acid × fiber source		0.112	0.403	0.240	0.009	0.297	0.728	0.289	0.948	0.223

SF = soluble fiber; IF = insoluble fiber; VH = villus height; CD = crypt depth.
[a, b, c] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).
[1] Rice hull as IF or sugar beet pulp as SF was supplemented at 30 g/kg to replace silica sand in the basal diet.

Table 6
Effects of dietary treatments on antibody titer and lymphoid organs.

Item		Antibody titer, log2			Lymphoid organ, % BW	
		IDV	NDV	SRBC	Spleen	Bursa of Fabricius
Organic acid, g/kg	Fiber source[1]					
0	No fiber	3.6^{c}	3.7	8.4	0.090	0.078
	SF	3.7^{bc}	3.9	8.6	0.102	0.074
	IF	3.7^{bc}	3.5	8.6	0.080	0.078
1	No fiber	3.6^{c}	3.2	8.4	0.104	0.076
	SF	3.8^{b}	3.8	8.5	0.088	0.070
	IF	4.0^{a}	3.9	8.5	0.074	0.064
Organic acid, g/kg						
0		3.0^{b}	3.5	8.6	0.090	0.076
1		4.1^{a}	3.8	8.3	0.088	0.070
Fiber source						
No fiber		3.2^{c}	3.8	8.7	0.097	0.077
SF		3.5^{b}	3.5	8.6	0.095	0.072
IF		4.1^{a}	3.6	8.1	0.077	0.071
Pooled SEM		0.029	0.12	0.18	0.015	0.007
P-value						
Organic acid		<0.001	0.176	0.367	0.798	0.439
Fiber source		<0.001	0.555	0.405	0.087	0.826
Organic acid × fiber source		0.318	0.323	0.734	0.331	0.826

SF = soluble fiber; IF = insoluble fiber; IDV = influenza disease virus; NDV = Newcastle disease virus; SRBC = sheep red blood cells.
[a, b, c] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).
[1] Rice hull as IF or sugar beet pulp as SF was supplemented at 30 g/kg to replace silica sand in the basal diet.

growth performance of broilers (González-Alvarado et al., 2007, 2010; Jiménez-Moreno et al., 2009, 2010, 2016; Adibmoradi et al., 2016), declined performance were observed in some other experiments on broilers (Janssen and Carré, 1985) and turkey (Sklan et al., 2003). These contradictory results may depend upon many factors such as dietary fiber source or supplemental fiber level and also health status of experimental animals. In the present experiment, high CF content of basal diet (4.7, 4.6 and 3.9 g/kg in starter, growing and finisher periods, respectively) likely resulted in lack of the supplemented fiber effect. In this respect, Jiménez-Moreno et al. (2009) declared that higher effect of fiber should be expected in diets with low CF content. There is still a need of further investigations on this subject. Dietary treatments had no effect on the feed consumption of broilers in this experiment. Generally, dilution of dietary energy content with fibrous materials causes higher feed intake of broilers in response to their low energy intake (Ferket and Gernat, 2006). However, in the current trial, experimental diets were formulated to be isocaloric and were not added as diluting factors which may avoid the change in DFI of broiler chickens. Abdominal fat deposition was lower in birds given diets added with SF and 1 g/kg OA than birds given SF-containing diets without OA supplementation. It seems that OA modified the effect of SF and led to decreased abdominal fat weight. Also, liver proportional weight was not affected by dietary treatments. This may stand to reason that acidification of the feed inhibits glycolysis, stimulates glycogenesis and consequently decreases abdominal fat deposition without any change in liver proportional weight (Fushimi et al., 2001). Analogous to our results, Abdel-Fattah et al. (2008) found that dietary acidification with 30 g/kg citric acid decreased abdominal fat while Dehghani-Tafti and Jahanian (2016)

Table 7
Effects of dietary treatments on ileal bacterial populations (log_{10} cfu/g).

Item		*Lactobacilli*	Coliforms
Organic acid, g/kg	Fiber source[1]		
0	No fiber	10.35[b]	7.64
	SF	11.35[b]	7.63
	IF	9.33[b]	8.68
1	No fiber	15.06[a]	11.46
	SF	8.61[b]	8.41
	IF	15.96[a]	11.03
Organic acid, g/kg			
0		10.34[b]	7.98
1		13.21[a]	10.30
Fiber source			
No fiber		12.71[a]	9.55
SF		9.98[b]	8.02
IF		12.65[a]	9.85
Pooled SEM		1.33	1.95
P-value			
Organic acid		0.002	0.002
Fiber source		0.021	0.029
Organic acid × fiber source		<0.001	0.021

SF = soluble fiber; IF = insoluble fiber.
[a, b, c] Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).
[1] Rice hull as IF or sugar beet pulp as SF was supplemented at 30 g/kg to replace silica sand in the basal diet.

reported no effect of a dietary OA blend on abdominal fat deposition. Proventriculus weight decreased when diets supplemented with IF. Similarly, Jiménez-Moreno et al. (2009) declared that proportional weight of proventriculus was reduced in response to dietary inclusion of 30 g/kg oat hulls.

