Evaluation of Oyster Mushroom (Pleurotus Ostreatus) as a Biological Growth Promoter on Performance, Humoral Immunity, and Blood Characteristics of Broiler Chicks

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This study was conducted to determine the impacts of two levels of oyster mushroom (Pleurotus ostreatus) powder on productive and carcass traits, humoral immune responses, and blood characteristics of Ross 308 male broiler chicks reared to 42 d of age in comparison with a prebiotic supplement. Two hundred and forty, day-old male broiler chicks (Ross 308) were randomly assigned to one of four treatments with four replicates of 15 chicks based on a completely randomized design. The dietary treatments consisted of the basal diet as control, prebiotic group receiving 1 g/kg A-Max® (Mannan-oligosaccharides), 10, and 20 g/kg oyster mushroom powder added to the basal diet. The obtained results showed that inclusion of 20 g/kg mushroom powder significantly improved body weight over the starter and grower (P<0.05) while feed efficiency was improved only over the starter (P<0.05) period compared to the control group. Considering the entire experimental period, (1-42 d) birds receiving prebiotic supplemented diets exhibited the highest body weight and lowest feed conversion ratio relative to the other treatments (P<0.05). Carcass yield and internal organs relative weights were not influenced by dietary treatments, but prebiotic supplementation significantly (P<0.05) decreased abdominal fat pad compared to the control group. Newcastle, influenza and sheep red blood cell antibody responses of chicks did not differ significantly at either level of inclusion of supplements. Chicks fed supplemented diets had the lowest serum triglyceride concentration at 42 day (P<0.05) compared to the control chicks, but other biochemical and hematological values tested including protein, albumin, globulin, high-density lipoprotein, low-density lipoprotein, and total cholesterol, red blood cell, white blood cell, hemoglobin and hematocrit were not markedly affected by treatments. In conclusion the obtained results indicated that oyster mushroom powder at an inclusion level of 20 g/kg of diet had favorable effects on performance criteria of chicks reared to 28 day of age while, prebiotic supplementation revealed its beneficial impact on chicks productive traits at slaughter age, besides reducing carcass abdominal fat and serum triglyceride concentration at 42 day.

Key words: blood metabolites, broiler, immunity, oyster mushroom, prebiotic

Introduction

The growing concerns about antibiotic residues in final livestock products and the risk of bacteria acquiring resistance to these specific antibiotics has arisen into a controversial issue around the world. Consequently, there has been a considerable interest in finding alternatives for in-feed antibiotics of plant origin to be proposed to livestock producers, particularly within the poultry industry.

Phytogenic additives, plant extracts, essential oils, prebiotics, and probiotics have largely been investigated as possible in-feed antibiotic substitutions, and some have proved satisfactory results. Mushrooms are known to have considerable health-promoting benefits based on their content of antioxidants, phenolic compounds, tocopherols, carotenoids, and antibacterial compounds (Zhou et al., 2010). Recently, a review by the University of Mississippi stated of an “evaluation of over 200 mushroom species, more than 75% of screened polypores showed strong antimicrobial activities (Zhou et al., 2010). Oyster mushroom, or Pleurotus ostreatus, is a common edible mushroom and studies with Pleurotus ostreatus have demonstrated various antimicrobial, antiviral and anticancer properties (Bobek and Galbavy,
2001; Jedink and Sliva, 2008). In addition, based on in vivo researches consumption of oyster mushrooms has been reported to lower cholesterol levels, since they naturally contain lovastatin (Khatun et al., 2007). Substances in mushrooms responsible for the favorable effects on growth performance and immune responses are a diverse class of oligosaccharides components (Xue and Meng, 1996). It has been hypothesized that some of the properties of plants or fungi are carried through a prebiotic effect due to their polysaccharide content (Cummings and Macfarlane, 2002). Prebiotics are defined as non-digestible food components or ingredients mainly including oligosaccharides like fructo-oligosaccharides (FOS), gluco-oligosaccharides (GOS), and mannan-oligosaccharides (MOS) which positively influence the host by their selective growth and/or activation of certain number of bacterial species present in intestines (Gibson and Roberfroid, 1995).

