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SYNERGISTIC EFFECT OF *Spirulina platensis* ON PERFORMANCE AND GUT MICROBIAL LOAD OF BROILER CHICKS

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Abstract

The experiment was carried out to evaluate the effects of different levels of *Spirulina platensis* on growth performance and intestinal microbiota in eight hundred, one-d-old, Ross 308 male broiler chicks raised from 1 to 36 days of age. There were 4 dietary treatments; (i) Control, (ii) 0.5% of *Spirulina platensis* (5 g/kg feed), (iii) 1% of *Spirulina platensis* (10 g/kg feed) and (iv) 1.5% of *Spirulina platensis* (15 g/kg feed). Weight gain and feed intake of the broilers were significantly influenced by the addition of 1% of *Spirulina platensis*. The results suggest that supplementation of 1% of *Spirulina platensis* to diets for growing broilers might enhance counts of LAB and yeast in the gut. In addition, the 1% of *Spirulina platensis* to diets might have a negative effect on *E. coli.*

Key words: *Spirulina platensis*, Intestinal microbiota, Broiler chicks and LAB.

1. Introduction

The use of antibiotics as growth promoters was completely banned in 1999 by the European Union (EU) (European Commission, 2001). This was due to increases in microbial resistance to antibiotics and residues in chicken meat products which might be harmful to consumers. Currently, in many parts of the world, feed additives, such as probiotics, prebiotics, are being experimented to alleviate the problems associated with the withdrawal of antibiotics from feed.

Probiotic microorganisms (non-pathogenic bacteria and/or yeast) are one of the alternatives for growth promoters in poultry. Functions of supplemental dietary microbial products in the digestive system are not known exactly, but some suggested mechanisms are; 1) they provide nutrient, 2) they aid digesting foods, and 3) they inhibit harmful bacteria in the gut (Owings *et al.*, 1990). Gastrointestinal normal flora plays an important role in the health and performance of poultry (Thongsong *et al*., 2008).

One such alternative is the addition of *Spirulina platensis* to poultry diets. *Spirulina* (blue-green alga) is one of the high quality natural feed additives that can be used in animal and poultry nutrition. There are two different species of *Spirulina* sp.: *Spirulina maxima* and *Spirulina platensis*, with varying distribution throughout the world (Oliveira *et al*., 1999). *Spirulina platensis* is more widely distributed and found mainly in Africa, Asia and South America (Vonshak, 2002). The blue-green algae (*Spirulina platensis*) have

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been used for hundreds of years as a food source for humans and animals due to the excellent nutritional profile and high carotenoid content. *Spirulina* is relatively high in protein with values ranged between 55 - 65% and includes all of the essential amino acids (Bourges *et al*., 1971; Anusuya Devi *et al*., 1981).

The available energy content of Spirulina has been determined to be 2.50 - 3.29 kcal/gram and its phosphorous availability is 41% (Yoshida and Hoshii, 1980; Blum *et al*., 1976). Also, *Spirulina* algae are rich in thiamin, riboflavin, pyridoxine, vitamin B12, vitamin C and carotenoids and have been used throughout the world as a feed component in broiler and layer diets to enhance yolk color and flesh (Colas *et al*., 1979; Brune, 1982; Ross and Dominy, 1985; Ross and Dominy, 1990). In addition, it is rich in nutrients such as vitamins, amino acids, gamma linoleic acid, phycocyanins, tocopherols, chlorophyll and β-carotenes (Abd El-Baky *et al*., 2003; Khan *et al*., 2005).

It has also been reported that *Spirulina* has health benefits in conditions such as diabetes mellitus and arthritis (Parikh *et al*., 2001; Rasool *et al*., 2006). It has also been shown that *Spirulina* has immuno-stimulatory effects and antiviral activity (Khan *et al*., 2005). *Spirulina* has been shown to enhance immune function, reproduction and increase growth. Less than 1% *Spirulina* added to chicken diets has been found to significantly enhance the defense systems for increased microbial killing, antigen processing and greater T-cell activity (Qureshi, *et al*., 1994). Qureshi (1995) observed that young poultry fed 1000 to 10,000 ppm SP had heavier spleen and thymus weights than poultry fed a control diet. Chicks fed SP also exhibited a higher clearance rate of *Escherichia coli* (intravenous inoculation) from their circulation than did chicks fed a basal diet. Therefore, the objective of the present study was to determine the effects of different levels *Spirulina platensis* supplementation on growth performance and intestinal microbiota in commercial broilers.

2. Materials and Methods

Eight hundred day-old Broiler chicks (Ross 308) were randomly assigned to 1 of 4 dietary treatments, consisting of eight replicates of 25 birds each. The experimental design was completely random, consisting of four dietary levels (0.5%, 1% and 1.5%) of *Spirulina platensis* and a control group (without *Spirulina platensis*) was formulated. Each treatment had eight replicates of 25 birds. Chicks fed four basal diets of Maize-soybean diets during four periods of 0-10 days birds fed with broiler Pre-starter, 11 - 20 birds fed with broiler Starter I, 21 – 30 days birds fed with broiler Starter II, 31 - 36 days birds fed with broiler Finisher. The diets supplemented with amino acids, minerals, and vitamins to meet the Ross 308 requirements.

