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EFFECT OF DIETARY TRYPTOPHAN LEVELS ON GROWTH PERFORMANCE OF BROILER CHICKENS REARED IN THE HOT SEASON UNDER TROPICAL ENVIRONMENT[1]

[EFECTO DE LOS NIVELES DE TRIPTOFANO DIETÉTICO SOBRE EL CRECIMIENTO DE POLLOS DE ENGORDE CRIADOS EN TEMPORADA CÁLIDA EN AMBIENTE TROPICAL]

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SUMMARY

This study was conducted to evaluate the effect of dietary levels of tryptophan on growth performance of broiler chickens reared under tropical environment. At the starter phase, a total of two hundred and eighty five day old mixed sex Arbor Acres broiler chicks were randomly allotted to five dietary treatments with three replicates each having nineteen (19) chicks. At the finisher phase, two hundred and seventy broilers were also allotted to five dietary treatments with three replicates each having 18 broilers per replicate. The dietary tryptophan levels at the starter phase were 0.15, 0.19, 0.23, 0.27 and 0.31% respectively while the diets for the finisher phase contained 0.13, 0.17, 0.21, 0.25 and 0.29% dietary tryptophan respectively. All other nutrient levels were constant. The experiment was conducted at 0 to 28d (starter phase) and 33 to 56d (finisher phase). Growth performance traits including weight gain, feed intake and feed conversion ratio were recorded at the end of each week. The results for the starter phase showed that chicks fed diet containing 0.23%, 0.27% and 0.31% dietary tryptophan had similar results in term of the weight gain, average daily weight gain, feed intake and average daily feed intake. For the finisher phase, the birds fed 0.21%, 0.25% and 0.29% tryptophan diets also had similar results in terms of final weight, weight gain, feed intake and average daily feed intake. Our results suggest that supplemental tryptophan was sufficient to have significant (P<0.05) effect on broiler performance. However, polynomial regression analysis reveals that the optimum performances were reached at 0.24% and 0.21% dietary tryptophan for the starter and finisher phases respectively. Therefore, it can be concluded that dietary tryptophan requirements during the hot season for the starter and finisher phases were 0.24% and 0.21%, respectively.

Key words: amino acid; bio-efficacy; broiler; season.

RESUMEN

Este estudio se realizó para evaluar el efecto de los niveles dietéticos de triptófano en el crecimiento de pollos de engorde criados en ambientes tropicales. En la fase inicial, se asignaron al azar un total de doscientos ochenta y cinco pollos de engorde de Arbor Acres de sexo mixto a cinco tratamientos dietéticos con tres réplicas cada uno con diecinueve (19) polluelos. En la fase final, doscientos setenta pollos de engorde también fueron asignados a cinco tratamientos dietéticos con tres réplicas cada uno con 18 pollos de engorde por repetición. Los niveles de triptófano en la dieta en la fase inicial fueron 0.15, 0.19, 0.23, 0.27 y 0.31% respectivamente, mientras que las dietas para la fase de finalización contenían 0.13, 0.17, 0.21, 0.25 y 0.29% de triptófano en la dieta, respectivamente. Todos los otros niveles de nutrientes fueron constantes. El experimento se realizó de 0 a 28 d (fase de inicio) y de 33 a 56 d (fase de finalización). Las características de rendimiento del crecimiento, que incluyen el aumento de peso, la ingesta de alimento y la tasa de conversión alimenticia, se registraron al final de cada semana. Los resultados para la fase inicial mostraron que los pollos alimentados con dieta con 0.23%, 0.27% y 0.31% de triptófano en la dieta tuvieron resultados similares en términos de aumento de peso, ganancia de peso diaria promedio, ingesta de alimento e ingesta promedio diaria de alimento. Para la fase de finalización, las aves alimentadas con 0.22%, 0.25% y 0.29% de las dietas de triptófano también tuvieron resultados similares en términos de peso final, ganancia de peso, ingesta de alimento e ingesta diaria promedio de alimento. Nuestros resultados sugieren que el triptófano suplementario fue suficiente para tener un efecto significativo (P <0.05) sobre el comportamiento del pollo de engorde. Sin embargo, el análisis de regresión polinomial revela que el comportamiento óptimo se alcanzó con 0.22% y 0.22% de triptófano

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en la dieta para las fases de inicio y finalización, respectivamente. Por lo tanto, se puede concluir que los requerimientos de triptófano en la dieta durante la temporada de calor para las fases de inicio y finalización fueron 0.24% y 0.21%, respectivamente.
Palabras clave: aminoácido; bioeficacia; pollo de engorde; estación.

