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EFFECT OF DIETARY SELENIUM SUPPRESSION ON DEXAMETHASONE INDUCED OXIDATIVE STRESS ON THE GROWTH PERFORMANCE AND HAEMATOLOGY OF BROILER CHICKENS.

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ABSTRACT

The experiment monitored the growth performance of broilers fed with varying levels of Dietary Selenium to suppress induced oxidative stress from Dexamethasone (Diodex) in their diets. Synthetic glucocorticoid (dexamethosone) administration mimics the adverse effects of increased corticosterone, as a mediator of prenatal stress, while supplementation of selenium in broiler diets is noted to increase antibodies and influence the immune responses through its incorporation into seleproteins such as the amino acid selencysteine. Moreover, supplementation of selenium concentration in the diet of broilers is essential for the normal development and functioning of the immune system of the chickens. A total of 240 day-old-broiler chicks of Oba Marshal strain were randomly assigned to four treatments in a completely randomized design (CRD), after two weeks of acclimatization. Each group was further sub-divided into three replicates of twenty (20) birds per replicate. Four experimental treatments were administered through intra-muscular injections such that treatment one (T_1 served as the control with 0.0mg/kg of diodex and selenium., Treatment Two (T_2), three (T_3) and four (T_4) received 0.3, 0.6 and 0.9 mg/kg body weight were of dexamethosone and selenium respectively for a period of seven (7) successive days, while the experiment lasted for 8weeks (56days). Commercial feed (Top feed) whose energy were about 3000MEkcal|kg and protein contents of 22% were used for the broilers while water was served *ad-libitum*. Response parameters evaluated were: Body weight gain, feed intake, feed conversion ratio (FCR), water intake as well as the haematological parameters. Data collected were subjected to analysis of variance (ANOVA) and differences in means were subjected to separation using Duncans New Multiple Range Test. Results showed that there were no significant differences ($P>0.05$) between the control diet (T_1) and diets two (T_2) and three (T_3). But, significant differences ($P<0.05$) existed between treatments four (T_4) and the other three treatments of one, two and three respectively in all the parameters monitored. The results of

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haematological and serum biochemical indices also followed similar trends. The results of this experiment suggest that treatment three ((T_3) which received 0.6mg/kg of body weight of selenium appeared to have been the best in performance of final body weight and daily gain compared to the control and diet Two (T_2); while diet four was the poorest. Treatment four birds had the highest feed intake which was not converted to gain due possibly to the uncontrolled stress from the dietary treatments. It can be concluded that dietary inclusions in treatment three (T_3) appeared to be the optimum level of inclusion. This was further substantiated from the lowest mortality result recorded from treatment three (T_3).

Keywords: Broiler, Oxidative stress, Selenium, Dexamethasone

INTRODUCTION

Broiler production in Nigeria and in all hot regions suffers great loss every year due to the effect of stress, particularly from sudden heat waves exceeding 30^{o}c which might occur during the dry seasons. Broilers are often subjected to stress such as fasting, transportation and high exposure to harsh environmental temperature (Eid *et al*., 2008). During stress, the endocrine system is markedly affected. Hypothalamus-pituitary-adrenal are seriously affected (Siegel, 1980). Stress reduces feed consumption, growth rate, feed efficiency, egg quality, fertility and chicks quality (Gross and Siegel, 1993; El-Lethey *et al*,., 2000).

Elevated levels of plasma glucocorticoids has also been used as an index of the response to stress in poultry (Freeman, 1971). High levels of circulatory glucocorticoids accelerate from the metabolic rates and elevate free radicals especially the reactive oxygen species (ROS). ROS can have beneficial roles, as in phagocytes where they protect against bacteria and parasites. However, if natural anti-oxidants are not adequate to quench excess oxygen radicals, they can react with cell structure and attack proteins, lipids, carbohydrates and nucleotides within the cells. This state is referred to as oxidative stress (Hidalgo *et al*., 1988).

