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Immune Response and Bone Marker Enzyme Activities of Broiler Birds Fed Graded Level Taurine-Supplemented-Diets

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Abstract

Taurine, a derived amino acid has been proven to play an important biological roles in enhancing bone strength and immune activities of broiler birds. This research investigated the effect of the different concentrations of graded level dietary taurine supplement on immune response of birds against Newcastle Disease Virus (NCDV) and Infectious Bursal Disease Virus (IBDV), as well as on enzymatic markers of bone metabolism and effect on mineral composition. A total of 300 unsexed day-old arbor acre broiler birds were used for this research. The birds were randomly distributed using a completely randomized design into five dietary treatment of six replicates with 10 birds per replicate. Treatment one (T1) served as the control group with 0% taurine supplement. Treatments 2, 3, 4, and 5 contained dietary taurine supplements at 0.002, 0.004, 0.006, and 0.008%. Antibody titre against NCDV and IBDV were determined according to standard procedures. The study lasted 42 days. Birds on 0.002% taurine had the highest antibody titre (128.38) against NCDV, while birds on the 0.006% taurine-supplemented-diet also portrayed a distinct ($p<0.05$) titre value (1029) against IBDV. Serum alkaline phosphatase and bone specific alkaline phosphatase (132.74 and 150.66) at the 42nd day were highest ($p<0.05$) for birds on 0.004 and 0.002% dietary taurine supplement respectively. The activity of serum tartrate resistant acid phosphatase (44.94) was notably highest ($p<0.05$) for birds on 0.008% taurine. Bone mineral contents showed that birds fed with 0.002% taurine-supplemented-diet had the highest percentage ($p<0.05$) of phosphorous (9.50), calcium (32.18) and phosphate (21.77) composition. Conclusively, inclusion of taurine as dietary supplement has proven useful not only in enhancing the birds' immunity against NCDV an IBDV, but also in boosting bone mineral composition of meat type poultry birds.

Keywords: Broiler birds, taurine, bone metabolism, Newcastle disease virus, Infectious Bursal disease virus.

1.0 Introduction

Taurine, a clear and an inodorous free amino acid, is found virtually in all animal tissues (Shim et al., 2006). It is an essential nutrient that plays notable roles in the cells of the body (Schaffer et al., 2010). Dietary taurine supplementation is very essential, mostly because of its' relatively inadequate endogenous synthesis (Kadam and Prabhasankar, 2010). It plays a key role in heart and brain functionality, as well as been a good immunomodulatory and anti-inflammatory derived amino acid (Huang et al., 2011).

Malnutrition have a severe effect on broiler birds' immune organs, especially if it occurs at the starter phase (Kirk and Barbul, 1992). Dietary supplementation of laying hens with taurine was able to reduce inflammation, hence assisting poultry birds' immune system (Ma et al., 2017). Infectious bursal disease and Newcastle disease viruses could undermine the immunity of chicks, especially in early days of their life, and could make them vulnerable to other infections (Ma et al., 2017). Taurine, been an immune booster is expected to boost the immunity of broiler birds against viral infections such as Newcastle disease and Infectious bursal disease viruses respectively.

Bone tissues are constantly been metamorphosed by osteoblasts and osteoclasts mainly for bone synthesis and bone resorption simultaneously. Constant bone formation by osteoblasts and bone resorption by osteoclasts helps in maintaining bone mass, and taurine has been proven to enhance bone strength and density of broiler birds (Choi, 2009).

The current research targeted monitoring the effect of graded level taurine supplemented diet on boiler birds' immunity against Newcastle disease and Infectious bursal disease viruses respectively, its effect on the weight of immune organs, as well as on on bone metabolism, density and mineral composition as a measure of bone quality in broiler birds.

2.0 Experimental

Source of Taurine

A 500 g well packed synthesized taurine supplement with pharmacy number 70403109AA, manufactured by Now food essential products, Schiedam Netherlands, was used for the research.

Experimental Design and Birds Management

A total of 300 unsexed day-old arbor acre broiler birds purchased from Zartech hatchery farm, Ibadan Oyo State were used for this research. The poultry unit was cleaned, disinfected, electrified and wood shaving were lightly spread in the cages to act as adsorbent of waste, mostly faeces. Facilities for an effective brooding such as thermometer and source of heat were adequately provided for, prior to the arrival of the birds. The birds were then group-brooded for a week, thereafter, they were randomly distributed using a completely randomized design into five dietary treatment of six replicates with 10 birds per replicate. Treatment one (T1) served as the control group with 0% taurine supplement. Treatments 2, 3, 4 and 5 contained dietary taurine supplements at 0.002, 0.004, 0.006, and 0.008% respectively. The birds were initially immunized against Newcastle disease virus (lasota vaccine) on the 8th day when maternal antibody has subsided (Ogunbode et al., 2013). The lasota vaccine was then repeated on the 28th day. The chicks were equally vaccinated against Infectious Bursal Disease (IBDV) virus on day 10 of age via drinking water and then repeated on the 18th day. The experiment ran for six weeks.

