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Effect of Spirulina platensis supplementation on performance, haematological and serum biochemical profiles of broiler chickens reared under tropical environment

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Target audience: *Poultry nutritionist, Livestock farmers, feed millers*

Abstract

The ban on feed grade antibiotic growth promoters and increasing demand for human protein food sources have resulted in a need for new feed materials which provide a safe source of nutrients for poultry. A study was conducted to evaluate the effect of dietary supplementation of spirulina on performance, haematological and serum profiles on broiler chickens. A total of 120 day old mixed sex broiler chicks were randomly assigned into 4 treatments with 3 replicate pens per treatment (10 birds/replicate pen) for 0-3 weeks and 4-7 weeks respectively. A reputable commercial diet was used as a basal diet (Treatment 1 without spirulina), while treatments 2, 3 and 4 had 6.00, 12.00, and 18.00 g/kg of spirulina supplemented in the commercial diet. The final weight, body weight gain, feed intake and feed conversion ratio were significantly ($P < 0.05$) higher in spirulina-supplemented groups compared with the control group for both the starter and finisher phases. For the starter phase it was observed that chicks fed 12.00g/kg spirulina diet performed better in terms of body weight gain while chickens fed 6.00g/kg spirulina diet had the best performance in terms of body weight gain at the finisher phase. Dietary treatment had significant ($P < 0.05$) effects on packed cell volume (PCV), haemoglobin count, red blood cells (RBC) and white blood cells (WBC). The values for total protein (TP), globulin, Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT) and Alkaline Phosphatase (ALP) were significantly ($P < 0.05$) higher for chickens fed diets containing spirulina compared to the chickens fed diet without spirulina. It is concluded that dietary supplementation of spirulina improves body weight gain, haematological parameters and blood serum biochemistry of broiler chickens.

Key words: *poultry; algae; blood.*

Description of problem

The increasing demand for human protein food sources has resulted in a need for new feed materials, which provide a safe source of nutrients for poultry and livestock. In addition, the animal feed manufacturers most importantly the poultry feed manufacturers are under increasing consumer pressure to reduce the use of antibiotic growth promoters as feed additive and find alternatives to antibiotic growth promoters in poultry diets (1,2, 3). Hence, the feed manufacturers are adopting new forms of natural feed additives that are the products of modern science (4, 5, 2). This new generation of growth enhancers include botanical additives like appropriate blends of herbs or plant extracts. Extracts from herbs and plants used as feed additives include many different bio-active ingredients such as alkaloids, bitters, flavonoids, glycosides,

mucilage, saponin and tannins (6). Herbs and plant extracts affect the appetite and intestinal microflora, stimulate the pancreatic secretions to increase endogenous enzyme activity and immune system (7). During the last ten years, the beneficial nutritional aspects of microalgae have been advertised extensively worldwide, and therefore the algae enterprises started to gain a clear interest among producers (8). Numerous studies have been conducted on exploring the contribution of Spirulina in poultry feeding (2, 3). Spirulina (blue-green alga) is one of the high quality natural feed additives that can be used in animal and poultry nutrition. Spirulina is relatively high in protein with values ranged between 55-65% and includes all of the essential amino acids. Results from the research conducted by (9), showed that hens fed diets containing Spirulina achieved greater productive performance than the control hens. (10), reported that feed conversion (FC), body weight gain (BWG), percentage of carcass yield improved by the dietary Spirulina platensis inclusion as compared the control. However, (11) reported that FC was improved for birds fed Spirulina platensis than the control birds. Moreover, it was revealed that Spirulina platensis enhances immune response and reproduction (12). Addition of less than 1% Spirulina in chicken diets significantly enhanced the defense systems for antigen processing, greater T-cell activity and increased microbial killing (12). Results from different studies about performance of broilers fed dietary levels of spirulina are contradictory and most studies which have been on the use of spirulina platensis, were mostly limited to 35^0C and in most cases in the temperate regions. On the other hand, the temperature rises to 45 to 50^0C in the tropical regions. As a result, the optimum inclusion level may differ in the tropical regions.

However, this present study was conducted to explore the bio-efficacy of Spirulina platensis supplemented in a commercial broiler feed on performance, haematological and serum biochemical profiles of broiler chickens reared from 0-3 weeks (starter phase) and 4-7 weeks (finisher phase) under tropical environment.

Material and Method

Experimental site

The experiment was carried out at the Poultry Unit of Kabba College of Agriculture, Ahmadu Bello University. Kabba is located within the Southern Guinea Savannah Zone on latitude $7^05^{'}$N, longitude $6^04^{'}$E and altitude of 640m above sea level. It has annual rainfall of 1500mm and rain starts between late April and early May to mid October. The dry season begins around the middle of November, with cold weather that ends in February. This is followed by relatively hot-dry weather between March and April just before the rain begins. The minimum daily temperature is from 14^oC-24^oC during the cold season while the maximum daily temperature is from 23^0C-45^0C during the hot season. The mean relative humidity during dry and wet seasons is 21% and 72%, respectively (14).

