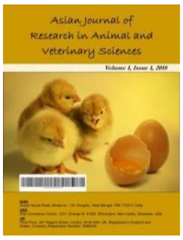

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Response of Broiler Chickens under Dexamethasone Induced Stress Conditions

L. A. Ademu[1], G. T. Erakpatobor-Iyeghe[2], P. P. Barje[2], O. M. Daudu[3] and R. J. Wafar[1*]

[1]Department of Animal Production and Health, Federal University Wukari, P.M.B 1020, Wukari, Taraba State, Nigeria.
[2]National Animal Production Research Institute, Shika, Zaria Kaduna State, Nigeria.
[3]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors LAA, PPB, GTEI and OMD designed the study. Author LAA performed the statistical analysis. Author RJW wrote the first draft of the manuscript. Authors GTEI and OMD wrote the protocol and managed the analyses of the study. Authors PPB and RJW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

An experiment was conducted to determine the response of broiler chickens under dexamethasone induced stress conditions. The experiment was conducted using two hundred and forty days old *Arbor acre* broiler chickens. There were four treatments each having three replicates with twenty birds per replicate. Concentrations of dexamethasone at 0, 1, 2 and 3 mg/ L of water were supplied daily. The experiment lasted 56 days. A decrease (P>0.05) in rectal temperature with an increase (P>0.05) in respiratory rate with increasing dose of dexamethasone was observed. Birds receiving 0 mg dexamethasone had the highest (P<0.05) final body weight, average daily weight gain, average daily feed intake and feed conversion ratio. Dexamethasone

E-mail: lawrenceademu@gmail.com

effect on tibia weight, length, weight/length index and robusticity index was significant ($P<0.05$) with the control group performing better than the dexamethasone groups. It is concluded that dexamethasone-induced stress had negative effects on broilers performance and tibia geometric properties.

Keywords: Dexamethasone; corticosterone; performance; tibia; broilers; stress.

1. INTRODUCTION

It has been estimated that world food consumption will double by 2050 and as more developing nations improve their economic status, per capital meat consumption will also increase [1]. Physiological stress is one of many concerns facing the modern broiler producer. When a stressor is actually causing a negative impact on the well-being of an animal, this can be defined as distress [2]. Extremes of ambient temperature is an important stressor that confronts poultry in many regions of the world and large economic losses can occur because of mortality and decreased production [3]. The thermoneutral zone for poultry is 18°C–24°C in the tropics and 12°C–26°C in the temperate zones, but this often gets exceeded in the tropics, resulting in heat stress [4,5]. When the hypothalamic-pituitary-adrenocortical axis is activated, the hypothalamus produces corticotrophin-releasing factor, which in turn stimulates the pituitary to release adrenocorticotropic hormone (ACTH). Secretion of ACTH causes the cells of the adrenal cortical tissue to proliferate and to secrete corticosteroids. The main active hormone of the axis is cortisol in cattle, sheep, pig, mink, fox and fish, and corticosterone in birds and rodents. These are cholesterol-derived steroids synthesized in the fascicular zone of the adrenal cortex under the control of the pituitary hormone. In chickens, adrenal corticosteroids are secreted shortly after exposure to stress and elevated plasma levels have been used as an index of the response to stress in poultry [6]. By elevating circulatory corticosteroids and decreasing thyroid activity, heat stress impairs broiler performance, especially adult birds, because the ability to dissipate heat decreases with age [7]. The drastic decline in feed intake occurs in heat-stressed birds as a physiological response to minimize intrinsic heat production and to maintain the thermal homeostasis, thus reducing the efficiency of feed utilization, live weight gain, and survival rates [8]. Corticosteroids have been directly implicated in the development of osteoporosis in stressed animals [9]. Similarly, synthetic glucocorticoid dexamethasone administration mimics the adverse effects of increased corticosterone. Dexamethasone (doses ranging from 0.2 to 4.0 mg/kg) was used as an immune suppressive agent [10], the mediator of prenatal stress [11] and to induce oxidative stress in laying hens [12] and in cockerels [13]. [14] demonstrated that administration of synthetic glucocorticoid, dexamethasone in doses of up to 6mg/kg had many effects on broilers similar to internal glucocorticoid.

