Single and Combinative Effects of Fructo-oligosaccharide and Organic Acid on Growth Performance and Immune Function of Broiler Chickens.

KAMRAN TAHERPOUR1* AND HOSSEIN ALI GHASEMI2
1Department of Animal Science, Faculty of Agriculture, Ilam University, Ilam, Iran.
2Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran.
*Corresponding Author: k.taherpour@ilam.ac.ir (Accepted: 14 Dec. 2013)

ABSTRACT
A 42 day trial was conducted to investigate the effects of prebiotic (Fructomix) and organic acids (Orgacid), either singly or in combination, on morphological and physiological performance of broiler chickens. The studied characteristics were growth performance, lymphoid organs weight, antibody response against sheep red blood cell (SRBC) and cell-mediated response to phytohaemagglutinin-P (PHA-P). Two hundred one day old male broiler chicks were randomly divided into 20 experimental units. Birds were offered either a corn-soybean meal basal diet (control) or the basal diet supplemented with 1 gkg\(^{-1}\) Fructomix (FM), gkg\(^{-1}\) Orgacid (OA) or a mixture of 1 gkg\(^{-1}\) FM plus 2 gkg\(^{-1}\) OA (FM+OA). The results showed that body weight gain and feed conversion ratio were significantly improved by FM and FM+OA diets compared to control diet from 0 to 42 days of age. On day 35, the toe web swelling (cell-mediated response to phytohaemagglutinin-P) in the birds fed with OA or FM+OA diets was significantly higher (p<0.05) than the control birds which were fed with corn-soybean meal basal diet. The spleen relative weight and secondary anti-SRBC titers in the birds fed with OA and FM+OA diets were also significantly higher than in birds fed the control diet, where the birds received FM+OA diet had the highest secondary antibody titers. However, feed intake, relative weights of thymus and bursa and primary antibody response against SRBC were not significantly affected by dietary treatments. In conclusion, the addition of FM or FM+OA in the diet of broiler chickens improve various growth performance, besides improving birds immunity system.

Keywords: Feed conversion ratio, feed intake, phytohaemagglutinin, prebiotic, productive performance, secondary antibody.

Abbreviations:
BWG: body weight gain; FCR: feed conversion ratio; FI: feed intake; FM: Fructomix; PHA-P: Phytohaemagglutinin-P; OA: Orgacid; SRBC: Sheep red blood cell.

INTRODUCTION
Normal microbial ecology in the gastrointestinal tract, ensure the gut health and improves growth performance in poultry. Antibiotics have been used in poultry industry during the past years not only for their antimicrobial property, but also for their growth-promoting property (Fature and Matanmi, 2008). However, antibiotics as a supplement are banned in many countries because of public concerns for the antibiotic residual effects and incidence of bacterial resistance in the consumers. Therefore, the interest in finding replacement for antibiotics in poultry feed has been a great concern during recent years. In this regard, prebiotics and organic acids have been recommended as the most important alternatives to antibiotics substitution (Chaveerach et al., 2004).

Prebiotics are indigestible substances that are used as additives in poultry foods in order to selectively stimulate the activity and/or proliferation of endogenous bacteria such as *Bifidobacteria*, which benefit the host. They also contain short-chain carbohydrates, which are non-digestible by the animal enzymes (Ashraf et al., 2013). Fructo oligosaccharides (FOS) are one of the common prebiotics with special chemical structure that can improve growth and overall intestinal health. Fructo-oligosaccharides such as inulin and oligofructose are naturally occurring indigestible carbohydrates that are readily fermented in the cecum and colon (Awad et al., 2011). Several studies have reported that administration of prebiotics can improve body weight gain, feed
intake and feed convention ratio in broiler chickens (Pelicono et al., 2004; Rodrigues et al., 2005). In contrast, some studies indicated that prebiotic supplementation do not affect growth performance (Stanczuk et al., 2005). In addition to growth promoting effects, prebiotic supplemental have been reported to stimulate immunity (Patterson and Burkholder, 2003).