Gutteridge (1993) reported that oxidative stress leads to development of many pathological processes. Various enzymes such as glutathione peroxidase (GPX) and superoxidase diamutase (SOD) play an important role in the protection of cell membrane against oxidative damage and may be used as indicators of anti-oxidative stress. Eid *et al*., (2003) had earlier indicated that glucocorticoid administration induced oxidative stress in chickens. Consequently, synthetic glucocorticiod dexamethasone administration mimics the adverse effect of increased corticosterone. Fowles, *et al*., (1992) had recommended dexamethasone doses ranging from 0.2 to 0.4mg/kg of body weight to serve as an immune suppressive agent and to act as a mediator of prenatal stress and can induce oxidative stress in birds such as laying hens and cockerels (Ei-Habbak *et al*., 2005; Eid *et al*., 2006b).

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Gross and Siegel (1985) realized that the heterophile to lymphocyte ratio could be used as an effective measure of stress in different strains of fowls. Also, the ratio was elevated by dexamethasone treatment (Huff et al., 2001). Treatment of chickens with dexamethasone results in increased white blood cell (WBC) counts, heterophil and monocytes. It has also been reported that stress increased serum glucose albumin, triglyceride, cholesterol and calcium concentration among others (Shahin and Kucuk, 2001). Against this backdrop, this work was designed to determine the effect of dexamethasone (diodex) induced oxidative stress and selenium suppression on the growth performance of broiler birds. The objective of this work was to (a) determine the effect of dexamethasone induced oxidative stress and selenium suppression on Growth performance of Broilers. (b) assess the haematological indices and serum biochemistry of birds treated with dexamethasone induced oxidative stress.

Significance of the study

Poultry production in Nigeria suffers significant losses every year because of oxidative stress which leads to economic losses to the Poultry Farmers (Rama Rio *et al*., 2011). Oxidative stress such as heat is important in intensive poultry operations especially in broilers, because their higher production performance and feed conversion efficiency make them more susceptible to oxidative stress than any other species (Lin *et al*., 2005).

Higher mortalities, decreased feed intake, lower body weight gain and poor feed efficiency are the common adverse effects of induced oxidative stress often seen in meat-type poultry flocks (Yegani, 2008). Supplementation of Selenium in chicken diets increase antibody tilters to Newcastle disease (Hegazy and Adachi, 2000). Furthermore, selenium influence responses through its incorporation into selenproteins such as the amino acid selencystein (Hoffmann, 2007). Ideal selenium concentration in the diet is essential for normal development and functioning of the immune system and for maintaining the systematic defence in broilers reared in the tropics (Koski and Marilyn, 2003).

MATERIALS AND METHODS

Experimental site

This experiment was conducted at the Department of Animal Science, Ebonyi State University Poultry Unit, Abakaliki, Nigeria. The site is located between Latitude 06 21N and Longitude 08 51E in the derived Savanna zone of the South-East agro-ecological zone of Nigeria, with an annual rainfall range of 1500 to 1800mm and temperature range of 21˚C to 30˚C. Ebonyi State is situated at an elevation of 59meters above sea level (Nwakpu, 2008). The experiment lasted for 9 weeks.

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Experimental birds and treatments

Two hundred and fourty (240) day-old-chicks of Oba Marshall Strain of broilers were sourced from a reputable farm at Ibadan, South-West Nigeria. The Experimental treatments comprised of 0, 0.3, 0.6 and 0.9mg/kg of body weight of dexamethasone (Diodex) injection was administered to the birds intramuscularly for seven (7) successive days as well as 0, 0.3, 0.6 and 0.9mg/kg of selenium added to two litres of water for the same period of time. Birds in treatment one (T1) received neither dexamethasone injection nor selenium in their water and served as the control treatment. Different doses of dexamethasone injection and selenium constituted treatment combinations as follows:

T_1 = zero mg/kg of body weight of dexamethasone and selenium (control)

T_2 = 0.3mg/kg of dexamethasone and selenium

T_3 = 0.6 mg/kg of dexamethasone and selenium

T_4 = 0.9 mg/kg of dexamethasone and selenium.

