EFFECTS OF SUPPLEMENTAL THREONINE ON THE PHYSIOLOGICAL RESPONSE, BONE MORPHOMETRY AND BONE MINERALS OF BROILER CHICKENS

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ABSTRACT
This study was carried out to evaluate the effect of supplemental threonine on the performance, physiological response, bone morphometry and bone minerals of Arbor Acres broiler chickens. One hundred and sixty (160) unsexed birds were randomly divided into four treatments. Each treatment had 4 replicates with each replicate having 10 birds. Thr1 (0 mg/kg), Thr2 (250 mg/kg), Thr3 (500 mg/kg) and Thr4 (750 mg/kg). Growth performance, physiological and bone health parameters were collected using standard protocols. Thr3 resulted in higher (P<0.05) final weight (2397.56g) compared to Thr1 (2322.08g), Thr2 (2327.04g) and Thr4 (2302.93g). Rectal temperature in Thr1 (42.76 0C) was significantly elevated (P<0.05) compared to Thr2 (42.58 0C), Thr3 (42.55 0C) and Thr4 (42.58 0C). Elevated respiratory rate (101.94 breaths/minute) and heart rate (216.92 beats/minute) were observed in Thr4. All bone minerals were significantly elevated (P<0.05) in Thr3. Density of the right femur in Thr2 (1.05) was higher (p<0.05) than that of Thr1 (0.09). Seedor index was also higher (p<0.05) in Thr1 (0.21) compared to Thr2 (0.18), Thr3 (0.17) and Thr4 (0.19). Thickness of left posterior epiphysis was significantly reduced (p<0.05) in Thr2 (11.10mm) compared to Thr3 (14.62mm) and Thr4 (15.04mm). The right anterior epiphysis was also influenced (p<0.05), Thr3 (13.00mm) and Thr4 (12.82mm) were thicker than Thr1 (8.46mm). The right posterior epiphysis had it width thicker (p<0.05) in Thr3 (16.07mm) compared to Thr1 (12.24mm). The study concluded that supplementing threonine at 500mg in diet of broilers improved the physiological status of the birds which in turn enhanced growth, thermo-response and bone health.
Keywords: Physiology, growth, temperature, bone health, bone minerals

INTRODUCTION
Performance of broilers depend on numerous factors but the obtainability of adequate amino acids that can be used for protein synthesis is important for normal physiological functioning (Waleed, 2017). Amino acids are necessary for optimum growth and development including bone and mineral deposition. Barkley and Wallis (2001) observed that increasing threonine concentration improved growth of broilers. Broilers grow very fast and require essential amino acids for optimal growth without compromising normal physiological functioning (Chen *et al*., 2016). A lot of attention is paid to methionine and lysine forgetting the aliphatic third limiting amino acid: threonine. Threonine is an important growth factor in broiler production as such formulating broiler diets to be adequate in threonine is critical, because threonine deficiency may decrease lysine utilisation. Lysine and threonine have been shown to interact in such a way that, at optimum levels, broiler weight gain and breast fillet yields are increased (Canogullari *et al*., 2009). Additionally, threonine plays a major role in intestinal development and function (Stoll *et al*., 2006). Threonine plays an important role in feather synthesis not only as a component of the feather protein but also as a precursor of glycine and serine. This implies an interaction between feather growth potential which plays an important role in thermoregulation of birds (Lemme, 2009).
Threonine is also involved in the production of immunoglobulins; the composition of mucin which plays a vital role in protecting the intestine from acidic chyme, pathogens and digestive enzymes as well as maintaining the intestinal integrity (Horn *et al*., 2009) and allows nutrients passing through the gut (Qaisrani *et al*., 2018). Diet lacking in threonine as well as other amino acids have been reported to result in diminished physiological function and poor growth (Kidd *et al*., 1999). Chickens cannot synthesise several amino acids, including threonine, hence this study evaluated the effects of supplemental threonine on performance, physiological responses, bone morphometry and bone minerals of Arbor Acres broiler chickens.

MATERIALS AND METHODS

This study was conducted at the Poultry Unit of the Institute of Agricultural Research and Training, Ibadan. The study involved 160 unsexed Arbor Acres broiler chickens. The birds were randomly divided into four treatment groups with each treatment having 10 birds per replicates. The chicks were fed *ad libitum* with a formulated starter and finisher diet. The diet contained 3,027 and 3,195.3 kcal/kg metabolic energy and 23 and 20% crude protein respectively. Threonine was added to the diet at 0, 250, 500 and 750 mg/kg and the study lasted 56 days.