Source of experimental birds and test ingredient (Spirulina)

The broiler chicks (Arbor Acre) used in this experiment was obtained from a commercial hatchery with good records of birds' performance. Spirulina platensis was purchased from Terrafertil Company, United Kingdom. The composition of nutrients in spirulina is as shown in Table 1.

Table 1: Nutritional composition of Spirulina platensis powder

Nutritional component	Amount
Energy	1.430kg/338kcal
Fat	1.2g
Carbohydrate	19.4g
Fibre	11.7g
Protein	68.3g
Salt	0.45g
Potassium	1.320mg
Calcium	327mg
Iron	85mg
Magnesium	290mg
Phosphorus	1.110mg
Vitamin A	19,900UI

Experimental diets

A reputable commercial broiler starter and finisher feeds were used in this study. Treatment 1 contained the commercial diet without spirulina, while treatment 2, 3 and 4 had 6.00, 12.00, and 18.00 g/kg of spirulina supplemented in the commercial diet respectively. All the diets were chemically analyzed according to (15) methods for their proximate composition (Table 2). The results from the analyses showed that the feeds met the major nutrient requirements as recommended by (16) for broiler chickens. The birds were fed the experimental diets from 0-3weekss for the starter phase and 4 – 7 weeks for the finisher phase.

Table 2: Proximate analyses for the commercial feed

	Commercial feed	
Parameter	Starter feed	Finisher feed
Dry matter	94.13	94.13
Crude protein	22.87	21.11
Crude fibre	7.03	6.06
Ether extract	4.93	4.76
Ash	5.81	6.61
NFE	60.04	56.03

NFE=Nitrogen Free Extract

Experimental design and management of birds

For the starter phase a total of one hundred and twenty day old mixed sex Arbo Acre broiler chicks were obtained from a reputable commercial farm in Nigeria. The chicks were placed into four treatments in a completely randomized design with three replicate each (10 birds per replicate). Chicks in Treatment 1 were fed commercial diets without spirulina, while treatment 2, 3 and 4 had 6.00, 12.00, and 18.00 g/kg of spirulina supplemented in the commercial diet respectively. However, at the finisher phase one hundred and twenty (120) chickens (26 days old) were pooled together and were given

a common diet for five days. At the end of the five day feeding the chickens were randomly allocated to 4 treatments on basis of equal weight. Chickens in Treatment 1 were fed commercial diet without spirulina, while treatment 2, 3 and 4 had 6.00, 12.00, and 18.00 g/kg of spirulina supplemented in the commercial diet respectively. The birds were reared in a deep clean floor litter system (10x10 feet). Heat and light were provided throughout the brooding period. Feed and water were provided *ad libitum*. The experiment was conducted during the hot season between March and April 2019.

Parameters measured

The parameters measured include: Final body weight and feed intake. From the primary data collected for feed intake and weight gain, data for feed conversion rate was generated. Mortality record was recorded as it occurred.

Haematological and blood serum evaluation

At day 21, 2ml of blood samples were collected from each of three birds per replicate via the wing veins into sterile tubes containing an anticoagulant (ethylene diamine tetra acetic acid, EDTA) for the determination of haematological parameters e.g. Packed Cell Volume (PVC), haemoglobin concentration (HB), red blood cells (RBC) and white blood cells (WBC). 2ml of blood was also allowed to clot and then centrifuged. The serum was separated and stored at -20°C until analyzed for serum parameters (albumin, total protein, glucose, globulin, triglyceride, Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT) and Alkaline Phosphatase (ALP) according to the methods described by (26) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was analyzed in triplicates.

Statistical analyses:

All data obtained were statistically analyzed using the General Linear Models (GLM) procedure of (17) for the analysis of variance. Duncan's multiple range tests (18) were used to determine differences among treatment means. Means were considered different at $P<0.05$.

General Linear Model

$Y_{ij} = \mu + K_{i+} e_{ij}$

Y_{ij}= Observation of the i^{th} level of spirulina platensis as shown by broilers performance

