



Haematoserological and Hormonal Responses of Barred Plymouth Rock Cocks Fed Monosodium Glutamate

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ABSTRACT

In a study that investigated the effects of monosodium glutamate on the hormonal profiles, haematological and serum biochemical indices, 300 sexually mature barred Plymouth rock cocks were randomly divided into six experimental diets containing MSG at 0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 g/kg diet. Each group was replicated five times with ten birds per replicate. The birds were fed *ad libitum* and clean water was provided regularly for a period of 16 weeks. Serum protein, hormones, electrolyte balance, metabolites, lipid profiles and enzymes were evaluated. Inclusion at 0.75 g/kg diet and above significantly ($P < 0.05$) influenced the serum proteins, enzymes, lipids, Na^+ concentration, metabolites and antioxidant status of the cocks adversely. There was significant ($P < 0.05$) elevation of the serum corticosterone concentration while there was a significant ($P < 0.05$) decrease in other growth and reproductive hormonal responses at the same level of inclusion. It can be concluded that inclusion of dietary MSG up to 0.50 g/kg could be utilized in cocks' diets to enhance palatability without any deleterious effect on the birds and their products.

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Introduction

Cocks have the potential of bridging the animal protein deficit gap in the nutrition of most developing countries. Their ability to utilize the non-conventional feed stuffs and convert such to meat is unparalleled. In sub-Saharan African countries where the cost of feeding broiler chickens to reach table size is beyond the reach of most citizens, especially, those in the rural settlements, cocks which can survive on non-conventional raw materials are fast becoming household means of providing animal protein in their diets (Olarotimi et al., 2018). There is, however, a major constraint to optimal utilization of these locally available and accessible feed resources which is lack palatability of most of the highly nutritious feed resources. The inclusion of phytochemical compounds in poultry feed, for instance, had been reported reduce palatability of diet due to pungent odour, which depress the feed intake (Windisch et al., 2008; Brenes and Roura, 2010).

Enhancing the palatability of such nutritious and easily accessible feed stuffs using feed additives will be a novel approach to enhance the performances and well-being of

cocks in these countries. Flavouring agents are supplements added to the ration to enhance feed intake as they improve the palatability and in turn, acceptability of feeds (Jay et al., 2010). Monosodium glutamate (MSG) which has been adjudged safe for human consumption with no specified average daily intake (Samuels, 1999) could be a useful taste enhancer in cock diets production. In some other quarters, MSG has been reported to alter the behavior and to cause suppressed blood count in male albino rat as well as reduced haemoglobin percentage (Hb), red blood cells (RBC) and white blood cell count (WBC) in female Swiss albino mice (Gasem, 2016). Though several reports have equally stressed the effects of MSG on biochemical components of blood, especially in rat models, there is dearth of useful information on its effects on serum enzymes, metabolites, proteins, hormones and cholesterol contents as far as domestic cocks are concerned. A significant increase in serum transaminases, total plasma cholesterol and low-density lipoprotein cholesterol at a higher dose of 1.0 g of MSG was previously reported

(Okediran et al., 2014). Therefore, studying the effects of MSG on the performance and blood profiles of cocks will equip the farmers with the baseline information on how to enhance the palatability of the feeds given to their chickens. Therefore, this study aims to determine the possible effects of MSG inclusion on the performance, haematological, serum biochemical and hormonal responses of cocks fed varied levels of dietary MSG.

Materials and Methods

Experimental Design and Animals

A total of three hundred (300) sexually matured barred Plymouth Rock cocks of twenty (20) weeks of age were purchased from a reliable farm in Nigeria. They were caged for four (4) weeks, before the onset of the experiment, for stabilization process. They were fed a commercial grower ration throughout the stabilization period with fresh and cool water given *ad libitum*. At the end of the stabilization period, the cocks were weighed and randomly allotted to the six (6) treatment groups (Table 1): A, B, C, D, E and F containing 0.00 (control), 0.25, 0.50, 0.75, 1.00 and 1.25 g MSG/kg diet respectively. Each treatment was replicated 5 times with 10 cocks per replicate in a completely randomized design. The experimental diets were given according to body weight twice daily and drinking water was also provided *ad libitum* throughout the sixteen weeks (16) period of the experiment. All required managerial practices such as strict bio-security measures were ensured as at and when due, appropriate vaccines and prophylactic treatments were administered. The birds were housed in an

open sided building in a thoroughly cleaned, washed and disinfected three tier cage system of 32 x 38 x 42 cm dimension. Two (2) birds were conveniently housed in a unit at the Poultry Unit of the livestock section of Teaching and Research Farm, The Federal University of Technology, Akure. The geographical coordinates of the location are between 7° 17' North and 5° 9' East with the rainfall of about 1524 mm per year and atmospheric temperature range of 28 °C to 31 °C and mean annual relative humidity of about 80% (Adu et al., 2017). The study was undertaken with approval from the institutional ethics committee for care and use of animal for research of the host institution. It was also conducted in accordance with the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750).

Blood Sampling

At the end of the experiment, five (5) birds per replicate were randomly selected and fasted overnight. Blood samples were collected from the jugular veins of the fasted birds into both heparinized tubes for determination of haematological parameters and dry clean plain centrifuged glass tubes to prepare the serum for determination of serum biochemical indices. Blood samples for serum analyses were left for 15 min at room temperature, and then the tubes were centrifuged for 10 minutes at 3000 rpm to obtain clean supernatant serum. The serum samples collected were kept frozen at -20°C until the determination of serum hormones, enzymes, metabolites, lipids, and proteins.

