Growth performance, nutrient digestibility and haematological indices of broiler chickens fed diets supplemented with riboflavin and pyridoxine

Jegede, A. V., Ogunsola, I. A., Fafiolu, A. O., Oluwatosin, O. O., Lawal, R. F., Odejaye, O. A. and Adeniran, A. C.

Department of Animal Nutrition, Federal University of Agriculture, Abeokuta

Corresponding author: vinbjegede@yahoo.com, +2348035397280

Abstract

A 56 days feeding experiment was conducted to investigate the growth performance, nutrient digestibility and blood characteristics of broiler chickens fed diets supplemented with riboflavin and pyridoxine. A total of two hundred and forty day old unsexed Marshall Broiler chickens were assigned to eight diets in a completely randomised design. The diets were such that diet 1 was the control (basal diet without vitamin supplementation), diets 2, 3 and 4 had basal diet supplemented with 4 mg/kg, 8 mg/kg and 12 mg/kg riboflavin, respectively, diets 5, 6, and 7 had basal diet supplemented with 3.5 mg/kg, 7 mg/kg and 10.5 mg/kg pyridoxine, respectively while diet 8 had basal diet with 4 mg/kg riboflavin + 3.5 mg/kg pyridoxine. Each dietary treatment was replicated three times with 10 birds per replicate. The growth parameters measured at day 28 were not significantly influenced ($P>0.05$) by the diets. At day 56, the final weight (1980 g) was highest in birds fed diets with 7 mg/kg supplemental pyridoxine, though the final weight value was similar ($P>0.05$) to those fed diets supplemented with 4 mg/kg (1793.30 g) and 12 mg/kg (1700.00 g) riboflavin. Higher protein retention values were recorded for broilers fed higher concentration of supplemental riboflavin and pyridoxine. The packed cell volume (PCV) and haemoglobin (Hb) concentration of broilers fed diets supplemented with 8 mg/kg riboflavin and 7 mg/kg pyridoxine (insert the values here) were higher than those on other diets. Higher levels of riboflavin and pyridoxine and their combination resulted in an elevated heterophil and decreased lymphocyte values. An elevated ($P<0.05$) mean corpuscular haemoglobin (MCH) (44.75 pg/cell) and mean corpuscular volume (MCV) (133.05 fL) concentration was observed in broilers fed diets containing added 3.5 mg/kg pyridoxine. The study revealed that 8 mg/kg riboflavin and 7 mg/kg pyridoxine is required for improved final weight, crude protein retention and good health of broiler chickens.

Keywords: Broilers, Digestibility, Haematology, Pyridoxine, Riboflavin

Introduction

Vitamins are essential micro-nutrients, occupying a central role in most metabolic processes. They are required for optimum health and normal physiological functions such as growth, development, maintenance, reproduction (Hubbard breeders, 2011). Riboflavin (vitamin B₂) is one of the B-vitamins required as part of many enzymes involved with metabolism of carbohydrate, fat and protein. If levels of riboflavin are low, the respiration process becomes less efficient, and 10% to 15% more feed is

required to meet energy needs (Christensen, 1983). The NRC (1994) requirement for broiler chicks is reported as 3.6 mg/kg of feed. However, Ruiz and Harms (1988) suggested that to prevent signs of leg paralysis in broilers fed a corn-soybean diet, the minimum requirement of 4.6 mg riboflavin per kg of feed is needed. Olkowski and Classen (1998) likewise suggested a level higher than NRC requirement for broilers (5 mg/kg). Pyridoxine (vitamin B₆) and its hydrochlorate salt are usually

supplemented to poultry diets as the source of this vitamin. The pyridoxine recommendations of NRC (1994) for poultry generally vary from 2.5 – 4.5 mg/kg of diet. When compared to other nutrients, there have been very few studies carried out in recent years to estimate the optimum levels of vitamins for broilers, and there is a huge variation in the levels used commercially. Most levels recommended by the NRC (1994) have been based on old studies, performed under controlled conditions, and using the minimum levels to avoid signs of deficiency, not evaluating the best performance under the challenge conditions found in the field. Moreover, modern breeds have a higher growth and production rate, and have higher nutritional requirements to express their genetic potential. Supplementation with higher levels than the minimum recommendations could result in higher production performance and nutrient utilization of broiler chickens, hence the need for this study.

