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 OA × 3 fiber sources). 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.

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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 (Ji 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
regard, Abazari et al. (2016) demonstrated that inclusion of rice husk in diets of broiler chickens increased the population of *Lactobacillus* 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 mucosal 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 by AOAC (1990), CP (Method 990.03), CF (Method 978.10), Ca and P (methods 968.08 and 965.17) 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. All experimental diets were fed in mash form and were formulated to be isoproteinous and isenergetic. 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
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 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 (1500 × g for 15 min) at room temperature. The hemagglutination inhibition assay method was used to measure the antibody titer against SRBC. Antibody titers against IDV and NDV were separately measured by hemagglutination inhibition (HI) method. The HI antibody 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 CO2, 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 ≤ 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 substrates 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

Duodrenal 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

| 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            | 1.8              |
| L-threonine         | 1.1            | 0.6            | 0.5              |
| Vitamin premix      | 2.5            | 2.5            | 2.5              |
| Mineral premix      | 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, M/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 phosphorus| 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 phosphorus    | 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 D₃ (cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K₁, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthotenic 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 (FeSO₄·7H₂O, 20.09% Fe), 50 mg; Mn (MnSO₄·H₂O, 32.48% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO₄·5H₂O), 10 mg; I (KI·58% I), 1 mg; Se (Na₂SeO₃, 45.56% Se), 0.2 mg.

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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 \((P < 0.05); \text{Table } 5\).

### 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-Tafit 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 3

| Item                        | 1 – 11 d | 12 – 28 d | 29 – 42 d | 1 – 42 d |
|-----------------------------|---------|----------|----------|---------|
| Dietary weight gain, g      |         |          |          |         |
| Organic acid, g/kg          |         |          |          |         |
| Fiber source                |         |          |          |         |
| No fiber                    |         |          |          |         |
| SF                          | 25.0    | 57.0     | 70.1     | 53.5b   |
| IF                          | 25.7    | 57.5     | 71.6     | 56.7ab  |
| 1                           |         |          |          |         |
| No fiber                    |         |          |          |         |
| SF                          | 26.5    | 55.6     | 75.9     | 57.7b   |
| IF                          | 26.6    | 58.4     | 70.6     | 56.9ab  |
| Organic acid, g/kg          |         |          |          |         |
| Fiber source                |         |          |          |         |
| No fiber                    |         |          |          |         |
| SF                          | 25.6b   | 56.0     | 71.0     | 55.1b   |
| IF                          | 26.5a   | 56.4     | 73.0     | 56.9a   |

### Table 4

| Item                        | Carcass yield | Abdominal fat | Heart Liver |
|-----------------------------|---------------|---------------|-------------|
| Organic acid, g/kg          |               |               |             |
| Fiber source                |               |               |             |
| No fiber                    | 71.60         | 0.98ab        | 0.41 2.00   |
| IF                          | 72.39         | 1.45a         | 0.46 1.97   |
| 1                           |               |               |             |
| No fiber                    | 70.60         | 1.00ab        | 0.42 2.16   |
| SF                          | 73.54         | 0.74a         | 0.41 2.03   |
| IF                          | 74.53         | 0.80a         | 0.41 2.15   |

SF = soluble fiber; IF = insoluble fiber.

\( a \) Values in the same column not sharing a common superscript differ significantly \((P < 0.05)\).

\( b \) Rice hull as IF or sugar beet pulp as SF was supplemented at 30 g/kg to replace silica sand in the basal diet.

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

\* Table 3: Effects of dietary treatments on performance of broiler chickens at different ages.
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 (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 acidity and dietary treatments might have different effects on energy and protein deposition without any change in liver proportional weight.

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 |          |         |       |          |         |       |          |         |       |
| 0    |          |         |       |          |         |       |          |         |       |
| No fiber | 1,384 | 233 | 6.1 | 1,060abc | 188 | 5.8 | 536 | 130 | 4.0 |
| SF   | 1,240 | 215 | 6.3 | 843bc | 192 | 4.4 | 569 | 130 | 4.3 |
| IF   | 1,472 | 207 | 7.3 | 780c | 188 | 4.3 | 603 | 171 | 3.9 |
| 1    |          |         |       |          |         |       |          |         |       |
| No fiber | 1,228 | 229 | 5.3 | 1,008bc | 191 | 5.5 | 664 | 127 | 5.2 |
| SF   | 1,512 | 230 | 6.8 | 1,088bc | 169 | 5.0 | 465 | 119 | 4.1 |
| IF   | 1,485 | 246 | 6.3 | 1,190abc | 230 | 4.7 | 582 | 162 | 3.5 |
| Organic acid, g/kg |          |         |       |          |         |       |          |         |       |
| 0    |          |         |       |          |         |       |          |         |       |
| No fiber | 1,365 | 219 | 6.6 | 894bc | 189 | 4.8 | 569 | 136 | 4.0 |
| SF   | 1,408 | 235 | 6.1 | 1,096abc | 196 | 5.0 | 570 | 144 | 4.3 |
| IF   | 1,396b | 231 | 5.7 | 1,034abc | 209 | 4.5 | 592 | 166a | 3.7 |