Although, some species of mushrooms like Shiitake mushroom (Lentinula edodes) (Guo et al., 2004b; Willis et al., 2007), and white button mushroom (Agaricus bisporus) (Giannenas et al., 2010) have been investigated as possible in-feed antibiotic alternatives, there is very limited information concerning the application of oyster mushroom in broiler diets. Thus, this study was carried out to evaluate the impact of two levels of dried oyster mushroom as a natural growth promoter agent in comparison with a commercial prebiotic supplement on performance, humoral immune responses, blood biochemical, and hematological profile of broiler chicks.

**Materials and Methods**

**Animals and Experimental Diets**

Two hundred and forty, day-old male broiler chicks (Ross 308) purchased from a local hatchery, were weighed on arrival and randomly assigned to one of four treatments with four replicates of 15 chicks based on a completely randomized design. The dietary treatments consisted of the basal diet as control, prebiotic group receiving 1 g/kg A-Max® (Mannan-oligosaccharides), 10, and 20 g/kg of feed oyster mushroom powder added to the basal diet. Table 1 lists the basal corn-soybean diets formulated to meet or exceed the nutrient requirements of broilers provided by Ross manual catalogue (2007); also, the same batch number of ingredients, which contained no antibacterial or anticoccidial supplements, was used to formulate the diets for different periods. Chicks were raised on floor pens covered by sawdust as litter.

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**Table 1. Ingredients and nutrient specifications of experimental diets applied in starter, grower, and finisher periods**

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>CON 1</th>
<th>M10</th>
<th>M20</th>
<th>CON 1</th>
<th>M10</th>
<th>M20</th>
<th>CON 1</th>
<th>M10</th>
<th>M20</th>
<th>CON 1</th>
<th>M10</th>
<th>M20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (8% CP)</td>
<td>540.7</td>
<td>529.7</td>
<td>516.7</td>
<td>539.6</td>
<td>527.6</td>
<td>515.6</td>
<td>565.5</td>
<td>556.9</td>
<td>545.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal (43% CP)</td>
<td>402</td>
<td>400</td>
<td>400</td>
<td>391</td>
<td>390</td>
<td>389</td>
<td>361</td>
<td>360</td>
<td>360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mushroom (13.7% CP)</td>
<td>14.0</td>
<td>17.0</td>
<td>20.0</td>
<td>12.1</td>
<td>12.1</td>
<td>12.1</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>13.4</td>
<td>13.4</td>
<td>13.4</td>
<td>14.6</td>
<td>14.6</td>
<td>14.6</td>
<td>13.9</td>
<td>13.9</td>
<td>13.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>16.9</td>
<td>16.9</td>
<td>16.9</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grower (14–28 d)</td>
<td>2840</td>
<td>2840</td>
<td>2838</td>
<td>2983</td>
<td>2982</td>
<td>2981</td>
<td>3043</td>
<td>3042</td>
<td>3042</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finisher (28–42 d)</td>
<td>2840</td>
<td>2840</td>
<td>2838</td>
<td>2983</td>
<td>2982</td>
<td>2981</td>
<td>3043</td>
<td>3042</td>
<td>3042</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Calculated Analysis**

| MEn (kcal/kg) | 2840 | 2840 | 2838 | 2983 | 2982 | 2981 | 3043 | 3042 | 3042 |       |     |     |
| Available P (g/kg) | 4.65 | 4.64 | 4.63 | 4.32 | 4.31 | 4.30 | 4.00 | 4.00 | 4.00 |       |     |     |
| Lys (g/kg) | 13.2 | 13.2 | 13.2 | 11.9 | 11.9 | 11.9 | 11.2 | 11.2 | 11.2 |       |     |     |
| Met+Cys (%) | 10.0 | 10.0 | 10.0 | 9.1 | 9.1 | 9.1 | 8.8 | 8.8 | 8.8 |       |     |     |

