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THE DYNAMICS OF DIETARY SUPPLEMENTATION OF THREONINE ON THE PERFORMANCE OF BROILER CHICKENS

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ABSTRACT

An experiment was conducted to evaluate the effect of three levels of supplemental threonine on the performance, carcass characteristics and blood parameters of broiler chickens. It was hypothesised that supplemental threonine would improve overall broiler chicken performance. One hundred and sixty day-old arbor acres broiler chicks were randomly allotted to 4 treatments. Each treatment was replicated 4 times and each replicate had 10 birds. The treatments were treatment 1 (control treatment): no supplemental threonine, treatment 2: 250 mg supplemental threonine, treatment 3: 500 mg supplemental threonine and treatment 4: 750 mg supplemental threonine. The experiment lasted 56 days. Birds on treatment 3 had significantly ($p < 0.05$) higher live weight than birds on the other treatments. No significant differences were observed in the weight gain, feed intake and feed conversion ratio of the birds on the different treatments. Platelet, white blood cell, red blood cell, heterophil counts and globulin values all differed significantly among birds on all the treatments. The results showed that supplemental threonine (500 mg) has the potential to enhance growth performance and blood health of broiler chickens.

Key words: Threonine, Broiler, Platelet, Blood cell, Globulin

INTRODUCTION

Due to the rapid growth of commercial chicken strains, the availability of amino acids for optimal growth, particularly muscle growth as well as physiological function is critical (Azzam *et al.*, 2011). Apart from methionine and lysine, the aliphatic amino acid threonine is an important growth factor in broilers (Baylan *et al.*, 2006) and is one of the basic amino acids needed for growth of domesticated birds (Bender, 2012). Threonine is typically the third limiting amino acid in broiler diets (Everett *et al.*, 2010) after methionine and lysine. It is also considered one of the important factors that affect poultry performance due to increase in the use of lysine and methionine in broiler diets (Gong *et al.*, 2005). Chickens do not have the ability to synthesise threonine; as such, it is a nutritionally essential amino acid. Threonine has a vital role in improving growth, intestinal morphology, barrier function, the mRNA expression level of mucin 2 (MUC2), immune system functions and antioxidant ability (Chen *et al*,. 2016 and Bi *et al.*, 2018). It is considered the second limiting amino acid for breast meat yield (Estalkhzir *et al.*, 2013). Its supplementation is therefore assumed to result in improved carcass characteristics. It is believed that threonine supplementation enhances feed intake, body weight gain and ultimately carcass weight (Estalkhzir *et al.*, 2013; Khan *et al.*, 2006).

Threonine is also considered a nutraceutical. Nutraceuticals are nutrients or constituents of animal diet that have nutritional and pharmaceutical importance by preventing various diseases, possessing immuno-modulatory potential, providing health benefits and consequently increasing productivity (Dhama *et al*. 2015; Aronson 2017; Helal *et al*. 2019; Waheed Janabi *et al*. 2020). They include nutrients and non-nutrients like amino acids, minerals, vitamins, fatty acids, enzymes, prebiotics, probiotics, synbiotics, pigments, medicinal herbs, herbal extracts, antioxidants, organic acids, flavouring agents, etc. (Narahari 2014;

Alagawany *et al*. 2018a; Elgeddawy *et al*. 2020).

The present study hypothesised that supplementing threonine to broiler chicken diet may have a positive impact on growth and carcass characteristics. The study therefore evaluated the dynamics of three levels of supplemental threonine on the performance parameters, carcass characteristics and blood profile of broiler chickens.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the poultry unit of the Institute of Agricultural Research and Training, Ibadan, Nigeria. Ibadan is in southwest Nigeria and lies on the geographical coordinates of 7° 23′ 16″N and 3° 53′ 47″ E. Ibadan has a mean annual rainfall of 1382mm, annual mean temperature range of 21.3 – 31.2℃, relative humidity of 60 – 90% and ranges in elevation between 150m – 275m above sea level (Adetayo and Dauda 2021).

