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Research Article

Use of *Bacillus Subtilis* and *Spirulina Platensis* in the Diet of Broiler Chickens

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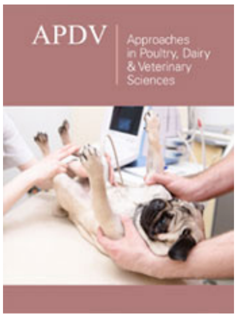

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Abstract

Ninety Cobb-500 straight run broiler chicks were allotted into three dietary groups; D_1 (Control diet), D_2 (diet with 0.5g *Bacillus Subtilis*/kg diet), D_3 (diet with 10g *Spirulina Platensis*/kg diet) having 3 replicates in each and 10 chicks/replicate. The birds were reared on a littered floor management system for 35 days of age and fed a starter diet (0-14 days) that contained 22% CP and 2900Kcal ME/kg diet and a finisher diet (15-35 days) contained 19% CP and 3000Kcal ME/kg diet. Dietary groups did not differ statistically for the body weight, feed intake, FCR, mortality, cost of production, and net profit ($p>0.05$). However, diet D_2 tended to perform the best among the 3 diets in terms of body weight, feed intake, FCR, mortality, production cost, and net profit. Of the two diets, D_1 tended to show lower production costs and a higher net profit compared to D_3. The highest dressed meat yield and heart weight were noted in D_1, followed by D_3 and D_2, respectively. Hence, diet D_2 was comparable to diet D_3 in the case of meat yield traits. Diets were found to be similar in total cholesterol, Triglyceride (TG), Low-Density Lipoprotein (LDL), and High-Density Lipoprotein (HDL) ($p<0.05$). However, D_2 tended to be the lowest for total cholesterol, and LDL, and the highest for HDL, followed by D_1 and D_3, respectively. Therefore, *Bacillus Subtilis* (0.5gBS/kg diet=D_2) may be beneficial for broiler production

Keywords: Broiler chicken; Growth; Lipid profiles; Meat yields; Probiotic; Spirulina

Introduction

Nowadays, broiler meat is very popular with consumers. Because it is easy and cheaper to produce broiler meat within the shortest possible period. Over and above, regardless of age and religion, everybody prefers broiler meat. To rear broiler chicken, different growth promoters and antibiotics are used in the diet of poultry to have maximum quality products, and control disease outbreaks [1]. Using for a long time of antibiotics in poultry feed developed a resistance to drugs [2] and residues [3] in the body of the birds and lowered the beneficial effect of microflora in the gut of the bird [4]. At present poultry industries have moved to reduce in use of antibiotics as medicine because of banding antibiotics in most of the countries to use in poultry rearing. Antibiotics have residual effects on the human body that develop antibiotic-resistant in humans [5]. Therefore, poultry scientists are working to introduce alternatives in poultry farming to have maximum growth without any hazards as well as maximum profit. In this regard, probiotics may be considered to add to the poultry diet. The genus of bacterial like lactobacilli and bifidobacteria are often used as probiotics even though the other beneficial groups of bacteria may be used in the diet that improves the immune system and inhibits the growth of harmful bacteria in the birds [6]. Therefore, the use of prebiotics in the poultry diet may be advantageous to produce quality, safe, and profitable poultry products.

Probiotics are cultures of microorganisms such as yeast and bacteria that have a beneficial effect on the growth and immune system of birds. It has been found in a study that probiotics impacted gut-related lymphoid tissues [7]. Probiotics are very active in developing a mucosal attachment, crucial nutrients, and antimicrobial complexes in the gut of the bird which prevent the growth of pathogenic microbes and their harmful effects [8]. As a result, using probiotics in the diet of bird increase the growth of birds. However, the efficacy of probiotics depends on strains, level of administration, application method, the composition of the diet, bird's age, survivability in the host, and duration of storage, etc. [9]. In this case, *Bacillus Subtilis* probiotic is a promising growth promoter in favor of the resistance of its spores in hot-humid climates [10].

