Effects of Probiotic (Bacillus subtilis) on Laying Performance,
Blood Biochemical Properties and Intestinal Microflora of Shaoxing Duck

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Abstract: The study was conducted to evaluate the effects of probiotic supplementation (Bacillus subtilis) in laying ducks (160 days old) on performance, blood properties and caecum microflora of Shaoxing duck. A population of 200 laying ducks was divided into two groups each having five replications. The control (T0) were fed on basal diet while T1 with Bacillus subtilis 1 x 10^8 CFU/kg in addition to basal diet for thirty five days. The results showed that for T1 group, the egg laying rate increased (3.79%) significantly, while a decrease of about 12.60%, 23.52% and 40.14% was observed in egg triglyceride, total cholesterol and malondialdehyde, respectively. However, the blood glucose content and alkaline phosphatase activity increased by 15.13% and 53.41%, significantly. Moreover, there was a decrease of blood cholesterol, uric acid and ammonia in T1 by 28.86%, 22.39% and 62.5%, respectively. As compared to T0, T1 showed a significant increased in the amount of caecal microflora, i.e. general aerobic bacteria (18.68%) and Lactobacillus (9.92%). In conclusion, supplementation of Bacillus subtilis in diets had significant effects on some aspects of egg composition, biochemical properties of blood and few microflora of Shaoxing ducks.

Key words: Probiotics, Bacillus subtilis, total cholesterol, shaoxing duck

INTRODUCTION
Probiotics are living microbes given orally to proliferate in the Gastrointestinal Tract (GI) of the host and create beneficial conditions for nutrients’ utilization (Nahashon et al., 2005; Jin, et al., 1996; Jin et al., 2000). Probiotics produce a positive balance of digestive microflora and limit the damage caused by pathogenic bacteria, improve epithelial cell integrity and increased immune response (Vanbella et al., 1990; Jin et al., 2000; Wenk, 2000; Panda et al., 2001; Linge, 2005). However, if microorganisms and/or substances, which contribute to the proper microbial balance, were added to the diet, the animal would continually receive a “boost” to establish the proper microbial population. Many investigators obtained data in the literature relating to the use of bacterial cultures to control and/or promote the proper environmental conditions for the establishment of an ideal microbial population in an animal’s digestive tract through application of cultured bacteria. Species that have traditionally been regarded safe probiotics are Enterococcus, Bifidobacterium and Bacillus (Ishibashi and Yamazaki, 2001). Preventive application of probiotics achieved better utilization of nutrients and they have a positive effect on environment of gastrointestinal tract (Capcarova et al., 2009). These act as growth promoters, feed savers, nutritional bioregulators and help in improving the performance and health (Vanbella et al., 1990; Jinet al., 1998), by releasing of proteases, beta-mannanase and many other enzymes, these germinating and outgrowing spores assist in the feed digestion process (Hooge, 2003). Several studies demonstrated that the supplementation of probiotics to poultry diets increased performance of the birds, stabilized the intestinal microbial flora and also reduced incidence of disease (Samanya and Yamauchi, 2002; Hooge et al., 2004). Therefore keeping in view the importance of the subject and rapid scarce of waterfowls the present study had been designed to evaluate the effects of probiotic (Bacillus subtilis) on performance, blood properties and caecum microbial population of the shaoxing ducks.

MATERIALS AND METHODS
The study was conducted to investigate the effects of supplementation (Bacillus subtilis) on the performance, biochemical changes in blood and caecum microflora of Shaoxing Ducks. A total of 200 shaoxing ducks (160-day-old at laying, (average weigh 1.72±0.02 kg) were randomly divided into two groups, with five replications each group and 20 ducks per replication. Ducks were rare; in standard farm with spreaded litter (wheat brown 2-3 inches thick) ducks had free access of feed and water with facility of playground and fresh water pond. Control group (T0) was only fed on basic diet (Table 1) for 35 days while treated group (T1) was fed on basic diet with Bacillus subtilis (1 x 10^8 cfu/kg). The nutrients in the...
The dishes containing more than 30 and/or fewer than 300 colonies were selected and counted using colony counter. The result was calculated using following formula:

$$N = \frac{\sum c}{(n_1 + 0.1 \times n_2) \times d}$$

3c : Sum of colonies counted on all the dishes retained.

$$n_1$$ : Number of colonies counted on all the dishes retained.

$$n_2$$ : Number of dishes retained in the first dilution.

d : Dilution factor corresponding to the first dilution.