2.1. Body weight and Feed Intake Measurement

Birds were group weighed for each replicate at 1, 10, 20, 30 and 36 days of age. Feed intake was monitored replicate wise at 10, 20, 30 and 36 days of age. From the replicate wise Feed intake and bodyweight measurements feed/gain ratios were calculated.

2.2. Intestinal Microbiology

Birds were killed by cervical dislocation while feeding normally. The abdominal cavity was opened, and all digest contents of ileum and cecum were immediately collected under aseptic conditions into sterile glass bags and put on ice, until they were transported to the laboratory for enumeration of microbial populations.

MRS agar (MERCK, 1.10660) was used for lactic acid bacteria (LAB) and malt extract agar (MERCK, 1.05398) was used for yeast, as the incubation medium. LAB and yeast counts of the ileum or cecum contents were obtained at 30°C degrees following 3 days incubation period. *E. coli* was grown on VRB agar (MERCK, 1.01406) aerobically at 37°C for 24 - 48 hours.

The bacterial colonies were enumerated, and the average number of live bacteria was calculated based on per gram of original ileal and cecal contents. All quantitative data were converted into logarithmic colony forming units (cfu/g).

2.3. Statistical analysis

All data were analyzed by analysis of variance (ANOVA) procedures (Steel and Torrie, 1980) appropriate for a completely randomized design by the GLM procedure of SAS (1995). The effects of *Spirulina platensis* on performance, blood constituents was the main effect. The level of statistical significance was preset at P = 0.05.

3. Results

3.1. Growth performance

The impacts of *Spirulina platensis* supplementation on performance of male broiler chicks are shown in Table - 1 to Table - 3. Weight gain ($P<0.01$), feed intake ($P<0.01$) and FCR ($P<0.01$) of the broilers in this study were significantly influenced by the addition 1% of *Spirulina platensis*. The lowest FCR was detected in the 1% of *Spirulina platensis* supplemented groups ($P>0.05$).

Table - 1: Effect of growth performance on Broiler Chicks fed with *Spirulina platensis* (g)

	10th day	20th day	30th day	36th day
T_1	271.44±14.126a	794.7 ±41.12a	1468.62 ±75.67b	1847.32 ±94.94b
T_2	307.04 ±4.03a	870.5 ±5.70a	1671.34 ±3.97a	2065.86 ±10.16a
T_3	310.06 ±2.85a	908.92 ±5.26a	1715.34 ±7.68a	2162.14 ±11.27a
T_4	393.70 ±13.95a	804.5 ±49.83a	1510.08 ±91.01b	1921.82±116.16b
Trt	892.06**			
Days	10.59**			
Txd	1.33 ns			

(T_1 - Control, T_2 - 5g/kg of *Spirulina*, T_3 - 1g/kg of *Spirulina*, T_4 - 15g/kg of *Spirulina*

** and ns, Significant at P< 0.01 and not significant respectively.

Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.# Mean ±S.E

Table – 3: Effect on Feed Conversion Rate on Broiler Chicks fed with *Spirulina platensis*

	10th day	20th day	30th day	36th day
T_1	1.157±0.05a	1.420 ±0.06a	1.582 ±0.05a	1.864 ±0.01a
T_2	1.110± 0.01a	1.416 ±0.04a	1.604 ±0.01a	1.868 ±0.09a
T_3	1.022 ±0.01a	1.256 ±96b	1.472 ±0.09a	1.716 ±0.09a
T_4	1.085 ±0.06a	1.455±0.07a	1.575 ±0.09a	1.897±0.07ab
Trt	70.32**			
Days	5.47**			
Txd	<1			

(T_1 - Control, T_2 – 5 g/kg of *Spirulina*, T_3 – 1 g/kg of *Spirulina*, T_4 – 15 g/kg of *Spirulina*

** and ns, Significant at P< 0.01 and not significant respectively.

Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.# Mean ±S.E

Table - 4: Effects of dietary treatments on ileal microbiota (log cfu/g cecal content) Ileum Microbiota

	Lactobacillus	Yeast	*E.coli*
T_1	5.833±14.126c	4.79±41.12b	4.273±75.67a
T_2	6.980±4.03b	5.030±5.70b	3.277±3.97b
T_3	8.283±2.85 a	5.643±5.26a	3.168±7.68b
T_4	7.050±13.95b	4.980±49.83b	3.090±91.01b
Trt		6.28**	
Days		238.04**	
Txd		10.21**	

T_1 - Control, T_2 – 5 g/kg of *Spirulina,* T_3 – 1 g/kg of *Spirulina,* T_4 – 15 g/kg of *Spirulina platensis*

**, Significant at P< 0.01

Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.