A gram-positive bacteria-*Bacillus Subtilis* can produce endospores resistant to hot environments and secrete enzymes; amylase, protease, and lipase that can degrade plant-based complex carbohydrates [11]. Bacillus can reduce the pH in the gut to maintain the micro ecologic balance in the animals' intestines and improve animal growth, Feed Conversion Efficiency (FCE), and immune responses [12,13]. It can reduce pH in the intestinal lumina maintain the equilibrium and stability and improve the intestinal microbiota [14]. Therefore, Bacillus is used as a safe and high-quality probiotic or feed additive in the diet of animals or poultry. Latorre et al. [15] suggested using *Bacillus Subtilis* in a broiler diet for its beneficial effects. As it has a beneficial effect and is non-toxigenic, the European Food Safety Authority (EFSA) proclaimed the safe animal feed additive of *B. subtilis* for an animal [16]. *Bacillus Subtilis* improved digestion, growth, and FCR [14,17].

Spirulina Platensis is a Cyanobacterium that is considered a good source of protein, essential Amino Acids, Vitamins; Vitamin B_{12}, Thiamin, Pyridoxine, Vitamin C, Riboflavin, Minerals, β-Carotene, essential Fatty Acids, antioxidants, pigments like carotenoids, and phenolic acids [18]. It has a beneficial effect on arthritis [19], immuno-system, and antiviral effect [20]. Besides, Spirulina has been found to influence immune function, and reproduction and improve the growth of birds. In addition, Ross & Dominy [21] reported superior broiler growth performance on a diet with Spirulina over a control diet. Spirulina promotes nutrient digestion and mineral absorption and protects against diarrhea [22]. Inborr [23] reported that algae meal in diets increased the concentration of carotene in the liver, adipose tissue, and breast muscle. As a result, birds have yellow pigment in their skin compared with the control group. The broilers in the diet with algae meal gained weight faster- utilizing feed significantly. Ross & Dominy [21] showed no adverse effect of dietary Spirulina on body weight and mortality of birds. As per the report of Kaoud [24] and Kharde et al. [25], it was found that birds had improved body weight, carcass yield, and Feed Conversion Efficiency (FCE) in the diet with *Spirulina Platensis*. *Spirulina Platensis* is a natural unconventional feed additive that may be beneficial for yielding safe and profitable broilers.

Considering the above facts, this study was aimed at assessing the effect of *Bacillus Subtilis* and *Spirulina Platensis* on the performances of growth, meat yield, and blood lipid profiles of broiler chickens to pick out a suitable dietary group for the safe and cost-effective broiler production.

Materials and Methods

Experimental site

The experiment was conducted at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) Livestock and Poultry farm, Gazipur-1706, Bangladesh in 2020-2021.

Collection of *Bacillus Subtilis* and *Spirulina Platensis*

Spirulina Platensis commercial name (Eskalina) was collected from a Poultry Medicine shop, Joydebpur, Gazipur, Bangladesh (Figure 1). *Bacillus Subtilis* probiotic (Figure 2) was collected from Challenge Group, 12 Zhongguancun South Street, Haidian District, Beijing 100081, P.R. China. These two feed items were used to prepare a broiler diet during the investigation.

Figure 1: *Spirulina platensis.*

Figure 2: *Bacillus subtilis.*

Preparation of house and diet

Before receiving the chicks, the house was prepared by cleaning and disinfecting correctly and then adjusting the brooding temperature and humidity. The experimental shed was fumigated using formalin and potassium permanganate @2:1 ratio for 12 hours after placing the necessary equipment and utensils to destroy all kinds of microorganisms. After 12 hours of fumigation, the windows of the shed were opened to remove the gas from the house. The 3 diets as mash were prepared using the tested and locally available feed ingredients every week as per the ration required for the broiler chicks (Table 1).

Table 1: Composition of control diet applied in the experiment.

D_1=Control diet (No *Spirulina platensis* or *Bacillus subtilis*)

D_2=Control diet supplemented with 0.5g *Bacillus subtilis*/kg diet

D_3=Control diet supplemented with 10g *Spirulina platensis*/kg diet

ME=Metabolizable energy.