**Analyzed composition (g/kg)**

| Crude protein (N×6.25) | 218 | 217 | 217 | 213 | 213 | 213 | 204 | 204 | 204 |       |     |     |
| Calcium | 11.80 | 11.80 | 11.90 | 9.60 | 9.65 | 9.65 | 8.80 | 8.80 | 8.80 |       |     |     |
| Total phosphorus | 7.60 | 7.60 | 7.60 | 7.20 | 7.20 | 7.26 | 6.80 | 6.83 | 6.85 |       |     |     |
| Crude fiber | 43.2 | 43.3 | 43.3 | 42.8 | 42.8 | 42.9 | 41.6 | 41.7 | 41.8 |       |     |     |
| Crude fat | 33.6 | 33.6 | 33.7 | 53.6 | 53.6 | 53.7 | 58.7 | 58.7 | 58.8 |       |     |     |

1 CON, M10, and M20 represent diet supplemented with dried Pleurotus ostreatus mushroom at the level of 0, 10, or 20 g/kg of feed, respectively.

2 Mushroom ME was considered 1270 kcal/kg as reported by Tonglian et al. (2004).

3 Monocalcium phosphate contained 22% phosphorous and 15% calcium.

4 Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 g; folic acid, 1 mg; choline chloride, 250 mg; 100 mg ethoxyquin as antioxidant.

5 Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe), 50 mg; Mn (MnSO4.H2O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO4.5H2O), 10 mg; I (KI, 58% I), 1 mg; Se (NaSeO3, 45.56% Se), 0.2 mg.
(10 birds/m²) for 6 weeks. Food and water were provided for ad libitum consumption throughout the experiment. The lighting program consisted of a period of 23 hours light and 1 hour of darkness. The house ambient temperature was initially set at 33°C by the first week and gradually decreased until 25°C was reached by the third week and was then kept constant.

**Mushroom Preparation**

Fresh oyster mushrooms (*Pleurotus ostreatus*) were obtained from Isfahan fruit and vegetable market center. The intact mushrooms were dried overnight at 60°C and ground through a 5-mm sieve before incorporation into the diet by thorough hand mixing. To determine major chemical analyses, mushrooms were freeze-dried and milled through a 1-mm sieve then crude protein, fat, fiber, and ash were determined according to the procedures described by AOAC international (1995). Kjeldahl method was used to determine total protein content, crude fat content was extracted from the samples with petroleum ether in a Soxhlet apparatus, crude fiber content was analyzed in a Dosi Fiber apparatus and ash was measured by incinerating dried samples at 600°C for about 6 h in a furnace and moisture by oven drying. The obtained values are as follow: Dry matter: 92.85%, crude protein: 13.73%, crude fat: 5.82%, crude fiber 8.33% and total ash content: 9.73%.

**Performance and Carcass Components**

Chicks were individually weighed at arrival and at 14, 28, and 42 d of age on a pen basis. Food consumption was measured per floor pen throughout the experiment and food conversion ratio (feed intake / weight gain) was calculated at the same intervals. All the pens were checked for mortality twice a day and the birds that died during the experiment from every group were weighed and sent to the pathology laboratory for necropsy, and feed intake was adjusted accordingly. At 42 d of age, three birds per replicate randomly chosen were weighed and slaughtered then abdominal fat, liver, pancreas, gizzard, heart, cecum, small intestine, bursa, and spleen were collected, weighed, and calculated as a percentage of live body weight. The organs were carefully examined to detect any pathological lesion or damages. The length of small intestine and caecum were also measured and recorded.