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

| Item | Antibody titer, log2 | Lymphoid organ, % BW |
|------|----------------------|----------------------|
|      | IDV                  | NDV                  | SRBC                 |
|      |                      |                      |                      |
| Organic acid, g/kg | Fiber source1 |          |         |          |         |       |          |         |       |
| 0    | No fiber | 3.6 | 3.7 | 8.4 | 0.090 | 0.078 |
| SF   | 3.7abc | 3.9 | 8.6 | 0.102 | 0.074 |
| IF   | 3.7abc | 3.5 | 8.6 | 0.080 | 0.078 |
| 1    | No fiber | 3.6c | 3.2 | 8.4 | 0.104 | 0.076 |
| SF   | 3.8b | 3.8 | 8.5 | 0.088 | 0.070 |
| IF   | 4.0b | 3.9 | 8.5 | 0.074 | 0.064 |
| Organic acid, g/kg | Fiber source1 |          |         |          |         |       |          |         |       |
| 0    | No fiber | 3.0c | 3.5 | 8.6 | 0.090 | 0.076 |
| SF   | 4.1a | 3.8 | 8.3 | 0.088 | 0.070 |
| IF   | 3.2 | 3.8 | 8.7 | 0.097 | 0.077 |
| 1    | No fiber | 3.5b | 3.5 | 8.6 | 0.095 | 0.072 |
| SF   | 4.1a | 3.6 | 8.1 | 0.077 | 0.071 |
| IF   | 0.029 | 0.12 | 0.18 | 0.015 | 0.007 |
| Organic acid |          |         |       |          |         |       |          |         |       |
| 0    | No fiber | <0.001 | 0.176 | 0.367 | 0.798 | 0.439 |
| SF   | <0.001 | 0.555 | 0.405 | 0.887 | 0.826 |
| IF   | 0.318 | 0.323 | 0.734 | 0.331 | 0.826 |

SF = soluble fiber; IF = insoluble fiber; VH = villus height; CD = crypt depth.

1 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 Dijger (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 (Teihlyńck 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) found that broilers consumed diets that contained formic acid or potassium diformate had lower cecal populations of total *clostridium* 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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References

Abazari A, Navidshad B, Mirzaei Aghjehgheshlagh F, Nikbin S. The effect of rice husk as an insoluble dietary fiber source on intestinal morphology and *Lactobacillus* and *Escherichia coli* populations in broilers. Iran J Vet Med 2016;10:217–24.
Abdel-Fattah SA, El-Sanhoury MH, El-Mednay NM, Abdel-Azeem F. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int J Poultry Sci 2008:7:215–22.
Adibmoradi M, Navidshad B, Faseleh Jahromi M. The effect of moderate levels of finely ground insoluble fibre on small intestine morphology, nutrient digestibility and performance of broiler chickens. Ital J Anim Sci 2016:15:310–7.
Adil S, Banday T, Bhat GA, Mir MS, Rehman M. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet Med Int 2010;2010:1–7.

Alp M, XocabaGL, Nahraman R, Bostan K. Effects of dietary supplementation with organic acids and zinc bacitracin on ileal microflora, pH and performance in broilers. Turk J Vet Anim Sci 1999;23:431–6.

Ao T, Cantor AH, Pescatore AJ, Ford MJ, Pierce JL, Dawson KA. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poultry Sci 2009;88:111–7.

AOAC. Official methods of analysis. Washington DC: Association of Official Analytical Chemist; 2000.

Aviagen. Ross 308 broiler: nutrition specification. Newbridge, Midlothian, Scotland, UK: Ross Breeders Limited; 2014.

Biggs P, Parsons CM. The effects of several organic acids on growth performance, nutrient digestibilities, andecal microbial populations in young chicks. Poultry Sci 2008;87:2581–9.

Corfield AP, Carroll D, Myerscough N, Probert CS. Mucins in the gastrointestinal tract in health and disease. Front Biosci 2001;6:D1321–57.

Debon SJ, Tester RF. In vitro binding of calcium, iron and zinc by non-starch polysaccharides. Food Chem 2001;73:401–10.