The experimental birds were randomly assigned to each treatment amounting to sixty birds/diet treatment and replicated three times. Each replicate contained twenty (20) birds. The treatments were administered to the birds at the age of four (4) weeks.

Care and management of experimental birds

Prior to the arrival of the birds, pens and all the poultry equipment were washed and thoroughly disinfected using disinfectants, Isol and Izal. Glucose and liquid vitamins were administered in water to the birds. The birds were fed commercial feeds (top feed) containing and served with safe drinking water *ad libitum*. All the necessary routine vaccinations and sanitation activities were strictly adhered to.

Data collection and analysis

Data were collected on the growth performance parameters of the birds as well as on the haematological and serum biochemical parameters. These parameters are (i) initial body weight; (ii) final body weight, (iii) body weight gain (iv) Feed intake (v) Water intake and (vi) Feed conversion ratio (FCR). Blood samples were taken from some selected birds per treatment and 2ml of blood sample, from the wing vein using well labeled sterilized bottles that contained Ethylene diaminetetra-acetic acid (ETDA) which served as anticoagulant. The blood samples were taken to Biochemistry Laboratory for haematological studies to evaluate the (i) Packed cell volume (PCV); (ii) Red Blood cells (RBC); (iii) White Blood Cells (WBC); (iv) Hemoglobin

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Counts (HBC); (v) Mean Corpuscular Volume (MCV); (vi) Mean Corpuscular Haemoglobin (MCH); according to Dein, (1984). From the red blood cell analysis, we were able to calculate the MCH, MCHC, and MCV.

All data were subjected to statistical analysis using Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD). Treatment means were separated statistically using Fisher's Least Significant Difference (F-LSD) according to Steel and Torrie (1980). The experimental model is as follows:

X_{ij}: $\mu + t_1 + \Sigma_{ij}$ where

X_{ij}: Any observation from the experimental units

μ: Overall population mean

t_1: Treatment effect

Σ_{ij} : Residual experimental error.

RESULTS AND DISCUSSION

Growth performance parameters

Results of the growth performance of broiler chickens induced with synthetic dexamethasone oxidative stress and dietary selenium are shown in Table 1 below. There was no significant ($P > 0.05$) differences in all the parameters v_{1z} ; the final body weight, body weight gain (BWG), daily weight gain (DWG), total feed intake, daily feed intake and daily water intake etc.

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Table1: Effect of dietary selenium suppression using dexamethasone-induced oxidative stress on growth performance of broiler chickens.

Parameters	T_1	T_2	T_3	T_4	S.E.M
Initial body weight(g)	81.96	81.31	83.68	84.50	±1.53
Final body weight $gain_{(g)}$	2555.56	2348.61	2408.33	2375.00	±365.98
Body weight $gain_{(g)}$	2473.60	2267.30	2324.65	2290.50	±209.92
Daily weight $gain_{(g)}$	44.17	40.49	41.51	40.90	±3.76
Total Feed intake(g)	7461.26	7008.17	7565.23	7630.58	±365.9
Daily Feed intake(g)	133.24	125.16	135.09	136.26	±6.54
Feed conversion ratio	3.02	3.16	3.32	3.33	±0.22
Total water intake(ml)	53846.67	52136.67	59893.33	55756.67	±574.69
Daily water intake (ml)	961.55	931.01	1069.52	995.65	±102.62