Although the administration of corticosterone or analogues of corticosterone may be a promising tool in the research of adaptation to stress by broiler chickens [15]; Majority of studies on corticosteroids have focused only on the immunosuppressive actions of the stress hormones [16, 17, 15, 18] and not on their effect on performance indices and mineral retention. One possible strategy for improvement in this area is to conduct nutritional research by using a model that induces physiological stress in broilers using a specific stressor. If researchers have a more information on the detrimental effects of physiological stress, then future research involving nutrition and more specific stressors could be conducted more efficiently. The two major criteria of an acceptable stress model are treatments that are exact and highly repeatable and a predictable set of stress responses that occur in a known temporal pattern. The aim of this study was to determine of the effect of different doses of dexamethasone on thermoregulatory parameters, Growth performance and tibia geometric properties of broiler chickens. This model will employ continuous delivery of a defined dosage level of dexamethasone for a defined time period via drinking water.

2. MATERIALS AND METHODS

2.1 Location of the Study

The experiment was conducted at the Teaching and Research farm of the Department of Animal

Science, Ahmadu Bello University Samaru, Zaria. The farm is located at latitude 11º 9' 45" N and longitude 7º 38' 8" E, at an altitude of 610 m above sea level [19]. The climate in the area is divided into two: dry and rainy seasons. The dry season is usually from November to March and the temperatures recorded are within an average of 28 ºC towards the end of the dry season. The rainy season is usually from April to October. The daily mean maximum temperature reaches a peak in April and a minimum occurs between December and January. The area enjoys a tropical savannah climate with the annual total rainfall of about 1099 mm [20]. The study was carried out during the rainy season between the months of May and July and lasted 56 days.

2.2 Experimental Design, Diets and Management

Two hundred and forty days old *Arbor acre* broiler chicks were used in this experiment. They were randomly allotted to four experimental treatments. Each treatment was replicated three times with twenty birds per replicate in a completely randomized design. A maize/soybean meal based broiler starter and finisher diet were formulated according to [21] nutrient requirement for broiler chickens (Tables 1 and 2) and fed to all birds. The concentration of dexamethasone at 0, 1, 2 and 3 mg/ L of water was supplied daily. Birds receiving no dexamethasone served as the control. Introduction of dexamethasone to the other treatments commenced after a two week adjustment phase and terminated after 28 days to allow for a two week readjustment phase after dexamethasone treatment.

The birds were raised on deep litter with feed and water provided *ad libitum*. All routine and management practices were strictly adhered to. Parameters measured include final weight, feed intake while weight gain and feed conversion ratio were calculated.

Mortality records were taken as they occurred. Indoor climatic conditions were recorded using electronic digital thermo-hygrometer. Indoor temperature readings were taken in the morning (8.00 am) and afternoon (3.00 pm) throughout the experimental period including rectal temperature and respiratory rate by direct counting of respiration (breath/minute) with the aid of a stopwatch. Temperature-humidity index (THI) was calculated according to [22]. They established several stages of thermal comfort values such as: absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0), using the following mathematical model:

$$THI = T°C - ((0.31 - 0.31 \times RH/100) \times (T°C - 14.4))$$

Where TºC is temperature and RH is relative humidity.