INTRODUCTION

The interaction between hot ambient temperature and dietary amino acids on the response of broilers chickens is not well defined. Shan *et al*. (2003) reported that on average, the threonine and tryptophan requirements of broiler chicks at 35°C were very similar to those kept at 25°C. Others reported interaction between dietary essential amino acids and rearing temperatures (Dozier *et al*., 2000; Ferguson *et al*., 2000; Duarte *et al*., 2013). There are also many reports on the tryptophan requirements of chickens; however, there is relatively little information on the influence of temperature on them. The scanty information about critical essential amino acid requirements in the tropical environment and its functions in protein synthesis, levels in feed ingredients, it's simple and straight forward analysis and economic feasibility as a supplement makes it unsatisfactory to make it the clear choice as the reference for growth, reproduction, feed efficiency and poultry health. The influence of hot ambient temperature and season of rearing might also affect amino acid requirements and broiler performance (Shan *et al*., 2003).

It is also important to know that specific nutritional requirement, measured at neutral conditions in one place, may not meet all the needs in other places around the world (NRC 1994). This might be due to the genotype of birds, age and seasons of rearing. Ambient temperatures can cause an inverse response in feed consumption; that is, the lower the temperature, the greater the feed intake and vice versa (NRC1994). This means that percentage requirements of protein and amino acids should be increased in warmer environments. In the tropical environment little research has been done with practical type diets to assess the requirements for many of the essential amino acids, other than methionine and lysine. Tryptophan and threonine are one of the critical essential amino acids and complex amino acids. Tryptophan is used to supplement dietary deficiencies in diets for growth (Kerr *et al*., 2005). Tryptophan serves as a precursor to serotonin, a neurotransmitter highly involved in feed intake regulation and also used as supplement through its requirement for protein synthesis (Kerr *et al*., 2005). Therefore, the present experiment was designed to evaluate the effect of dietary levels of tryptophan on growth performance of broiler chickens reared in the hot season under tropical environment.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Poultry Unit of Kogi State Ministry of Agriculture, Kabba, located within the Southern Guinea Savannah Zone on latitude $7^0 5'$N, longitude $6^0 4'$E and altitude of 640m above sea level. It has an 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 cool 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 14ºC - 20ºC during the cool season while the maximum daily temperature is from 28^0C- 45^0C during the hot season. The mean relative humidity during dry and wet seasons is 21% and 72%, respectively. Nigerian Meteorological Agency 2015.

Source of Experimental Birds and Testing Ingredient (Tryptophan)

The mixed sex broiler day old chicks (Arbor Acre) used in this experiment were obtained from a commercial hatchery with good records of birds' performance. Synthetic tryptophan was purchased from Ajinomoto Eurolysine S. A. S, from France under Licence from Ajinomoto. Inc. Japan.

Experimental Diets

The experiment was divided into two phases. For the starter phase (d 0-28) five dietary tryptophan levels contained 0.15, 0.19, 0.23, 0.27 and 0.31%, respectively (Table 1) while for the finisher phase (d 33 -56) , five dietary tryptophan treatments contained 0.13, 0.17, 0.21, 0.25 and 0.29 %, respectively, (Table 2). All other nutrient levels were kept constant. The diets were formulated to be isocaloric and isonitrogenous (CP=23%, ME=3008 Kcal/Kg) for the starter phase and (CP=21%, ME=3056 Kcal/Kg) for the finisher phase.

Table 1: Composition of Broiler Starter Diets Containing Graded Supplemental Levels of Tryptophan During the Hot Season (0 - 28 d)