S.E.M: Standard error of means

Stress susceptibility of chickens is a major production problem in the modern poultry industry. Birds are often subjected to stresses such as fasting, transportation and high exposure to harsh environmental temperature (Eid etal., 2008). Stress reduces feed consumption, growth rate, feed efficiency, egg quality, fertility and chicken quality (Gross and siegel, 1993., El-lethey *et al*, 2000). In this experiment, the intramuscular injection of dexamethasone (DEX) oxidative stress on dietary selenium suppression at different combination doses (0, 0.3 ,0.6 and 0.9mg of body weight), did not significantly ($p>0.05$) improve the final body weight gain, body weight gain(BWG) and daily weight gain (DWG) more than the control treatment. The highest weight gain value was observed among the broiler birds in the control (T_1) diet, while T_2, T_3, and T_4 representing 0.3, 0.6 and 0.9 mg/kg body weight of dexamethasone and selenium at the same proportion of doses had the lowest values of weight gain. Compared to the control diet, diet T_2 recorded the least weight gain whereas, T_3 had the highest numerical value of 2408.33g, although was not higher than the control diet (T_1).

This implies that, the intramuscular injection of dexamethasone and selenium at 0.3—0.9mg/kg body weight for seven successive days induced oxidative stress on the birds thereby impairing the growth performance and weight gains of broiler birds when compared to the control. Eid *et al*.,(2008) had earlier reported lack of significant ($p>0.05$) differences in the body weight of laying hens induced with dexamethasone oxidative stress and lipid peroxidation by dietary vitamin E supplementation. The present result agrees with Fowles *et.al*, (1993), Welberg and

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Seckl (2001), and Maccari *et al*, (2003) who reported that dexamethasone (doses ranging from 0.2 to 0.4mg/kg body weight) was used as an immune suppressive agent and as a mediator of prenatal stress and used to induce oxidative stress in laying hens (El-habbaket etal.,2005), and in cockerels (Eid *et al*., 2006b).

This results are also in accordance with the earlier submissions of Pourreza and Edriss (1992) who stated that stress is very disruptive for broiler chickens and reduces feed intake, weight gain, carcass weights, feed intake, feed conversion ratio, abdominal fat and increases mortality. Similarly, Mezes (1999) studied the effect of anti-oxidant vitamins in poultry nutrition and showed that it served as a biological anti-oxidant which plays an important protective role against the negative effect of heat stress and resulted in better feed conversion ratios. The stress triggered by dexamethasone injection affected water intake among the birds although not significantly. Increased values recorded in the water intake observed in T_3 and T_4 could be to reduce the effect of stress by cooling the body temperature and reducing feed intake to maintain homeostasis. Therefore, it can be said that, the stress induced by dexamethasone was maintained by the broiler birds through the addition of selenium to their drinking water.

Haematological Parameters:

The results of the haematological indices of broiler chickens induced with dexamethasone oxidative stress on dietary selenium suppression is shown in Table 2. Results indicate lack of significant ($p>0.05$) differences in the packed cell volume (PCV), haemoglobin concentration (HBC), white blood cell (WBC), red blood cell (RBC), mean corpuscular haemoglobin(MCH) and mean corpuscular haemoglobin concentration (MCHC).

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Table 2: Effect of dietary selenium suppression using dexamathasone induced oxidative stress on haematological indices of broiler chicks.

Parameters	T_1	T_2	T_3	T_4	SEM
Packed cell volume(PCV)	26.67	27.00	27.67	25.67	l.00
Haemoglobin concentration	8.87	9.03	9.20	8.57	0.34
White blood cell (WBC)	19.73	22.9	23.77	18.93	1.82
Red blood cell (RBC)	2.87	2.83	2.97	2.63	0.24
Mean corpuscular volume (MCV)	136.30	161.03	162.63	139.53	8.36
Mean corpuscular Haemoglobin	30.87	33.60	31.07	33.13	3.68
Mean corpscular Haemoglobin concentration (MCHC)	33.23	33.47	33.23	33.40	0.06

S.E.M: Standard error of means.