μ= Overall mean

K_i =i^{th} effect of spirulina

e_{ij} = Random error

Results and Discussion

The effect of spirulina platensis on growth performance of broiler chicks during the periods of 0 to 3 weeks are shown in Table 3. Supplemental spiruluna had significant effects on final weight, weight gain and average daily weight gain, total feed intake and feed conversion ratio of chicks. The performances of chickens fed diets supplemented with spirulina were similar and significantly ($P<0.05$) better than those on the control diet in terms of the final weight, weight gain and feed intake. There were trends of improved performance in final weights and weight gains observed as the levels of dietary spirulina increased. Chicks fed 12g/kg had the best results in terms of final weight and body weight gain. This observation is in agreement with the results of (8 19, 20, 21), who reported that birds fed diets containing Spirulina had useful impacts on productive performance as a result of increased feed intake. The improvements observed in terms of final weight and body weight may be due to the absorption of adequate amino acids, minerals and vitamins by chicks (22, 21). Also the increased performance observed, may be related with the microbial population balance

in the gastrointestinal tract. Hence, enhancing, the absorbability of dietary vitamins and minerals by broiler chicks (21). The poor growth observed in treatment 4 with 18g/kg spirulina and the control treatment without spirulina in terms final weight and weight gain (642.32g and 699.32g) respectively, may be due to poor mineral and vitamin absorption as a result of antagonistic nutrient interactions. Chicks fed 12g/kg spirulina consumed the highest feed and this was evident in their increased weight gain. This result is similar to the findings of (21), who reported that different levels of Spirulina improved feed consumption. Chicks fed 6g/kg spirulina supplemented diet had the best feed conversion ratio; although it was observed that the results were similar for all the spirulina supplemented diets compared to the control diet. This result is in consonance to the findings of (8), who reported a significant improvement as the levels of spirulina increased across the treatment groups compared to the control diet without spirulina.

Table 3: Performance of broiler starter chicks fed graded levels of Spirulina (0- 3 Weeks)

	Spirulina (g/kg)				
Parameters	0.00	6.00	12.00	18.00	SEM
Initial weight (g)	40.01	40.01	40.00	40.01	0.02
Final weight(g)	739.33^{b}	748.00^{b}	790.67^{a}	682.33^{c}	3.14
Ave weight gain (g)	699.32^{b}	707.99^{b}	750.66^{a}	642.32^{c}	3.15
Ave daily gain (g)	33.30^{b}	33.71^{b}	35.75^{a}	30.59^{c}	0.15
Ave feed intake (g)	1620.83^{b}	1579.16^{bc}	1818.35^{a}	1500.00^{c}	45.25
Ave feed intake (g/b/d)	77.18^{b}	75.19^{bc}	86.61^{a}	71.43^{c}	2.11
FCR	2.32^{b}	2.23^{a}	2.42^{ab}	2.34^{ab}	0.06
Mortality (%)	0.00	0.00	0.00	0.00	0.00

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
FCR = Feed conversion ratio

At the finisher phase no significant differences were found among the treatments for mortality of broiler chickens during the experimental period, but the final weight, body weight gain, feed intake and feed conversion ratio were significantly ($P < 0.05$) higher in spirulina supplemented groups than those of the control group (Table 4). A linear increase in growth performance was observed as the levels of spirulina increased and began to drop at 18g/kg spirulina supplementation in the diet. Although, it was observed that chickens fed 6.00g/kg and 12g/kg of spirulina supplemented diets were similar in terms of body weight gain compared to the control diet. This result is similar to the findings of (23) who reported that final body weight, weight gain, and feed efficiency were significantly affected by dietary supplementation of spirulina at different levels in the broiler diet. The increased body weight gain possibly could the as a result of effective utilization of potential nutrients such as amino acids, minerals and vitamins in spirulina which has been reported to affect positively the growth rate of broiler chickens.

The observed increase in feed intake for broiler chickens fed diet without spirulina platensis supplementation could be as a result of chickens searching for missing nutrients. Hence, it is expected that chickens lacking in nutrients will always consume more to meet up with the body's nutrient requirements. Chickens fed 6g/kg spirulina supplemented

diets had the best feed conversion ratio as compared to chickens fed diet without (0g) or chickens fed the highest concentration of spirulina. This might due to the nutritional value of spirulina as it is rich in protein, essential amino acids, essential fatty acids, minerals and vitamins (24, 25).

Table 4: Performance of broiler finisher chickens fed graded levels of Spirulina (4- 7 weeks)

	Spirulina (g/kg)				
Parameters	0.00	6.00	12.00	18.00	SEM
Initial weight (g)	750.01	750.34	749.84	750.00	0.20
Final weight(g)	2217.33^{b}	2450.00^{a}	2452.67^{a}	2177.67^{b}	46.93
Ave weight gain (g)	1467.32^{b}	1699.66^{a}	1702.83^{a}	1427.67^{b}	46.77
Ave daily gain (g)	52.40^{b}	60.70^{a}	60.82^{a}	50.99^{b}	1.67
Ave feed intake (g)	4081.11^{a}	3176.67^{c}	3675.00^{b}	3564.00^{b}	18.94
Ave feed intake (g/b/d)	145.75^{a}	113.50^{c}	131.25^{b}	127.28^{b}	0.68
FCR	2.78^{c}	1.87^{a}	2.16^{b}	2.56^{bc}	0.08
Mortality (%)	0.00	0.00	0.00	0.00	0.00