Ingredient (kg)	Treatments				
	1 Try 0.00%	2 Try 0.04%	3 Try 0.08%	4 Try 0.12%	5 Try 0.16%
Maize	50.66	50.66	50.66	50.66	50.66
Groundnut cake	25.00	25.00	25.00	25.00	25.00
Soya cake	12.59	12.59	12.59	12.59	12.59
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm oil	3.69	3.69	3.69	3.69	3.69
Limestone	0.90	0.90	0.90	0.90	0.90
Bone meal	2.75	2.75	2.75	2.75	2.75
Common salt	0.30	0.30	0.30	0.30	0.30
Premix **	0.30	0.30	0.30	0.30	0.30
Lysine	0.32	0.32	0.32	0.32	0.32
Methionine	0.21	0.21	0.21	0.21	0.21
Tryptophan	0.00	0.04	0.08	0.12	0.16
Threonine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME (Kcal/kg)	3008	3008	3008	3008	3008
Crude protein (%)	23.00	23.00	23.00	23.00	23.00
Ether extract (%)	8.11	8.11	8.11	8.11	8.11
Crude fibre (%)	3.70	3.70	3.70	3.70	3.70
Calcium (%)	1.28	1.28	1.28	1.28	1.28
Lysine (%)	1.26	1.26	1.26	1.26	1.26
Methionine (%)	0.56	0.56	0.56	0.56	0.56
Available P (%)	0.57	0.57	0.57	0.57	0.57
TSAA (%)	0.90	0.90	0.90	0.90	0.90
Tryptophan (%)	0.15	0.19	0.23	0.27	0.31
Threonine (%)	0.95	0.95	0.95	0.95	0.95
Glycine (%)	1.69	1.69	1.69	1.69	1.69
Arginine (%)	1.69	1.69	1.69	1.69	1.69
Phenylalanine (%)	1.07	1.07	1.07	1.07	1.07
Leucine (%)	1.85	1.85	1.85	1.85	1.85
Isoleucine (%)	0.99	0.99	0.99	0.99	0.99
Valine (%)	1.05	1.05	1.05	1.05	1.05

Total sulfur amino acid =TSSA, Try = Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Experimental Design and Management of Birds

For the starter phase (0 to 28 d) two hundred and eighty five healthy day olds broiler chicks with an average body weight of 40.01 ± 0.02 g were divided randomly into 15 pens of 19 chicks each. They were allotted to 5 dietary treatments with 3 replications of each. At the end of the starter phase, all the birds in the various groups were pooled together and fed a common diet for 5 days. They were randomly re-allocated after sorting into 15 pens with 3 replications, each having 18 chickens and were allotted to 5 dietary treatments. The broilers were housed in pens under the deep litter system of (45 cm × 36 cm × 36 cm) in a completely randomized design. The room temperature was maintained at 30 to 35°C from d 1-7, and then gradually reduced 2°C per week until a final temperature of 25°C was reached. Relative humidity was maintained at 55% to 60%. Chicks were exposed to 22 h of light and 2 h of darkness from d 1-8 and 16 h of light and 8 h darkness from d 9-56. The experiment was conducted during the hot season between April and May (2015). The maximum temperatures varied from 29^{0}C to 45^{0}C. Routine vaccinations and all necessary medications were administered as described by the Faculty of Veterinary Teaching Hospital, Ahmadu Bello University, Nigeria. Feed and water were provided *ad libitum*.

Table 2: Composition of Broiler Finisher Diets Containing Graded Supplemental Levels of Tryptophan During the Hot Season (33- 56 Weeks)

	Treatments				
	1	2	3	4	5
Ingredient (kg)	Try 0.00%	Try 0.04%	Try 0.08%	Try 0.12%	Try 0.16%
Maize	56.33	56.33	56.33	56.33	56.33
Groundnut cake	22.00	22.00	22.00	22.00	22.00
Soya cake	10.00	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm oil	3.50	3.50	3.50	3.50	3.50
Limestone	1.00	1.00	1.00	1.00	1.00
Bone meal	2.80	2.80	2.80	2.80	2.80
Common salt	0.30	0.30	0.30	0.30	0.30
Premix **	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.21	0.21	0.21	0.21	0.21
Tryptophan	0.00	0.04	0.08	0.12	0.16
Threonine	0.29	0.29	0.29	0.29	0.29
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME (Kcal/kg)	3056	3056	3056	3056	3056
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
Ether extract (%)	7.79	7.79	7.79	7.79	7.79
Crude fibre (%)	3.43	3.43	3.43	3.43	3.43
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Lysine (%)	1.14	1.14	1.14	1.14	1.14
Methionine (%)	0.54	0.54	0.54	0.54	0.54
Available P (%)	0.57	0.57	0.57	0.57	0.57
TSAA (%)	0.85	0.85	0.85	0.85	0.85
Tryptophan (%)	0.13	0.17	0.21	0.25	0.29
Threonine (%)	0.88	0.88	0.88	0.88	0.88
Glycine (%)	1.52	1.52	1.52	1.52	1.52
Arginine (%)	1.51	1.51	1.51	1.51	1.51
Phenylalanine (%)	0.98	0.98	0.98	0.98	0.98
Leucine (%)	1.72	1.72	1.72	1.72	1.72
Isoleucine (%)	0.90	0.90	0.90	0.90	0.90
Valine (%)	0.96	0.96	0.96	0.96	0.96

Total sulfur amino acid =TSSA, Try = Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Parameters Measured

The final body weight and feed intake were measured weekly. From the primary data collected for feed intake and weight gain, data for feed conversion ratio was generated. Mortality record was recorded as it occurred.