Performance characteristics such as initial body weight, final body weight were determined by weighing the birds weekly. Weight gain was determined by deducting the initial weight from the final weight while average feed intake was determined weekly by deducting remnant from feed supplied. The feed conversion ratio (FCR) was calculated by dividing the feed intake by the weight gain.

Determination of Physiological parameters

Physiological parameters determined twice weekly between 1:00pm and 2:00pm include rectal temperature (RT) determined using a digital thermometer which was inserted into the cloaca of the birds, respiratory rate (RR) measured by observing and counting the number of breaths taken/minute while the heart rate was determined using a stethoscope placed on the flank to listen and count the no of heart beats/minute.

Determination of bone parameters

At the end of the experimental period, 8 birds were randomly picked from each treatment and slaughtered. The right and left thighs of the chickens were excised during carcass evaluation and immersed in petroleum ether for 15 minutes to loosen adhering tissues. Thereafter the femur bones were separated from the thighs. Bone samples were air-dried for 24 hours at room temperature.

Determination of bone morphometric parameters

Morphometric parameters: weight, length and epiphyseal diameter (width) of femur samples were evaluated. Bone weight was measured using a digital weighing balance. Femur length and width (thickness) were measured using a digital pair of vernier callipers and a digital micrometer according to the method described by Samejima (1990). Femur length was measured from the proximal end of the shaft to the lateral condyle and the width at the medial diaphysis. Geometric parameters (Seedor index and robusticity index) of the femur were s calculated using the following formulae :

Seedor index = bone weight/ bone length (Seedor *et al*., 1991)

Robusticity index = bone length/cube of bone weight (Reisenfeld, 1972)

Bone density assessment

Density of the femurs were evaluated according to the principle of Archimedes. Bone sample was placed in a graduated measuring cylinder (100 mL) filled to 70 mL mark with deionised water. Volume of deionised water displaced by bone samples were recorded. Density of each bone sample was calculated using the formula:

BD = BW/ (FV-IV)

Where,BD = bone density (g/mL); IV = volume of deionised water (70 mL); BW = bone weight (g); FV: Final volume obtained due to displacement of water by bone sample.

Bone ash and mineral determination

Femur samples were oven-dried at 105 °C for 24 hours cooled in a dessicator and weighed. Thereafter, bone samples were ashed in a muffle furnace at 600°C for 24 hours in crucibles according to method recommended by AOAC (2000). Ash content of the femur samples were expressed as percentage of dry bone weight. For mineral content analysis, ash samples were subjected to digestion using a mixture of nitric acid (65% HNO_3) and perchloric acid ($HClO_4$) at 2:1 v/v (Oyegunle *et al*., 2010). Following digestion, samples were allowed to cool at room temperature. Contents of calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), iron (Fe), potassium (K) and phosphorus (P) in the digested samples were determined as described by Oyegunle *et al*. (2016) using atomic absorption spectrophotometry.

All data obtained were statistically analysed by the General Linear Model procedures of SAS (2002) and means, where significant, were separated using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

According to Table 1, addition of threonine at Thr1 (500mg/kg) resulted in increase ($P<0.05$) in final weight (2397.56g) compared to Thr1 (2322.08g), Thr2 (2327.04g) and Thr4 (2302.93g).

Table 1: Performance of broiler chickens fed varying levels of supplemental threonine

Parameters	Thr1 (0mg/kg)	Thr2 (250 mg/kg)	Thr3 (500 mg/kg)	Thr4 (750 mg/kg)	SEM	P-value
Initial weight (g)	39.35	39.16	38.90	38.60	0.184	NS
Final weight (g)	2322.08^{b}	2327.04^{b}	2397.56^{a}	2302.93^{b}	13.064	0.05
Weight gain (g)	2282.73	2288.44	2358.38	2264.03	27.263	NS
Feed Intake (g)	4931.90	4852.00	4798.80	4783.40	52.046	NS
Feed conversion ratio	2.16	2.13	2.04	2.12	0.024	NS

Means with different superscripts (±SEM) in the same row are significantly different (p<0.05).