Materials and methods

This study was carried out at the Poultry unit of the University Farms, Federal University of Agriculture, Abeokuta, Alabata Road, Nigeria. Alabata is in Odeda Local Government Area of Ogun State. It falls within the rainforest vegetation zone of the south-west Nigeria on the latitude 7° S 13' 49.66'N and longitude 3° 26'11.98 E and 76 metres above sea level (Google earth, 2017). The climate is tropical humid with a mean annual rainfall of 1037mm

Experimental design

240, 1 day-old unsexed Marshall broiler chicks were randomly assigned to 8 dietary groups in a Completely Randomized Design (CRD). Each dietary group consisted of 30 birds with 3 replicates of 10 birds each. The study was conducted in two

phases of starting (0-28 d and finishing (29-56 d).

Dietary treatment

A corn-soybean based diet was formulated to meet the nutrient requirement of birds (NRC, 1994) for the starter broilers (0 - 28 d) and finisher broilers (29 - 56 d). Eight diets were formulated from a basal diet such that: diet 1 was control (without added riboflavin and pyridoxine), diets 2, 3, 4 were basal diets supplemented with 4, 8 and 12 mg/kg riboflavin, diets 5, 6, 7 were basal diets supplemented with 3.5, 7 and 10.5 mg/kg pyridoxine and diet 8 was basal diet supplemented with 4.0 mg/kg riboflavin + 3.5 mg/kg pyridoxine. The ingredients and basal diets composition is presented in Table 1.

Birds and management

240 d-old unsexed Marshall Broiler chicks were purchased from a commercial hatchery in Ibadan. The birds were weighed and reared in 24 floor pens (each measuring 2.0m x 1.5 m) containing fresh wood shavings. Each unit housed 10 birds. Brooding was done for 21 days at 36°C pen temperature for the first one week and then reduced by 3°C per week until 27°C was reached and maintained during the remaining rearing period. All necessary vaccinations and medication were duly administered. Feed and fresh water were supplied *ad libitum* throughout the rearing period.

Chemical analysis

The proximate composition of the basal diets was determined by the method described by AOAC (1995).

Measurement of apparent nutrient digestibility

At day 56, three birds per pen (making a total of 9 birds per treatment) were randomly selected and housed individually in metabolic cages. A 3-day acclimatization

Table 1: Basal composition of experimental diets (broilers) g/kg

Ingredient	Broiler starter	Broiler finisher
Maize	500	540
Fish meal (72%)	25	20
Soybean meal	300	200
Groundnut cake	60	60
Wheat offal	60.5	126.5
Bone meal	30	30
Oyster shell	15	15
Methionine	2.5	2.0
Lysine	2.0	1.5
*Vitamin premix	2.5	2.5
Salt (NaCl)	2.5	2.5
Total	1000.00	1000.00
Determined Values		
ME(Kcal/kg)	2848.50	2855.60
Crude protein (%)	23.54	20.10
Fat (%)	3.72	3.76
Fibre (%)	3.34	3.47
Calcium (%)	1.47	1.45
Available phosphorus	0.58	0.5

*2.5 kg Premix supply the following per ton of feed: Vitamin A 12,000,000 I U; Vitamin D3 3,000,000 I.U; Vitamin E 30,000,000; Vitamin K 2,500mg; folic acid 1,000mg; Niacin 40,000mg; pantholelic acid 10,000mg; Vitamin B12 20mg; Vit amin B 12,000mg; Vitamin B6 3,500mg; Biotin 80mg; Antioxidant 125,000; Colbact 250mg; Selenium 250mg; iodine 200mg, Iron 40,000mg; Manganese 70,000mg; Copper 8,000mg; Zinc 60,000mg; chlorine 200,000mg

period was allowed prior to a 4-day collection period. Birds were fed with the quantity of feed which exceeded their daily feed intake. Daily excreta voided per bird was collected and dried at 65°C in an air-draft oven to a constant weight. Excreta obtained from each replicate were pooled together and ground to pass through 1 mm screen. Ground feed and faecal samples were used to determine their respective proximate constituents (AOAC 1995).

Blood collection and analysis

At day 56, blood samples were collected from the birds by severing the jugular veins; 2.5mL of blood samples were collected from 2 birds per replicate (6 birds per treatment) into tubes containing ethylenediamine- tetra-acetate (EDTA). The blood in the EDTA bottles were used to determine haematological parameters (packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) The PCV was determined by microhaematocrit method

(Baker and Silvertown, 1985) and Hb and RBC were determined using colorimetry cyanomethaemoglobin and improved Neubauer haemocytometer methods respectively (Jain, 1986).

Data collection

Data on growth indices such as body weight, weight gain feed intake and feed conversion ratio were collected weekly

Statistical analysis

Data collected were subjected to one-way analysis of variance in a Complete Randomized Design using SAS (2000) while significance means were separated according to Tukey's test of the SAS package and were considered to be significant when $P < 0.05$.