Table 1. Ingredient composition of the experimental cock diets

Ingredients (kg)	Inclusion Level of MSG					F
	A	B	C	D	E	
Maize	39	39	39	39	39	39
Groundnut cake	4	4	4	4	4	4
Soya Bean Meal	0.7	0.7	0.7	0.7	0.7	0.7
Bone Meal	1.5	1.5	1.5	1.5	1.5	1.5
Limestone	1.1	1.1	1.1	1.1	1.1	1.1
Salt	0.3	0.3	0.3	0.3	0.3	0.3
MSG	0	0.025	0.05	0.075	0.1	0.125
Wheat Bran	44	44	44	44	44	44
Palm Kernel Cake	9	9	9	9	9	9
Lysine	0.08	0.08	0.08	0.08	0.08	0.08
Methionine	0.07	0.07	0.07	0.07	0.07	0.07
Layer Premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100.025	100.05	100.075	100.1	100.125
Calculated Nutrients						
ME (Kcal/Kg)	2481.53	2481.53	2481.53	2481.53	2481.53	2481.53
Crude Protein (%)	14.81	14.81	14.81	14.81	14.81	14.81
Calcium (%)	1.02	1.02	1.02	1.02	1.02	1.02
Phosphorus (%)	0.42	0.42	0.42	0.42	0.42	0.42
Lysine	0.91	0.91	0.91	0.91	0.91	0.91
Methionine	0.33	0.33	0.33	0.33	0.33	0.33
Crude Fibre (%)	5.53	5.53	5.53	5.53	5.53	5.53

*Composition of premix: 2.5 kg of premix contains: Vit. A (10000000 iu), Vit. D3 (2500000 iu), Vit. E (12000 iu), Vit. B1 (2000 mg), Niacin (15000 mg), Vit.B6 (1500 mg), Vit.B12 (10 mg), Vit. K3 (2000 mg), Biotin (20 mg), Folic Acid (600 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (150000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10 mg), Zinc (60000 mg), Selenium (150 mg), Iodine (1000 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g)

Hematological Parameters Measurements

Packed cell volume (PCV, %) or haematocrit (Hct, %) was determined by the micro haematocrit method, haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan, 1958) while red blood cell (RBC, cells $\times 10^6 \text{ml}^{-1}$) and white blood cell counts (cells $\times 10^3 \text{ml}^{-1}$) were determined using a haemocytometer with the improved Neubauer slide (Douglas and Harold 2004). Mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin (MCH, pg) and mean corpuscular hemoglobin concentration (MCHC, g/dl) were calculated from PCV, RBC, and Hb using equations from Tazawa et al. (2011). Leucocyte differential counts (heterophils, lymphocytes, eosinophils, basophils, and monocytes) were carried out on blood smears stained with May-Grunwald-Giemsa stain. Erythrocyte sedimentation rate (ESR) was determined by the Westergren method (Ritchie et al., 1994) from blood diluted with 4 parts citrate solution (3.3% sodium citrate) allowed to stand in Westergren tube.

Blood viscosity Determination

Blood viscosity (BV) was determined using the remaining blood after haematological measurements. It was well stirred to prevent sedimentation and 0.5 mL of blood was placed into the sample cup of a Wells-Brookfield Viscometer (Model LVTD-CP, Middleboro, MA, USA) and maintained at $38.0 \pm 0.1^\circ \text{C}$ with an Isotemp 1006S re-circulating water bath (Fisher Scientific, Waltham, MA, USA).

Serum Proteins, Metabolites, and Enzymes Analyses

The serum total protein (TP) was determined by biuret method, globulin (GLB) was determined by bromocresol green method as described by Tietz (1995) and albumin (ALB) was calculated as the difference between the TP and ALB while creatinine, bilirubin, and urea were estimated by deproteinization and Urease-Berhelot colorimetric methods using a commercial kit (Randox Laboratories Ltd, U.K). The serum enzymes: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were obtained using auto analyzing test kits from Randox Laboratories, Crumlin, UK. The results were expressed as mg/dl.

Serum Lipids Analyses

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were assayed by standard enzymatic endpoint method (Roschlan et al., 1974), precipitation method (NCEP, 2001) and colorimetric method (Tietz, 1995) coupled with spectrophotometry using commercially available assay kit supplied by Randox Laboratories, Crumlin, UK. The serum very-low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were estimated by employing the formula postulated by Friedewald et al. (1972): $VLDL-C = TG/5$. The LDL-C was estimated as the difference between TC and the sum of VLDL-C and HDL-C. $LDL-C = TC - (VLDL-C + HDL-C)$. The results were expressed as mg/dl.

Serum Hormonal Assay

Serum insulin, corticosterone (CS), insulin-like growth factor (IGF), growth hormone (GH), triiodothyronine (T3) and thyroxine (T4) concentrations were determined by double-antibody RIA using commercially available RIA kits (China Institute of Atomic Energy, Beijing, China) as described by (Darras et al., 1992).

Serum Electrolytes Measurements

Serum electrolytes (Na^+ , K^+ and Cl^-) were analyzed by auto analyzer (Kodak Ektachem; Eastman Kodak Company, Rochester, New York).

Sodium Ion (Na^+)

The serum Na^+ concentration was evaluated as described by Terri and Sesin (1958). Sodium ion was calculated using the following formula:

$$\text{Na}^+ \left(\frac{\text{mEq}}{\text{L}} \right) = \frac{\text{Abs. blank} - \text{Abs. S}}{\text{Abs. blank} - \text{Abs. Std}} \times \text{Conc. Std.}$$

Abs = Absorbance

S = Sample

STD = Standard

Potassium Ion (K^+)

The serum K^+ concentration was determined using the method Terri and Sesin (1958).

$$\text{K}^+ \left(\frac{\text{mEq}}{\text{L}} \right) = \frac{\text{AS} \times \text{Conc. of Standard}}{\text{Absorbance of Standard}}$$

AS = Absorbance of Sample

Potassium Standard: Equivalent to 4 mEq/L

Chloride Ion (Cl^-)

The serum Cl^- concentration was evaluated by the method described by Skeggs and Hochstrasser (1964).

$$\text{Cl}^- \left(\frac{\text{mEq}}{\text{L}} \right) = \frac{\text{AS} \times \text{Conc. of Standard}}{\text{Absorbance of Standard}}$$

AS = Absorbance of Sample

Absorbance of Standard

Chloride Calibrator: Sodium Chloride: 100mEq/L.