Dehghani-Tafti N, Jahanian R. 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 Technol 2016;211:109–16.

Emami NK, Naeini SZ, Ruiz-Feria CA. Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus deficient diets supplemented with microbial phytase and organic acids. Livest Sci 2013;157:506–13.

Ferket PR, Gernat AG. Factors that affect feed intake of meat birds: a review. Int J Anim Feed Sci 2010;8:156–66.

González-Alvarado JM, Jiménez-Moreno E, González-Sánchez D, Lázaro R, Mateos GG. Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 days of age. Anim Feed Sci Technol 2010;162:37–46.

González-Alvarado JM, Jiménez-Moreno E, Lázaro R, Mateos GG. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poultry Sci 2007;86:1705–15.

Gural M, Vayli G, Kaya O, Karahan N, Sulak O. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int J Poultry Sci 2006;5:149–55.

Houshmard M, Azhar K, Zulkiifli R, Mateos GG. Effect of type of dietary fiber and fat on performance and digestive traits of broilers. Br Poult Sci 2016;57:162–71.

Houshmard M, Azhar K, Zulkiifli R, Mateos GG. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poultry Sci 2009;88:2562–74.

Jiménez-Moreno E, González-Alvarado JM, Jiménez-Serrano A, Lázaro R, Mateos GG. Effects of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poultry Sci 2010;89:2197–212.

Jiménez-Moreno E, González-Alvarado JM, Jiménez-Serrano A, Lázaro R, Mateos GG. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. Poultry Sci 2009;88:2562–74.

Kalmendal R, Elwingr K, Holm L, Tauson R. High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens. Br Poult Sci 2011;52:86–96.

Khan SH, Iqbal J. Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res 2016;44:339–69.

Mateos GG, Jiménez-Moreno E, Serrano MP, Lázaro RP. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J Appl Poultry Res 2012;21:156–74.

Montagne L, Piel C, Lalle JP. Effect of diet on mucus kinetics and composition: nutrition and health implications. Nutr Rev 2004;62:105–14.

Montagne L, Pluske JR, Hampson DJ. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Aniim Feed Sci Technol 2003;108:95–117.

Panda AK, Rao SVR, Raju M, Sunder GS. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian Aust J Anim Sci 2009;22:1026–31.

Parmitter HK, Baehrens R, Savelkoul HFJ, Dorny P, Deney B, Berkvens D. Serum haemolytic complement activities in 11 different MHC (B) typed chicken lines. Vet Immunol Immunopathol 2004;100:25–32.

Ragaa NM, Korany RMS. Studying the effect of formic acid and potassium dichromate on performance, immunity and gut health of broiler chickens. Anim Nutr 2016;2:296–302.

Rezaei M, Karimi Torshizi MA, Rouzbahien Y. The influence of different levels of micronized insoluble fiber on broiler performance and litter moisture. Poultry Sci 2011;90:2008–12.

Rodríguez-Lecompte JC, Yitbarek A, Brady J, Sharif S, Cavanagh MD, Crow G, Guenter W, House JD, Camelio-James G. The effect of microbial-nutrient interaction on the immune system of young chicks after early probiotic and organic acid administration. J Anim Sci 2012;90:2246–54.

Sadeghi A, Toghyan M, Ghesari A. Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poultry Sci 2015;94:2734–43.

Sklan D, Smirnov A, Plavnik I. The effect of dietary fibre on the small intestines and apparent digestion in the Turkey. Br Poult Sci 2003;44:735–40.

Teirlynck E, Bjerrum L, Eeckhaut V, Huygebaert G, Pasmans F, Haesebrouck F. The effect of dietary microbial phytase on performance and digestive health in broiler chickens. Br Poult Sci 2011;52:1453–61.

Van der Wielen PWJ, Biesterveld S, Lipman LJA, van Knapen F. Inhibition of a glucose-limited sequenced fed-batch culture of Salmonella enterica serovar Enteritidis by volatile fatty acids representative of the ceca of broiler chickens. Appl Environ Microbiol 2001;67:1979–82.

Van Soest PJF, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.

Wegmann TG, Smithies O. A simple hemagglutination system requiring small amounts of red cells and antibodies. Transfusion 1965;6:67–73.

Wiis-Plnot EL, Dilger RN. Combined dietary effects of supplemental threonine and purified fiber on growth performance and intestinal health of young chicks. Poultry Sci 2013;92:726–34.

Zhang WH, Jiang Y, Zhu QF, Gao F, Dai SF, Chen J, Zhou GH. Sodium butyrate maintains growth performance by regulating the immune response in broiler chickens. Br Poultry Sci 2011;52:292–301.