Ethical Approval

All rules and guidelines of ethics involving animal experimentation in research were followed according to laid down rules and regulations. Ethical approval was obtained from the ethical review of the University of Ilorin with the approval number; FERC/ASN/2020/101

Sample Collections for Analysis

Anti-Newcastle Disease Virus Haemagglutination Assay

A total of 24 birds were selected per treatment group (n=24) to screen for baseline antibody titre against Newcastle disease virus (NCDV). Non-heparinized blood samples were aseptically collected for haemagglutination assay on the 1st, 14th, 28th, and 42nd days through the jugular vein of the birds. The blood samples were initially kept at room temperature for 2 hours in test tubes, then centrifuged at 8000 revolution per minute for 10 minutes, and serum pippeted into a well-labelled eppendorf tube for sera storage. Serum prepared from sequential blood collection was heated, inactivated at 56°C for 30 minutes and stored at -20°C. The level of NCDV antibodies in the serum samples were determined by using haemagglutination inhibition (HI) test as described by Awad *et al*. (Awad et al., 2015). Antibody level for each serum sample was expressed as Log to the base of two (log_2) and recorded as the titre value. Published cut-off for selective serum protection level against NCDV (HI to log_2 antibody titres ≥ 3.0) as suggested and reported by Allan and Gough, 1974 was adopted for use (Echeonwu et al., 2008).

Serology and Infectious Bursal Disease Virus (IBDV)

A total of 24 birds were equally selected per treatment group (n=24) to screen for baseline antibody titre against IBDV. Non-heparinized blood samples were as well aseptically collected via the jugular vein for the determination of antibody titre against IBDV using Enzyme linked immunosorbent assay kit (ELISA) on the 1st, 14th, 28th, and 42nd day. Serum was separated by centrifugation (8000 revolution per minute for 10 minutes) and antibodies specific for IBDV was detected in the sera using an ELISA kit (ProFLOK^{R}, Synbiotics Corporation, CA, USA) according to the manufacturer's instructions. One hundred microliters of each sample were used for the assay. Absorbance was measured at 405nm using an ELISA reader (SUNRISE Absorbance Reader, TECAN. Option Touchscreen Color - Switzerland) by standard procedures, (Snyder et al., 1984) so as to monitor the effect of the experimental diets on the immune response of the birds.

Measurements of enzymes activities

Alkaline phosphatase (ALP) activities represents the rate of hydrolysis of 4-nitrophenylphosphate to 4-nitrophenol, described as follows:

4- Nitrolphenylphosphate [4-NPP] + H_20 ⟶ 4- Nitrolphenol + Phosphate ion

The enzyme assay was carried out by method described by Glogowski *et al*. (2002), but with minor modifications. Five (5) test tube containing 0.45 µl dilutions of 4, 3.2, 2.4, 1.6 and 0.8mM of 4-Nitrolphenylphosphate [4-NPP] solution was introduced into 5 corresponding test tubes containing 110 µl of 0.1 M carbonate buffer. The enzyme reaction was started by adding 10µl of the aliquot of the mixture (50 µl of the serum sample diluted with 50 µl of distilled water) into the various test tubes. At a regular interval of 5min for 30 min, 100 µl of 1 M NaOH was added to the assay mixture to terminate ALP activities. The mixture was transferred into a cuvette and absorbance was measured with a spectrophotometer (SPECTRONIC 20, Labtech- Digital Blood Analyzer (R) at maximum wavelength of 405nm. The concentration of 4-NP Produced in the various tubes were interpolated from standard curves (Njoku et al., 2011).

Serum bone specific alkaline phosphatase quantification (BALP) is analysed for using the method described by (Jila & Soleiman, 2012), Activities of Bone specific acid phosphatase (BALP) was determined by adding 200 µl of 11 mM phenylalanine to 50 µl of the diluted sample to inhibit the activities of other ALP isoforms, then incubated at 37 °C for 20 minutes. 50 µl of 1M NaOH was added and absorbance read at 410 nm.

