Efficiency of different levels of mushroom (*Agaricus bisporus*) in comparison with an antibiotic growth promoter on intestinal morphology and microflora composition in broiler chickens

N. Landy¹*, A. Kavyani² and M. Toghyani³

¹Young Researchers Club, Khorasgan Branch, Islamic Azad University, Isfahan, Iran.
²Young Researchers Club, Shahrekord Branch, Islamic Azad University, Chaharmahalobakhtyari, Iran.
³Department of Animal Science, Khorasgan Branch, Islamic Azad University, Isfahan, Iran.

**Corresponding author:** n_landy1984@yahoo.com (N. Landy)

**Abbreviated Title:** Mushroom as an antibiotic alternative

This experiment was conducted to examine the effects of different levels of edible mushroom (*Agaricus bisporus*) in comparison with an antibiotic growth promoter (flavophospholipol) on intestinal morphology and microflora composition in broiler chicks. In this trial 180 nine-day-old mix sex broiler chicks (Ross 308) were weighed and randomly allocated to 1 of 6 experimental treatments. The 6 treatments were as follow: Basal diet; Basal diet + antibiotic (4.5 mg flavophospholipol/kg diet); and Basal diet supplemented with either levels of 5, 10, 20 or 30 g of dried mushroom/kg of feed. The results of this trial showed that dietary mushroom supplementation did not significantly affect crypt depth, villus height and epithelial thickness except for the goblet cell numbers that decreased in the broilers fed diets containing 30 g mushroom/kg. *Lactobacilli* spp. population in birds supplemented with mushroom at the level of 30 g/kg none significantly was higher than other groups at 45 d of age. *Escherichia coli* loads significantly decreased in broilers fed diets containing 5 g mushroom/kg. In conclusion, the results indicated that supplementing broiler diet with 30 g mushroom/kg could induce favorable influences on intestinal health of broiler chicks.

**Keywords:** *Agaricus bisporus*, Broiler chickens, Intestinal morphology, Intestinal bacteria populations, Mushroom.

**Objective**

For the past several decades, different strategies have been applied to improve the poultry productivity and profitability. Most important of them were always directed towards maintaining health, reducing disease outbreak and improving general immunity. Changes in the microbiota population in gastrointestinal tract may potentiate animal performance by different mechanisms such as nutrient sparing, control of clinical diseases, protective effect against the production of toxins in the gastrointestinal tract, and metabolic effects (MELLOR, 2000). Antibiotics can be used preventively, therapeutically, or as performance enhancers, promoting positive effects on animal production. However, issues such as loss of antibiotic efficiency along time and risk of residues in food of animal origin, with the possible development of bacterial resistance in humans, have concerned consumers creating a great problem for poultry production (NOLLET, 2005). Today, the non-prescription use of antibiotics in poultry feeds has been eliminated or severely limited in many countries, thus as a result of restrictions to the use of antibiotic growth promoters have stimulated the search for alternative additives. Natural medicinal products originating from fungi or herbs have been used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (GUO, 2003). Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (BREEENE, 1990). The fungi have a wide range of activities and have been used for centuries to combat disease outbreaks in many parts of the world such as Asian and Mediterranean countries (CHANG and BUSWELL, 1996; GUE et al., 2003).
Mushrooms and its different derivatives contain a variety of active substances like ergothioneine (DUBOST et al., 2007), phenolic antioxidants, variegatic acid and dibiviquinone (KASUGA et al., 1995). Effective compounds, representing in the mushrooms possesses antioxidant, antibacterial, immune-enhancing, and stress reduction activities (DALLOUL et al., 2006; DALLOUL and LILLEHOJ, 2006). The present study was designed to compare the efficacy of different levels of Agaricus bisporus mushroom dry powder as an antibiotic growth promoter on intestinal morphology and microflora composition in broiler chicks

**MATERIALS AND METHODS**

**Animals and dietary treatments**

A total of 180 nine-d-old mix sex broiler chicks (Ross 308) were randomly allocated to 6 experimental treatments. Each treatment consisted of 3 replicates of 10 birds. The experiment lasted for 36 d. To meet the nutrient requirements of the broiler chicken over this period, a complete basal diet was formulated for each of the 2 stages of growth: grower (9 to 28 d), and finisher (29 to 45 d). The diets were formulated to meet or exceed the nutrient requirements of broilers (AVIAGEN, 2007). The birds within the control group were given the basal diet for the respective growth stage. The other 5 groups were given experimental diets based on the basal diets but contained an additional 5, 10, 20, or 30 g/kg of ground dried A. bisporus mushroom at the expense of corn in comparison broilers fed basal diet supplemented with 4.5 g/kg flavophospholipol.