Table 1. Ingredient and Chemical Composition of the Diet for Starter Broiler Chickens (0-4 weeks)

Ingredients	
Maize	55.00
Soyabean cake	24.00
Groundnut cake	14.00
Maize offal	2.00
Limestone	1.00
Bone meal	3.00
Common salt	0.30
Vitamin premix	0.30
Lysine	0.20
Methionine	0.20
Calculated analysis	
ME kcal/kg	2860
Crude protein	23.06
Ether extract	4.62
Crude fibre	4.25
Calcium	1.22
Available phosphorus	0.52
Lysine	1.24
Methionine	0.60

Nutrivitas broiler premix provides per 1kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg

2.3 Determination of Tibia Geometric Properties

The left tibia of three birds per replicate was removed. Tibiae were then labelled and immersed in boiling water (100°C) for 15 minutes according to the procedure described by [23] to complete tissue removal. Length of each bone was measured using a meter rule. The distance from cranial to distal extremities of each tibia was taken as the tibia length. The bone weight was obtained using a digital precision weighing balance (Satorius ENTRIS). The bone weight/length index was obtained by dividing the tibia weight by its length [24]. The Robusticity index was determined using the formula described by [25].

$$\text{Robusticity Index} = \frac{\text{Bone length}}{\text{Cube root of bone weight}}$$

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to procedures described by [26]. The percentage ash was then determined relative to dry weight of the tibia.

Table 2. Ingredient and chemical composition of the diet for finisher broiler chickens (5-8 weeks)

Ingredients	
Maize	62.5
Soya cake	18.0
Groundnut cake	15.0
Limestone	0.50
Bone meal	3.00
Common salt	0.30
Vitamin premix	0.30
Lysine	0.20
Methionine	0.20
Calculated analysis	
ME kcal/kg	2950
Crude protein	21.05
Ether extract	4.48
Crude fibre	3.77
Calcium	1.03
Available Phosphorus	0.51
Lysine	1.09
Methionine	0.58

Nutrivitas broiler premix provides per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg

2.4 Data Analysis

All data collected from the experiment were subjected to analysis of Variance (ANOVA) using the general linear model procedure of [27] according to the following model:

$$Yi = \mu + \delta i + \mathcal{E}i$$

Where Y_i is the individual observation, μ is the overall mean of the population, δ_i is the effect of the ith dexamethasone level and $\mathcal{E}_i$ is the random error.

Where the result of ANOVA was statistically significant, [28] test for multiple comparisons was performed to compare results of all groups.

3. RESULTS AND DISCUSSION

3.1 Thermo-Regulatory Parameters

The temperature humidity index (THI) inside the poultry house for the experimental period is shown in Fig. 1. THI in the mornings averaged 25.7 while the evenings THI averaged 31.1. In general, the results obtained indicated that THI in the afternoons was higher by 17.4% than THI in the morning indicating the absence of heat stress in the morning and the presence of severe heat stress in the afternoon [22]. Thermal sensitivity to high temperature increases with body weight [29]. Extremes of ambient temperature is an important stressor that confronts poultry in many regions of the world and large economic losses can occur because of mortality and decreased production [3]. The effect of varying doses of dexamethasone on rectal temperature of broiler chickens is shown in Fig. 2. The rectal temperature across the treatment groups ranged between 41.62°C to 42.28°C (difference of 0.66°C) and was not significant (P>0.05). A decreasing trend in rectal temperature with increasing doses of dexamethasone was observed with birds on 3 mg dexamethasone having the lowest rectal temperature. [14] reported a decrease in rectal temperature in broilers receiving between 1-5 mg/kg dexamethasone. This may be a successful consequence of the birds combating the effect of dexamethasone and attempting a return to homeostasis. Rectal temperature may be considered one of the indicators of metabolic rate in broilers, and increases when birds are exposed to high ambient temperature. The effect of varying doses of dexamethasone on respiratory rate of broiler chickens is shown in Fig. 3. The respiratory rate across the treatment groups ranged between 201.33 and 215.00 breaths per minute (difference of 13.67) and was not significant (P>0.05). An increasing trend in respiratory rate with increasing doses of dexamethasone was observed. This agrees with [14] who administered dexamethasone to broiler chickens. This increase is similar to birds under stress and this helps birds reduce body temperature (Fig. 2) through evaporative heat loss to return to homeostasis. When the environmental temperature exceeds the comfort limit, chickens suffer heat related stress resulting in physiological and metabolic changes [30]. In addition, in this study, the phenomena of feather dishevelment and diarrhea were also observed in broilers receiving dexamethasone. This also showed that dexamethasone can simulate glucocorticoid-induced stress successfully. Reduced feather coverage, either by decreased number or by modified shape, may help broilers to dissipate internal heat more efficiently [31].