Short-chain fatty acids, as organic acids, are also considered as potential alternatives to antibiotic-growth promoters (Smulikowska et al., 2010). An important aim of dietary acidification is the reduction of possible toxic bacterial metabolites and an inhibition of intestinal bacteria competing with the host for available nutrients. In this regard, a number of researches have suggested that organic acid supplements influence the populations of bacteria in the small intestine and ceca (Cengiz et al., 2012). However, it has been shown that dietary organic acids have no effects on body weight or weight gain during starter, grower and finisher periods, but birds consumed less feed intake when diets were supplemented with butyrate relative to the control birds (Lesson et al., 2005). The use of organic acids, such as fumaric acid, citric acid and lactic acid, can induce an acidic environment (pH 3.5 to 4.0) in the host gut that favors the development of lactic acid bacteria and inhibits the growth of pathogenic bacteria such as Escherichia coli, Salmonella (Chowdhury et al., 2009).

However, the comparative effects of prebiotic and organic acids on the immune response in associated with their growth promoting activities in broiler chicks are still unclear. Therefore, the present study was designed to investigate the single or combined effects of a prebiotic (fructooligosaccharide) and an organic acid blend on growth performance, lymphoid organs weight, haemaggutumin antibody titer against sheep red blood cell (SRBC) and cutaneous basophil hypersensitivity reaction to phytohemagglutinin-P (PHA-P) in broiler chickens.

MATERIALS AND METHODS

Treatments and Experimental Designs:

In the present study, 200 one-day-old male broiler chicks (Ross 308) were used during a 42-day period. The birds were randomly allotted to 20 floor pens covered with fresh shaving (10 birds/pen) and fed with regular starter (0-10 d), grower (11-28 d) and finisher (29-42 d) diets as mentioned in table 1. All diets were formulated to provide the nutrient requirements of broilers according to the recommended reference levels (Aviagen, 2002). From day one, chickens were fed either a corn-soybean meal basal diet (control) or the basal diet supplemented with 1 gkg⁻¹ Fructomix (FM), 2 gkg⁻¹ Orgacid (OA) or mixture of 1 gkg⁻¹ FM and 2 gkg⁻¹ OA (FM+OA). Fructomix is a natural fructooligosaccharide produced from plant sources of chicory and topinambour. The chain length and polymerization degree of Fructomix is 5–14. The organic acids blend (Orgacid) used in this experiment was a mixture of six kinds of organic acids (formic, citric, malic, lactic, tartaric and orthophosphoric acid). All floor pens measured 1.3 × 1.5 m. Each pen had one bell-type waterer and one tube feeder. Birds were vaccinated routinely against infectious bronchitis, Newcastle and Gambbaro diseases, but no medication administered during the entire experimental period. All chickens had free access to water and feed. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were recorded at the end of each experimental period and analyzed.

### Table 1. Composition of broilers' basal diet.

<table>
<thead>
<tr>
<th>Ingredient (% of diet)</th>
<th>0–10d</th>
<th>11–28d</th>
<th>29–42d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>53.19</td>
<td>59.28</td>
<td>64.90</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>31.55</td>
<td>20.78</td>
<td>22.96</td>
</tr>
<tr>
<td>Gluton meal (60% CP)</td>
<td>7.13</td>
<td>4.49</td>
<td>4.22</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Dicalkium Phoshpat</td>
<td>2.04</td>
<td>1.80</td>
<td>1.85</td>
</tr>
<tr>
<td>Oyster shells</td>
<td>1.41</td>
<td>1.28</td>
<td>1.29</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>DL-Methionin</td>
<td>0.29</td>
<td>0.25</td>
<td>0.30</td>
</tr>
<tr>
<td>L-Lysin,HCL</td>
<td>0.53</td>
<td>0.46</td>
<td>0.52</td>
</tr>
<tr>
<td>Vitamin PremixⅠ</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral PremixⅠ</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Calculated composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME (kcalkg⁻¹)</td>
<td>3010</td>
<td>3150</td>
<td>3200</td>
</tr>
<tr>
<td>CP</td>
<td>2.3</td>
<td>20.10</td>
<td>18.50</td>
</tr>
<tr>
<td>Ca</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Non Phytate Phosphorus (NPP)</td>
<td>0.5</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Na</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.44</td>
<td>1.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Methionine + Cysteine</td>
<td>1.09</td>
<td>0.94</td>
<td>0.80</td>
</tr>
</tbody>
</table>

1 Supplied per kilogram of diet: 600 IU vitamin A, 800 IU vitamin D, 83 mg vitamin E, 2.2 mg vitamin K3, 2 mg vitamin B6, 8 mg vitamin B12, 10 mg Nicotine amid, 0.3 mg Folic acid, 20 mg D-Biotin and 160 mg Choline Chloride.