Acid phosphatase (ACP) specific activity was analysed for as described by (Mudasir & Ashaq, 2016), by adding 50 µl of distilled water to 50 µl of the sample. Sodium acetate (0.1M, P^H = 4.5) was used as buffer. 10 µl of 0.1M magnesium sulphate (MgSO4) was then added and incubated at 37°C for 10 minutes, this was followed by addition of 25 µl 10 mM PNPP (Para-nitro phenyl phosphate), incubated at 37 °C for 30 minutes. Lastly was the addition of 100 µl of 1 M NaOH to each sample mixture to stop the assay reaction and the absorbance was read at 405nm.

Tartrate resistant acid phosphatase (TRAP) specific activity which represents the rate of conversion of p-NPP to p-nitrophenol by **s**odium tartrate in TRAP buffer was analysed for by adding about 200 µl of distilled water to 50 µl of the sample. 100 µl of TRAP buffer containing 2.5 mM p-nitrophenyl phosphate (p-NPP), 0.1 M sodium acetate, 0.2 M KC, 0.1% Triton X-100, 10 mM sodium tartrate, 1mM ascorbic acid and 100 µM $FeCl_3$ were then added. This was incubated at 37°C for 60 minutes. The p- nitrophenol liberated after incubation at 37°C for 1h was converted into p- nitrophenolate by addition of 50 µl of 1M NaOH to stop the assay. The absorbance was read at 405nm.The absorbance of each well was determined using a microplate spectrophotometer at 405nm. Enzyme activities and specific activities of the enzyme was calculated (Jae-won et al., 2009).

A total of eight birds per treatment were sacrificed by severing the jugular vein for bone quality determination. The birds were sacrificed by severing the jugular vein, the tibia and femur of the birds were carefully removed and manually de-fleshed. Thereafter, the fresh bones were weighed to obtain fresh weights of bones. The lengths of the tibia and femur bones were measured and bone density was calculated = $\frac{Bone\ weight}{Bone\ length}$ (Almeida and Bruno, 2006).

Bone digestion was analysed for according to the method of Siemowit *et al.* (2018). The tibia bone was ashed and mineralized in a muffle furnace at 750 °C and left for 24 hrs until a white ashed colour was gotten (Siemowit et al., 2018). About 2g of the ashed sample was weighed into 100 ml digestion flask and immediately 30 ml of Triple acid (i.e. nitric, sulphuric, perchloric acid at a ratio of 650:70:20 ml) were added. The flask and its contents were transferred into a hot digestion block for 1 hr. The digestion was completed when the white thick fumes of the perchloric acid cease to appear and the volume of the digest eventually reduced to 5 ml. The digest was allowed to cool for 30 mins by adding some amount of distilled water after which it was transferred into a 50 ml plastic bottle and then diluted to 50 ml. This serves as the digestion volume. Homogeneous mixture was subsequently obtained by shaking the bottle.

The concentrations of calcium and magnesium in the bone were determined using Atomic Absorption Spectrophotometer (AAS) method of analysis according to the method described by Skoog *et al*. (2006). An air-acetylene flame for Ca analysis, and a nitrous oxide-acetylene flame for Mg analysis were used. Different standard solutions were produced from pure stock solutions containing 1,000 ppm of the elements (Merck 1.09943 Titrisol, Merck 1.09949 Titrisol, and Merck 1.09953 Titrisol for Ca and Mg, Merck KGaA, Darmstad, Germany). In the Ca analysis, a strontium chloride solution (50 g/L; Merck 1.07865, Merck KGaA) was used as a releasing agent (Palma et al., 2015).

Actual concentration=

$$\frac{AAS\ Reading\ \times\ Digestion\ volume\ \times\ Dilution\ Factor}{weight\ of\ the\ sample.}$$

Concentration in part per million (ppm) or mg/kg was expressed in percentage by dividing by ten thousand to get the percentage elemental calcium and magnesium in the bone.

Total phosphorus content of the samples was determined by colorimetric method using vanadomolybdate method of phosphorus determination. Briefly described, Ammonium molybdate reacts under acid conditions to form heropoly acid, molybdate reacts under acid conditions to form molybdo phosphoric acid. In the presence of vanadium, yellow vanadomybdo phosphoric acid is formed. The intensity of the yellow colour is proportional to phosphate concentration.

Procedure: To about 0.5 ml of the pipetted sample, 4 ml of Vanadate-molybdate reagent (25g ammonium molybdate + 1.25g NH_3 metavanadate + 330 ml HNO_3 + 300 ml distilled water) was added as the colour developing reagent. The colour was allowed to develop for 30 min. The optical density of the sample was determined and the result was computed in part per million and expressed in percentage by dividing by ten thousand (Palma et al., 2015).