This result corresponds with the observation of Waleed (2017) which underscores the importance of threonine in growth and physiological development of chickens. Threonine however did not influence weight gain, feed intake and feed conversion ratio. The trend observed in the final weight in this study could be attributed to better nutrient uptake as a result of nutritional enrichment which most likely led to better intestinal conditions as outlined by Horn *et al*. (2009) and Qaisrani *et al*. (2018). According to Rezaeipour *et al*. (2015), increasing the amount of dietary threonine improved morphological traits of the small intestine in broiler chickens and these traits resulted in increased growth rate.

Table 2: Effect of supplemental threonine on physiological parameters of broiler chickens

Parameters	Respiratory Rate (breaths/minute)	Rectal Temperature (^{0}C)	Heart Rate (Beats/minute)
Thr1 (0 mg/kg)	99.21^{ab}	42.76^{a}	214.29^{a}
Thr2 (250 mg/kg)	93.04^{b}	42.58^{b}	206.69^{b}
Thr3 (500 mg/kg)	95.27^{ab}	42.55^{b}	211.58^{ab}
Thr4 (750 mg/kg)	101.94^{a}	42.58^{b}	216.92^{a}
±SEM	3.670	0.043	2.430
P-value	0.01	0.05	0.05

Means with different superscripts (±SEM) in the same column are significantly different (p<0.05).

According to Table 2, threonine supplementation resulted in higher (P<0.05) respiratory rate in birds on Thr4 (101.94 breaths/minute) compared to birds on Thr2 (93.04 breaths/minute). The rectal temperature was highest (P<0.05) in birds on Thr1 (42.76 ^{0}C) while birds on Thr2 (42.58 ^{0}C), Thr3 (42.55 ^{0}C) and Thr4 (42.58 ^{0}C) were lower. Elevated (P<0.05) heart rate was observed in Thr1 (214.29 beats/minute) and Thr4 (216.92 beats/minute) compared to Thr2 (206.69 beats/minute). The high respiratory and heart rate observed in birds on Thr4 suggests that inclusion of threonine above 500mg could have a detrimental effect on physiological response of broiler chickens. This differs from observations of Waleed (2017) who recommended inclusion of 900mg of threonine for optimisation of growth. Rectal temperature, an indicator of thermal stress or ill health was highest in the control group (Thr1). This indicates that threonine had a positive effect on the physiology of the birds which corroborates the report of Lemme (2009) that threonine plays an important role in poultry thermoregulation.

Table 3: Effect of supplemental threonine on bone minerals of broiler chickens

Parameters	Thr1 (0mg/kg)	Thr2(250 mg/kg)	Thr3(500 mg/kg)	Thr4(750 mg/kg)	±SEM	P-Value
Sodium (%)	0.87^{c}	0.89^{b}	0.94^{a}	0.90^{b}	0.002	0.05
Potassium (%)	1.23^{d}	1.24^{c}	1.47^{a}	1.32^{b}	0.024	0.05
Calcium (%)	18.75^{d}	18.96^{c}	19.61^{a}	19.14^{b}	0.268	0.05
Magnesium (%)	0.33^{c}	0.38b^{c}	0.54^{a}	0.42^{b}	0.017	0.05
Iron (mg/kg)	8.67^{d}	8.81^{c}	9.64^{a}	9.21^{b}	0.381	0.05
Zinc (mg/kg)	14.30^{d}	14.73^{c}	16.24^{a}	16.00^{b}	1.792	0.05
Phosphorus (%)	10.17^{d}	10.42^{c}	11.26^{a}	10.78^{b}	0.445	0.05

Means with different superscripts (±SEM) in the same row are significantly different (p<0.05).
Results in Table 3 showed that supplementation influenced (P<0.05) bone minerals. All bone minerals were elevated (P<0.05) in Thr3. This result suggests that including threonine in broiler diet was indeed necessary for adequate bone formation (Chen *et al*., 2016). The drop observed in minerals levels at Thr4 (750mg) further affirms the detrimental effect of threonine when 500mg/kg is exceeded which opposes the submission of the 900mg recommendation of Waleed (2017).