Antioxidant Status Indicators Measurement Malondialdehyde (MDA)

The determination of the serum MDA was done by thiobarbituric acid (TBA) assay method as described by Baliga et al. (2018). The absorbance is determined as:

$$\text{MDA} \left(\frac{\text{nmol}}{\text{mL}} \right) = \frac{\text{Abs. Sample} - \text{Abs. Control}}{\text{Abs. Standard} - \text{Abs. Blank}} \times \text{CST}$$

CST = Concentration of standard (nmol/mL)

Glutathione Peroxidase

The serum glutathione peroxidase enzyme activity was measured using the method described by Flohe and Gunzler (1984). GSH- P_x concentration was calculated as:

U/l of Haemolysate (The haemolysate was prepared by adding equal volumes of the reagent into normal-saline-washed packed red cells and mixing for five minutes) = $8412 \times \Delta A_{340} \text{ nm} / \text{minute}$

Superoxide Dismutase

The serum superoxide dismutase (SOD) activity was determined as highlighted by Oyanagui (1984).

$$\text{SOD content } \left(\frac{\text{nmol}}{\text{mL}} \right) = \frac{A_1 - A_2}{3}$$

Total Antioxidant Concentration

The serum total antioxidant concentration was determined using colorimetric method as described by Lussignoli et al. (1999).

Total Antioxidant Conc. was calculated as:

$$\text{Factor} = \frac{\text{conc of standard}}{(\Delta A \text{ blank} - \Delta A \text{ standard})}$$

$$\text{mmol/l} = \text{Factor} \times (\Delta A \text{ Blank} - \Delta A \text{ Sample})$$

Statistical Analysis

All experimental data obtained were subjected to One-Way Analysis of Variance (ANOVA) using GraphPad Prism, software version 6.01 (2012). Significant differences between the treatment means were compared using the Tukey's Honestly Significant Difference (HSD) option of the same software at 5% level of significance.

Results

Haematology

The haematological responses of the cocks fed diets containing various inclusion levels of MSG are as shown in Tables 2. All the studied haematological parameters were not significantly ($P > 0.05$) different except the erythrocyte sedimentation rate (ESR) which was significantly ($P < 0.05$) highest among the birds on 1.25 g

MSG/kg diet. Though the cocks fed 1.00 g MSG/kg diet recorded the highest PCV, RBC and Hb values, there was no significant difference ($P > 0.05$) when compared with cocks on other diets. While those fed 0.25 g MSG /kg diet recorded the highest non significant ($P > 0.05$) values for MCH and MCV when compared with others, cocks on all treatments had statistically ($P > 0.05$) similar value for MCHC. For the WBC differentials, the heterophils, eosinophils, basophils and lymphocytes values across the treatment groups were not significantly ($P > 0.05$) different. However, the cocks on 1.25 g MSG/kg diet recorded highest value for heterophils while those on 0.75, 0.05 and 1.00 g MSG/kg diets recorded the highest means for basophils, lymphocytes and eosinophils respectively. For monocytes, significant differences ($P < 0.05$) were observed when the means of birds on all other diets were compared with those on 1.25 g MSG/kg diet which also recorded the highest significant ($P < 0.05$) value. Furthermore, cocks fed 1.00 g MSG/kg diet recorded the highest non-significant ($P > 0.05$) values for both whole blood and blood plasma viscosities while those on 1.25 g MSG/kg diet had the highest non-significant ($P > 0.05$) blood serum viscosity. On the other hand, while the cocks on 0.50 g MSG/kg diet had the lowest whole blood and blood serum viscosities, those on 1.25 g MSG/kg diet recorded the least value with non-significant differences ($P > 0.05$) observed among the treatment levels.

Serum Proteins

The results of the serum biochemical indices and antioxidant status with electrolyte balance are presented in Table 3 and 4, respectively. It was observed that the cocks on Diet A (control) recorded the highest significant ($P < 0.05$) value for albumin (ALB) while the birds on 0.25 to 0.50 g MSG/kg diet showed statistical similarities ($P > 0.05$) in serum ALB levels with those on the control diet. It was further discovered that MSG inclusion rate at 0.75 to 1.25 g/kg diet significantly ($P < 0.05$) reduced the serum albumin level with the least value recorded by cocks on 1.25 g MSG/kg diet.

Table 2. Haematological Response of Cocks Fed Diets with Different Levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
PCV (%)	31.30±0.73	31.70±1.01	32.00±1.04	31.30±0.33	32.70±0.93	31.30±0.44	0.8114 ^{ns}
RBC ($\times 10^6 \text{ mm}^3$)	2.91±1.54	2.51±1.11	2.89±0.68	2.76±0.96	2.97±2.95	2.91±0.66	0.3128 ^{ns}
MCHC (g/dl)	33.30±0.04	33.30±0.04	33.30±0.03	33.30±0.02	33.30±0.03	33.30±0.02	0.3569 ^{ns}
MCV (fl)	91.10±0.88	92.70±0.34	91.10±0.39	91.50±0.49	92.20±1.54	90.80±0.09	0.4864 ^{ns}
MCH (pg)	37.11±0.29	42.04±0.12	37.00±0.13	38.33±0.17	40.17±0.51	36.09±0.03	0.4890 ^{ns}
Hb (g/dl)	10.40±0.24	10.60±0.34	10.70±0.35	10.40±0.11	10.90±0.31	10.40±0.15	0.8101 ^{ns}
ESR (mm) ¹	2.31±0.19 ^{bc}	2.79±0.25 ^b	2.05±0.26 ^c	1.86±0.13 ^c	3.09±0.27 ^{ab}	3.43±0.29 ^a	0.0132 [*]
Heterophils (%)	23.70±2.74	24.00±4.58	27.00±2.36	24.00±2.36	24.00±4.01	29.00±4.09	0.6611 ^{ns}
Eosinophils (%)	2.00±0.29	2.00±0.50	1.67±0.17	2.33±0.17	2.67±0.33	1.67±0.33	0.2104 ^{ns}
Basophils (%)	2.64±0.17	2.33±0.17	2.33±0.17	2.67±0.17	2.33±0.17	2.33±0.17	0.3905 ^{ns}
Lymphocytes (%)	71.70±3.03	70.00±4.62	77.30±2.33	68.00±2.52	70.70±4.70	73.00±5.350	0.6565 ^{ns}
Monocytes (%) ²	3.00±0.29 ^{bc}	3.33±0.33 ^{bc}	2.67±0.33 ^c	4.00±0.58 ^b	2.00±0.00 ^d	5.00±0.76 ^a	0.0005 [*]
Whole	1.94±0.04	1.95±0.05	1.91±0.04	1.94±0.04	1.97±0.03	1.96±0.04	0.6891 ^{ns}
Serum	1.33±0.04	1.35±0.03	1.33±0.02	1.33±0.03	1.35±0.04	1.36±0.04	0.5322 ^{ns}
Plasma	1.05±0.02	1.06±0.03	1.02±0.04	1.06±0.01	1.07±0.03	1.0±0.06	0.1689 ^{ns}