Phosphorus (ppm or mg/kg) = $\frac{D.F\ \times\ DV\ \times G\ \times R}{Wt}$

Elemental phosphorus (% P) = $\frac{Phosphorus\ (mg/kg)}{10{,}000}$

Dilution factor (D.F) = $\frac{Final\ sample\ developed}{Volume\ of\ aliquot\ used}$

Dilution volume (DV) = $Final\ sample\ developed\ +\ aliquot\ used$

DV = 30

Where G = Constant value (90.91) s

R = Reading (Absorbance) from colorimeter

Wt = Weight of the sample used.

Statistical analysis

Data for all analysis (alkaline phosphatase, bone specific alkaline phosphatase quantification, acid phosphatase, tartrate resistant acid phosphatase, bone quality determination and bone mineral composition) were presented as means ± standard error of the mean (SEM). Continuous variables were tested for normality and then analysed using one-way ANOVA procedure of SAS. Significant difference between treatment means were separated using Duncan's Multiple Range Test (SAS, 2000). $p < 0.05$ was considered as significant.

3.0 Results

Experimental feed

Table 1 shows the gross composition (kg/100g) of the experimental diets. The only ingredients (added over) that was varied was taurine, which was the main target of the research. The diet were adequately compounded to meet up with the basic energy and protein requirement, as well as the basic mineral compositions of the birds.

Calculated crude protein content of the diet ranging between 18.05 and 22.57% is within the range recommended by NRC 1994. The crude fibre (4.38 to 5.40%), ether extract (4.4 to 5.10%) and ash (3.74) were all also within the NRC recommended ranges. The analysed proximate composition in Table 2b commensurate the calculated nutrient composition in table 2a.

Immunonutrition

Heamagglutination inhibition titre (HI - Log_2) against NCDV (Table 3) showed that the geometric mean titre, or simply put, antibody titre taken as basal titre value on day 1 were uniform ($p>0.05$). On day 14, birds on 0.002% taurine-supplemented diet had a distinct ($p<0.05$) antibody titre value. The titre value for birds on the control diets and those on 0.006% taurine supplements were not totally different from the titre value of birds on 0.004% supplement against NCDV. Birds on 0.008% dietary supplement had the least antibody titre value against NCDV on day 14. The pattern of the performance for titre values on the 28th day followed suit exactly with what was obtained on the 14th day, with birds on 0.002% taurine having the highest antibody titre against NCDV. Also on the 42nd day, birds on the 0.002% supplementary diets had the highest titre value against NCDV. The titre value of birds on the control diet did not differ ($p>0.05$) from those of the birds on 0.004% supplementary diet. In the same vein, no noticeable differences (in the titre value of birds on 0.006% and 0.008% against NCDV. Overall, the antibody titre value increases as the birds ages.

Antibody titre against IBDV (Table 4) showed a progressive improvement in the titre values of the birds as they aged. The birds had similar basal titre value ($p>0.05$) at day old. At the 14th day, birds placed on 0.008% dietary taurine supplement had the best titre value ($p>0.05$) against IBDV. At the 28th day, birds placed on 0.006% dietary supplement had a distinct titre value ($p<0.05$) against IBDV. This followed suit with what was obtained on the 42nd day, birds on the 0.006% taurine-supplemented-diet also portrayed a distinct ($p<0.05$) titre value, followed by the titre value of the birds on 0.004% taurine-supplemented-diet. Throughout the experimental period, with the exception of the basal titre value, birds placed on 0% dietary supplement had the least titre value against IBDV.

The result of the liver and the immune organs to body weight ratio (Table 5) revealed that there were no differences ($p>0.05$) among the liver to body weight ratio of birds on diet 2, diet 3, and diet 4 (0.002%, 0.004%, and 0.006%) taurine-supplemented-diets**,** respectively when compared with those of the birds on diet 1 (0% taurine-supplemented-diets). Birds on diet 5 (0.008% taurine-supplemented-diets) had a significant ($p<0.05$) liver weight to body weight ratio when compared with those of the birds in diet 1 (0% taurine-supplemented-diets). The spleen to body weight ratio of birds on diet 3 and 4 (0.004 and 0.006) % taurine-supplemented-diets were not so much different, but noticeably different ($p<0.05$) from the spleen to body weight ratio of the birds on 0% taurine-supplemented-diets. Birds on diet 5 (0.008%) taurine-supplemented-diets had a significant spleen to body weight ratio when compared with those of the birds on the control diets (0% taurine-supplemented-diets). The bursal of fabricius weight to body weight ratio for birds 0.002 and 0.004% taurine-supplemented diets were slightly different from the weight of same organ for birds on 0% and 0.006% taurine-supplemented diets and glaringly ($p<0.05$) different from the weight of the organ for birds on 0.008% of experimental diet.