**Immune Parameters**

Birds were intramuscularly vaccinated against influenza and Newcastle viruses at day 18 of experiment. At 22 d of age, 12 banded birds from each treatment groups were injected intravenously with 1 ml of a 1% suspension of sheep red blood cell (SRBC) prepared in phosphate-buffered saline. To assess the systemic antibody response to influenza, Newcastle, and SRBC blood samples were collected from brachial vein of vaccinated (three birds per replicate), and challenged birds on d 24 and 28 respectively. Blood samples were kept at room temperature for 2 hours and then at 4°C overnight. Blood samples were centrifuged at 2000 × g for 10 minutes to obtain serum (SIGMA 4-15 Lab Centrifuge, Germany); serum was isolated and stored at −80°C. Antibody titers against Newcastle disease (ND) and influenza viruses were measured using Hemagglutination Inhibition Test. Anti-SRBC titers were measured by the microtiter procedure of Wegmann and Smithies (1966). All titers were expressed as the log2 of the reciprocal of the highest dilution giving visible hemagglutination. At day 42, three birds per replicate were selected and blood samples were collected by syringes containing heparin to avoid blood clot formation. Blood films were air dried (unfixed) and stained in concentrated May-Greenwald stain for 6 min, 1:1 May-Greenwald stain distilled water for 1.5 min and 1:9 Geisma stain for 15 min (Robertson and Maxwell, 1990). A minimum of one hundred leukocytes per samples were counted by heterophil to lymphocyte separation under an optical microscope (Nikon, Japan) with 100x oil immersion lens, then heterophil to lymphocyte (H/L) ratio was calculated and recorded.

**Serum Biochemical Metabolites and Hematological Parameters**

After 12 h fasting, on day 42 of the experimental period, 3 ml of blood was collected by puncturing the brachial vein from three birds per pen. Serum samples were isolated by centrifugation at 2000 × g for 10 minutes. Individual serum samples were analyzed for total protein, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglyceride, by an automatic biochemical analyzer following the instructions of the corresponding reagent kit (Pars Azmoon Co., Tehran, Iran). Albumin concentration of serum was measured via bromcresol green (BCG) assay method. Subsequently, globulin concentration in serum was computed by subtracting albumin concentration from total protein, and consequently albumin to globulin ratio was calculated.

At day 42, blood samples were collected from 12 birds in each treatment into vials containing EDTA to prevent blood clot formation. The red blood cell (RBC) and white blood cell (WBC) counts were determined by a hemocytometer method using Natt-Herrick solution; hematocrit and hemoglobin values were measured by microhaematocrit and cyanmethaemoglobin methods, respectively.

**Statistical Analysis**

Performance data (derived from pen means) were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedure of SAS Institute (2008). Data analysis for the other sampled traits were performed using Mixed Model procedure of SAS followed by considering birds within experimental unit as repeated measures. Tukey-Kramer method was used to assess any significant differences at the probability level of $P \leq 0.05$ among the experimental treatments.

**Results**

**Performance and Carcass Traits**

The performance indices of control and supplemented chicks are summarized in Table 2. Chicks in prebiotic group significantly exhibited a higher feed intake over 14–28 d period compared to control chicks ($P < 0.05$) while, consider-
ing the entire period (1-42 d) inclusion of 20 g/kg mushroom in the diet none significantly increased feed intake compared to chicks receiving control diet. At 14 and 28 d, birds fed mushroom powder particularly 20 g/kg were heavier \((P < 0.05)\) than other treatments but at d 42 of age, prebiotic fed chicks had the greatest body weight \((P < 0.05)\). Mushroom supplementation at 20 g/kg inclusion rate significantly improved feed efficiency over the starter period \((P < 0.05)\) but regarding the finisher and entire periods, prebiotic supplementation markedly reduced feed conversion ratio of chicks \((P < 0.05)\). Mortality rate was not influenced by experimental treatments over any phases.