Ingredients	Amounts (kg)	
	Starter diet (0-2 weeks)	Finisher diet (2-5 weeks)
Maize	56.60	63.50
Soybean meal	25.50	18.50
Rice polish	8.00	8.50
Protein concentrate	7.50	7.00
Limestone	2.00	1.50
Soybean Oil	-	0.50
Salt	0.50	0.50
Chemical Composition		
ME (kcal/kg)	2906.02	3027.34
Crude protein (%)	21.94	19.24
Calcium (%)	1.32	1.08
Phosphorus (%)	0.63	0.6
Lysine (%)	0.91	0.74
Methionine (%)	0.29	0.27
Tryptophan (%)	0.20	0.17

Feeding trial

A total of 90-1-day-old broiler chicks of Cobb 500 were collected from Nourish Agro Ltd. and randomly assigned into three diets; D_1 (control or basal diet), D_2 (diet with 0.5g *Bacillus Subtilis*/kg diet), D_3 (diet with 10g *Spirulina Platensis*/kg diet) having 3 replications each and 10 chicks/replicate (Table 1). A starter ration or diet contained 22% CP and 2900Kcal ME/kg diet for 0-14 days and a finisher ration or diet contained 19% CP and 3000Kcal ME/kg diet was offered to the birds for 15-35 days. The experimental birds were brought up on a saw-dust-based littered floor for 35 days of age. Standard management practices were followed during the investigation. No synthetic amino acids, vitamins, minerals, antibiotics, etc. were fed to the birds during the investigation.

Data recording

Following are the growth performance traits recorded during the experimental period: Body weight and feed intake (replication-wise): Every week. Dead birds: when happened. The following traits were calculated using the formula given by Onunkwo & Okoro [26].

$$Feed\ conversion\ ratio\ (FCR) = \frac{(Feed\ intake)}{(Live\ weight)}$$

Production cost (Taka/kg live weight): calculated considering the cost of chick, feed, labor, litter, vaccine, etc. Net profit (Taka/kg live weight)=Price (Taka/kg live weight)-Production cost (Taka/kg live weight).

Meat yield traits of broiler chickens fed diets with *Bacillus Subtilis* and *Spirulina Platensis*

At 35 days of age, a total of 9 birds from 3 dietary groups had 3 replications each, and 1 bird/replication was taken randomly. The birds were slaughtered, de-feathered, eviscerated, and made cut-up parts to record meat yield traits. The meat yield traits were recorded during the investigation and then calculated as a percentage: Live weight, blood, feather, head, dressed meat, breast meat, dark meat, wings, thigh, drumstick, heart, gizzard, liver, and skin weight. The meat samples of slaughtered bird replication wise were taken and measured dry meat weight using the oven at a temperature of 105 °C for 24hrs.

Lipid profiles of broiler chickens fed diets included *Bacillus Subtilis* and *Spirulina Platensis*

The blood samples (10ml/bird) at 35 days of age of the bird during slaughter for recording meat yield were collected replication-wise and then centrifuged at 2400rpm for 15 minutes. The supernatant (blood serum) was transferred into the Eppendorf tube to measure blood lipid profiles of cholesterol, Triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL) in a spectrophotometric method using the cholesterol test kit (Crescent diagnostic cholesterol kit: Cat No. CS 603).

Statistical analysis

The collected data were analysed by ANOVA in a simple Completely Randomized Design (CRD), applying the Statistix10 computer package program.

Statistical model

The following statistical model was followed for data analysis

$$Yij = \mu + Di + eij$$

Where Yij is the observation of the j^{th} replication of the i^{th} dietary groups.

μ is the overall mean.

Di is the fixed effect of the i^{th} dietary groups (i=1, 2, 3).

eij is the random error

Animal welfare and ethical approval

The study was approved by the Institutional Committee on Animal Care and Use in Research (ICACUR) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (No. BSMRAU/DEAN/FVMAS/25/ICACUR/19).