After merciful killing, weight of internal organs, such as liver, spleen, pancreas and heart was recorded. Ten eggs from each replicate were randomly selected to collect the yolk. The egg yolk cholesterol was extracted by the method of Folch et al. (1956) as modified by (Washburn and Nix, 1974) and their chemical composition was determined according to the methods of Association of Official Analytical Chemists (AOAC, 1990). Caecal contents were also collected for microbial analysis.

Blood samples were collected from carotid artery blood-letting using 23 gauge needle. Serum was separated and purified by 10 minutes centrifugation (5,500 x g), then aspirated by pipette and kept in 1.5 ml, eppendorf tubes at -80°C for further analysis. Biochemical blood indexes i.e. triglyceride, total cholesterol, glucose, uric acid, serum ammonia, alkaline phosphatase, glutamic oxaloacetic transaminase, Glutamic pyruvic transaminase were also analyzed by RX Dayton auto analyzer using (Roche Diagnostics commercial) kits.

**Microbial analysis**

**Preparation of test sample:** Ceacum content (1 g) was diluted in sterile diluents peptone water solution (9 ml) to make primary dilution ($10^0$). Then a series up to $10^5$ dilution was prepared by transferring primary dilution (1 ml) into test tube containing sterile diluents (9 ml) to obtain $10^2$ dilution and repeating the operations with sterile diluents (9 ml) using the $10^2$ and further dilutions to obtain $10^3$, $10^4$ and/or $10^5$. The caecal content samples from each group were used to enumerate the count of total aerobic bacteria, total anaerobic bacteria, *Bifidobacterium*, *E. coli*, *Lactobacillus* and *Bacillus* by selective culture medium after collection of caecal contents.

Pre prepared test sample (1 ml) of $10^2$, $10^3$ and/or $10^5$ dilutions was transferred into petri dishes containing selective media in duplicate through dispensing pipette (100 µl) with sterile plastic tips and warm sterile agar medium (15 ml) was mixed with inoculums. The mixture was allowed to solidify and incubated. Parallel to that, control plates were also prepared using similar medium (15 ml) to check the sterility.

**Counting of cecal microflora:** The dishes containing more than 30 and/or fewer than 300 colonies were selected and counted using colony counter. The result was calculated using following formula:

$$N = \frac{\sum c}{(n_1 + 0.1 \times n_2) \times d}$$

3c : Sum of colonies counted on all the dishes retained.

$$n_1$$ : Number of colonies counted on all the dishes retained.

$$n_2$$ : Number of dishes retained in the first dilution.

d : Dilution factor corresponding to the first dilution.

basal feed were analyzed by the method of Naumann and Bassler (1993) (Table 1). Nutrient levels of the basal diet were analyzed for CP, Lys, Met, Ca and P by the methods of AOAC (1990).

**Sampling, testing and observations:** Feed consumption, Egg number and weight were recorded daily during the whole test period. Egg laying rate, mean egg weight, daily egg mass, feed-egg ratio were determined using formula, 1, 2, 3 and 4 respectively.

**Table 1: Ingredients and nutrient levels of basal diet**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
<th>Nutrients (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>46.5</td>
<td>DE (MJ/kg) 11.40</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>23.0</td>
<td>CP 18.00</td>
</tr>
<tr>
<td>Rapeseed cake</td>
<td>8.0</td>
<td>Ca 3.00</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>10.0</td>
<td>P 0.75</td>
</tr>
<tr>
<td>Mono calcium phosphate</td>
<td>1.5</td>
<td>Lys 0.95</td>
</tr>
<tr>
<td>Limestone</td>
<td>6.7</td>
<td>Met 0.35</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Premix compound each kilogram</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

1Premix compound each kilogram contained: vitamin A, 5,000 IU; cholecalciferol, 1,500 IU; tocopherol acetate, 11 IU; menadione, 1.1 mg; thiamine•HCl, 3.0 mg; riboflavin, 5.0 mg; pyridoxine•HCl, 2.2 mg; cyanocobalamin, 0.66 meq; niacin, 44 mg; Ca pantothenate, 12 mg; choline chloride, 220 mg; folic acid, 0.55 mg; D-biotin, 0.11 mg; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg. Premixes were formulated to meet recommended levels for minerals and vitamins (NRC, 1994). Composition was analyzed by AOAC (1990).
Liver 47.03±2.39 48.81±2.64
Heart 10.36±0.60 10.67±0.53
Pancreas 5.24±0.27 5.19±0.15
Spleen 0.53±0.06 0.73±0.11

Means in columns with different letters are significantly different from each other (p<0.05)

Statistical analysis: Data was processed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Paired-samples t test was chosen as compare mean to analyze index differences between group T0 and T1. The probability of (p<0.05) was considered as statements of statistical significance.