Results

The growth performance of broiler chickens fed diets supplemented with riboflavin and pyridoxine at the starter phase is shown in Table 2. Final weight, total weight gain, average weight gain, total

Table 2: performance characteristics of broiler chickens fed diets supplemented with riboflavin and pyridoxine (0 to 56 d)

Parameters	Control	Riboflavin (4mg/kg)	Riboflavin (8mg/kg)	Riboflavin (12mg/kg)	Pyridoxine (3.5mg/kg)	Pyridoxine (7mg/kg)	Pyridoxine (10.5mg/kg)	Riboflavin(4mg/kg)+ pyridoxine(3.5mg/kg)	SEM
0-28 d									
Initial weight g/b/d	34.67	36.00	37.00	33.67	34.00	34.00	34.33	34.67	0.47
Final weight g/b/d	466.00	462.67	436.00	472.67	432.67	482.67	479.33	452.67	9.05
Total weight gain g/b/d	431.33	426.67	399.00	439.00	398.67	448.67	445.00	418.00	9.23
Total feed intake g/b/d	1063.90	1283.30	1222.50	1117.60	1182.00	1053.80	1162.80	1248.20	33.56
Feed conversion ratio	2.45	2.99	3.12	2.55	2.97	2.35	2.65	3.00	0.09
29-56 d									
Initial Weight g/b/d	466.00	462.67	436.00	472.00	432.67	482.67	479.33	452.67	9.05
Final Weight g/b/d	1666.70 ^b	1793.30 ^{ab}	1600.00 ^b	1700.00 ^{ab}	1596.70 ^b	1980.00 ^a	1663.30 ^b	1653.30 ^b	33.33
Total weight gain g/b/d	1200.70	1330.70	1164.00	1227.30	1164.00	1497.30	1184.00	1200.70	32.25
Total feed intake g/b/d	3217.80	3395.50	2894.60	3229.20	2980.70	3146.70	3002.70	2952.10	63.61
Feed conversion ratio	2.68	2.56	2.53	2.66	2.57	2.12	2.56	2.47	0.07

^{ab}Means in the same row having different superscripts are significantly different (P<0.05)

Table 3: Nutrient digestibility of broiler chickens fed diets supplemented with riboflavin and pyridoxine (56 d)

Parameters (%)	Control	Riboflavin (4mg/kg)	Riboflavin (8mg/kg)	Riboflavin (12mg/kg)	Pyridoxine (3.5mg/kg)	Pyridoxine (7mg/kg)	Pyridoxine (10.5mg/kg)	Riboflavin Pyridoxine (4mg/kg)+(3.5mg/kg)	SEM
Dry matter digestibility	72.90	72.64	70.02	70.40	77.30	71.87	72.70	74.68	1.57
Crude protein retention	70.35 ^c	73.01 ^b	76.93 ^a	77.16 ^a	73.15 ^b	78.01 ^a	74.80 ^{ab}	74.50 ^{ab}	0.82
Crude fibre digestibility	70.08	76.15	74.33	74.20	74.99	79.57	77.94	79.80	1.97
Ether extract digestibility	71.175	70.355	70.205	72.585	71.885	74.245	72.635	76.78	0.25
Ash digestibility	74.85	74.09	76.55	79.93	73.94	77.09	75.25	74.60	0.68

pyridoxine (56 d)

^{a,b,c}Means in the same row having different superscripts are significantly different (P<0.05)

Table 3: Blood characteristics of broiler chickens fed diets supplemented with riboflavin and pyridoxine (56 d)