Dietary Taurine and effect on bone marker enzymes

Serum bone formation markers (Table 6) revealed an initial surge in the activities of the enzymes ($p<0.05$) in the serum of the birds on the different taurine-supplemented-diets when compared with that of the control within the first few weeks of life, then later decreased as the birds ages. Specifically, the serum alkaline phosphatase and bone specific alkaline phosphatase at the 42nd day were highest ($p<0.05$) for birds on diet 2, 3, and 4 (0.002, 0.004, and 0.006%) dietary taurine supplement and lowest ($p>0.005$) for birds on diet 1 and 5 (0 and 0.008%).

Serum bone resorption marker (Table 7) revealed a relatively stable serum acid phosphatase concentration, though at the end of the feeding trial, birds on 0.008% taurine-supplemented diet had the highest ($p<0.05$) activities of the enzyme, and the least concentration was found in birds on the control (0%) diet. The concentration of the serum tartrate resistant acid phosphatase increased relatively as the birds ages, at the end of the feeding trial, the activity of the enzyme was notably highest ($p<0.005$) for birds on 0.008% taurine and least also for birds on the control diet.

The bone density (tibia and femur bone morphometry) of the birds is as shown in (Table 8). The tibia bone weight of the birds on 0.002% and 0.008% dietary supplement were the densest ($p<0.05$). This is followed by the tibia bone weight of birds placed on 0.004% and 0.006% dietary supplement. Birds on the control diet (0% taurine-supplemented diet) had the least tibia bone weight. There were no striking differences ($p>0.05$) amongst the length of the tibia bone with respect to the dietary taurine treatment inclusion in birds' feeds, but numerically, birds on 0.006% dietary-taurine-supplement had the longest tibia bone length. On the other hand, birds on 0.002% and 0.004% taurine supplement had the densest ($p<0.05$) femur bone weight. This is followed by the femur bone weight of birds placed on 0.006% and 0.008% dietary-taurine-supplement. In the same vein, birds on the control diet had the least femur bone weight. The femur bone length also showed no marked difference ($p>0.05$), but numerically, birds placed on 0.006% taurine-supplemented-diet also had the longest femur bone length. The bone density is a ratio of bone weight to length, hence followed the same pattern just as tibia and femur bone morphometry report.

Bone mineral contents (Table 9) showed that birds fed with 0.002% taurine-supplemented-diet had the highest percentage ($p<0.05$) of phosphorous, calcium and phosphate composition, birds on 0%, 0.004% and 0.006% dietary supplements had the highest magnesium composition ($p<0.05$). Birds on 0.008% dietary supplement also contained a higher percentage ($p<0.05$) of calcium composition. Birds on the control diet had the least amount of phosphorus and phosphate composition. The calcium composition of birds on 0.006% taurine is similar ($p>0.05$) to that of the birds on the control diet.

Table 1: Gross composition (kg/100g) of experimental diets

	Experimental phase		
Ingredient (kg)	Pre-starter	Starter	Finisher
Taurine supplement (%)		0.002 - 0.008	
Maize	44.60	51.38	56.00
Oil	0.30	0.40	2.00
Soybeans meal	32.30	21.29	18.00
Water	10.80	11.00	9.00
Groundnut cake	9.00	13.00	2.00
Lime stone	1.90	1.55	11.22
Dicalcium phosphate	0.33	0.41	2.00
Common salt	0.38	0.35	0.4
Methionine	0.14	0.17	0.37
Lysine	0.00	0.20	0.62
Premix	0.25	0.25	0.14
Total	100	100	100

Table 2: Calculated Nutrient Composition (%) and Proximate Analysis (g/100 g DM) of Experimental diets.

Ingredients	Pre-starter	Starter	Finisher
Crude protein (C)	22.57	20.12	18.05
Crude Protein (P)	22.97	20.34	18.38
Crude fibre (C)	5.40	4.85	4.38
Crude fibre (P)	5.33	4.93	4.52
Ether extract (C)	5.10	4.84	4.40
Ether extract (P)	4.99	4.72	4.21
Ash (C)	3.74	3.74	3.74
Ash (P)	3.82	3.72	3.93

C = calculated and P = proximate

Table 3: Heamagglutination inhibition titre (HI - Log2) against Newcastle Disease Virus (NCDV) of broiler birds fed graded level taurine-supplemented-diets.