RESULTS AND DISCUSSION

The effect of supplemental probiotic *Bacillus subtilis* on the laying performance of Shaoxing ducks is presented in Table 2. Live body weight of birds was not significantly improved comparatively to control group; results showed that the supplementation of 1 x 10^8 *Bacillus subtilis*/kg diet brought about a significant (p<0.05) improvements in intensity of egg laying in supplemented group as compared to control groups. However, slight numerical improvements were observed in means of daily egg collection, feed conversion ratio and average egg weight, when birds were fed with 1 x 10^8 *Bacillus subtilis*/kg diet.

It is of interested to note that egg weight did not decline by the increase of intensity of egg laying in *Bacillus subtilis* fed ducks. These results are in conformity with the findings of Zewel and Ismail (1998) and Abdel-Azeem et al. (2005), who also indicated that egg production, egg weight and egg mass were improved in laying hens fed probiotic-supplemented diets. The positive effects of *Bacillus subtilis* supplementation to Shaoxing ducks could be due to decrease in the multiplication of harmful bacteria resulting from improvement in gut environment and enhanced nutrient utilization (Miles, 1993). *Bacillus subtilis* may also, enhance enzymatic activity in the digestive tract resulting in improving nutrient utilization. The positive effects of probiotic supplementation, observed in the present study, are in agreement, with by El-Sheikh (2006).

The effects of the dietary supplementation with probiotic on relative weight of some internal organs of Shaoxing ducks are summarized in Table 3. These results suggested that the effect of probiotic supplementation as a feed additive has not significantly (p>0.05), affective on liver, spleen, heart and pancreas weights respectively. Jin et al. (1998) and Bozkurt et al. (2005) also found that dietary prebiotic and probiotic supplementation did not stimulate the internal organ weight of broilers. The present results are in disagreement with the findings of Hill et al. (1957), Visek (1978), Henry et al. (1986) and Engberg et al. (2000) indicated that dietary inclusion of feed grade antibiotics, given as growth promoters, reduced liver, spleen and intestine weight by thinning the intestinal wall evoked particularly by antimicrobial activity in gut lumen. However, a series of reports suggested similar antimicrobial mode of action for prebiotics (Iji et al., 2001; Shane, 2001; Ferket, 2004); organic acids (Broek, 2000; Dibner, 2004) and probiotics (Vanbella et al., 1990; Yeo and Kim, 1997; Wenk, 2000). Moreover, definitive data are lacking with respect to effects of dietary probiotics on the internal organs of poultry.

Analysis of the egg quality traits of shaoxing ducks after supplementation of *Bacillus subtilis*, data showed in Table 4. Probiotic (*Bacillus subtilis*) inclusion did not influence significantly on the egg weight, shell thickness, horizontal-vertical egg yolk color and haugh unit (p>0.05), are in agreement with the findings of Cerniglia et al. (1983), Mohan et al. (1995), Haddadin et al. (1996) and Chen and Chen (2003). Complementary reports by the Nahashon et al. (1996) and Haddadin et al. (1996) suggested that additional concentration of biological additives did not influence the egg quality significantly (p>0.05). But, present results are entirely different as reported by Nahashon et al., 1992; Tortuero and Fernandez, 1995, showed that using vital biomass of probiotic supplements affects the egg quality significantly (p<0.05) that might be related to the concentration, strain of bacteria, induction root and the form of application (viability, dry or Liquid and their products). Damron et al. (1976) and Jensen et al. (1978) also found significant (p<0.05) improvements in interior

Table 3: Effect of supplemental *Bacillus subtilis* on weights of selected organs

<table>
<thead>
<tr>
<th>Organs(g)</th>
<th>T0</th>
<th>T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>47.03±2.39</td>
<td>48.1±2.64</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.53±0.06</td>
<td>0.73±0.11</td>
</tr>
<tr>
<td>Heart</td>
<td>10.36±0.60</td>
<td>10.67±0.53</td>
</tr>
<tr>
<td>Pancreas</td>
<td>5.24±0.27</td>
<td>5.19±0.15</td>
</tr>
</tbody>
</table>

Means in rows with same letters are non-significant (p>0.05). T0 = Control, T1 = *B. subtilis* treated

Table 4: Effect of supplemental *Bacillus subtilis* on egg quality of Shaoxing ducks