In the current experiment, intestinal morphometric features were altered in response to dietary application of an OA mixture at 1 g/kg, and this effect is even more when diets were added fibrous materials compared with the same diet but without OA inclusion. Generally, short-chain fatty acids are able to stimulate the proliferation of crypt cells and consequently enhance turnover and maintenance of healthy tissue. In line with our results, Panda et al. (2009) reported that supplementing various levels of butyrate (2, 4 or 6 g/kg) in diets of broilers improved duodenal villus height and crypt depth. Furthermore, Adil et al. (2010) indicated that villus height in duodenum, jejunum and ileum of broilers increased following dietary administration of either 30 g/kg butyric acid, 30 g/kg fumaric acid, or 20 g/kg fumaric acid. Feeding broilers diets added with RH increased duodenal villus height and ileal crypt depth without any effect on VH:CD. Similar to our results, Rezaei et al. (2011) demonstrated the increased ileal villus height with dietary consumption of IF substances. Furthermore, Wils-Plotz and Dilger (2013) observed the increased duodenal crypt depth in broiler chickens fed diets containing cellulose. In contrast, the reduction of intestinal villus height was observed in response to dietary inclusion of high fiber sunflower cake (Kalmendal et al., 2011) and rice husk (Abazari et al., 2016) while Jiménez-Moreno et al. (2013) failed to find any significant effect of oat hull on the jejunal morphometric features. The VH:CD ratio is an indicator of the absorptive capacity in the small intestine (Teirlynck et al., 2009), suggesting why growth performance of broilers did not change after dietary supplementation with IF.

In this study, interaction results suggested that supplemental OA in the feed enhanced antibody titer against IDV when diets contained IF. In this respect, enhancement in the antibody titer against NDV was observed by Houshmand et al. (2012) when broiler diets were supplemented with 1.5 g/kg of an OA. The beneficial impact of OA on humoral immune responses might be applied through the increased population of *Lactobacillus* bacteria and the reduction in the count of Gram-negative bacteria in the GIT. Therefore, intestinal microbial populations were studied in this experiment. The underlying reason for the effect of IF on immune related parameters is the generation of an equilibrium and interaction between commensal microflora and gut associated lymphoid tissue, which is regarded as a primary mechanism of the host against invading pathogens (Montagne et al., 2003). It seems that IF increase mucin maturity and consequently colonize beneficial bacteria that might increase the acquired immunity. It also has been shown in human studies that many diseases are associated with changes in mucin production (Corfield et al., 2001). Further research on the effect of fibrous materials on the antibody titer against IDV is warranted.

In the present experiment, dietary supplementation of 1 g/kg OA in diets containing IF increased population of *Lactobacillus* bacteria, suggesting that OA and fiber interacted to modulate GIT microflora. This is supported by the improved antibody titer against IDV when OA or fibrous materials were supplemented in the diet. The mode of action for the effect of OA on pathogenic bacteria is via penetration in a certain types of bacteria cell wall in non-dissociated form and disruption in the normal physiology of them, whereby they cannot tolerate a wide internal and external pH gradient (Khan and Iqbal, 2016). In other words, OA reduce the GIT level of some pathogenic bacteria in poultry and control the population of those compete with birds for nutrients. Similarly, Ragaa and Korany (2016) founded that broilers consumed diets that contained formic acid or potassium diformate had lower cecal populations of total *clostridia* and *salmonella* spp. On the other hand, IF has abrasive effects in the small intestine which stimulate the secretion of mucous (Montagne et al., 2004). Intestinal mucous has a dynamic nature and is involved in protection, creating fluidity, and nutrients absorption. In harmony with our results, Abazari et al. (2016) reported that supplemental rice husk in the feed improved the growth of *Lactobacillus* bacteria and reduced the population of *Escherichia coli* in the ileum and cecum of broiler chickens.

5. Conclusion

Feeding broilers OA dietary supplementation at 1 g/kg improved growth performance of broilers across the entire rearing period, particularly in diets without fiber supplementation. The lack of supplemental fiber effect on the performance of broiler chickens is likely due to high CF content of the basal diet. Effect of OA on jejunal villus height was more pronounced in diets containing fibrous materials. Antibody titer against IDV increased with supplementation of OA in IF RH-containing diets, which is supported by the increased intestinal *Lactobacillus* bacteria population in birds fed 1 g/kg OA in RH-containing diets.

Conflicts of interest

Authors declare no conflict of interest.

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