Management of experimental birds, diets and design

One hundred and sixty (160) day-old Abor Acres broiler chicks were tagged, weighed and randomly allotted using an allotment software into 4 treatments of 4 replicates each. Each replicate had 10 birds. The birds were reared in a well ventilated and illuminated poultry house on deep litter. Routine management procedures were followed while fresh feed was supplied *ad libitum* and the birds had access to cool clean water. Commercial poultry feed was fed to the birds. The experiment lasted 56 days which comprised the starter phase (day 1 – day 28) and the finisher phase (day 29 – day 56). Routine vaccinations for broilers was carried out. Body weight was taken and recorded every week along with feed intake on a daily basis till the end of the experiment. Feed intake, feed conversion ratio (FCR) and body weight gain were calculated. Threonine was included in the feed at the following rate/100kg of feed:

Treatment 1: No supplemental threonine (control group)

Treatment 2: 250 mg supplemental threonine

Treatment 3: 500 mg supplemental threonine

Treatment 4: 750 mg supplemental threonine

The experimental design was Completely Randomised Design.

Measurement of carcass traits

At the end of the feeding trial, 2 birds were randomly selected from each replicate for carcass trait evaluation. Feed was withdrawn overnight before slaughter so as to reduce the potential for carcass contamination from crop and intestinal contents. Each bird was exsanguinated by cutting the jugular vein and allowed to bleed for approximately 2 minutes. The weights of breast, drumsticks, thighs, wings were taken. Relative weights were then calculated as percentages of live body weight.

Blood collection

At the end of the experiment, 5 millimeters of blood was collected from 2 randomly selected birds from each replicate via the jugular vein into specimen bottles. Blood samples for haematological analysis were collected into sterilised bottles containing ethylene diamine tetra acetic acid (EDTA) as anti-coagulant while those used for serum biochemical analysis were collected into tubes without EDTA and centrifuged before analysis. Packed cell volume (PCV) was determined using the microhaematocrit method while the haemoglobin content was determined with the cyanomethaemoglobin method. Red blood cell count was determined using the Neubrauer haemocytometer while Aspartate transaminase (AST) and Alanine transaminase (ALT) were determined with a spectrophotometer.

Statistical analysis

All data were subjected to one-way Analysis of Variance (ANOVA) using the General linear model of SAS (1999) and means, where significant, were separated using the Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Performance characteristics of broiler chickens fed diets with supplemental threonine

The performance characteristics of the birds on the different dietary treatments are shown in Table 1. The final live weight of the birds differed significantly with birds on Treatment 3 (500mg threonine) having significantly ($p < 0.05$) higher live weight than birds on the other treatments. This is in line with reports of Zaghari *et al.* (2011) who found that the inclusion of threonine in diets can improve broiler performance. Weight gain, feed intake and feed conversion ratio however did not differ significantly. According to Dozier *et al*. (2000) and Rama Rao *et al*. (2011), no improvement in performance was observed in broilers fed with diets supplemented with crystalline threonine. Several authors have proposed different dietary inclusion rates of threonine adequate for broiler chickens. Corzo *et al*. (2003) found that ideal total dietary threonine level was 0.69% for growth performance and 0.71% for feed conversion ratio during finisher period (30 to 42 days) in broilers. In contrast, Kidd *et al*. (2004) reported that total dietary threonine requirement was 0.74% for body weight gain and 0.71% for breast meat yield during grower and finisher (21 to 42 days) period. Li (2000) estimated that the requirement of the total threonine was 0.66% from 3 to 6 weeks of age. Threonine requirements for feed intake were 0.79% during starter and grower phase and 0.72% for finisher phase (Samadi and Liebert, 2006). Ciftci and Ceylan (2004) reported that ideal total dietary threonine levels for growth performance were 0.68 to 0.75% for starter (0 to 21 days) and 0.65 to 0.68% for grower (22 to 42 days) periods. The present study however showed that 500 mg threonine supplementation in broiler diet appears to be adequate to sustain the performance compared to other treatments. This underscores the important role of threonine in promoting growth and muscularity (Kidd *et al.,* 1999). Rezaeipour *et al.* (2012) observed an improvement in the growth performance of broilers as a result of the effect of different inclusion rates of dietary threonine. Valizade *et al*. (2016) investigated the effect of 0.675 and 0.843% of total dietary threonine on growth performance of broilers in comparison with a control diet containing 0.641% threonine. The authors found out that the diet containing 0.843% dietary threonine supported better growth performance in broilers with a 1.0% increase in body weight gain compared with those fed control diet. According to the authors, the improved growth performance with higher level of threonine may be due to supplementation of higher level of threonine required for an ideal growth performance.