¹WBC Differentials, ²Blood Viscosity (mPa. s), Values are means ± SEM; Means in a row without common superscripts are significantly ($P < 0.05$) different. Level of significance = ns (not significant) = $P > 0.05$; * = $P < 0.05$. PCV = Packed Cell Volume, RBC = Red Blood Cells, MCHC = Mean Corpuscular Haemoglobin Concentration, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, Hb = Haemoglobin, ESR = Erythrocyte Sedimentation Rate, WBC = White Blood Cells, mPa .s = one millipascal-second, MSG levels in g/kg diet.

Table 3. Serum Biochemistry of Cocks Fed Diets with Different Levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
SP	16.21±0.07 ^a	14.95±0.37 ^{ab}	15.22±0.39 ^{ab}	9.91±0.55 ^b	9.57±0.37 ^{bc}	8.83±0.10 ^c	0.0107 [*]
GL	14.63±0.00 ^a	14.93±0.01 ^a	13.88±0.02 ^{ab}	8.62±0.00 ^c	8.59±0.01 ^c	7.46±0.01 ^d	<0.0001 [*]
TP	30.84±0.04 ^a	29.88±0.03 ^a	29.10±0.03 ^a	18.53±0.01 ^b	18.16±0.01 ^b	16.29±0.11 ^c	<0.0001 [*]
ALP	136.75±0.24 ^c	136.36±0.20 ^c	137.81±0.29 ^c	141.57±0.22 ^b	157.81±0.29 ^a	151.30±0.49 ^{ab}	<0.0001 [*]
AST	44.21±0.14 ^c	45.83±0.09 ^{bc}	46.08±0.06 ^{bc}	52.04±0.29 ^b	56.70±0.25 ^{ab}	56.99±0.01 ^a	0.0005 [*]
ALT	15.65±0.03 ^c	16.04±0.03 ^c	15.91±0.01 ^c	17.74±0.01 ^{bc}	27.57±0.03 ^b	30±0.15 ^a	<0.0001 [*]
TC	147.20±0.12 ^a	141.31±0.09 ^{ab}	139.52±0.10 ^{ab}	126.66±0.09 ^b	122.66±0.09 ^c	114.38±0.09 ^d	<0.0001 [*]
HDL-C	91.34±0.08 ^a	87.30±0.12 ^{ab}	86.81±0.01 ^{ab}	85.80±0.06 ^b	83.24±0.04 ^c	77.37±0.01 ^d	<0.0001 [*]
TG	94.61±0.03 ^d	94.18±0.10 ^d	94.60±0.07 ^d	119.67±0.02 ^c	122.55±0.04 ^b	129.35±0.04 ^a	<0.0001 [*]
VLDL	18.92±0.21 ^c	18.84±0.03 ^c	18.92±0.03 ^c	23.93±0.31 ^b	24.51±0.03 ^{ab}	25.87±0.06 ^a	0.0014 [*]
LDL-C	36.93±0.15 ^a	35.19±0.10 ^{ab}	33.79±0.01 ^{ab}	16.96±0.29 ^c	14.91±0.10 ^{cd}	11.15±0.03 ^d	<0.0001 [*]
CR	0.19±0.02 ^{bc}	0.17±0.02 ^c	0.25±0.01 ^{ab}	0.25±0.02 ^{ab}	0.27±0.01 ^a	0.27±0.01 ^a	0.0015 [*]
BLR	2.67±0.01 ^{bc}	2.46±0.02 ^{bc}	3.72±0.01 ^b	4.13±0.01 ^{ab}	4.19±0.02 ^{ab}	5.08±0.03 ^a	<0.0001 [*]
U	8.10±0.06 ^b	7.90±0.05 ^b	9.50±0.03 ^{ab}	9.50±0.04 ^{ab}	12.00±0.11 ^a	12.60±0.03 ^a	<0.0001 [*]

SP: Serum Proteins (g/dl) Albumin, GL: Globulin, TP: Serum Enzymes (U/L), ALT: Serum Lipids (mg/dl), TC: (mg/dl), HDL-C: (mg/dl), TG: (mg/dl), VLDL-C: (mg/dl), LDL-C: (mg/dl) Serum Metabolites (mg/dl), CR: Creatinine (mg/dl), BLR: Bilirubin (mg/dl), U: Urea (mg/dl), Values are means ± SEM, Means in a row without common superscripts are significantly (P<0.05) different. Level of significance = ns (not significant) = P>0.05; * = P<0.05, TP (Total Protein); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Alkaline phosphatase (ALP); Total Cholesterol (TC); High Density Lipoprotein Cholesterol (HDL-C); Triglyceride (TG); Very Low Density Lipoprotein Cholesterol (VLDL-C); Low Density Lipoprotein Cholesterol (LDL-C), MSG levels in g/kg diet.