**Mushroom preparation and supplementation**

The whole mushrooms were dried out at 60°C and were added to experimental diets of broilers after carefully grinding. Table 1 presents the proximate analysis and total phenolic content of the mushroom Agaricus bisporus.

**Table 1. Proximate analysis and total phenolic content of the mushroom Agaricus bisporus**

<table>
<thead>
<tr>
<th>Composition of mushroom Agaricus bisporus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein (N × 6.25), g/kg</strong></td>
<td>230.0</td>
</tr>
<tr>
<td><strong>Total phenolic content,</strong> ( \text{mg of GAE/g} )</td>
<td>8.83</td>
</tr>
<tr>
<td>Lysine (g/kg)</td>
<td>9.0</td>
</tr>
<tr>
<td>Methionine (g/kg)</td>
<td>9.0</td>
</tr>
<tr>
<td>Cysteine (g/kg)</td>
<td>6.6</td>
</tr>
<tr>
<td>Calcium (g/kg)</td>
<td>13.3</td>
</tr>
<tr>
<td>Total phosphorus (g/kg)</td>
<td>12</td>
</tr>
<tr>
<td>Sodium (g/kg)</td>
<td>0.33</td>
</tr>
<tr>
<td>Chlorine (g/kg)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

1Phenolic content is expressed as dry weight basis in milligrams of gallic acid equivalents per gram (mg of GAE/g).

**Collection of intestinal tissue samples**

At 45 d of age, 2 birds per replicate were randomly chosen, based on the average weight of the group and slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter, and the digestive tract was carefully excised. After removing the intestinal contents, approximately 5 cm lengths of jejunum (mid point of jejunum) were removed for gut morphological measurements. Intestinal samples were immersed in formalin, before fixation in Bouin’s solution and paraffin embedding. The samples were then transferred into 70% ethanol after 24 h.

**Histology of the jejunum**

Histological examinations were carried out according to the method of IJI et al. (2001).

**Enumeration of bacteria populations in ileum**
Intestinal samples were collected and fresh digesta samples from ileum were taken for bacterial analyses within an hour from collection. Digesta samples were serially diluted in 0.85% sterile saline solution for enumeration of Lactobacilli spp and E. coli by conventional microbiological techniques using selective agar media. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. Lactobacilli spp. were anaerobically assayed using MRS agar (Fluka 80961). In cases of doubt confirmation of Lactobacilli spp. was performed by using API 50 CH kit (Biomerieux, SA, Marcy-l’Etoile, France). E. coli were enumerated through the use of Plate Count MUG Agar (Fluka 80961) and TBX Agar (Fluka 92435). Results were expressed as base-10 logarithm colony-forming units per gram of ileal.

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Means were compared using Tukey test. Statements of statistical significance are based on P < 0.05.

Results

Morphometric analysis of the jejunum

In this trial the treatments failed to induce any significant effect on crypt depth, though it tended to decrease in broilers fed diets containing 30 g mushroom/kg. The lowest goblet cell numbers obtained in broilers fed diet containing 30 g mushroom/kg. Reduced goblet cell numbers may be expected to lower mucin production and endogenous protein losses (JUNQUEIRA et al., 1995). Changes in intestinal morphology, such as shorter villi and deeper crypts have been associated with the presence of toxins (YASON et al., 1987) or higher tissue turnover (MILES et al., 2006). In this trial, no increase in jejunum villus height in mushroom fed chickens was found. In other trial in broilers GIANNENAS et al. (2010b) reported that, use of Agaricus bisporus mushroom had not any significant effect on villus height and crypt depth. In other trial in turkey poults villi height increased by Agaricus bisporus mushroom supplementation in all intestinal section, but use of mushroom had not any effect on crypt depth (GIANNENAS et al., 2011).