Table 1: Performance characteristics of broiler chickens fed diets with supplemental threonine

Parameter	T1	T2	T3	T4	SEM	p-value
Initial weight (g)	39.35	38.60	39.18	38.90	0.19	0.54
Final weight (g)	2322.08^{b}	2327.04^{b}	2397.56^{a}	2302.93^{b}	10.30	0.034
Weight gain (g)	2282.73	2288.44	2358.38	2264.03	28.68	0.68
Feed intake (g)	4931.9	4852	4798.4	4783.4	55.72	0.78
FCR	2.16	2.13	2.04	2.12	0.02	0.35

^{ab} Means along the same row with different superscripts differ ($p < 0.05$) significantly
FCR: feed conversion ratio

Carcass characteristics of broiler chickens fed diets with supplemental threonine

Results of the carcass characteristics of birds on the different dietary treatments are shown in Table 2. The values for the wings and

gizzards differed significantly while all other parameters measured did not differ significantly. This result is at variance with reports of Al-Hayani (2017) who reported a significant improvement in the dressing percentage and relative breast weight of birds fed with a diet supplemented with threonine compared with those fed with a diet without threonine supplementation. Authors like Shirzadegan *et al*. 2015 however reported that 0.75% L-threonine in diet improved the intestinal morphology and consequently body weight gain of broilers under hot and humid status but did not have any significant effect on carcass traits. Rezaeipour *et al.* (2012) also reported that threonine fortification did not improve carcass characteristics in a significant manner. Dozier *et al.* (2000b, 2001) indicated that total carcass yield was not affected by dietary threonine concentration. Probably higher inclusion levels of threonine could have elicited better growth and improved carcass characteristics in this study.

Table 2: Carcass characteristics of broiler chickens fed diets with supplemental threonine

Parameter	T1	T2	T3	T4	SEM	p-value
Live weight (g)	2079^{b}	1956.25^{c}	2252.25^{a}	1966^{c}	2.30	0.001
Defeathered weight	95.3	96.82	93.69	94.76	0.73	0.52
Eviscerated weight	81.72	79.43	81.98	82.1	0.62	0.42
Breast	23.33	23.29	25.6	24.33	0.46	0.30
Wings	9.25^{a}	9.1^{a}	7.84^{b}	8.19^{b}	0.14	0.012
Thighs	11.96	12.16	12.41	12.13	0.16	0.79
Drumsticks	9.51	9.92	10.1	9.82	0.16	0.61
Gizzard	2.12^{ab}	2.30^{a}	1.98^{b}	2.03^{ab}	0.05	0.15
Liver	2.46	2.57	2.27	2.31	0.08	0.54
Heart	0.50	0.50	0.49	0.48	0.12	0.98
Kidneys	0.16	0.24	0.13	0.14	0.02	0.29
Lungs	0.52	0.46	0.57	0.51	0.03	0.57

[ab] Means along the same row with different superscripts differ ($p < 0.05$) significantly

Serum biochemical and haematological assay of broiler chickens fed diets with supplemental threonine

Analysis of hematological parameters could help to evaluate the general health status of animals while serum biochemical profile details animals' immune system and general health. Results of the serum biochemical and haematology indices of the birds are shown in Table 3 and Table 4 respectively. For the serum biochemistry parameters, it was observed that only the globulin values of the birds differed significantly with birds on treatment 4 having significantly ($p < 0.05$) higher globulin concentration than birds on the other treatments. This could be attributed to the inclusion level of threonine in the diet as low levels of threonine is inadequate for globulin production. Threonine is a major component of plasma globulin in poultry, rabbits and human beings (Tenenhouse and Deutsch, 1966). Also Min *et al.* (2017) reported that adequate levels of threonine enhanced globulin production in broiler chickens hence higher content of it could explain the trend observed in this study. The other parameters (total protein, albumin, AST, ALT, ALP, glucose) however did not differ significantly. All the values for the parameters measured fell within the normal range for clinically healthy broiler chickens. This suggests that the diets were adequate for the birds irrespective of supplemental threonine levels otherwise an increase (higher than normal range) in the serum AST, ALT

and ALP levels could indicate liver, kidney and/or heart damage.