### Immunological Tests:

At 42 days of age, two birds per replicate randomly chosen were slaughtered and thymus, spleen and bursa were collected, weighed and calculated as a percentage of live body weight and used as indicators of immune function.

Two birds for each replicate were injected (via the brachial vein) by 0.1 ml of a 5% suspension of SRBC in phosphate-buffered saline (PBS) at 14 and 25 days of age. Six days after each injection, blood samples were collected from the birds and kept for at least two hours at the ambient temperature to separate serum from the blood clot. The serum samples were stored in a -20°C freezer until analyzed. Antibody titers against SRBC were determined by a hemagglutination method into the 96-well, U-bottomed microtiter plates. Briefly, the serum samples were placed in a water bath for 30
min at 55° C to deactivate the complement. Serum samples (25 μL) were then added and serially double diluted in the wells from columns 2 to 12. The first columns (PBS only) of wells were considered as blank. Then, 25 μL of 1% SRBC suspension in PBS was added to all wells (final volume of 50 μL). Subsequently, the plates were covered with plastic bags and stored at room temperature for 2 h to allow antibody-antigen reaction. The hemagglutination titer for each serum sample was the reciprocal of the highest serum dilution that gave 100% agglutination.

The cell-mediated immune response was determined by a cutaneous basophil hypersensitivity test using PHA-P (Corrier and De Loach, 1990). At d 32, the toe-web (between the second and the third digit) of the left foot (2 birds from each pen) were measured with a constant tension caliper. Immediately after measurement, 100 μg of PHA-P was injected into the toe web. The toe-webs thickness was measured 24 h after injection. The response was calculated as the differences between the toe-web thickness at time 0 and the mean thickness of toe-web 24 h after dermal injection.

Statistical Analysis:
Data were analyzed in a completely randomized design using the General Linear Model (GLM) procedure of SAS (2001). Means were compared for significant (p≤0.05) differences using Duncan’s multiple range tests.

RESULTS
The impact of different experimental diets on growth performance is shown in Table 2. FM and FM+OA diets significantly improved FCR (P<0.05) for the grower period (11-28 d) and also for the total experimental period. An improvement in FCR (P<0.05) during the finisher period (29-42 d) and whole experimental period (0-42 d) was also found when the birds fed diets FM and FM+OA. However, feed intake (FI) was not statistically affected (P>0.05) by the mentioned treatments.

The weights of lymphoid organs are summarized in Table 3. The different feed additives in the current study failed to induce any significant impact (P > 0.05) on the relative weights of thymus and bursa. However, the relative weight of the spleen was significantly (P < 0.05) higher in birds fed diets OA or FM+OA compared with control diets, where the birds fed diet FM+OA had the highest spleen relative weight.
The effect of different treatments on antibody response against SRBC (20 and 31 d of age) in broilers are presented in Figure 1. The birds fed diets OA or FM+OA showed the highest (P<0.05) total secondary antibody titer compared to control group, where the birds received FM+OA diet had the highest (P<0.05) secondary antibody titers. Although, no significant difference (P>0.05) was observed between treatments regarding the primary response of anti-SRBC titer, but a tendency (P=0.087) found to increase total antibody titers by diet FM+OA.

As Figure 2 shows, feeding diets OA or FM+OA to broilers were also suppressed (P<0.05) the cell-mediated immune response to PHA-P.