Table 4. Serum Electrolytes and Antioxidant Status of the Cocks Fed Diets with Different Levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
EB	142±0.30 ^c	141±0.46 ^c	142±0.12 ^c	153±0.10 ^b	157±0.31 ^{ab}	169±0.29 ^a	< 0.0001 [*]
K ⁺ (mEq/L)	4.80±0.03 ^{ab}	4.99±0.04 ^a	5.10±0.07 ^a	5.00±0.03 ^a	4.50±0.03 ^{ab}	3.01±0.03 ^c	< 0.0001 [*]
Cl ⁻ (mEq/L)	102±0.29 ^a	100±0.30 ^{ab}	98±0.16 ^{ab}	96±0.29 ^{ab}	92±0.10 ^b	85±0.09 ^c	< 0.0001 [*]
AS	230±0.31 ^a	210±0.30 ^{ab}	210±0.02 ^{ab}	190±0.16 ^b	130±0.089 ^c	120±0.18 ^d	< 0.0001 [*]
SOD (μmol/ml)	130±0.15 ^a	130±0.29 ^a	128±0.24 ^{ab}	110±0.16 ^b	81±0.06 ^c	74±0.03 ^d	< 0.0001 [*]
T-AOC (μmol/ml)	6.90±0.02 ^a	6.20±0.04 ^{ab}	6.10±0.06 ^{ab}	4.30±0.03 ^b	2.40±0.02 ^c	1.90±0.06 ^d	0.0001 [*]
MDA (nmol/ml)	2.20±0.03 ^c	2.30±0.02 ^c	2.90±0.00 ^{bc}	4.00±0.03 ^b	4.00±0.06 ^b	4.70±0.01 ^a	0.0001 [*]

EB: Electrolytes Balance Na⁺ (mEq/L), AS: Antioxidant Status GSH-Px (μmol/ml), Values are means ± SEM, Means in a row without common superscripts are significantly (P<0.05) different. Level of significance = ns (not significant) = P>0.05; * = P<0.05, Glutathione Peroxidase (GSH-Px); Total Antioxidant Activity (T-AOC); Malondialdehyde (MDA); Superoxide Dismutase (SOD); Sodium (Na⁺); Potassium (K⁺); Chloride (Cl⁻), MSG levels in g/kg diet.

For serum globulin (GLB), the cocks on treatments 0.25 and 0.50 g MSG/kg diet showed statistical (P>0.05) similarities with those on the control diet with birds on diet B (0.25 g MSG/kg) recording the highest significant value. It was further observed that the birds on the control diet recorded the highest significant (P<0.05) value for total protein (TP) with no significant (P>0.05) difference with the means recorded by the birds on diets B and C. An inclusion level of MSG above 0.50 g/kg diet significantly (P<0.05) reduced the total protein of the treated birds. Generally, progressive significant (P<0.05) reductions were observed in serum proteins at an inclusion level from 0.75 g MSG/kg diet.

Serum Enzymes

There were significant (P<0.05) increases in the activities of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) among the cocks fed diets containing 0.75 to 1.25 g MSG/kg when compared with those on the control diet while this occurred in the activity of alanine aminotransferase (ALT) among the cocks on 1.00 and 1.25 g MSG/kg diet. The highest significant (P<0.05) means for ALT and AST were recorded among the cocks on diet containing 1.25 g MSG/kg while that of ALP was observed among the cocks on 1.00 g MSG/kg diet.

Serum Lipids

Cocks on the control diet recorded the highest significant (P<0.05) means for the total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), whereas, those on diet F (1.25 g MSG/kg) recorded the highest significant (P<0.05) means for triglyceride (TG) and very-low-density lipoprotein cholesterol (VLDL-C).

Serum Metabolites

The mean values for bilirubin, creatinine and urea were found to be significantly (P<0.05) higher among the cocks on diet containing 1.25 g MSG/kg when compared with those on other diets. The mean values for those on diets containing 0.25 and 0.50 g MSG/kg were not significantly (P>0.05) different from those on the control diet.

Serum Electrolytes Balance

It was observed that MSG inclusion levels up to 0.50 g/kg diet did not significantly (P>0.05) affect the serum Na⁺ concentration of the cocks when compared with the control group (Table 4). However, above this inclusion level, significant (P<0.05) increase were observed from 0.75 to 1.25 g MSG/kg diet inclusion levels. For serum Cl⁻ concentration, a significant (P<0.05) decrease was noted among the birds on diets containing 1.00 and 1.25 g MSG/kg

when compared with those on the control diet but for serum K^+ , there was a significant decrease among the cocks fed 1.25 g MSG/kg diet when compared with those on the control and other diets. For the three serum electrolytes studied, the birds on the control diets were discovered to record the highest significant means with a progressive decrease in response to increasing levels of MSG.

Serum Antioxidant Status

The mean values for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and total antioxidant activity (T-AOC) followed the same trend (Table 4). The cocks on the control diets recorded the highest significant ($P<0.05$) means for the three antioxidant parameters. Cocks on diets containing 0.25 and 0.50 g MSG/kg showed statistical similarities ($P>0.05$) with those on the control diets. Inclusion levels of 0.75 g MSG/kg diet and above were observed to significantly ($P<0.05$) reduced the mean values of the studied parameters. However, an inverse relationship was observed between the means of Malondialdehyde (MDA) and the other three antioxidant status parameters as a significant ($P<0.05$) increase was observed when 0.75 to 1.25 g MSG/kg diet were fed to the birds. The birds on 1.25 g MSG/kg diet recorded the highest significant ($P<0.05$) value.