Table 4: Effect of supplemental Threonine on bone morphometric parameters (Femur)

Parameters	LFW (g)	RFW (g)	LFV (ml)	RFV (ml)	LFD	RFD	LFL (mm)	RFL (mm)	SI	RI
Thr1 (0mg/kg)	18.50	18.00	20.00	20.00	0.93	0.90^{b}	102.09	101.35	0.21^{a}	0.02
Thr2 (250mg/kg)	18.50	20.50	20.00	19.50	0.93	1.05^{a}	102.84	104.65	0.18^{b}	0.01
Thr3 (500mg/kg)	23.50	22.50	24.00	23.00	1.00	0.98^{ab}	111.56	110.52	0.17^{b}	0.01
Thr4 (750mg/kg)	19.00	18.50	18.50	18.50	1.02	1.00^{ab}	109.58	109.65	0.19^{b}	0.02
±SEM	1.837	2.622	3.553	2.872	0.080	0.0377	4.447	3.386	0.002	0.001
P-value	NS	NS	NS	NS	NS	0.05	NS	NS	0.05	NS

Means with different superscripts (±SEM) in the same column are significantly different (p<0.05).
LFW - Weight of left femur, RFW - Weight of right femur, LFV - Volume of left femur, RFV - Volume of right femur, LFD - Density of left femur, RFD - Density of right femur LFL- Length of left femur, RFL - Length of right femur, SI - Seedor index, RI - Robusticity index.

According to Table 4, density of right femur in Thr2 (1.05) was higher (p<0.05) than those of birds in Thr1 (0.90) which affirms the influence of supplemental threonine on bone indices (Chen *et al*., 2016). The Seedor index is a bone density indicator. Low values signify greater tibial bone density (Abdelaziz *et al*., 2018). Seedor index was higher (p<0.05) in birds on Thr1 (0.21) than those on Thr2 (0.18), Thr3 (0.17) and Thr4 (0.19). This suggests better bone fill in threonine-supplemented groups which quite differs from the reports of Abdelaziz *et al*. (2018).

Table 5: Effect of supplemental threonine on bone thickness parameters of broiler chickens

Parameters	LAE (mm)	Shaft (left) (mm)	LPE (mm)	RAE (mm)	Shaft (right) (mm)	RPE (mm)	LFC (mm)	RFC (mm)
Thr1 (0 mg/kg)	9.45	6.28	13.07^{ab}	8.46^{b}	6.60	12.24^{c}	30.50	30.00
Thr2 (250 mg/kg)	10.04	7.21	11.10^{b}	10.37^{ab}	6.71	12.62^{cb}	30.00	31.75
Thr3 (500 mg/kg)	13.72	9.37	14.62^{a}	13.00^{a}	8.32	16.07^{a}	32.50	34.50
Thr4 (750 mg/kg)	11.30	8.34	15.04^{a}	12.82^{a}	7.25	14.24^{b}	26.00	26.50
±SEM	3.416	1.893	1.529	2.206	0.507	0.42	13.25	13.781
P-Value	NS	NS	0.05	0.05	NS	0.05	NS	NS

Means with different superscripts (±SEM) in the same column are significantly different (p<0.05)
LAE - Anterior epiphysis (Left), LPE - Posterior epiphysis (Left), RAE - Anterior epiphysis (right), RPE - Posterior epiphysis (right), LFC - Femur circumference (Left), RFC - Femur circumference (right)

According to Table 5, thickness of left posterior epiphysis (LPE) was lower (p<0.05) on Thr2 (11.10mm) than Thr3 (14.62mm) and Thr4 (15.04mm). Also, right anterior epiphysis (RAE) differed (p<0.05), birds in Thr3 (13.00mm) and Thr4 (12.82mm) were thicker than Thr1 (8.46mm). The right posterior epiphysis (RPE) was thicker (p<0.05) in birds on Thr3 (16.07mm) than those on Thr1 (12.24mm). The result suggests that supplemental threonine had beneficial effect on bone thickness. This result validates reports of Kidd (1999) that diet lacking in threonine as well as other amino acids can lead to diminished physiological functions and poor growth.

CONCLUSION

It was concluded that supplemental threonine at 500mg in diet of broiler chicken improved the physiological status of the birds which in turn enhanced growth, thermo-response and bone health.

RECOMMENDATION

An inclusion of 500mg/kg of threonine is recommended for optimal growth, physiological response and bone health of broiler chicken.

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