As Table 3 displays carcass yield was not influenced by dietary treatments. A significant reduction of abdominal fat pad was observed in birds fed prebiotic compared to control birds \((P < 0.05)\). No statistical effects of treatments were observed on internal organ weights, small intestine, and cecum length of broilers.

### Immune Parameters

According to the data on Table 4, none of immune related parameters of broiler chicks measured at different ages was significantly influenced by the experimental treatments \((P >\)
Diet supplementation slightly improved antibody response to ND and influenza viruses, but SRBC response was measured to be marginally higher in control birds. Heterophile/lymphocyte (H/L) and albumin/globulin (A/G) ratios none significantly tended to be lower in birds fed supplemented diets.

**Serum Biochemical Metabolites and Hematological Parameters**

There was no statistical impact of diet supplementation on the concentration of serum metabolites including protein, albumin, globulin, LDL, HDL, and total cholesterol (Table 5). Triglyceride concentration of the serum was markedly reduced by prebiotic and mushroom inclusion in the diet in comparison with the control diet \((P < 0.05)\). As exhibited in Table 5, no particular trend was detected on hematological parameters and the tested values were not statistically different among treatment groups.

**Table 4. Effect of experimental diets on antibody titers against Newcastle and influenza at d 24 and SRBC at d 28, heterophil to lymphocyte and albumin to globulin ratios at d 42**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Prebiotic</th>
<th>10 g/kg mushroom</th>
<th>20 g/kg mushroom</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newcastle ((\log_2))</td>
<td>5.62</td>
<td>5.75</td>
<td>5.25</td>
<td>6.37</td>
<td>0.60</td>
<td>0.52</td>
</tr>
<tr>
<td>Influenza ((\log_2))</td>
<td>3.62</td>
<td>5.00</td>
<td>5.00</td>
<td>4.50</td>
<td>0.58</td>
<td>0.61</td>
</tr>
<tr>
<td>SRBC ((\log_2))</td>
<td>6.00</td>
<td>5.12</td>
<td>5.12</td>
<td>5.00</td>
<td>0.89</td>
<td>0.62</td>
</tr>
<tr>
<td>H/L</td>
<td>0.39</td>
<td>0.34</td>
<td>0.32</td>
<td>0.31</td>
<td>0.05</td>
<td>0.64</td>
</tr>
<tr>
<td>A/G</td>
<td>0.85</td>
<td>0.62</td>
<td>0.51</td>
<td>0.65</td>
<td>0.21</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Mean values and SEM are based on (Repeated Measure) 3 observations per subject (4 subjects per Treatment).

**Table 5. Effect of experimental diets on blood biochemical and hematological parameters of broilers at d 42**

<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>Control</th>
<th>Prebiotic</th>
<th>10 g/kg mushroom</th>
<th>20 g/kg mushroom</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/dL)</td>
<td>3.34</td>
<td>3.44</td>
<td>3.49</td>
<td>3.65</td>
<td>0.34</td>
<td>0.31</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.8</td>
<td>1.4</td>
<td>0.31</td>
<td>0.46</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>2.10</td>
<td>2.20</td>
<td>2.37</td>
<td>2.25</td>
<td>0.16</td>
<td>0.40</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>142</td>
<td>94b</td>
<td>109b</td>
<td>111b</td>
<td>11.55</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>112</td>
<td>124</td>
<td>118</td>
<td>111</td>
<td>12.61</td>
<td>0.62</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>32</td>
<td>35</td>
<td>35</td>
<td>31</td>
<td>7.23</td>
<td>0.78</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>71</td>
<td>85</td>
<td>74</td>
<td>73</td>
<td>5.64</td>
<td>0.71</td>
</tr>
<tr>
<td>RBC ((\times 10^6/\muL))</td>
<td>2.58</td>
<td>2.39</td>
<td>2.73</td>
<td>2.48</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>WBC ((\times 10^3/\muL))</td>
<td>17.05</td>
<td>19.63</td>
<td>18.77</td>
<td>17.47</td>
<td>2.93</td>
<td>0.66</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>10.6</td>
<td>9.7</td>
<td>10.5</td>
<td>10.7</td>
<td>0.49</td>
<td>0.58</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>30</td>
<td>29</td>
<td>31</td>
<td>32</td>
<td>1.3</td>
<td>0.96</td>
</tr>
</tbody>
</table>

\(a^b\) Tukey values in rows with no common superscripts differ significantly \((P \leq 0.05)\).

