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RESEARCH ARTICLE

DIETARY ADMINISTRATION OF SPIRULINA PLATENSIS AS PROBIOTICS ON GROWTH PERFORMANCE AND HISTOPATHOLOGY IN BROILER CHICKS

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ABSTRACT

A trial was conducted to investigate the effects of dietary supplementations of *Spirulina platensis* on broiler performance, and Histopathology. 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. Commercial mash starter and finisher diets were supplemented with 0%, 0.5%, 1%, and 1.5% of *Spirulina platensis*. The Body Weight Gain, Feed Conversion Ratio and Villi length, were significantly increased by the dietary inclusion of the 1% of *Spirulina platensis* supplementation significantly increased Body weight gain, decreased Feed Conversion Ratios and increases the villi height. The *Spirulina platensis* offers a good natural alternative to improve poultry production.

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INTRODUCTION

The intestinal epithelium acts as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen. Stressors, pathogens, and chemical substances, among others, cause disturbances in the normal microflora or in the intestinal epithelium that may alter the permeability of this natural barrier, facilitating the invasion of pathogens and prejudicial substances, modifying the metabolism, the ability to digest and absorb nutrients, and leading to chronic inflammatory processes at the intestinal mucosa (Hofstad, 1972; Podolsky, 1993; Oliveira, 1998). Consequently, there is decrease in the villus, increase in the cell turnover and decrease in the digestive and absorptive activities (Visek, 1978).

The action of probiotics can be explained by some mechanisms such as the production of antimicrobial substances and organic acids, protection of the villi and absorptive surfaces against toxins produced by pathogens, as well as the stimulation of the immune system (Dobrogosz *et al.*, 1991; Ewing and Cole, 1994; Walker and Duff, 1998; Pelicano *et al.*, 2002).

Spirulina is the oxygenic photosynthetic bacterium belongs to *Cyanobacteria* and *Prochlorales* according to the classification in Bargey's Manual of Determinative Bacteriology. In this classification, sequence of the Rrna sub-unit 16S is considered. In 1989, these microorganisms were classified into two genera –

Spirulina platensis and *Spirulina maxima*, according to suggestion by Gomont (1892) and Castenholz and Waterbury (1989). This classification is accepted currently. At the present time, there are some discrepancies about classification of *Spirulina*. Botanists are identifying this microorganism as microalgae because of their photosynthetic attitude. On the other hand, bacteriologists include this microorganism into bacteria after they determined the main difference as a phospholipidic membrane between prokaryotes and eukaryotes (Tomaselli *et al.*, 1996).

On the other hand, prebiotics effects are based on reduction of the growth of many pathogenic or nonpathogenic intestinal bacteria by means of the pH reduction that results from increased lactic acid levels in the ceca (Choi *et al.*, 1994). Some bacteria may recognize binding sites in such molecules as if they were on the mucosa surface, and the intestinal colonization by pathogenic bacteria is thus reduced. Therefore, there is lower incidence of infectious processes, and the functions of secretion, digestion and absorption of nutrients can be appropriately performed by the mucosa (Iji and Tivey, 1998). The present study evaluated the effects of different levels of *Spirulina platensis* and their association on the histological of the intestinal mucosa of broilers.

MATERIALS AND METHODS

Eight hundred day-old broiler chicks (Ross 308) were randomly assigned to 1 of 4 dietary treatments, consisting of eight

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replicates of 25 birds each. Commercial mash diets were supplemented with 0%, 0.5%, 1%, and 1.5% of *Spirulina platensis*. Chicks fed with four basal diets of Maize-soybean diets during four periods of 0-10 days birds fed with broiler Prestarter, 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 all the Ross 308 broiler chicken requirements.

anterior to Meckel's diverticulum) was removed, rinsed in Tris-buffered saline, cut into 5 equal pieces, and and fixed in 10% neutral buffered formalin. Each intestinal pieces was subsequently cut into 5-mm sections and placed into tissue cassettes.

Cassettes were embedded in paraffin, cut into thicknesses of 5μ m, and mounted onto slides. Tissue slides were stained using hematoxylin and eosin Sun *et al.*, (2005)

Table 1 Effect of growth	performance on B	Broiler Chicks fed	with Spirulina	platensis (g)
LUDIC L Diffect of growth	periorinance on D	fond chiefes ica	min spinitenter	pronono (5)

	10 th day	20 th day	30 th day	36 th day
T1	271.44±14.126a	794.7 ±41.12a	1468.62 ±75.67b	1847.32 ±94.94b
T2	307.04 ±4.03a	870.5 ±5.70a	1671.34 ±3.97a	2065.86 ±10.16a
T3	310.06 ±2.85a	908.92 ±5.26a	1715.34 ±7.68a	2162.14 ±11.27a
T4	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	
TT2 1 /1	6 G · 1 TO 15 4	6 6 1 1		

(T1-Control, T2-5g/kg of Spirulina, T3-1g/kg of Spirulina, T2-15g/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 2 Effect of Feed Intake on Broiler Chicks fed with Spirulina platensis (g)

	10 th day	20 th day	30 th day	36 th day
T1	319.24±13.14a	1154.60 ±6.57a	2362.56 ±12.11b	3497.92 ±24.05b
T2	343.0 28.21a	1238 ±6.9a	2682 ±10.47a	3465 ±23.75a
T3	318 ±3.99a	1144.96 ±96a	2530 ±15.9ab	3207 ±20.6ab
T4	324.28 ±4.63a	1184.649±17.25a	2595.84 ±26.01ab	3260.80±96.64ab
	Trt		985.28**	
	Days		2.85**	
	Txd		<1	