DISCUSSION

In the current study, it was found that BWG and FCR could be improved by a dietary regime supplemented with FM or FM+OA. Moreover, a slight improvement in performance traits was observed in broilers fed the diet containing organic acids compared to control birds. The differences in growth parameters among experimental groups can be associated with additives effects, because nutrient composition and feed ingredients of their diets were exactly same, as shown in Table 1. Therefore, the results of this experiment confirmed that using prebiotic alone or mixed with organic acids improves broiler performance. Fructooligosaccharides display special chemical structures, which may help promote gut health of the host, consequently improving growth performance. In different studies that were performed to evaluate the impact of fructooligosaccharide on broilers growth performance, contradictory results have been obtained. For example, Williams et al. (2008) reported reductions in BWG and FI and improvements in FCR in broiler chickens fed prebiotic-supplemented diets (600 mg kg⁻¹). This has been while other researchers (Yang et al., 2008; Kim et al., 2011) reported improvements in BWG of broilers fed with fructo-oligosaccharide, with no changes in FCR. Improvement in growth performance by prebiotics in broiler chickens may largely be due to the development and improvement of intestinal microbial flora balance (Patterson and Burkholder, 2003). Growth promoting effects of prebiotics have also been reported to be related to some positive changes in digestive enzymes and immune system (Houshmand et al., 2011). Furthermore, supplementation with prebiotics can positively change mucosal architecture and increase villi height (Yang et al., 2007).

Similar to

In terms of organic acid effects on broilers performance, we found that butyric acid application in the diet of broiler has no effect in BWG of broilers. This finding is in accordance to Leeson et al.’s (2005) report. They also realized that by using butyric acid in the broiler diet, BWG of broilers would not be affected. However, Chowdhury et al. (2009) found a positive influence on broilers performance after supplementing their diet with organic acids blend at levels up to 2%. Inconsistent results of using prebiotic and organic acid may be due to the additive dose used in the diets, differences in supplement preparation methods, variations in feedstuffs and nutrient levels and different experimental condition. Variations in the specific acids used in the organic acid blend, which may have different impacts on the growth performance of the birds, may also have an impact on the results.
The relative weight of the spleen also increased by OA and OA+FM supplementation of diets, whereas the relative weights of thymus and bursa were not affected significantly by experimental diets. Positive effects of organic acid supplements on broilers immunity has been reported previously. In this regard, Abdel-Fattah et al. (2008) reported that the dietary supplementation of organic acids resulted in a higher immune response. In their research, the birds fed an organic acid supplemented diet had heavier immune organs and also a higher level of globulin in their blood. The spleen, as a secondary lymphoid organ, plays important roles concerning erythrocytes and immune system. It also synthesizes antibodies in its white pulp and removes antibody-coated blood cells and antibody-coated bacteria by means of blood and lymph node circulation (Mebius and Kraal, 2005). The response in antibody-mediated immunity was similar to that of spleen weight observed in this study. Therefore, it can be stated that the positive effects of OA and OA+FM diets supplementation on spleen relative weight may be associated with improved antibody-mediated immunity, which could be a result of a promotion of the immunoglobulins production.

The results of the current study also demonstrated that dietary supplementation of OA, as well as a mixture of FM and OA, could significantly improve secondary antibody titers against SRBC and cutaneous basophil hypersensitivity reaction, but no significant effects were identified in the primary anti-SRBC titers. One of the possible factors for the lack of effect of OA on growth parameters could be the stimulation of the immune response. By stimulation of immune response, nutrients will be applied for production of immunoglobulins and therefore, growth will be retarded (Khodambashi-Emami et al., 2012). The improvement in immune function might be due to a direct antimicrobial effect of organic acids as reported by Gunal et al. (2006). Gunal et al. (2006) found that the application of OA in broiler chickens significantly reduced the total bacterial population, specially the gram-negative bacteria. Organic acids may also influence the cell macromolecules or integrity of microbial cell membrane or interfere with energy metabolism and nutrient transport causing bactericidal effect (Ricke, 2003). Furthermore, Abdel-Fattah et al. (2008) stated that OA supplementation has the pH-decreasing properties in different gastrointestinal segments of broilers. The low pH conditioning is favorable for the growth of beneficial bacteria, especially lactic acid bacteria, while it is unsuitable for the growth of pathogenic bacteria such as Escherichia coli and Salmonella (Chowdhury et al., 2009). The direct antimicrobial and pH-decreasing properties of OA might result in toxic bacterial metabolites diminution and as a result, the reduction in bacterial fermentation (Kopecky et al., 2012). The later might result in immune function improvement.

**CONCLUSION**

Based on the results of the current study, it could be concluded that dietary supplementation of FM alone or in combination with organic acid improves growth performance. This is while the immune response performance was not affected by FM supplementation. Moreover, the addition of OA or FM+OA to broiler’s diet improves their immune function by increasing secondary anti-SRBC titers or Cutaneous basophil hypersensitivity response to PHA-P.

**REFERENCES**