DIETS	DAY 1		DAY 14		DAY 28		DAY 42	
	Geometric mean titre	Antibody titre	Geometric mean titre	Antibody titre	Geometric mean titre	Antibody titre	Geometric mean titre	Antibody titre
DIET 1	1.87[a]	2^1	8.00[ab]	2^3	8.42[ab]	2^3	64.50[b]	2^6
DIET 2	1.75[a]	2^1	8.38[a]	2^3	16.13[a]	2^4	128.38[a]	2^7
DIET 3	1.63[a]	2^1	4.37[b]	2^2	8.25[b]	2^3	64.75[b]	2^6
DIET 4	1.75[a]	2^1	8.13[ab]	2^3	8.88[ab]	2^3	32.75[c]	2^5
DIET 5	1.75[a]	2^1	4.00[b]	2^2	8.00[b]	2^3	32.75[c]	2^5
SEM	0.12		0.68		2.12		4.67	

Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively. SEM: Standard Error of Mean. [abc] are means within same column with different superscripts that are significantly different ($p < 0.05$).

Table 4: Antibody Titre against Infectious Bursal Disease Virus (IBDV) of broiler birds fed graded level taurine-supplemented-diets.

DIETS	1st Day	14th Day	28th day	42nd day
DIET 1	10.11±1.430[a]	16.88 ± 2.295[e]	85.00 ± 5.720[e]	143.00.± 6.380[e]
DIET 2	10.25±1.330[a]	49.00 ± 8.952[d]	134.75 ± 12.719[d]	210.00±7.834[d]
DIET 3	9.99±1.440[a]	134.38 ± 6.657[c]	508.75 ± 26.090[b]	885.00±9.342[b]
DIET 4	10.31±1.330[a]	209.62 ± 22.071[b]	669.12 ± 71.620[a]	1029.00±5.432[a]
DIET 5	10.02±1.340[a]	283.62 ± 10.680[a]	372.00 ± 2.847[c]	532.00±4.796[c]

Mean ± SEM (Standard Error of Mean). Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively.
[abcde] are means within same column with different superscripts that are significantly different (p < 0.05).

Table 5: Liver and immune organs to body weight ratio of broiler birds fed graded level taurine-supplemented-diets.

Parameter(g)	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	SEM
Liver	1.86[b]	1.80[b]	1.90[b]	1.94[b]	2.13[a]	0.07
Spleen	0.09[c]	0.11[b]	0.12[ab]	0.12[ab]	0.14[a]	0.04
Bursa of fabricius	0.07[ab]	0.04[b]	0.03[b]	0.06[ab]	0.19[a]	0.04

Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively. SEM: Standard Error of Mean.
[abc] are means within same column with different superscripts that are significantly different (p < 0.05).

Table 6: Serum bone formation marker of broiler birds fed graded level taurine- supplemented-diets.

	Alkaline phosphatase (mM/min/mg-protein)				Bone specific alkaline phosphatase (mM/min/mg-protein)			
DIETS	**1ST DAY**	**14TH DAY**	**28TH DAY**	**42ND DAY**	**1ST DAY**	**14TH DAY**	**28TH DAY**	**42ND DAY**
DIET 1	3.25[d]	171.83[a]	70.35[c]	35.19[d]	3.92[d]	181.75[b]	130.80[c]	119.50[c]
DIET 2	5.45[b]	140.50[c]	103.39[b]	102.79[b]	6.06[b]	167.26[c]	162.61[b]	150.66[a]
DIET 3	4.98[bc]	161.56[ab]	111.36[a]	132.74[a]	5.21[c]	177.21[bc]	159.76[b]	133. 27[b]
DIET 4	3.75[c]	150.00[b]	106.62[ab]	116.90[ab]	7.30[ab]	200.85[a]	162.04[b]	130.74[b]
DIET 5	7.96[a]	112.41[d]	66.11[c]	52.40[c]	9.31[a]	199.04[ab]	204.18[a]	121.37[c]
SEM	0.50	0.54	4.76	6.17	0.40	3.84	3.74	2.66

Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively. SEM: Standard Error of Mean.
[abcd] are means within same column with different superscripts that are significantly different (p < 0.05).

Table 7: Serum bone resorption marker of broiler birds fed graded level taurine supplemented diets.