3.2 Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens

Growth performance of broiler chickens fed varying doses of dexamethasone is presented in

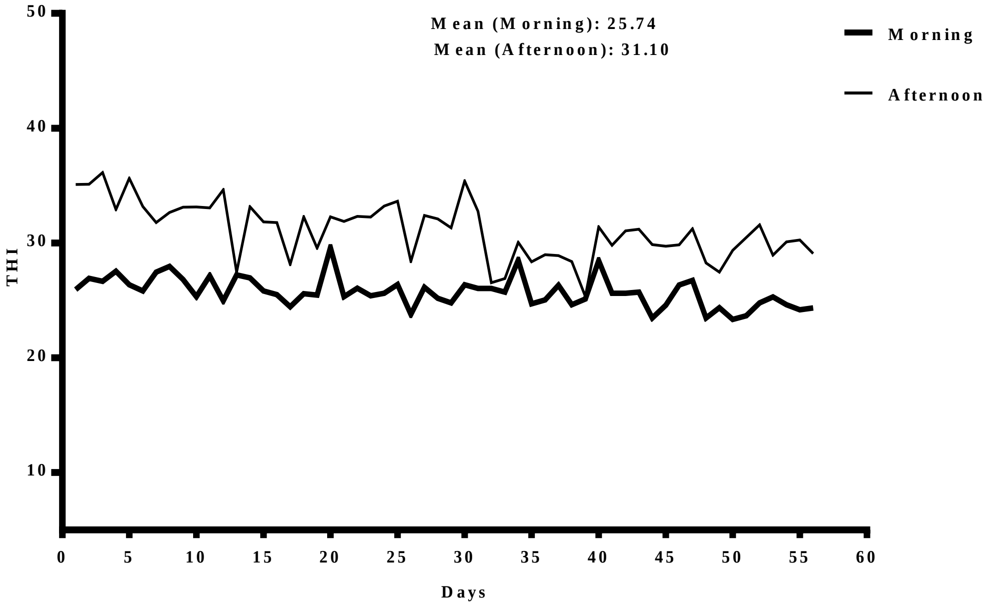

Figure 1: Daily THI inside the Poultry House During the Experiment

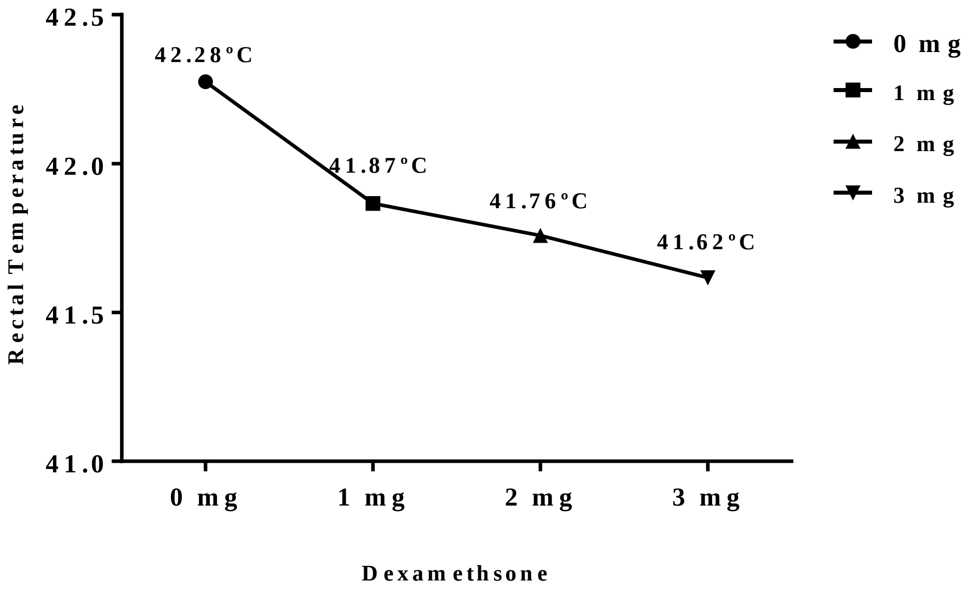

Figure 2: Effect of Varying Doses of Dexamethasone on Rectal Temperature of Broiler Chickens

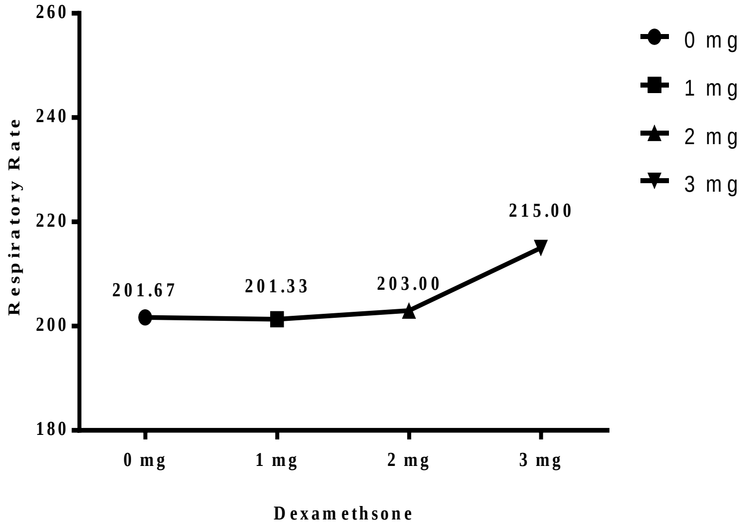

Figure 3: Effect of Varying Doses of Dexamethasone on Respiratory Rate of Broiler Chickens

Table 3. The average final weight was significant (P<0.05), with chickens fed 0 mg dexamethasone having the highest final weight compared to other dietary treatments. Final weights obtained in this study is in agreement with a study by [32] who simulated chronic stress conditions in broilers, from day 1 to 49 days of age. The average daily feed intake varied from 90.76 to 118.21 g/bird/day across the dietary treatments. Birds on dexamethasone had significantly (P<0.05) lower feed intake compared to those in the control (0 mg) with birds on 3 mg/l having the lowest (P<0.05) feed intake. It has been established that decreased feed intake is a primary cause of reduced growth rate in broiler chickens. The result of feed intake is in agreement with the report of [33] who reported that dexamethasone caused a decrease in feeding and appetite.

The effect of dexamethasone on the average weight gain ranging from 36.59 to 54.37 g was also significant (P<0.05). Birds on the control had significantly (P<0.05) higher daily weight gain than those on the dexamethasone treatments. The effect of dexamethsone on weight gain clearly showed a decrease in weight gain with increasing doses of dexamethasone. This is in agreement with the report of [34] that found that when chickens received dexamethasone, it caused muscular dystrophy and reduced growth.

Feed conversion ratio differed significantly (P<0.05) among the treatment groups ranging from 2.18 to 2.48 with birds on the control performing best. A decreasing trend in performance was observed with increasing levels of dexamethasone. The increase in feed conversion ratio indicates that the administration of dexamethasone-induced physiological stress where glucose metabolism is favoured over protein synthesis which had a negative impact on feed to tissue conversion and is in agreement with other studies [35,36,29,37] where corticosterone was administered.