Statistical Analyses:

All data obtained were statistically analyzed using the General Linear Models (GLM) procedure of SAS software, (2001) for the analysis of variance. Duncan's (1955) multiple range tests were used to determine differences among treatment means. Means were considered different at P<0.05.

General Linear Model

$Y_{ij} = \mu + K_i + e_{ij}$

Where:

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

μ= Overall mean

K_i =i^{th} effect of tryptophan

e_{ij} = Random error

RESULTS AND DISCUSSION

The optimal use of commercially available synthetic essential amino acids (EAA) in broiler feeds can satisfy broiler requirements for EAA (Moran and Stilborn, 1996). However, the requirements of animals, including poultry, for energy, protein (amino acids), vitamins, and minerals may all be influenced by environmental temperature (Shan *et al*., 2003). This study was conducted to determine the effect of dietary levels of tryptophan on performance of broiler chickens reared during the hot season under tropical environment. For the starter phase dietary treatments had significant (P<0.05) effects on final body weight, weight gain, average daily weight gain and feed intake. An increased trend in weight gain was observed as the levels of tryptophan increased up to 0.23% and began to decline at 0.31% (Table 3). This result is in agreement with the results obtained by Harms and Russel (2000), Rosa *et al*. (2001), Fatufe *et al*. (2005), Emadi *et al*. (2010), Campos *et al.* (2010) and Yu *et al*. (2010). They reported an increase in body weight gain in broiler chickens which received the high concentrations of dietary tryptophan compared with those which received low concentration. This increase in body weight gain may be partly due to the increased feed intake and dietary essential amino acid balance. Although a polynomial regression analysis predicted 0.24% tryptophan to be the optimum requirement for broiler chicks (Figure 1). This value disagreed with the 0.20% estimated by NRC (1994). Reasons for this may be due to genotype and seasons of rearing. The bird's response can vary with the essential amino acid, the extent of its inadequacy, and existing relationships among the remainder.

Chicks fed diet without supplemental tryptophan (basal diet) of 0.15% had the least performance in terms of the body weight gain and feed conversion ratio compared to those receiving higher concentrations of tryptophan in their diets. These results are in agreement with the findings of Rosa *et al*. (2001) who reported depressed body weight and high feed conversion ratio of broiler chicks fed the lowest concentration of tryptophan. This may be due to reduced levels of potassium or altered ionic balance, lack of sufficient nitrogen pool to provide for synthesis of dispensable or nonessential amino acids, and imbalances among certain amino acids. A steady increase in feed consumption was observed as the dietary level of tryptophan increased between 0.15 and 0.27% and thereafter decreased. This result is similar to the findings of Harms and Russel (2000), Corzo *et al*. (2005a) and Wei *et al*. (2011), who reported reduced feed intake of broiler chickens, fed the low concentration tryptophan diets. The increased feed intake observed may partially be the function of tryptophan as a precursor of neurotransmitter of serotonin which is responsible for increased appetite. In this study, tryptophan deficient diet had the poorest feed conversion ratio of 1.55 compared with chicks fed supplemental tryptophan. This result is similar to the findings of Rostagno *et al*. (1995), Duarte *et al*. (2013). They reported increase in feed conversion ratio and a gradual decrease as the levels of dietary tryptophan increased. This may be due to dietary amino acids imbalance.