Growth Hormones

It was observed that increase in inclusion level of MSG in the diets progressively increases corticosterone levels of the cocks (Table 5) with those fed diet containing 1.25 g MSG/kg recording the highest significant ($P<0.05$) value. Statistically, birds on diets containing 0.00 to 0.50 g MSG/kg have similar values while those on 0.75 and 1.00 g MSG/kg diet were also not significantly different ($P>0.05$). The highest significant ($P<0.05$) value for insulin was recorded on the diet containing 0.25 g MSG/kg. It was observed that the inclusion level from 0.50 g MSG/kg diet and above significantly ($P<0.05$) decreased the serum insulin values. The values for the diets containing 0.00 and 0.50 as well as 0.75 and 1.00 g MSG/kg, respectively, were not significantly ($P>0.05$) different. It was also observed that inclusion of MSG up to 0.50 g MSG/kg diet significantly ($P<0.05$) increases the values of serum growth hormone (GH), insulin-like growth factor (IGF) and triiodothyronine (T3) (though not significantly). Thyroxine (T4) and the T3:T4 ratio were not significantly different across all the treatment groups when compared ($P>0.05$). Any inclusion level above 0.50 g MSG/kg diet was discovered to lead to a progressive and significant ($P<0.05$) decrease in serum values of GH, IGF and T3.

Table 5. Hormonal Responses of Cocks Fed Different Levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
GC	531.00±4.10 ^c	526.00±3.88 ^c	527.00±3.18 ^c	572.00±1.32 ^b	594.00±0.73 ^b	640.00±17.30 ^a	< 0.0001*
I	3.96±0.03 ^b	4.32±0.14 ^a	4.00±0.03 ^b	3.88±0.05 ^{bc}	3.76±0.03 ^{bc}	3.64±0.02 ^c	< 0.0001*
GH	110.00±0.73 ^c	125.00±1.44 ^a	129.00±1.17 ^a	117.00±0.76 ^b	107.00±1.36 ^c	107.00±1.01 ^c	< 0.0001*
IGF	28.10±0.27 ^b	29.80±0.14 ^a	29.90±0.14 ^a	27.10±0.28 ^c	24.70±0.19 ^d	23.50±0.19 ^e	< 0.0001*
T3	1.56±0.02 ^a	1.60±0.03 ^a	1.60±0.01 ^a	1.56±0.01 ^a	1.42±0.01 ^b	1.37±0.02 ^b	< 0.0001*
T4	16.70±0.00	16.70±0.02	16.70±0.02	16.60±0.05	16.40±0.09	16.00±0.13	0.1850 ^{ns}
T3 : T4	0.09±0.01	0.10±0.02	0.10±0.01	0.09±0.01	0.09±0.00	0.09±0.01	0.0671 ^{ns}
LH	3.60±0.01 ^a	3.58±0.03 ^{ab}	3.58±0.02 ^{ab}	3.59±0.01 ^{ab}	3.58±0.01 ^{ab}	3.51±0.02 ^b	0.0002*
PR	1.52±0.05	1.48±0.00	1.48±0.04	1.48±0.03	1.48±0.03	1.48±0.01	0.0791 ^{ns}
OE	456±0.29	455±0.75	455±1.52	452±0.82	453±1.31	454±1.63	0.2322 ^{ns}
TES	24.40±0.15 ^a	23.30±0.42 ^{ab}	23.60±0.34 ^{ab}	22.90±0.20 ^b	22.80±0.20 ^b	23.00±0.29 ^b	0.0020*
FSH	4.35±0.01 ^a	4.35±0.01 ^a	4.35±0.01 ^a	4.30±0.02 ^{ab}	4.33±0.01 ^{ab}	4.28±0.02 ^{ab}	0.0049*

GC: Growth Corticosterone (ng/mL), I: Insulin (μ U/mL), GH: (ng/mL), IGF: (ng/mL), T3: (ng/mL), T4: (nmol/L), T3 : T4: Reproductive, LH: (IU/L), PR: Progesterone (ng/mL), OE: Oestrogen (ng/dL), TES: Testosterone (nmol/L), FSH: (IU/L), Values are means $\pm$ SEM; Means in a row without common superscripts are significantly ($P<0.05$) different. Level of significance = ns (not significant) = $P>0.05$; * = $P<0.05$; Luteinizing hormone (LH), Growth hormone (GH), Insulin-like growth factor (IGF), Triiodothyronine (T3), Thyroxine (T4), MSG levels in g/kg diet.

Reproductive Hormones

Luteinizing hormone (LH) was found to be significantly highest ($P<0.05$) among the cocks on the control diet but only differ significantly ($P<0.05$) when compared with those on the diet containing 1.25 g MSG/kg. The cocks on the diet containing 1.25 g MSG/kg were discovered to be significantly ($P<0.05$) lowest in value while cocks on all other diets recorded values that were statistically similar with the control. Estrogen and progesterone, also, recorded the highest values among the cocks on the control diet but they showed values that were insignificant ($P>0.05$). Testosterone among the cocks on the control diet was significantly ($P<0.05$) highest and also statistically ($P<0.05$) similar to what was obtained among the cocks on diets containing 0.25 and 0.50 g MSG/kg while MSG inclusion of 0.75 to 1.35 g MSG/kg diet significantly influenced serum testosterone concentration. For follicle-stimulating hormone (FSH), cocks on the control diet, 0.25 and 0.50 g MSG/kg diet were statistically

($P>0.05$) similar as they had the significantly ($P<0.05$) highest means. Cocks on the diets containing 0.75, 1.00 and 1.25 g MSG/kg diet recorded similar ($P>0.05$) values which were not significantly ($P>0.05$) different from what were recorded by those on the control diet, 0.25 and 0.50 g MSG/kg diets.