Result and Discussion

Growth performance of broiler-fed diets with *Spirulina Platensis* and *Bacillus Subtilis*

No significant difference was found among the diets for body weight, feed intake, FCR, mortality, production cost, and net profit ($p>0.05$) (Table 2). Evidently but not significantly diet D_2 performed the best among the diets in terms of the traits; body weight, feed intake, FCR, mortality, production cost, and net profit. Of the two diets, diet D_1 had a tendency to show higher net profit than diet D_3. Despite the diet was not different statistically, diet D_2 tended to be better than Diet D_1 or D_3 in terms of growth performance which was consistent with Liu et al. [27] and Vimon et al. [28]. They found improved body weight in the diet with *Bacillus Subtilis* compared to the control diet. In this study, diet D_3 had a lower net profit despite having a higher body weight and a lower FCR because of the high price of the Spirulina which affected the production cost as well as net profit. This was supported by Rawshon et al. [29]. They observed the highest body weight and the lowest FCR in the diet containing 8g Spirulina/kg diet. Joya et al. [30] reported the improved body weight of broiler chicken and FCR in diet with individual use of *Spirulina Platensis* and *Bacillus sibtilis*.

Table 2: Growth performance of broiler fed diets with *Bacillus subtilis* and *Spirulina platensis* at 35 days of age. $^+D_1$=Control diet; D_2=Control diet supplemented with *Bacillus subtilis*; D_3=Control diet supplemented with *Spirulina platensis*; NS, P>0.05.

Traits	Diet (D)			LSD value and level of significance+
	D_1	D_2	D_3	D
Body weight (g/bird)	1582.00	1658.00	1663.00	207.120^{NS}
Feed intake (g/bird)	2822.30	2715.70	2840.30	331.530^{NS}
FCR (Feed intake/live weight)	1.78	1.65	1.71	0.265^{NS}
Mortality (%)	6.66	3.33	6.66	χ^2 =0.900^{NS}
Production cost (Tk/kg live bird)	113.75	108.36	115.81	17.359^{NS}
Net profit (Tk/kg live bird)	11.25	16.64	9.19	17.359^{NS}

Meat yield traits (%) of broiler fed the diet with *Bacillus Subtilis* and *Spirulina Platensis*

The diet was significantly different for dressed meat yield (p<0.01) and heart weight (p<0.05) but not different for live weight, blood, head, shank, breast meat, dark meat, wings, thigh, drumstick, gizzard, liver, skin, and dry meat yield (p>0.05) (Table 3). The highest dressed meat yield and heart weight were recorded in D_1, followed by D_3 and D_2, respectively. The breast meat yield tended to be higher in diet D_2, followed by D_3 and D_1, respectively. Nevertheless, diet D_3 tended to show higher dark meat and drumstick meat yield than D_2 or D_1. Dry meat yield was statistically similar among dietary groups. However, D_1 tended to show the highest dry meat yield, followed by D_2 and D_3, respectively.

Table 3: Meat yield traits (%) of broiler fed diets with *Bacillus subtilis* and *Spirulina platensis* at 35 days of age. $^+D_1$=Control diet; D_2=Control diet supplemented with *Bacillus subtilis*; D_3=Control diet supplemented with *Spirulina platensis*; NS, p>0.05; *, P<0.05; **, p<0.01

Traits	Diet (D)			LSD Value and Level of Significance+
	D_1	D_2	D_3	D
Live weight (g/bird)	1662.70	1805.30	1872.00	315.200^{NS}
Blood weight	4.55	4.00	4.24	1.697^{NS}
Feather weight	2.18	2.59	2.58	0.925^{NS}
Head weight	2.82	2.75	2.75	0.387^{NS}
Shank weight	4.33	3.96	3.88	0.973^{NS}
Dressed meat weight	75.34	68.01	68.18	3.657**
Breast meat weight	14.38	15.82	14.65	4.770^{NS}
Dark meat weight	46.89	46.92	47.91	2.946^{NS}
Wings weight	6.08	5.96	6.74	1.157^{NS}
Thigh weight	9.69	8.71	9.37	1.613^{NS}
Drumstick weight	8.17	7.81	8.24	1.397^{NS}
Heart weight	0.81	0.55	0.61	0.164*
Gizzard weight	1.56	1.51	1.32	0.283^{NS}
Liver weight	2.54	2.59	2.12	0.699^{NS}
Skin weight	9.79	10.96	8.01	3.067^{NS}
Dry meat yield	27.65	26.87	24.21	4.328^{NS}