(T1-Control,T2-5g/kg of Spirulina, T3-1g/kg of Spirulina, T2-15g/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

	10 th day	20 th day	30 th day	36 th day
T1	1.157±0.05a	1.420 ±0.06a	1.582 ±0.05a	1.864 ±0.01a
T2	$1.110 \pm 0.01a$	1.416 ±0.04a	1.604 ±0.01a	1.868 ±0.09a
T3	1.022 ±0.01a	1.256 ±96b	1.472 ±0.09a	1.716 ±0.09a
T4	1.085 ±0.06a	1.455±0.07a	1.575 ±0.09a	1.897±0.07ab
	Trt		70.32**	
	Days		5.47**	
	Txd		<1	

(T1-Control,T2-5g/kg of Spirulina, T3-1g/kg of Spirulina, T2-15g/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 4 Effect of Spirulina on Histopathology in Broiler at 36th Day

	T1	T2	Т3	T4
Day 36 th	270.95±14.36c	331.68±14.78b	362.01±14.53a	306.27±4.84b
TXD		16.16**		

Body weight and Feed Intake Measurement

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

Tissue Sampling and Measurement of villus height

Tissue Sampling

On day 36, six chicks from each treatment were killed by cervical dislocation for measurement of intestinal villus height by method of Sun *et al.*, (2005). Five centimeter a section of jejunum (medial portion posterior to the bile ducts and

Measurement of Villus Height

In the jejunum (4 sections for each segment per bird), the villus height was measured from the villus tip to the bottom, not including the intestinal crypt.

The measurement was done with the Scion Image Program (Scion Corporations, Frederick, MD). The mean villus heights from 15 birds were expressed as a mean villus height for 1 treatment groupfor each as per Zentek *et al.*, (2002)

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 effect of *Spirulina platensis* on growth performance, feed gain ratio and villus height were the main effect. The level of statistical significance was preset at P_0.05.

RESULTS AND DISCUSSION

Growth performance

The present study showed Body Weight Gain (Table 1), Feed Intake (Table 2) and Feed Conversion Ratio (Table 3) of broiler chicks fed different levels of Spirulina platensis at 10th, 20th, 30th and 36th days of age. Results showed that chicks fed with 1% of Spirulina platensis had the higher body weight gain and improved feed conversion ratio compared with control group or other dietary treatments. The 1% of Spirulina platensis (Table 1) supplemented group had a greater Body weight gain (2162.14 \pm 11.27a) compared with control birds (1847.32 \pm 94.94b), Feed Intake (Table 2) was lower for birds supplemented with 1% Spirulina platensis (3207 ±20.6ab) compared with the control $(3497.92 \pm 24.05b)$. Feed Conversion Ratio (Table 3) was lower for birds supplemented with 1% Spirulina platensis (1.716 $\pm 0.09a$) than control birds $(1.864 \pm 0.01a)$, The obtained results confirmed the previous findings of several researchers including Razafindrajaona et al., (2008). Also in agreement with our study, Toyomizu et al., (2001) reported that Spirulina platensis confirmed these results when Spirulina was introduced at the rates of 40 and 80g/kg in broiler diets. Birds feeding with spirulina platensis shows significant difference (P<0.01). This result was agreed with Hussein and Kaoud (2012). Ross and Dominy (1990), Mariey et al., (2012) and Nikodémusz et al. (2010) also reported that birds fed dietary Spirulina had benefit effects on productive performance.

Histopathology

Effects of dietary *Spirulina* supplementation on villi height is presented in Table 4. Feeding dietary *Spirulina* had a significant (P<0.01) increase in the height of villi as Spirulina levels in diets increased up to 1%. The increased height of villi length may be related to the high protein contents in *Spirulina* (with values ranging from 55-65% and includes all of the essential amino acids).

Intensive livestock production systems may be associated with multiple stressful incidents that negatively impact immune response and animal performance. The high metabolic rate during intensive feeding is accompanied by an increased production of free radicals, and any imbalance between production of these molecules and their safe disposal may culminate in oxidative stress, which can damage cells and tissues (Miller *et al.*, 1993; Lykkesfeldt and Svendsen, 2007). Therefore, under oxidative stress conditions, there is an increased demand for antioxidants to reduce the deleterious effects of free radicals on the immune system (Carroll and Forsberg, 2007).

Interestingly, feeding natural, rather than synthetic, antioxidant could be advantageous to animal welfare and consumer safety (Call *et al.*, 2008; Makkar *et al.*, 2007). The blue-green algae, *Spirulina platensis*, have been considered as a suitable natural antioxidant and immune-stimulant to humans and animals with fewer side effects and more cost effectiveness than synthetic products (Abdel-Daim *et al.*, 2013; Belay, 2002; Khan *et al.*,

2005). Recently, the impact of dietary Spirulina supplementation on animal health and productivity has been reported (Holman and Malau-Aduli, 2012).

CONCLUSION

The results show that dietary *Spirulina platensis* could improve intestinal villi and epithelial cells, resulting in improved growth performance. The fact that a synergistic effect was observed with regard to growth performance and intestinal histology at 1% level of *Spirulina platensis* suggests a good supplementary partner for probiotics.

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