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
FCR = Feed conversion ratio

The effect of graded levels of spirulina supplemented diets on haematological parameters and serum biochemical indices for broiler finisher chickens were as presented in Table 5 and 6 respectively. Dietary treatments had significant (P<0.05) effects on packed cell volume, haemoglobin count, white blood cells and red blood cell. The packed cell volume (PCV) and haemoglobin counts were significantly (P < 0.05) higher for broiler chickens fed diets containing spirulina compared to broiler chickens fed diet without spirulina (Control diet). The number of white blood cells (WBC) was significantly higher (P < 0.05) in broilers fed diet without spirulina compared with diets containing spirulina, and the number of red blood cells (RBC) was also significantly higher (*P* < 0.05) in broiler chickens fed 12g/kg spirulina, although a trend was not observed. The mean value for packed cell volume (27.97- 36.52%) obtained in this study fell within the normal range of values (21-45%) as reported by (27), indicating that the diets were ideal and adequate for broiler finisher chickens. It also indicated that the birds were not anemic. The mean values of 7.33-10.07 for heamoglogin count fell within the normal value of 8.50g/dl reported by (27). The mean values of 9.37 - 11.20 for heamoglogin count obtained in this study were higher than normal value of 8.50g/dl reported by (28). The WBC of 4.20-6.17 was above the normal range of 1.58- 2.05 x 10^{6}ul reported by (29), the discrepancies in the values may be due to the season of rearing, species, sex and age of birds. The RBC values of 3.07- 4.20 (x 10^{6}ul) obtained from this study fell with the normal values of 3.82 for RBC reported by (29) and 2.99- 3.06 (x 10^{6}ul) reported by (28). This shows that the dietary treatment had no adverse effects on the chickens. Chickens fed diets supplemented with Spirulina at levels of 6.00, 12.00 and 18.00g/kg diet had higher values of globulin, glucose, AST, ALT, ALP, Triglycerides and Total protein compared with the control diet. This observation is contrary to the findings of (30) who reported that the level of serum ALT, AST, triglycerides and total

cholesterol decreased (P≤0.05) significantly in chickens fed high levels of dietary Spirulina platensis compared with the control diets. Reasons for this may be due to genotype and seasons of rearing.

Table 5: Haematological parameters of broiler chickens fed diets supplemented with Spirulina platensis (4 – 7weeks)

	Spirulina Platensis Levels (%)				
Parameters	0.00	6.00	12.00	18.00	SEM
PCV (%)	27.97^{b}	35.00^{a}	36.52^{a}	36.33^{a}	2.90
Hb (g/dl)	9.37^{c}	11.20^{b}	13.52^{a}	11.00^{b}	0.83
RBC (x10^{6}/l)	3.63^{b}	3.23^{bc}	4.20^{a}	3.07^{c}	0.28
WBC (x10^{6}/l)	6.03^{a}	6.17^{a}	4.70^{b}	4.20^{b}	0.29

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
PCV= Packed cell volume
HC=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells

Table 6: Serum biochemical indices of broiler chickens fed diets supplemented with Spirulina platensis (4 – 7weeks)

	Spirulina Platensis Levels (%)				
Parameters	0.00	6.00	12.00	18.00	SEM
Globulin(mg/dl)	5.40^{b}	5.73^{a}	5.60^{ab}	5.60^{ab}	0.14
Glucose(mg/dl)	6.30^{c}	8.30^{a}	8.13^{a}	7.00^{b}	0.37
AST(u.i/l)	20.67^{c}	25.67^{b}	47.67^{a}	24.33^{bc}	2.14
ALT(u.i/l)	19.00^{b}	20.33^{b}	32.00^{a}	22.33^{b}	2.18
ALP(u.i/l)	17.00^{c}	19.00^{bc}	38.00^{a}	23.00^{b}	2.13
Triglycerides(mg/dl)	102.00^{c}	124.00^{b}	160.33^{a}	169.33^{a}	11.27
Albumin(g/dl)	3.70	4.37	3.90	4.80	0.62
Total protein(g/dl)	6.47^{b}	6.86^{b}	9.00^{a}	6.90^{b}	0.52

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
AST=Aspartate Amino-Transferase
ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

Conclusion and Applications

1. Dietary supplementation of spirulina improves body weight gain of broiler chickens.
2. The inclusion of up to 12g/kg spirulina in commercial diet improved growth performance of broiler chicks while 6g/kg spirullaahich was might improve growth performance.
3. Supplementation of up to 6g/kg spirulina in commercial diet of broiler finisher chickens might improve growth performance.
4. The inclusion of up to 6g/kg of spirulina in the conventional diets of broiler chickens might improve haematological and serum biochemistry of broiler chickens.

Declaration of interest

The authors declare no potential conflicts of interest associated with this research.

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