For the finisher phase, dietary treatments had significant effects on final weight, weight gain, average daily weight gain, feed intake and average daily feed intake. Dietary treatments had no significant (P<0.05) effect on the feed conversion ratio and mortality. A steady increase in weight gain was observed as the levels of dietary tryptophan increased from 0.13 – 0.21%. A sudden decline was observed at 0.29% dietary tryptophan (Table 4). This result also agreed with the report of Rostagno *et al*. (1995), Shan et al. (2003), Xi *et al*. (2009) and Wang, *et al*. (2014)., who stated that supplemental tryptophan alleviated body weight decline. A polynomial regression analysis showed that at 0.21% dietary tryptophan, the optimum weight gain was attained (Figure 2). Chickens fed tryptophan deficient diets (0.13%) had the least weight gain. This result is similar to the findings of Corzo *et al*. (2005b), Rosa *et al*. (2001), Xi *et al*. (2009) and Liu *et al*. (2015). Who reported depressed body weight gain, feed intake and feed conversion ration. The observed body weight decline could be related to possible stress-induced insufficiency of dietary tryptophan, amino acid imbalance and the season of rearing as the chickens were raised during the peak of hot season.

Improved the final weight, weight gain and average daily weight gain was observed at 0.21% dietary tryptophan. This result disagreed with the 0.18% suggested by NRC (1994). Birds fed diet supplemented with tryptophan consumed more feed compared to those receiving lower dietary concentrations of tryptophan. This result is similar to the findings of Harms and Russel (2000), Corzo *et al*. (2005b) and Huang *et al*. (2007), who reported reduced feed intake of broiler chickens, fed the low concentration of tryptophan. The increased feed intake observed may partially be the function of tryptophan as a precursor of neurotransmitter of serotonin which is responsible for increased appetite. The feed conversion ratio was observed to be best for chickens fed higher concentrations of dietary tryptophan. This result was similar to the findings Rostagno *et al*. (1995), Neto *et al*. (2000) who reported increase in feed conversion ratio and a gradual decrease in feed conversion ratio as the levels of dietary tryptophan increased. Although there was no significant differences observed in terms of the feed conversion ratio. This result is similar to the findings of Si *et al*. (2001), Corzo *et al*. (2005b), who reported that digestible tryptophan levels did not influence feed conversion ratio. Mortality was low and was not significantly different among treatment groups. Also, some of the mortality was not related to treatment effect (e.g.,legs trapped in the floor wire of the cage). The results of this experiment have a direct practical consequence. Even if broiler diets were formulated on the basis of other amino acids to yield suboptimal performance, it may be beneficial to provide the diet with an optimal amount of tryptophan to improve feed intake and performance.

Table 3: Performance of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Hot season (0-28 d)

Parameters	Tryptophan Levels %					SEM
	0.15	0.19	0.23	0.27	0.31	
Initial weight (g)	40.02	40.02	40.01	40.00	40.01	0.01
Final weight(g)	725.99[c]	824.98[b]	905.27[a]	904.95[a]	904.01[a]	14.21
Weight Gain (g)	685.98[c]	784.96[b]	865.25[a]	864.95[a]	864.00[a]	14.21
Ave daily gain (g)	24.50[c]	28.03[b]	30.91[a]	30.89[a]	30.86[a]	0.51
Feed Intake (g)	1062.31[b]	1099.56[ab]	1159.26[ab]	1218.52[a]	1177.78[ab]	42.95
Feed Intake (g/b/d)	37.94[b]	39.27[ab]	41.40[ab]	43.52[a]	42.06[ab]	1.53
FCR	1.55	1.41	1.34	1.41	1.36	0.06
Mortality (%)	5.60	1.85	0.00	3.70	1.85	2.90

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

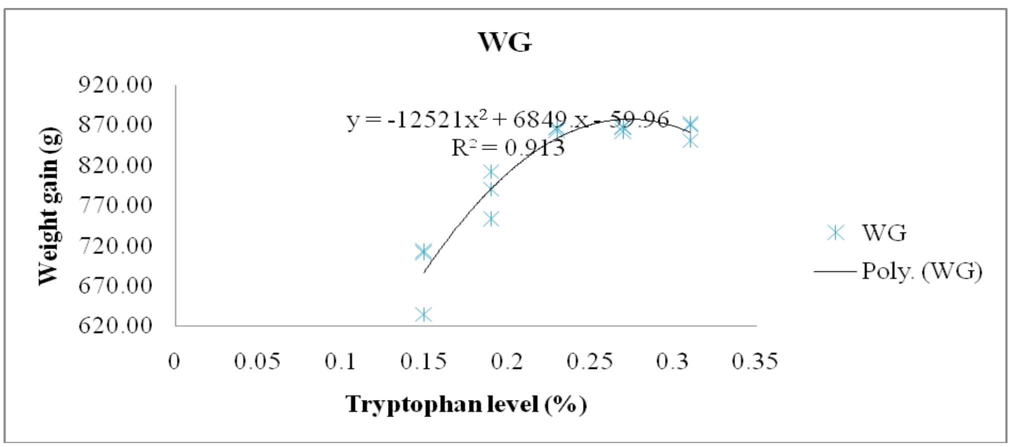

Figure 1: Polynomial regression showing the optimum dietary tryptophan level to maximize weight gain for broiler chicks during the hot season under tropical environment (0-28 d).