Mean values and SEM are based on (Repeated Measure) 3 observations per subject (4 subjects per Treatment).

1RBC = red blood cells; WBC = white blood cells; HDL = high-density lipoproteins; LDL = low-density lipoproteins.

0.05). Diet supplementation slightly improved antibody responses to ND and influenza viruses, but SRBC response was measured to be marginally higher in control birds. Heterophile/lymphocyte (H/L) and albumin/globulin (A/G) ratios none significantly tended to be lower in birds fed supplemented diets.

**Discussion**

**Performance and Carcass Traits**

In this study, diet supplementation with mushroom powder, particularly at 20 g/kg inclusion rate, improved body weight and feed efficiency of broilers at younger ages; while prebiotic supplementation displayed its beneficial impact on body weight (BW) and feed conversion ratio (FCR) in finisher period. During the first weeks of the experiment, no marked differences were observed in the feed intake of birds in mushroom and control groups, this could indicate that the improvement in BW during this period may have partially been due to improved nutrient digestibility and better nutrient absorption as reflected by improved feed efficiency. Similarly, Willis et al. (2007) found an improved BW gain of broilers with the supplementation of mushroom *L. edodes* extract to 21 d of age; however, this supplementation did not sustain improved weight gains up to d 49 when the trial was completed. Our findings are also consistent with the recent work of Giannenas et al. (2010) who showed that incorpo-
ration of the dried mushrooms *A. bisporus* in chicken diets improved both BW and feed efficiency compared with the none supplemented treatment.

The favorable results of mushroom on body weight and feed efficiency of birds (Table 2; compared to control group) could be explained by the positive impact of mushroom inclusion on the microflora balance of the intestinal tract of chicken resulting in a more efficient use of nutrients from diet. The work of Guo et al. (2004b) indicating that mushroom and herbal extract supplemented diets reduced *Bacteroides* spp., *Enterococci*, and *E. coli* numbers, but increased numbers of *Bifidobacteria* and *Lactobacilli* in broilers gut; could further support our inferences. In addition, it has been demonstrated that some bacteria in the large intestine are more specialized in the hydrolysis of large molecular carbohydrates such as oligo- and polysaccharides, producing small molecular weight carbohydrates from large polymers and then fermenting them, which can lead to greater bacterial numbers. Fermentation end-products such as short-chain fatty acids lower intestinal pH, which can depress harmful bacteria and stimulate beneficial bacteria (Gibson and Robertfriod, 1995). This impact is particularly pronounced at a critical age when young birds are more susceptible and prone to gut pathogens, and the facts that older birds’ nutrient requirements decrease with age and they have a better developed digestive tracts and organs. Interestingly, in our previous studies peppermint (Toghyani et al., 2010), cinnamon and garlic (Toghyani et al., 2011) powders were found to exhibit their beneficial effects on productive traits of broilers over the starter and grower periods. These results are consistent with those reported by Zhou et al. (2009) who showed that dietary supplementation with 14 g/kg of chitoooligosaccharide enhanced chicks BW by 6.2% in the starter period.