Diet D_1 was found to be better than D_2 or D_3 in terms of dressed meat and heart weight in which D_2 and D_3 were almost similar for these traits. The other meat yield traits and live weight did not differ among the dietary groups which contradicted Kaoud [24] and Molner et al. [31]. Kaoud [24] reported an improved carcass yield in the diet with (*Spirulina Platensis* 1kg/ton of feed). Molner et al. [31] showed improved carcass and thigh meat in the diet with *Bacillus Subtilis* over the control diet. However, in this study, the diet with *Spirulina Platensis* (D_3) and the diet with *Bacillus Subtilis* both showed a lower carcass yield in comparison with the control diet which also contradicted Joya et al. [30]. They reported the improved carcass yield in the diet with *Spirulina Platensis* and *Bacillus Subtilis* at 42 days of age of the broiler chickens. It has also been found in this study that the control diet (D_1) tended to be better than diet D_2 or D_3 in terms of the percentage of dry meat yield. Of the two diets, diet D_2 tended to be better than D_3 in terms of the dry matter percentage of meat yield. No previous literature was found on the percentage of the dry meat yield affected by *Spirulina Platensis* or *Bacillus Subtilis*.

Lipid profiles of broiler chickens fed diets with *Bacillus Subtilis* and *Spirulina Platensis*

Lipid profiles: Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL) were statistically similar among the diets (p>0.05) (Table 4). However, diet D_2 tended to show the lowest amount of TC and LDL and the highest amount of HDL, correspondingly in D_1 and D_3, respectively.

Table 4: Lipid profiles of broiler fed on diets with *Bacillus subtilis* and *Spirulina platensis* at 35 days of age. +D_1=Control diet; D_2=Control diet supplemented with *Bacillus subtilis*; D_3=Control diet supplemented with *Spirulina platensis*; NS, p>0.05; TG=Triglycerides, HDL=High-density lipoprotein, LDL=Low-density lipoprotein.

Traits	Diet (D)			LSD value and level of significance+
	D1	D2	D3	D
Total cholesterol	228.72	225.64	247.18	52.403^{NS}
TG	166.84	152.28	151.27	62.125^{NS}
HDL	87.22	97.09	85.21	37.749^{NS}
LDL	108.13	98.10	131.71	67.451^{NS}

Despite there being no significant difference among the diets for the blood lipid profiles of the birds, diet D_2 was found to be the best among the diets because it contained the lowest amount of blood TC and LDL and the highest amount of HDL. Of the rest diets, diet D_1 performed better than diet D_3 in terms of the blood lipid profiles of the birds. Therefore, it is assumed that diet D_2 may be the most suitable diet among the dietary groups in terms of the blood lipid profiles of the birds, corroborated by Aliakbarpour et al. [32]. They found a lower amount of TG, LDL, and TC and higher amount of HDL in the diet supplemented with *Bacillus Subtilis* (50g/kg of feed) over the control diet. The present study is also consistent with Santroso et al. [12]. They showed a lower amount of blood cholesterol and TG of the birds in the diet supplemented with *Bacillus Subtilis* (1%). Joya et al. [30] reported that *Bacillus Subtilis* (0.05%) affected to decrease TC and TG in the blood serum of broilers.

Conclusion

The present study reveals that diet D_2 (*Bacillus Subtilis* @0.5g/kg diet) may be the best-performer dietary group among the diets considering the growth and blood lipid profiles (total cholesterol, LDL, and HDL) of the bird. Meat yield traits except for dressing yield and heart weight were statistically similar among the dietary groups. Diet D_1 (control) showed higher dressing yield and heart weight compared to diet D_2 or D_3. Hence, D_2 was comparable to D_3 for meat yield traits. Diet D_2 had the lowest amount of blood cholesterol and low-density lipoprotein, and the highest amount of high-density lipoprotein among the dietary groups, followed by D_1 and D_3, respectively. Therefore, D_2 (0.5g *Bacillus Subtilis*/kg diet) may be considered a beneficial dietary group among the diets for the growth, meat yield traits, and blood lipid profiles of the broilers. However, more studies are needed using *Bacillus Subtilis* and *Spirulina Platensis* at different levels in the diet to produce safe and profitable broilers.

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