	Acid phosphatase (mM /min /mg protein)				Tartrate resistant acid phosphatase (mM /min /mg-protein)			
DIETS	**1ST DAY**	**14TH DAY**	**28TH DAY**	**42ND DAY**	**1ST DAY**	**14TH DAY**	**28TH DAY**	**42ND DAY**
DIET 1	9.05[a]	8.96[a]	3.83[d]	5.04[d]	10.38[ab]	21.67[c]	17.39[bc]	28.61[c]
DIET 2	7.32[b]	8.88[a]	5.37[c]	6.11[c]	11.06[a]	13.33[d]	19.94[b]	31.65[bc]
DIET 3	7.03[b]	6.97[b]	5.81[bc]	5.03[d]	10.07[ab]	26.99[b]	15.97[c]	34.04[b]
DIET 4	5.30[c]	5.98[b]	7.23[b]	9.21[a]	8.83[b]	23.71[bc]	16.35[c]	35.78[ab]
DIET 5	2.72[d]	5.69[b]	7.68[a]	9.08[b]	7.30[c]	30.80[a]	20.50[a]	44.94[a]
SEM	0.50	0.49	0.49	0.68	0.60	1.53	1.00	2.26

Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively. SEM: Standard Error of Mean.
[abcd] are means within same column with different superscripts that are significantly different (p < 0.05).

Table 8: Bone density of broiler birds fed graded level taurine-supplemented-diets

	Tibia Bone Morphometry			Femur-Bone Morphometry		
DIETS	**Weight (g)**	**Length(cm)**	**Density (g/cm)**	**Weight (g)**	**Length(cm)**	**Density (g/cm)**
DIET 1	11.62[b]	7.04[a]	1.64[b]	13.25[c]	6.90[a]	1.93[c]
DIET 2	14.25[a]	7.03[a]	1.79[a]	17.38[a]	6.92[a]	2.50[a]
DIET 3	13.13[ab]	7.25[a]	1.84[a]	17.00[a]	7.25[a]	2.36[a]
DIET 4	12.63[ab]	7.44[a]	1.70[ab]	15.00[ab]	7.43[a]	2.04[ab]
DIET 5	14.00[a]	6.93[a]	2.04[a]	13.88[b]	6.98[a]	1.98[b]
SEM	1.34	0.25	0.18	1.40	0.20	0.17

Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively. SEM: Standard Error of Mean.
[abc] are means within same column with different superscripts that are significantly different (p < 0.05).

Table 9: Bone-chemical-composition of broiler birds fed graded level taurine-supplemented-diets

Parameters (%)	DIET 1	DIETS 2	DIETS 3	DIETS 4	DIETS 5	SEM
Phosphorus	7.990^{c}	9.500^{a}	9.000^{ab}	8.400^{bc}	8.800^{b}	0.200
Calcium	27.630^{b}	32.180^{a}	23.930^{c}	28.140^{b}	35.400^{a}	1.340
Magnesium	0.027^{a}	0.016^{b}	0.029^{a}	0.026^{a}	0.024^{ab}	0.003
Phosphate	18.310^{c}	21.770^{a}	20.630^{ab}	19.250^{bc}	20.150^{b}	0.460

Diet 1 - 0%, Diet 2 - 0.002%, Diet 3 – 0.004%, Diet 4 – 0.006%, Diet 5 – 0.008% taurine-supplemented-diets respectively. SEM: Standard Error of Mean. ^{abc} are means within same row with different superscripts that are significantly different ($p < 0.05$).

4.0 Discussion

Infectious bursal disease and Newcastle disease viruses (IBDV AND NCDV) are broiler birds' viral infections that are capable of grossly affecting birds' health, production and income of poultry farmers if not well handled. The efficacy of taurine as an immunological agent against common diseases in broiler birds were experimented against the two diseases, and were found to be effective as it boost**s** and generally improve the antibody titre against the diseases as the birds ages. Dietary betaine (Bet) supplementation of heat stressed broiler birds' diet have been proven by Rao *et al.* (Rao et al., 2011) to increase primary antibody titre of birds against IBDV and NCDV. Kettunen *et al*. (Kettunen et al., 2001) and Klasing *et al*. (Klasing et al., 1991) reported that dietary Bet supplementation (1g/kg) enhanced broiler birds' humoral immunity. Hassan *et al*. (Hassan et al., 2011) also concluded that dietary supplementation of broiler birds with Bet enhanced the antibody titers against sheep red blood cell in heat stressed New Zealand White rabbits. Tsiagbe *et al*. (Tsiagbe et al., 1987) on the other hand noticed that supplementation of broiler birds' feed with Bet (0.121% of the diet) did not have any noticeable effect on the humoral immunity of broilers at six weeks posthatch.

The bursa of Fabricius is an important lymphoid organ, its' presence is germane to the development and maturation of B-lymphocytes (Li et al., 2005; Wang, 2009). Schuller-Levis *et al*. (Schuller-Levis et al., 1990) and Wang *et al.* (2014) both reported that taurine supplementation at various levels usually enhance the weight of immune organs like spleen, thymus and bursal of fabricius. The favourable effect of taurine on the development and growth of the immune organs especially at 0.008% supplementation level can be a good signal to its' ability at enhancing the overall immune functions.