Table 2. Effects of dietary treatments on villus height, crypt depth, epithelial thickness, goblet cell number (per 100 villus height) in jejunum at d 45 of age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Flavophospholipol</th>
<th>5 g mushroom/kg</th>
<th>10 g mushroom/kg</th>
<th>20 g mushroom/kg</th>
<th>30 g mushroom/kg</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus height (μm)</td>
<td>1312.5</td>
<td>1212.5</td>
<td>1362.5</td>
<td>1250.0</td>
<td>1250.0</td>
<td>1312.5</td>
<td>160.1</td>
</tr>
<tr>
<td>Crypt depth (μm)</td>
<td>156.6</td>
<td>173.3</td>
<td>150.0</td>
<td>135.0</td>
<td>163.3</td>
<td>126.6</td>
<td>21.8</td>
</tr>
<tr>
<td>Goblet cell number</td>
<td>6.6a</td>
<td>4.5b</td>
<td>5.6b</td>
<td>4.8b</td>
<td>4.3b</td>
<td>3.1b</td>
<td>1.34</td>
</tr>
<tr>
<td>Epithelial thickness (μm)</td>
<td>17.5</td>
<td>17.5</td>
<td>18.3</td>
<td>20.0</td>
<td>15.8</td>
<td>21.6</td>
<td>3.22</td>
</tr>
</tbody>
</table>

a,bValues in the same row not sharing a common superscript differ (P < 0.05).

Standard error of mean.

Enumeration of intestinal microflora composition

Data on ileum bacteria populations of broiler chicks at d 45 of age are summarized in Table 3. The Lactobacilli spp. population in birds supplemented with mushroom at the level of 30 g/kg none significantly was higher than other groups at 45 d of age.

Table 3. Effects of dietary treatments on ileum bacteria populations of broiler chicks at d 45 of age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Flavophospholipol</th>
<th>5 g mushroom/kg</th>
<th>10 g mushroom/kg</th>
<th>20 g mushroom/kg</th>
<th>30 g mushroom/kg</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>6.47a</td>
<td>5.63b</td>
<td>4.32c</td>
<td>5.95c</td>
<td>6.28e</td>
<td>6.50d</td>
<td>0.95</td>
</tr>
<tr>
<td>Lactobacilli spp.</td>
<td>6.80</td>
<td>6.12</td>
<td>6.32</td>
<td>7.13</td>
<td>6.07</td>
<td>7.46</td>
<td>0.62</td>
</tr>
</tbody>
</table>

a,bValues in the same row not sharing a common superscript differ (P < 0.05).

Standard error of mean.

A Results are given as means of groups (n = 3 = subgroups).
Escherichia coli loads significantly decreased in broilers fed diets containing 5 g mushroom/kg. The antibiotic had not any significant effect on Escherichia coli loads, though it tended to decrease. GIANNENAS et al., (2011) reported that, lactobacilli spp. Population in the ileum of turkeys poults supplemented with Agaricus bisporus mushroom were higher, and E. coli counts were lower than control group. Similarly, in other trial the broilers supplemented with Agaricus bisporus mushroom at the level of 20 g/kg diet had higher Lactobacilli spp. Population in the caecum and ileum compared with control group; however, other measurements of bacteria loads were similar amongst the dietary treatments (GIANNENAS et al., 2010b). REHMAN et al. (2006; 2007a, b) reported that lactic acid producing bacteria may improve gastrointestinal function, feed digestibility and animal performance. Also, lactobacilli may produce organic acids and other bactericidal substances (NEAL-MCKINNEY et al., 2012). Furthermore, GIANNENAS et al. (2011) reported that fermentable polysaccharides content of mushrooms may improve growth of lactobacilli and bifidobacteria populations and inhibited that of E. coli. In other trial STANLEY et al. (2000) reported that coliform bacteria decreased in turkeys intestinal content when turkeys were supplemented with mannan oligosaccharides. Similarly, SIMS et al. (2004) showed that supplementation of turkeys with mannan oligosaccharides may potentiate caecal populations of lactobacilli and bifidobacteria and reduce the caecal population of E. coli. In addition, findings of our trial showed that supplementation of broilers with Agaricus bisporus mushroom led to a favorable shift into microbiota composition in ileum of birds.

**Conclusion**

In conclusion, the results indicate that supplementing broiler diet with 30 g mushroom/kg could induce favorable influences on intestinal health of broiler chicks.

**References**


GIANNENAS, I., E. TSALIE, E.F. CHRONIS, S. MAVRIDIS, D. TONTIS, I, KYRIAZAKIS I. 2011: Consumption of Agaricus bisporus mushroom affects the...


NOLLET, L. 005: AGP alternatives-part I. EU close to a future without antibiotic growth promoters. World Poult. 21, 14-15.