These values corresponded to the range (24.9-45.2%) identified as normal range of PVC for birds; 8.57-9.20g/dl which is between the range identified as optimal (7-8g/dl) haemoglobin (Hb) for chicken, 9.20-31.00 x 10^{-3}/µl) for white blood cells as identified by Mitruka and Rawnsley (1977) as normal for chickens. The range from RBC from the experiment is between 2.63-2.97 x 10^6/µl) while the outlined normal range is between 1.58-4.10x10^6/µl. The mean corpuscular volume (MCV) of the experiment ranged between 136.30-162.62ul; while the outlined normal range is between 100-120ul; mean corpuscular haemoglobin concentration from the experiment ranged between 33.23-33.47 while the normal for chicken ranged between 19-36g/dl. All the haematological indices observed were within the optimal ranges outlined by Mitruka and Rawnsley (1977) as normal for chickens. The implication of this is that the oxidative stress induced on the birds under the influence of dexamethasone had no side effect on the health status of the birds owing to the suppressive effects of selenium inclusion in the diet.

Serum Biochemistry and Mortality Parameters:

The data on the serum biochemical indices and mortality of the broiler chickens are presented in Table 3. Results showed lack of significant ($p>0.05$) differences among the treatments. The

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glucose, total protein, albumin, total cholesterol, urea, creatinine and uric acid were statistically similar in spite of the treatments.

Table 3: Effect of Dietary Selenium Suppression using dexamethasone induced oxidative stress on serum biochemical parameters of broiler chickens.

Parameters	T_1	T_2	T_3	T_4	SEM
Glucose (mg/dl)	175.33	170.67	184.00	189.33	14.18
Total Protein (g/dl)	6.67	6.40	6.77	7.10	0.17
Albumin (g/dl)	3.33	3.13	3.73	3.63	0.68
Total Cholesterol (mg/dl)	179.67	177.00	168.67	186.33	9.39
Urea (mg/dl)	13.17	12.77	12.20	11.97	0.41
Creatine (mg/dl)	0.40	0.45	0.45	0.39	0.04
Uric acid (mg/dl)	4.00	3.80	3.90	4.20	0.40
Mortality	13.33^a	13.33^a	6.67^b	6.67^b	0.67

Despite the lack of significant differences ($P>0.05$) among the parameters monitored, result of the experiment showed that, there was increment in the glucose level as the level of the treatment decreased. Treatment 4 that received the highest dosage (0.9mg/kg) had the highest level of glucose 189.33mg/dl where as treatment2 (0.3mg/kg) had the least Glucose of 170.67mg/dl. Similar trend was observed in the protein (TP), albumen, total cholesterol and urea content of the birds. Urea is a function of protein quality and high urea level depicts low protein quality feed. The low level of urea (12.20 and 11,97mg/dl) observed in T_2, T_3 and T_4 respectively compared to the control (T_1) that had 13.17mg/dl could be a good indication of protein utilization from the feed inspite of the stress factor. The contract was observed in the value of creatinine where T_1 and T_4 had lower values and higher values for T_2 and T_3 respectively. T_4 recorded the highest uric acid (4.20mg/dl) while T_2 recorded the least (3.80mg/dl) compared to the control. This means that there was no joint inflammation and pains-a characteristic of gout and free kidney stones that could damage the kidney. Mortality was higher in the control birds and T_2 and were statistically ($P<0.05$) different from T_3 and T4 respectively.

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CONCLUSION

Intramuscular injection of dexamethasone to induce oxidative stress and supplementations of selenium to control/suppress the stress showed negative effect in the growth performance of the birds, particularly as the doses increased from 0.3-0.9mg/kg body weight. The dexamethasone induced stress had no adverse effect on the haematology and biochemical indices of the broiler birds, since all values observed were within the range of optimum for broiler chickens. Mortalities recorded also favored the control diet and diet2 that had the lowest level of dexamethasone, suggesting that the higher the level of selenium supplementation, the higher the immune status of the broiler birds. It is therefore concluded that selenium supplementation in the diets of stressed broiler birds can help to mitigate the stress factors for improved performance and reduced mortalities.

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