Discussion

Haematology

Based on the results of this research, it could be noted that MSG did not significantly influence red blood cells parameters as well as white blood cells differentials of the cocks except the monocytes and erythrocyte sedimentation rate. Therefore, the results of this study suggest that MSG at the doses administered was not hazardous to the health of the cocks since there were no significant changes in packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell (RBC), mean corpuscular haemoglobin

concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and white blood cell (WBC) differentials when MSG-treated cocks were compared with the control group. This showed that the immune status of the cocks was not compromised by any of the experimental diets. This corroborates the findings of Oyetunji (2013) which reported no significant change in all the haematological and biochemical parameters studied in Wistar rats treated with 5 to 15 mg MSG/kg body weight and Enemali et al. (2015) which treated the rats with 0.5 to 1.5 g MSG/kg diet but countered the results obtained by Zafar and Shrivastava (2017) which reported significant reduction in haemoglobin percentage, red blood cells and white blood cells in female Swiss albino mice treated high dose of MSG.

The results of the present study was also at variance with the finding of Ashaolu et al. (2011) and Ghadhban (2017) which reported that MSG had a significant effect on the neutrophil and lymphocyte counts in Wistar rats compared to the control. Neutrophils and monocytes functionally provide the defense against invading micro organism, toxic substances, and foreign substances (Hall, 2011). The reduction, though insignificant, recorded in the PCV, Hb, RBC, MCV, MCH and MCHC values as well as an elevated ESR among birds on diets 1.25 g MSG/kg were all indicative of an anemic condition. Blood viscosity is an important blood property, which plays a key role in maintaining vascular homeostasis. Increased whole blood viscosity was implicated with increased risk of morbidity and mortality of several life-threatening diseases, including cardiovascular and cerebrovascular disease (Naghedi-Baghdar et al., 2018). The results of the whole blood, plasma and serum viscosity recorded in this study further strengthens that the inclusion levels used in the diets were not hazardous to the health of the cocks.

Serum Protein

From the present study, the serum albumin (ALB), globulin (GLB) and total protein (TP) levels were significantly influenced by MSG inclusion at varied rates. Serum ALB, GLB and TP were all adversely affected at an inclusion level of 0.75 g MSG/kg diet and above. This present experiment showed that the inclusion levels of MSG up to 0.50 g/kg diet would not adversely influence the serum TP, ALB and GLB concentrations of cocks. It further indicated that interference of MSG with protein metabolism would only occur in the cocks if the inclusion level of MSG exceeds 0.50 g/kg diet. The significant decrease observed in serum TP, ALB and GLB above this tolerable level was indicative of problems in the liver or kidney (Quinlan et al., 2010). The lowest concentrations of TP, ALB and GLB recorded among the birds on the diet containing 1.25 g MSG/kg is suggestive of adverse impact of high dose of MSG on these organs. These results affirmed the reports of Oladipo et al. (2015) in that there was a decrease in the activity levels of the albumin and total protein dose-dependently in the serum. Since the liver functions primarily in the synthesis of blood proteins, the lowest concentration of TP recorded among the cocks on diet containing 1.25 g MSG/kg is indicative of disturbance of protein synthesis which resulted as a consequence of impaired hepatic function and consequently led to a decrease in their TP concentration (Okediran et al., 2014).

Serum Lipids

The decrease in the serum high density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL-C) and total cholesterol (TC) in response to increasing level of MSG observed in the present study with a significant influence among the birds fed diets containing 0.75 g MSG/kg and above was in disagreement with the findings of Alwaleedi (2016) and El Malik and Sabahelkhier (2019) who reported increase at an increasing dosage of MSG in rats for the studied parameters. However, this study agreed with Sani et al. (2015) who observed a significant decrease in both TC and LDL-C in response to increasing level of MSG. This result, however, varied with theirs on the ground that they reported a non-significant influence of MSG on serum HDL-C of rats at an elevated dosage. The recorded significant increase in the serum levels of triglyceride (TG) and very low lipoprotein cholesterol (VLDL-C) among the birds on the diets containing 0.75 g MSG/kg and above when compared with the control were consistent with the findings of Alwaleedi (2016) and El Malik and Sabahelkhier (2019). The decreased levels of serum TC, HDL-C and LDL-C in response to increasing level of MSG, were indicative of impairment of cholesterol synthesis. The recorded elevated level of serum TG and VLDL-C is suggestive of hyperlipoproteinemia and this might be due to the excessive dietary intake of fat and carbohydrates beyond the body's need as induced by excessive feed intake occasioned by the MSG containing diet. This must have led to their hepatic conversion to TG, which is later converted into VLDL-C for export to the various tissues (Nduka, 1999). With the increase in serum TG and VLDL-C levels and reducing serum HDL-C, TC and LDL-C levels, and the cocks stands the risk of atheroma development and subsequent atherosclerosis if prolonged MSG feeding above 0.50 g/kg diet is maintained.

Serum Enzymes

Hepatocellular damage had been adjudged to be responsible for the high level release of the aminotransferases into the blood stream. Therefore, the concentration of these liver enzymes is a sensitive biomarker of liver damage (Al-Mamary, 2002). A significant elevation in serum enzymes activities such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) is a pointer to alterations in liver functions (Kim et al., 2008). The present investigation revealed that there was a sign of hepatocellular damage induced by MSG among the cocks fed diets containing MSG inclusion in excess of 0.50 g/kg diet. This result was corroborated the findings of Inuwa et al. (2011) who reported hepatocellular damage in Wistar rats treated with 200 to 400 mg MSG/kg body weight as well as Onyema et al. (2006) and the reported significant elevation in the serum aminotransferases in male albino rats due to high dose of monosodium glutamate consumption (Egbonu et al., 2009).

Serum Metabolites

This study presented significant differences in the serum creatinine, bilirubin and urea levels among the MSG treated cocks when compared with those on the control diet. An elevation in the concentrations of serum creatinine

and urea which is a result of decline in the ability of the kidney to filter fluid within the body (Edwards, 1991) was evident among the cocks that received diets containing 1.00 g MSG/kg and above. This revealed there was possibility of renal damage among the cocks that were placed on diets containing above 0.50 g MSG/kg diet. Elevated levels of serum total bilirubin also indicate liver damage or disease. The statistical similarities in the total bilirubin concentrations observed among the cocks fed up to 1.00 g MSG/kg diet and the control group revealed that normal liver function was only impaired by the inclusion levels above 1.00 g MSG/kg diet.