Prebiotic group marginally consumed more feed compared with control group, throughout the experimental period. The improvement in feed intake by dietary prebiotic supplementation has been reported to account for the improved growth performance of broiler chicks. However, the principle effects of prebiotics have been reviewed by Cummings and Macfarlane (2002) and include improvement of calcium and magnesium absorption, production of short-chain fatty acids, and selective increases in the population of lactate producing bacteria like *Lactobacillus* and *Bifidobacterium*. It has been shown that increased lactate concentration often decreases intestine pH and is a potent anti-microbial substance to several pathogenic species such as *E. coli* (Samli et al., 2007). Thus, prebiotic helps to balance the intestinal microflora of poultry, consequently an improved utilization of diet nutrients (protein and energy) and higher feed intake leading to better performance criteria. In accord to our findings, several studies have shown that addition of prebiotics to the diet of broilers, leads to improved performance through improving gut microflora and feed utilization (Spring et al., 2000; Xu et al., 2003). However, Midilli et al. (2008) failed to observe any improvement on productive traits of broilers fed prebiotic supplemented diets. Eventually, the mode of action of both mushroom powder and prebiotic (MOS) appear to be related to a reduction on the microbial load in gut and consequently improved utilization of dietary nutrients as being reflected by enhanced growth and improved feed efficiency compared to control birds.

In the present study, experimental treatment did not affect carcass yield and relative organ weights of birds slaughtered at 42 d, but abdominal fat content was significantly influenced. The impact of mushroom on the reduction of carcass fat content was marginal and more pronounced by increasing its inclusion rate, while birds fed prebiotic diets exhibited the lowest fat pad weight. Fat deposition in the abdominal area of broilers is regarded as waste in the poultry industry; since it represents a loss in the market and consumer acceptability, and enhances expense during the treatment of effluent produced when processing broilers. The obtained results of this study indicate that prebiotic supplementation of broilers diet has the potential to lessen this type of waste by reduction of the fat content in the abdominal area of birds. In accord to our results, Mohamed et al. (2008) reported that the highest abdominal fat percentage value was recorded for birds fed the control diet (2.21%) while the lowest value was recorded for birds fed the MOS supplemented diet (1.78%). Similarly, they did not notice any significant impact of supplements on dressing percentage, liver, heart, and gizzard relative weight.

No clear mechanisms have been reported responsible for the reduction of lipid synthesis by prebiotics and herb oligosaccharides. It might in part be due to increasing beneficial bacteria such as *Lactobacillus* that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis. The results reported by Zhou et al. (2009) also agree with our findings, these scientists reported that application of chitoooligosaccharide in diet reduced abdominal fat pad of broiler chicks. In addition, it has been reported that diet supplementation with mushroom extract plus probiotic result in a marked reduction of fat pad in male and female broilers slaughtered at 49 d (Willis et al., 2007).

**Immune Parameters**

Antibody responses have been used as measures of the humoral immune status of birds and a viable part of the immune system (Scott, 2004). In the present study, broiler chickens fed supplemented diets had a marginally enhanced antibody titers production against ND and influenza viruses compared to those fed control diets. Substances in mushrooms responsible for up regulating the immune responses may include polysaccharides, glycosides, alkaloids, volatile oils, and organic acids (Yang and Feng, 1998; Willis et al., 2007). Research studies indicate that poly and oligosaccharides present in mushroom could affect both innate and adaptive immunity, including cellular and humoral responses (Xue and Meng, 1996). In the study of Guo et al. (2004a), *E. tenella*-infected birds fed with polysaccharide extracts showed significantly higher specific antibody responses at 14 and 21 d post infection. In addition, some prebiotics may reduce pathogen translocation in the intestine for instance MOS has been reported to act by binding and removing pathogens from the intestinal tract and stimulation of the immune system (Spring et al., 2000).
The antibody response of birds to SRBC challenge was not quite as expected, so that control birds none significantly exhibited higher responses. In contrasts to our observations, Lilburn et al. (2000) reported that inclusion of 0.5 and 1 g/kg MOS enhanced serum antibody titer against sheep erythrocyte antigen. Also, in the study of Savage et al. (1996), inclusion of MOS at 1 g/kg of diet enhanced both IgG and IgA serum antibody levels in turkey pouls, and statistically significant increases were detected at 7.5 week of age. Nevertheless, similar to current findings and in contrast to previous reports of enhanced plasma antibody titers due to MOS treatment Shafey et al. (2001) and Silva et al. (2009) reported no such improvement in the antibody titers against infectious bursal disease and ND viruses in broilers fed MOS and yeast extract supplemented diets.