For the haematology parameters, red blood cell, white blood cell, platelet, lymphocyte and heterophil counts differed significantly among the birds on the different dietary treatments. Packed cell volume, haemoglobin, monocytes and eosinophil counts did not differ significantly but their values fell within the normal range for clinically healthy broilers. Red blood cell counts of birds on treatment 2 (2.88) was significantly ($p < 0.05$) higher than that of birds on the other treatments. Birds on the control treatment (without threonine supplementation) had the least value of RBC (2.22). Threonine supplementation probably enhanced RBC counts as birds on the control diet had RBC counts which fell below the normal range for healthy broilers (2.5 – 3.5/4.5). White blood cell counts differed significantly with birds on treatment 1 having significantly ($p < 0.05$) higher count than birds on the other diets. Birds on treatment 2 had the lowest count. Birds on the supplemented diets had WBC counts that fell within the normal range for clinically healthy broilers (5,000 – 15,000 c/cu mm) while birds on the control diet had WBC count that was slightly elevated (15, 693.8). This value could indicate disease or stress for birds on the control diet. Ahmed *et al.* (2020) reported that application of threonine above NRC requirements resulted in enhanced immunity. Supplemental threonine probably enhanced the immunity of the birds on diets 2, 3 and 4.

Lymphocyte counts differed significantly with birds on treatment 2 having significantly ($p < 0.05$) higher counts than birds on the other treatments. The lymphocyte counts fell within the normal range for clinically healthy broiler chickens. Lymphocytes are an important part of the immune system. An increase in its count could mean chronic infection while a decrease could indicate viral infection. Since the lymphocyte counts fell within the normal range, it means the birds were healthy. Platelet and heterophil counts also differed significantly among the different dietary treatments but they fell within the normal range for clinically healthy broilers.

Table 3: Serum biochemical parameters of broiler chickens fed diets with supplemental threonine

Parameter	T1	T2	T3	T4	SEM	p-value
Total protein (g/dl)	6.38	5.95	6.2	6.38	0.09	0.33
Albumin (g/dl)	1.73	1.39	1.33	1.39	0.07	0.23
Globulin (g/dl)	4.65^{b}	4.56^{b}	4.88^{ab}	4.99^{a}	0.05	0.42
AST (IU/L)	194.75	183.25	190.75	190.75	1.87	0.23
ALT (IU/L)	31.75	26.25	25.25	27.5	1.07	0.21
ALP (IU/L)	285.75	245	286.5	267.75	10.57	0.48
Glucose (mg/dl)	181	176.75	180.25	185.25	2.1	0.57

[ab] Means along the same row with different superscripts differ ($p < 0.05$) significantly
AST - Aspartate transaminase
ALT - Alanine transaminase
ALP – Alkaline phosphatase

Table 4: Haematology parameters of broiler chickens fed diets with supplemental threonine

Parameter	T1	T2	T3	T4	SEM	p-value
PCV (%)	24.43	26.13	25	24.88	0.42	0.55
Hgb (g/100ml)	7.95	8.43	8.3	8.13	0.15	0.69
RBC ($x10^3$/µl)	2.22^{b}	2.88^{a}	2.58^{ab}	2.70^{ab}	0.10	0.17
WBC ($x10^3$/µl)	$15,693.8^{a}$	$13,300^{c}$	$14,318.8^{b}$	$13,843.8^{bc}$	94.34	0.001
Platelets ($x10^3$/µL)	$176,125^{a}$	$157,750^{c}$	$166,875^{b}$	$152,375^{c}$	978.45	0.001
Lymph(%)	59.63^{ab}	67.25^{a}	60.00^{ab}	59.63^{ab}	1.36	0.11
Hetero (%)	34.25^{a}	25.00^{b}	34.75^{a}	35.25^{a}	1.29	0.05
Mono (%)	3.00	3.50	3.50	3.13	0.22	0.79
Eos (%)	3.25	3.50	3.50	4.38	0.31	0.60

[ab] Means along the same row with different superscripts differ ($p < 0.05$) significantly
PCV – Packed cell volume
Hgb – Haemoglobin
RBC – Red blood cells
WBC – White blood cells
Lymph – Lymphocytes
Hetero – Heterophils
Mono – Monocytes
Eos - Eosinophils

CONCLUSION

The study found out that broiler chickens responded positively to supplemental threonine in their diet. It was observed that it had the potential to improve performance as indicated by its effect on final body weight. It also had a positive effect on the blood health and consequently overall health of the birds. It is however recommended that the inclusion rate of supplemental threonine be increased in order to harness the optimal advantages.

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