Table 3. Effect of varying doses of dexamethasone on performance of broiler chickens

Parameters	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Initial weight (g/b)	101.67	102.50	102.50	103.33	0.93	.6722
Final weight (g/b)	2765.59^{a}	2212.72^{b}	2178.10^{b}	1896.28^{c}	51.63	<.0001
Weight gain (g/b/d)	54.37^{a}	43.07^{b}	42.36^{b}	36.59^{c}	1.04	<.0001
Feed intake (g/b/d)	118.21^{a}	102.45^{b}	103.05^{b}	90.76^{c}	1.49	<.0001
Feed conversion ratio	2.18^{a}	2.38^{b}	2.43^{b}	2.48^{b}	0.04	.0046
Mortality (%)	0.20^{b}	0.20^{b}	0.62^{a}	0.16^{b}	0.14	0.0251

^{a,b,c} *Means with different superscript on the same row differ significantly (P<0.05), SEM= Standard error mean*

Table 4. Effect of varying doses of dexamethasone on tibia geometric properties of broiler chickens

Tibia compositions	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Tibia weight (g)	12.21^{a}	11.03^{ab}	9.73^{bc}	8.63^{c}	0.35	<0.0001
Tibia length (cm)	10.98^{a}	10.20^{b}	9.68^{c}	9.27^{d}	0.09	<0.0001
Tibia weight/length index (g/cm)	1.11^{a}	1.08^{a}	1.00^{ab}	0.93^{b}	0.03	0.0049
Robusticity index ($\mathrm{cm/g^{1/3}}$)	4.77^{a}	4.59^{ab}	4.54^{b}	4.52^{b}	0.05	0.0125
Ash (%)	38.25	36.62	37.56	38.79	3.42	0.9729

^{a,b,c} Means with different superscript on the same row differ significantly (P<0.05), SEM= Standard error mean

3.3 Effect of Varying Doses of Dexamethasone on Tibia Geometric Properties

The result showing the effect of varying doses of dexamethasone on tibia geometric properties of broiler chickens is presented in Table 4. Dexamethasone effect on tibia length was significant (P<0.05), with tibia length decreasing with increasing levels of dexamethasone. The percentage decrease relative to the control group for tibia length was 7.1 % (1 mg), 11.8% (2 mg) and 15.6% (3 mg), representing an increase in tibia length reduction with increasing dose of dexamethasone.

The effect of dexamethasone on tibia weight also showed a trend similar to tibia length, with birds in the control having the highest tibia length while birds on 3 mg dexamethasone had the lowest tibia length. The percentage decrease in tibia weight relative to the control group was 9.7% (1 mg), 20.3% (2 mg) and 29.3 % (3 mg). This is in agreement with [38] who reported a decrease in tibia length and weight with increasing doses of dexamethasone in mice. In a study by [39], the bones from younger turkeys were more susceptible to corticosteroid-induced stunting of growth. This may be as a result of the reduction in growth rate observed among doses of dexamethasone in relation to the control which was more severe with higher doses of dexamethasone.

Dexamethasone effect on robusticity index was significant (P<0.05), decreasing with increasing doses of dexamethasone. However, there was no significant (P>0.05) difference among the dexamethasone containing groups. Studies by [39] found that the synthetic corticosteroid, dexamethasone, decreased bone strength of turkeys, and the severity of the effect increased with age.

Dexamethasone effect on Tibia weight/length index ranged from 0.93 to 1.11 and showed a significant (P<0.05) decrease as the level of dexamethasone increased. Birds treated with 3 mg dexamethasone gave a significantly (P<0.05) lower index than other treatments. The higher the index, the denser is the bone [40]. On the contrary, low robusticity index indicates a strong bone structure [25]. Effect of varying doses of dexamethasone on ash composition ranged from 36.62 to 38.79 and was not significant (P>0.05). This could indicate dexamethasone has no effect on bone mineralization. Bone mineralization provides compressional strength to bone, the bone ash content or bone mineral densities have been used as the indices of bone strength [39].

4. CONCLUSION

These results demonstrate that dexamethasone had a negative effect on performance and tibia geometric properties of broiler chickens.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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