A/G ratio has been used as an indicator of immune responses so that high globulin level and low A/G ratio signify better disease resistance and immune responses (Grimmer, 1986). In addition, the reliability of H/L Ratio as a biological index of stress in avian species is also, well documented (Maxwell, 1993). Although the lower H/L and A/G ratios observed in supplemented groups did not reach statistical significance compared to control birds but it could imply the positive influence of additives on stress and immunity.

The lack of statistically significant effects of the mushroom and MOS on immune responses might be related to the inclusion levels of the additives in the diets. Mushroom supplementation levels in the present experiment may not be optimal for enhancing immune responses in chickens. It seems that immunomodulatory activities of oligosaccharides classified as prebiotics in enhancing the antibody titer in response to soluble antigens such as SRBC are highly dependent on the antigen, immunization regimen, type, and source of prebiotics, and genetic background of the host. Nevertheless, the health status of birds, hygienic status of experimental site, external challenges and basal diets composition and digestibility may to a great extent account for the contradictory and inconsistent reports in the literature regarding the immunomodulatory impact of mushrooms and oligosaccharides.

Serum Biochemical Metabolites and Hematological Parameters

Blood parameters are of diagnostic significance and have been shown to be major indices of physiological, pathological, and nutritional status of an organism and could be used to interpret the effects of therapeutic or nutritional management in human and veterinary medicine. Consumption of oyster mushrooms and prebiotic supplementation have been reported to lower cholesterol concentration in blood (Khatoon et al., 2007; Li et al., 2007). However, most of the biochemical and hematological parameters tested in this study were not influenced by dietary treatments; only a significant reduction of triglyceride concentration in blood serum was observed in response to inclusion of prebiotic (MOS) and mushroom in the diet. Oligosaccharides particularly MOS are considered as substrates for lactic acid producing bacteria like Lactobacillus spp. in the gut (Van Loo, 2004). These bacteria can effectively reduce the activity of acetyl coenzyme A carboxylase (the enzyme limiting the synthesis rate of fatty acids) leading to decreased lipid synthesis, and consequently reduction of serum triglyceride.

These results are in line with the effects observed on carcass fat of chicks fed prebiotic. Our findings agree with the results of earlier studies in which chitosan (used as a prebiotic supplement) was found to lower the concentration of blood lipids of broiler chicks (Tang et al., 2005; Li et al., 2007). Also, Kannan et al. (2005) reported that MOS fed broilers had statistically reduced serum triglyceride concentration than the control broiler at 5 weeks of age. Similar to current results, Zhou et al. (2009) did not find any marked changes in the concentration of serum total protein, albumin, total cholesterol, or LDL cholesterol and white blood cells counts of boiler chicks fed diets supplemented with Chitooligosaccharide. However, the authors reported significant increase of RBC counts and HDL cholesterol and decreased triglyceride concentration in response to treatment with Chitooligosaccharide compared to control birds.

Conclusion

The results of the current study showed that application of oyster mushroom powder as a growth promoter improved performance indices over the starter and grower periods but chicks did not sustain this improvement until the slaughter age. This is probably due to the relatively suitable digestibility of the basal diet (conventional ingredients) or the levels of additives applied; since mushroom fed broilers (particularly 20 g/kg inclusion rate) were numerically heavier than control chicks at 42 d. Thus, oyster mushroom could be considered as a potential natural growth promoter in the absence of in-feed antibiotics, but further researches are to be conducted to optimize its inclusion rate.

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