Serum bone specific alkaline phosphatase is very specific for bone formation. The enzyme is secreted by osteoblasts for biological deposition of or conversion into calcium carbonate or some other insoluble calcium compounds (Osyczka and Leboy, 2005). Structural composition of bone mostly is a function of the genetic make-up of the birds in term of strain or could equally be linked to the quality of feed. Modern broiler breeds targeting maximal meat production do have various skeletal deformities like abnormal cartilage development in the proximal end of the tibia, excessive inward and outward angulation of the distal segment of a bone or joint and other related crisis, mostly because they reach table size as early as five to eight weeks (Ammann et al., 2000). Research has shown that amino acids generally are capable of improving the structure and quality of bone formation (Ammann et al., 2000). Notable amount of taurine is moved into bone tissues, and has been proven to aid bone anabolic effect by halting bone resorption. It equally plays a stimulatory role on the activity of alkaline phosphatase and the formation of collagen (Koide et al., 1999; Park et al., 2001; Yasutomi et al., 2002). Alkaline phosphatase is a wide spread quaternary enzyme attached to glycosyl-phosphatidylinositol moieties found situated at the external surface of a cell membrane (Park et al., 2001). More activities of the enzymes in the serum in this case could indicate poor bone formation, while lesser amount in the serum could indicate better bone formation. Lesser amount of the serum enzymes activities in birds fed with 0.008% taurine-based diet is an indication of the ability of taurine at this concentration in preventing against bone loss, hence halting bone resorption and encouraging bone osteoblasts metabolism through stimulation of extracellular signal regulated protein kinase phosphorylation (Park et al., 2001; Ahmed and Hamza, 2009).

Serum tartrate resistant-acid phosphatase is closely related to bone resorption. It is the only enzyme secreted by osteoclasts (Nordin, 1978; Kleerekoper et al., 1994; Kirstein et al., 2006). Significant increased amount of serum bone formation and bone resorption markers of birds at 0.006% supplementation level is an indication of effective reformation and dissolution of bone which requires asynchronous action of bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts) occurring together simultaneously during the process of bone metabolism (remodelling) so as to maintain skeletal homeostasis by acting as reservoir for minerals and guiding against excessive bone loss (Hazarina,. 2017).

Taurine has been proven to impact bone mineral density and bone mineral content (Lubec et al., 1997; Koide et al., 1999). The marked improvement in the tibia cum femur weight and length (bone density) of birds on the experimental diets (0.002 to 0.008%) negate the findings of Choi and Seo, 2013 which reported that taurine had no effect on the femur bone mineral density in experimental male rats. The authors emphasised that the femur bone mineral density per weight (FBMD/wt) were not significantly different between experimental groups, control group inclusive.

Enhanced bioavailability of trace elements/minerals usually assist the morphology of bone cartilages (Bao et al., 2009; Sharideh et al., 2015). Phosphorus is very important for a number of cellular activities used to maintain homeostasis in animals such as the formation of nucleic acids, bioactive signaling proteins, phosphorylating enzymes, and a naturally occurring mineral form of calcium apatite (hydroxyapatite)

(Berndt et al., 2005). Calcium is used in many vital cellular functions as well including mediating nerve transmission, muscle function, intracellular signaling, secretion of vital chemical substances, and widening of blood vessel. Both minerals are usually monitored through their concentrations in the blood and the surrounding tissue. Free calcium in the blood is majorly used for bone formation in growing broilers (Tucker et al., 2007). The marked improvement in the bone mineral composition of birds on the various concentration of taurine supplemented diet is in accordance with the findings of Choi and Seo, 2013; Choi and DiMarco 2009 which reported a higher femur bone mineral contents per weight (FBMC/wt) of the rats fed taurine-supplemented diet than in those rats fed with the control diet. The improvement in bone mineral content has been proven to reduce osteoporosis, atherosclerosis and inflammation in experimental animal models (Belury, 2002). This unique feature has given taurine an edge at addressing and managing various chronic diseases (Hayes and Stuiman, 1981).

Conclusion

Inclusion of taurine as dietary supplement within the range of 0.002 to 0.008% has proven useful not only in enhancing the birds' immunity against NCDV and IBDV, but also in maintaining bone strength of meat type poultry birds that are always encountered with bone malformation as result of the meat to bone ratio proportion. Taurine has also been found useful in maintaining a good phosphorous to calcium ratio in broiler birds, another strong determinant of bone strength.

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Conflict of interest

Authors declared no conflict of interest.

Authors' Contributions
Conception: (OSM, SAK)
Design: (OSM, BRA, SAK)
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