Serum Electrolytes

Sodium ion (Na^+) regulates the total amount of water in the body and plays a major role in neuronal and nerve signaling. In the present study, an elevated blood Na^+ level was observed among the cocks on diets containing 0.75 to 1.25 g MSG/kg diet when compared with those on the control and other diet groups. The values recorded were also above the reference values of 135-145 mEq/L (Jain, 1993). This is suggestive that high dose of MSG in cocks' diets above 0.50 g/kg diet could result in hypernatremia. The results of this finding agreed with the report of Ilegbedion et al. (2013) who documented an elevation in the blood Na^+ level of Wistar rats fed high dose of MSG. A low blood protein level had been explained as a possibility for high blood sodium level (Kliegman et al., 2015). The reduction observed in serum protein levels in the present study might also contribute to the hypernatremia experienced by the treated birds.

Potassium ion (K^+) is essential for the proper functioning of the hearts, kidneys, muscles, nerves, and digestive system. This result showed that inclusion of MSG at 1.25 g/kg diet caused a significant decline in the serum K^+ concentration level when compared with those on the control and other treatment diets. The results of this finding disagreed with the report of Ilegbedion et al. (2013) who documented an elevation in the blood K^+ level of Wistar rats fed high dose of MSG.

Renal vasoconstriction and reduced medullary blood flow are linked with hypokalemia (Reungjui et al., 2008). Hypokalemia has been reported to also increase renal ammonia metabolism and can cause the development of an acid-base disorder: metabolic alkalosis (condition in which the pH of tissue is elevated beyond the normal range: 7.35–7.45) Han et al. (2011). Hence, feeding cocks treated diets above 1.00 g MSG/kg may predispose them to metabolic alkalosis.

Chloride ion (Cl^-) plays a critical role in keeping the proper balance of body fluids and maintaining the body's acid-base balance. In this study, cocks on diets containing 1.00 and 1.25 g MSG/kg recorded hypochloremia. This is indicative of defective renal tubular absorption. Vomiting, diarrhea, and metabolic acidosis can also lead to hypochloremia. Symptoms of hypochloremia are similar to those of hyponatremia and could result in general weakness.

Serum Antioxidant Status

The results obtained in the present study revealed that dietary MSG inclusion above 0.50 g/kg diet had significant effects on antioxidant and peroxide formation in cocks.

Lipid peroxidations, measured as malondialdehyde (MDA) levels, were significantly increased in response to increasing levels of MSG inclusion. There were no significant differences among the birds on 0.25 and 0.50 g MSG/kg diet and those on the control diet. However, a significant elevation in MDA value was observed among the cocks on diet containing 0.75 g MSG/kg and above. This could be attributed to the significant decrease observed in the total antioxidant activity (T-AOC) of the birds fed MSG above 0.50 g/kg diet inclusion rate. Increase in the levels of MDA favours oxidative stress while increase in T-AOC protects against free radicals and peroxides. This result agreed with the opinion that there is always an inverse relationship between lipid peroxidation and antioxidant capacity (Jimoh et al., 2018). This result also supported the claim by Bertolin et al. (2011) that MSG is a very reactive substance and induced lipid peroxidation, leading to formation of reactive substances of low molecular weight, such as MDA. Farombi and Onyema (2006) equally recorded an increased formation of MDA in the liver and brain of albino rats administered MSG intraperitoneally at 4 mg/g of body weight.

In the present study, antioxidant enzyme activity assayed revealed that superoxide dismutase (SOD) which is the first line of defense was negatively influenced by varied levels of MSG inclusion. Functionally, SOD converts superoxides to hydrogen peroxides (H_2O_2) while glutathione peroxidase (GSH-Px) converts H_2O_2 to water and gaseous oxygen (Egbunu and Ejike, 2017). The significant decrease observed in SOD activity among the birds fed 0.75 g MSG/kg diet and above confirmed increased involvement of SOD in antioxidant defense response following MSG-induced oxidative stress and this was in consonance with the position of (Manal and Nawal, 2012).

The significant depletion of GSH-Px observed among the cocks fed 0.75 g MSG/kg diet and above was indicative of its role as a second line of antioxidant defense mechanism. The decreased GSH-Px observed in this study as MSG inclusion increases was in consistent with the findings of (Egbunu and Ejike, 2017). A decrease in GSH-Px activity induced by MSG consumption had also been explained to favour lipogenesis by increasing the level of glutamine (Kushwaha and Bharti, 2015). GSH-Px uses glutathione as a substrate to catalyse the conversion of H_2O_2 to water and gaseous oxygen thereby protecting mammalian cells against oxidative stress (Singh and Ahluwalia, 2012). It is, therefore, suggestive that low activity of this enzyme may render the tissue more susceptible to lipid peroxidation damage.

Conclusions

The study revealed that higher doses of MSG above 0.50 g/kg diet caused physiological imbalance due to the adverse effects on the serum proteins, enzymes, lipids, Na^+ concentration, and antioxidant status of the birds as well as hormonal imbalance. The inclusion of 0.25 – 0.50 g MSG/kg diet enhanced improved hormonal functions such as lowered corticosterone level in the blood which reduced the stress on the bird and ensured better functioning of the reproductive hormones, growth hormone and hormones of the thyroid gland. It could also be stated that higher MSG

inclusion level above 0.50 g/kg diet had no effects on the immunity of the cocks due to non-significant effects of higher inclusions on the blood counts and white blood cell constituents of the cocks. However, the physiology of the bird could become compromised resulting in pathological conditions due to the imbalance in the serum Na⁺ concentration and antioxidant status of the birds. There is every tendency of renal and hepatotoxicity induction at higher inclusion rates of MSG above 0.50 g/kg diet as indicated by decreased liver protein concentrations, marked increase in liver enzymes and accumulation of urea, creatinine and bilirubin in the blood.

Conflicts of interest

The authors declare no conflicts of interest

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