Parameters	Control	Riboflavin (4mg/kg)	Riboflavin (8mg/kg)	Riboflavin (12mg/kg)	Pyridoxine (3.5mg/kg)	Pyridoxine (7mg/kg)	Pyridoxine (10.5mg/kg)	Riboflavin(4mg/kg)+ Pyridoxine(3.5mg/kg)	SEM
Haematological indices									
Packed cell volume (%)	30.5 ^{cd}	34.5 ^b	36.5 ^a	34.5 ^b	28.5 ^d	35.0 ^{ab}	31.00 ^{cd}	35.0 ^b	0.60
Haemoglobin (g/dL)	10.00 ^{cd}	10.70 ^{bcd}	12.40 ^a	11.45 ^{abc}	9.60 ^d	12.05 ^{ab}	10.35 ^{cd}	11.83 ^b	0.23
Red blood count (10 ⁶ /mm ³)	2.80	2.80	3.15	2.80	2.15	3.10	2.60	2.90	0.07
White blood count (10 ⁶ /mm ³)	10.75	7.42	12.10	10.40	9.30	11.65	11.15	12.20	0.46
Heterophil (%)	28.50 ^e	30.50 ^{bc}	32.50 ^{abc}	31.50 ^{abc}	29.50 ^{bc}	33.50 ^{ab}	33.50 ^{ab}	36.00 ^a	0.56
Lymphocyte (%)	69.00 ^a	68.50 ^a	65.00 ^b	68.50 ^a	70.00 ^a	64.00 ^b	64.00 ^b	63.50 ^b	0.57
Eosinophil (%)	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.06
Monocyte (%)	1.5	1.0	2.0	0.0	0.0	0.0	0.0	1.5	0.21
MCH (pg/cell)	36.15 ^c	38.20 ^{bc}	39.30 ^{bc}	40.95 ^{abc}	44.75 ^a	39.00 ^{bc}	39.85 ^{abc}	42.25 ^{ab}	1.80
MCV (fL)	109.60 ^b	116.05 ^{ab}	115.85 ^{ab}	123.75 ^{ab}	133.05 ^a	113.40 ^b	119.35 ^{ab}	124.50 ^{ab}	0.62
MCHC (g/dL)	32.50	43.87	33.95	33.20	33.70	34.40	33.40	33.90	1.35

^{a,b,c,d} Means in the same row having different superscripts are significantly different (P<0.05)

feed intake, average feed intake and feed conversion ratio were not significantly influenced ($P>0.05$) by the diets. At finisher phase, birds fed diet containing pyridoxine at 7 mg/kg (1980 g) had the highest final live weight, while those fed control diet (1666.70 g) and diets containing 8 mg/kg riboflavin (1600.00 g), 3.5 mg/kg (1596.70 g) and 10.5 mg/kg pyridoxine (1663.30 g) and combination of the two vitamins recorded the least final weight values. Those fed diets containing 4 mg/kg riboflavin and 12 mg/kg riboflavin (1653.30 g) had statistically similar values. The Nutrient digestibility of experimental diets fed to broiler chickens is shown in Table 3. The crude protein retention of birds fed diets with higher levels of riboflavin, pyridoxine and their combination were higher ($P<0.05$) than those on control diet and those fed diets with low levels of riboflavin and pyridoxine. Other nutrient digestibility parameters were not significantly ($P>0.05$) influenced by the diets.

The results of the blood characteristics of broiler chickens fed diets supplemented with added dietary riboflavin and pyridoxine is shown in Table 4. Birds fed 8mg/kg added pyridoxine had highest ($P<0.05$) PCV and Hb values compared to broilers on control diet and diets with added 3.5 mg/kg and 10.5 mg/kg pyridoxine which had the least PCV and Hb values. The heterophil of birds fed diet with added 4 mg/kg riboflavin + 3.5 mg/kg pyridoxine was higher ($P<0.05$) than birds on control diet and those on diets containing lower levels of riboflavin and pyridoxine. The heterophil values of birds on higher levels of riboflavin were similar ($P>0.05$) to birds on diets with 4mg/kg riboflavin and 3.5 mg/kg pyridoxine. Birds fed control diet and those on lower levels of riboflavin and pyridoxine had elevated lymphocyte values

compared to those on higher levels of riboflavin and pyridoxine and their combination. The MCH of birds fed 3.5 mg/kg pyridoxine and combination of riboflavin and pyridoxine were higher ($P<0.05$) compared to those on other diets except those on 12 mg/kg riboflavin. The MCV of birds on 3.5 mg/kg added pyridoxine was higher ($P<0.05$) than those on the control diet, while those on other diets recorded a similar values.

Discussion

The growth performance of the starter broilers in this study were neither improved nor adversely affected by dietary supplementation of riboflavin and pyridoxine beyond NRC recommendation. This suggests that addition of pyridoxine and riboflavin beyond NRC recommendation (NRC, 1994) is not necessary for growth of starter broilers. Ruiz and Harms (1988) reported that 3.5mg of added riboflavin was needed to optimise the growth of turkey poults fed with corn-soya bean meal diet. Also a similar report of 3.6mg/kg dietary riboflavin was reported to be satisfactory for growing broilers (Deyhim *et al.*, 1993). However 4 mg/kg riboflavin and 7 mg/kg pyridoxine seems adequate for improved final weight. Whitehead (2000) reported 7mg/kg of riboflavin to broiler chickens when exposed to chronic heat stress. The improved final live weight of broiler chickens fed 7mg/kg pyridoxine can be attributed to effective utilization of the pyridoxine at the inclusion level due to age and physiological condition of the bird. This agreed with the result of Kidd *et al.* (2001); Quentin *et al.* (2005) which states that there are evidences that essential amino acid levels and pyridoxine in the feed higher than NRC specification is needed to achieve optimal growth performance, immuno-competence and

disease resistance.