Mean ±S.E

Table - 5: Effects of dietary treatments on cecal microbiota (log cfu/g cecal content) Ileum Microbiota

	Lactobacillus	Yeast	*E.coli*
T_1	4.303±14.126c	4.410±41.12b	6.758±75.67a
T_2	7.047±4.03b	6.323±5.70a	5.118±3.97bc
T_3	8.050±2.85 a	6.380±5.26a	4.662±7.68c
T_4	7.633±13.95ab	5.897±49.83a	5.697±91.01b
Trt	21.90**		
Days	13.71**		
Txd	23.06**		

T_1 - Control, T_2 – 5 g/kg of *Spirulina,* T_3 – 1 g/kg of *Spirulina,* T_4 – 15 g/kg of *Spirulina platensis*

** Significant at P< 0.01

Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.

Mean ±S.E

3.2. Microbiology study

The effects of dietary treatments on ileal microbiota (log cfu/g ileal content) are shown in Table - 4. In ileal digesta, LAB counts were consistently increased (P<0.01), whereas *E. coli* numbers were consistently decreased compared to control groups (P<0.01). The Table - 5 reveals the effects of dietary treatments on cecal microbiota (log cfu/g cecal content). In cecal digesta, LAB counts were significantly increased for the birds fed with 1% of *Spirulina platensis,* whereas *E. coli* were significantly decreased compared to control groups (P<0.01). An increase in the population of yeast in ileal and cecal digesta were observed for 1% of *Spirulina platensis* (P<0.01).

4. Discussion

The present study revealed that the addition of 1% of *Spirulina platensis* improved the weight gain and feed intake (Table - 1 & 2). These results are in agreement with those obtained by Ross *et al*. (1994), who found that there was no adverse effect of dietary *Spirulina* on final body weight. However, Ross and Dominy (1990) and Nikodemusz *et al*. (2010) reported that birds fed dietary *Spirulina* had benefit effects on productive performance. The significant ($P<0.05$) effect of dietary treatment on weight gain of hens fed 0.20% *Spirulina* diet may be brought about through improving the efficiency of feed utilization. In this regard, Raju *et al*. (2005) concluded that dietary inclusion of *Spirulina* at a level of 0.05% can partially offset the adverse effects of aflatoxin on growth rate of broiler chickens.

Besides, FCR (Table - 3) is tended to decrease by 1% of *Spirulina platensis*. These results agreed with the findings of several investigators, who reported that addition of *Spirulina* to the diet improved feed conversion of laying hens (Ross and Dominy, 1990; Ross *et al*., 1994; Nikodemusz *et al*., 2010). All previous traits were significantly ($P<0.05$) improved by dietary treatment, in particular, with the highest level of *Spirulina* (0.2%). It is of interest to note that such improvement was associated with insignificant differences in daily feed intake of hens. In line with the present results, several authors reported that laying hens fed *Spirulina* - containing diets, especially those fed 0.2% *Spirulina* - containing diet attained the best means of egg production and feed conversion compared with those of the control group (Ross and Dominy, 1990; Ross *et al*., 1994; Nikodemusz *et al*., 2010).

The results of the present study showed that the supplementation *Spirulina platensis* positively influenced the ileal microbiota. Counts of LAB and yeast were significantly higher ($P<0.01$) in 1% *of Spirulina platensis* groups compared with the control groups (Table - 4). One per cent of *Spirulina platensis* were the most efficient group in the LAB count in ileal digesta. Also, addition of the 1% of *Spirulina platensis* significantly reduced *E. coli* count ($P<0.01$).

One per cent of *Spirulina platensis* supplementation substantially increased the population of lactic acid bacteria and yeast in the cecum content. The population of *E. coli* was significantly decreased by the addition of one per cent of *Spirulina platensis* in cecum ($P<0.01$). Savage and Zakrzewska (1996) reported that the removal of potential pathogens from the intestinal tract of growing animals may provide a more favorable environment for the digestion, absorption, and metabolism of growth - enhancing nutrients.

This improvement may be related with the balanced microbial population in the gastrointestinal tract which has an important role in the health and performance of the broilers (Thongsong *et al*., 2008). The probiotic activity of some bacterial strains with the ability to colonize the intestinal epithelium contributing to stabilize the intestinal microflora, especially after antibiotic treatment, has been described in several reports (Gasson, 1993). Because, the gut microbiota can play a major role in health, there is currently some interest in functional feed ingredients that may stimulate beneficial lactic acid bacteria. It was previously reported that *Spirulina* contains growth promoters, in the form of unique polysaccharides that enhances the growth of lactic acid bacteria (Parada *et al*., 1998). Feeding *Spirulina* containing diets may increase the *Lactobacillus* population and enhance the absorbability of dietary vitamins (Tokai *et al*., 1997).

5. Conclusion

The present study demonstrated that supplementation of 1% of *Spirulina platensis* might improve growth performance and enhance the growth of lactic acid-fermenting bacteria (LAB) and yeast in the gut. In addition to these, the 1% of *Spirulina platensis* supplementation might have a negative effect on *E. coli.*

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