<table>
<thead>
<tr>
<th>Contents</th>
<th>T0</th>
<th>T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg weight (g)</td>
<td>63.26±2.38a</td>
<td>62.63±1.57a</td>
</tr>
<tr>
<td>Shell thickness (mm)</td>
<td>0.39±0.01a</td>
<td>0.40±0.009a</td>
</tr>
<tr>
<td>Horizontal-vertical</td>
<td>0.77±0.01a</td>
<td>0.76±0.01a</td>
</tr>
<tr>
<td>Egg yolk color</td>
<td>11.20±0.37a</td>
<td>11.00±0.00a</td>
</tr>
<tr>
<td>Haugh unit</td>
<td>82.16±9.3a</td>
<td>83.37±2.12a</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>712.45±22.12a</td>
<td>622.66±28.95a</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>126.96±2.79a</td>
<td>97.09±2.29a</td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>943.92±38.68a</td>
<td>564.99±39.99a</td>
</tr>
</tbody>
</table>

Means in rows with different letters are significantly different from each other (p<0.05). T0 = Control, T1 = *B. subtilis* treated
Table 5: Effect of supplemental Bacillus subtilis on biochemical parameters of blood in Shaoxing ducks

<table>
<thead>
<tr>
<th>Contents</th>
<th>T₀</th>
<th>T₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (GLU, mmol/L)</td>
<td>25.84±2.679*</td>
<td>30.45±1.159*</td>
</tr>
<tr>
<td>Triglyceride (TG, mmol/L)</td>
<td>4.88±0.200*</td>
<td>3.95±0.345*</td>
</tr>
<tr>
<td>Total cholesterol (T-CHL, mmol/L)</td>
<td>9.20±4.044*</td>
<td>7.05±1.698*</td>
</tr>
<tr>
<td>Uric acid (UA, mg/L)</td>
<td>90.27±4.044*</td>
<td>63.00±13.096*</td>
</tr>
<tr>
<td>Serum ammonia (SA, μmol/L)</td>
<td>168.00±30.108*</td>
<td>33.58±1.548*</td>
</tr>
<tr>
<td>Glutamicoxaloacitate Transaminase (GOT)</td>
<td>31.76±2.03*</td>
<td>43.32±1.75*</td>
</tr>
<tr>
<td>Glutamic Pyruvic Transaminase (GPT)</td>
<td>43.32±1.75*</td>
<td>43.05±0.892*</td>
</tr>
</tbody>
</table>

Means in rows with different letters are significantly different from each other (p<0.05). T₀ = Control, T₁ = B. subtilis treated

Table 6: Effect of supplemental Bacillus subtilis on cecal microbe’s concentration of Shaoxing ducks

<table>
<thead>
<tr>
<th>Microbes (cfu/g)</th>
<th>T₀</th>
<th>T₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic bacteria</td>
<td>8.31±0.161*</td>
<td>10.22±0.121*</td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td>10.54±0.452*</td>
<td>10.42±0.276*</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>10.34±1.23*</td>
<td>10.77±0.565*</td>
</tr>
<tr>
<td>E. coli</td>
<td>8.27±1.05*</td>
<td>7.61±0.294*</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>7.97±0.120*</td>
<td>8.86±0.244*</td>
</tr>
<tr>
<td>Bacillus</td>
<td>9.62±0.079*</td>
<td>9.69±0.248*</td>
</tr>
</tbody>
</table>

Means in rows with different letters are significantly different from each other (p<0.05). T₀ = Control, T₁ = B. subtilis treated

Addition of probiotics in feed improves the overall body biochemical factors, but stress of additional dietary supplement may be cause of increase on glucose in blood. Triglyceride in blood did not show significant difference in control and treatment group. However, alkaline phosphatase was significantly higher in treatment group. No significant difference was observed in glutamicoxaloacetate transaminase and glutamic pyruvic transaminase between control and treatment groups. The present results are in agreement with Abdel-Fattah et al. (2008), who reported non significant (p>0.05) difference in the SGPT and SGOT levels. Application of Bacillus subtilis as a feed supplement affects the microbes of caecum significantly (p<0.05) increased the population of aerobic microbes and lactobacillus (Table 6). The presented results are in agreement with the findings of Dimcho et al. (2005). In addition, no numerical improvement and degradation was observed in caecum general anaerobic bacteria, Bifidobacterium, E. coli and Bacillus. These results resemble with Hristev et al. (2004) who found that, probiotics did not influence on concentration of Enterococci growth in Muskoy ducks. The results of current study gives line to observe further study about the probiotics on quality improvements. Moreover, Pandy et al. (2010) concluded that dietary supplementation of probiotics in the diet of poultry could be given without producing any adverse effect.

REFERENCES


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