Improved crude protein retention of birds fed diets containing higher levels of riboflavin and pyridoxine attest to the roles of these vitamins in protein metabolism. Riboflavin and pyridoxine plays significant roles in the metabolism of protein, carbohydrate and fat. They are required as part of many enzymes involved with metabolism of carbohydrate, fat and protein. Riboflavin in these coenzyme forms (FMN and FAD) are called flavoproteins and act as intermediaries in the transfer of electrons in biological oxidation-reduction reactions. If levels of riboflavin are low, the respiration process becomes less efficient, and 10% to 15% more feed is required to meet energy needs (Christensen, 1983). Fuller (1964) reported that deficiency of pyridoxine led to impairment in protein utilization. 8 mg/kg riboflavin and 7 mg/kg pyridoxine improved crude protein utilization. This value is higher than 6 mg/kg reported by (Oloyo, 2001).

The values of packed cell volume and haemoglobin recorded in this study are within the normal range of 25-45 % PCV and 7 – 13 mg/dL Hb for broiler chickens reported by Ross *et al.* (1978). However, the PCV and Hb of broilers fed 3.5 mg/kg diet (Table 4) are at the lower end of the range of normal values reported by Ross *et al.* (1978). This study therefore revealed that a value of up to 7 mg/kg pyridoxine would be more appropriate for good health of broiler chickens.

It is concluded that the NRC recommendation of 4 mg/kg riboflavin and 3.5 mg/kg pyridoxine may no longer realistic for modern day broiler production. An upward review of up to 8 mg/kg riboflavin and 7 mg/kg pyridoxine for improved broiler weight, crude protein retention and health status of the modern

day broiler chickens is recommended.

References

- A. O. A. C. 1995.** Association of Official Analytical Chemists. Official Methods of Analysis 16th ed. Washington D.C.
- Baker, F. J. and Silverton, R. E. 1985.** Introduction to Medical Laboratory Technology, 6th ed., pp. 523—531 (London, Butterworth & Co).
- Christensen, K. 1983.** Diagnostic and Statistical Manual (DSM) in Animal Nutrition and Health. www.dsm.com.
- Deyhim, F. and Tetter, R. G. 1993.** Dietary vitamin and /or trace mineral premix effects on Performance, humeral mediated immunity and carcass composition of broilers during thermo neutral and high ambient temperature distresss. *Journal of Applied Poultry Resource*, 2:347-355
- Fuller, B. L. 1964.** Vitamin B6 in farm animal nutrition and pets. *Vitamins and Hormones* 22: 659-676.
- Google Earth, 2017.** <http://www.Google.earth>.
- Hubbard breeders, 2011.** Breeders nutrition guide 2011, Available at <http://www.hubbardbreeders.com>
- Jain, M. C. 1986.** Scalon's Veterinary Haematology, 6th ed., pp. 178—207 (Philadelphia, Lea and Fabinger).
- Kidd, M. T., Gerard, P. D., Herger, J., Kenr, B. J. and Rowe, D. E. 2001.** Threonine and crude protein response in broiler chicks. *Animal Feed Science and Technology* 94: 57-64 Doi: 10.16/50377-8401(01)00301-7

- Olkowski, A. A and Classen, H. L, 1999.** The study of riboflavin requirement in broiler chickens. *Internatonal Journal of Vitamin Nutrition.*-1998- Res.68:316-327
- Oloyo, R.A. 2001.** Pyridoxine requirement of broilers on fed guinea corn – palm kernel meal based diet. *Pertanika Journal of Tropical Agricultural Science.* 24(2) 159-164
- Quentin, M., Bouvarel, I. and Picard, M. 2005.** Effects of *starter diet, light intensity and essential amino acids level on growth and carcass composition of broilers.* *Journal Applied Poultry Resources*, 14: 69-76. 9. SAS Institute.
- Ross, J. G., Christie, G., Halliday, W. G. and Jones, R. M. 1978.** Haematological and blood comparative values for clinical pathology in Poultry. *Veterinary Record.* (102) 29-31
- SAS, 2000.** Statistical Analysis System user's guide. Version 6. SAS Institute. Cary. N.C., USA.
- Ruiz, N. and Harms, R. H. 1988.** Riboflavin requirement of broiler chicks fed a corn-soybean diet. *Poultry Science.* 67:794.

Received: 4th November, 2017

Accepted: 10th February, 2018