CONCLUSION

It can be concluded that broiler chickens reared in the tropical region may require 0.24% dietary tryptophan for the starter phase (d 0-28) and 0.21% dietary tryptophan for the finisher (d 33- 56) phase. However, more research is needed to alleviate contradictory findings as the effects of supplemental tryptophan in improving broiler production are speculative at this time in the tropical environment.

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Table4: Performance of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season (33- 56 d)

	Tryptophan Levels %					
Parameters	0.13	0.17	0.21	0.25	0.29	SEM
Initial weight (g)	1170.88	1170.61	1170.41	1171.67	1170.00	0.65
Final weight(g)	2453.12[b]	2623.39[b]	2776.14[a]	2771.43[a]	2763.22[a]	49.27
Weight Gain (g)	1282.40[b]	1452.80[ab]	1605.70[a]	1599.80[a]	1434.90[ab]	89.67
Ave daily gain (g)	61.06[b]	69.18[ab]	76.46[a]	76.18[a]	68.33[ab]	4.27
Feed Intake (g)	3012.00[b]	3183.80[ab]	3627.60[a]	3715.70[a]	3390.30[ab]	175.15
Feed Intake (g/b/d)	143.45[b]	151.61[ab]	172.75[a]	176.94[a]	161.44[ab]	8.34
FCR	2.34	2.20	2.26	2.32	2.40	1.09
Mortality (%)	8.33	0.00	0.00	0.00	0.00	5.80

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

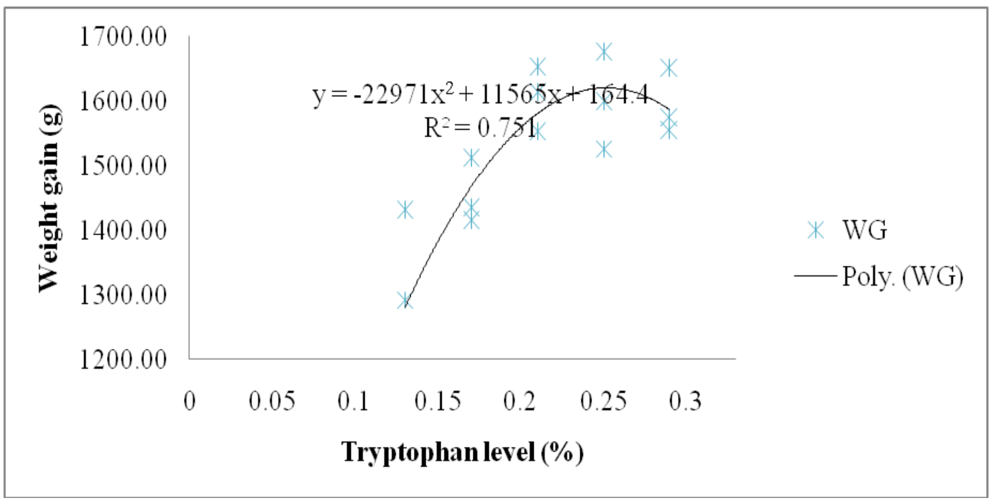

Figure 4: Polynomial regression showing the optimum dietary tryptophan level to maximize weight gain for broiler finisher chickens during the hot season under tropical environment. (33-56 d).

Ethical approval

Protocols for these experiments were approved by the Animal Care Committee of Ahmadu Bello University, Nigeria and birds were reared and cared for according to the Veterinary Teaching Hospital, Ahmadu Bello University, Nigeria. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This experiment was monitored by the Department of Animal Science, Ahmadu Bello University, Nigeria.

Conflict of Interest Statement

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

O.E. (Ahmadu Bello University) performed most of the experiments, including the planning of the study and the analyses of data, as a PhD-student. O. S.O. (Ahmadu Bello University), B.G.S. (Ahmadu Bello University) and O.P.A (Ahmadu Bello University) made contributions in study conception, data analysis and interpretation as well as to drafting of manuscript.

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