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SEMEN CHARACTERISTICS, GONADAL AND EXTRAGONADAL SPERM RESERVES IN COCKS FED DIETS CONTAINING DIFFERENT INCLUSION LEVELS OF MONOSODIUM GLUTAMATE

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ABSTRACT

The investigation was carried out to assess the semen characteristics, gonadal and extragonadal sperm reserves of cocks fed dietary monosodium glutamate (MSG) at varied inclusion levels (0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 g.kg^{-1} diet). A total of 240 sexually matured barred Plymouth Rock cocks of twenty 24 weeks of age were used in a 16-week feeding trial. The cocks were weighed and allotted to the 6 treatment diets. Each treatment was replicated 5 times with 8 cocks per replicate in a completely randomized design (CRD). At the end of the feeding trial, 4 cocks per replicate were humanely sacrificed and their reproductive tracts were dissected. The testes and epididymides were carefully sampled, weighed and processed. The data collected were subjected to analysis of variance followed by Tukey's Honestly Significant Difference ($\alpha_{0.05}$) where significant differences occurred. The results showed that all the semen characteristics were significantly and negatively affected by inclusion of MSG above 0.50 g.kg^{-1} diet. The paired epididymides and *vas deferens* weights were significantly ($P<0.05$) reduced by the inclusion levels of MSG above 0.75 g.kg^{-1} diet, while their lengths were significantly ($P<0.05$) reduced above 0.50 g MSG.kg^{-1} diet. Nevertheless, the testicular parameters were not significantly ($P>0.05$) affected by the varying inclusion levels of MSG when compared with the control. The paired testicular sperm reserves (TSR/testis and TSR/g testis) were not significantly ($P>0.05$) influenced by the MSG inclusion when compared with the control diet. However, the paired epididymides sperm reserves were significantly lowered at the inclusion levels of 1.00 and 1.25 g MSG.kg-^{1} diet. This study suggests that dietary MSG has the potential to significantly affect the sperm characteristics and sperm reserves of cocks when administered above 0.50 g.kg^{-1} diet.

Key words: cocks; sperm reserves; characteristics; epididymis; testis; monosodium glutamate

INTRODUCTION

An important feed factor to give a deserved attention is feed palatability and acceptability. Non-palatability nature of feeds could prevent birds from consuming adequate quantity required for optimum performance. The purpose of investing so much on the feed will be totally defeated if the feeds are not acceptable, non-palatable and inadequately utilized by the animals. This may predispose the animal to feed refusal and/or feed wastage and this will confer a serious economic risk on the part of the farmers. Feed additives may be included into diets for the purpose of improving performance such as weight gain, increased laying performance, enhancing the sperm production capacity for breeding purposes, improving hatchability, preventing diseases, enhancing palatability and

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providing enhanced digestibility of the feed materials (Windisch, 2008). The additive of interest in this study to enhance feed palatability is monosodium glutamate (MSG).

Therefore, fortifying poultry feeds with taste enhancers, such as MSG, in order to increase the palatability of such feeds for better acceptability and subsequent improved productivity, such as increased body weight, improved hen day production, enhanced reproductive potentials, among others, will be highly welcomed (Olarotimi *et al*., 2019). MSG has also been reported to have a toxic effect on the testis by causing a significant oligozoospermia and increase in abnormal sperm morphology in a dose-dependent fashion in male Wistar rats (Onakewhor *et al.*, 1998). It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Oforofuo *et al.*, 1997). Another study also reported that there were no gross morphological changes on the physical examination of testes of Wistar rats administered 250 mg – 2 $g.ml^{-1}$ MSG solution with only slight variations observed in the sperm concentration of all the treatment groups (Kianifard, 2016). Progressive motility of sperm reduced equally with all the treatment groups having fair forward directional movement, compared to the control, which had an excellent forward directional movement (Kianifard, 2016). Since the potential of MSG as useful and cheaper taste enhancer both for animal and human feeding has been previously emphasized, this study, therefore, aims to evaluate the possible effects it may confer on cocks' sperm characteristics, gonadal and extragonadal sperm reserves, which are traits used in assessing good quality sperm for breeding purposes.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Poultry Unit, Teaching and Research Farm, the Federal University of Technology Akure, Nigeria. The geographical coordinates of the location are between 7° 17' North and 5° 9' East (Mapzoom, 2015). The climatic condition of Akure follows the pattern of southwest Nigeria where the climate is influenced mainly by the rain-bearing southwest monsoon winds from the ocean and the dry northwest winds from the Sahara desert. The rainy season lasts for about seven months (April to October). The rainfall is about 1524 mm per year. The atmospheric temperature ranges between 28 °C and 31 °C and mean annual relative humidity of about 80 % (Ajibefun, 2011). It was conducted in accordance with the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750).

Experimental design and diet

Six (6) experimental diets (Table 1) were formulated with varying inclusion levels (0.00, 0.25, 0.5, 0.75, 1.00 and 1.25 $g.kg^{-1}$) of MSG (Ajinomoto brand) sourced from the open market. The diets met the nutrient requirements of adult cocks according to NRC (1994) recommendations. The proximate analyses of the diet samples were carried out with the use of a Near-Infra Red Spectrophotometer. The metabolizable energy (ME) of the feed samples was calculated using the prediction equation by Pauzenga (1985) as follows:

ME = (37 x CP + 81.8 x EE + 35.5 x NFE).

Where:

CP = crude protein, EE = ether extract, NFE = nitrogen free extract

Experimental cocks and management

A total of 240 sexually matured barred Plymouth Rock cocks of twenty-four (24) weeks of age were used for the study. They were caged for two weeks before the onset of the experiment for stabilization. They were fed 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 experimental treatments. Each treatment was replicated 5 times with 8 cocks per replicate in a completely randomized design. The experimental diets were given according to the body weight twice daily and drinking water was also provided *ad libitum* throughout the sixteen weeks 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

Table 1. Composition of the cocks' experimental diets

	Diets with MSG inclusion (g.kg^{-1})					
Ingredients (kg)	T1	T2	T3	T4	T5	T6
Maize	330	330	330	330	330	330
Soya Bean Meal	28	28	28	28	28	28
Ground Nut Cake	35	35	35	35	35	35
Corn Bran	110	110	110	110	110	110
Palm Kernel Meal	160	160	160	160	160	160
Wheat Offal	300	300	300	300	300	300
Limestone	15	14.75	14.5	14.25	14	13.75
Bone Meal	14	14	14	14	14	14
Lysine	1	1	1	1	1	1
Methionine	1	1	1	1	1	1
Salt	3.5	3.5	3.5	3.5	3.5	3.5
Layer Premix	2.5	2.5	2.5	2.5	2.5	2.5
MSG	0	0.25	0.5	0.75	1	1.25
Total	1000	1000	1000	1000	1000	1000
ANALYSED VALUES						
Crude protein (%)	15.15	15.2	15.19	15.23	15.21	15.11
ME (kcal.kg^{-1})	2520.73	2516.96	2520.37	2516.17	2513.69	2519.8
Ca (%)	1.3	1.26	1.18	1.23	1.31	1.19
Crude fibre (%)	9.45	10.19	10.08	10.22	9.98	10.01
Moistiure (%)	11.5	10.88	11.01	10.89	10.99	11.47
Crude fat (%)	2.19	2.13	2.25	2.02	2.22	2.34
NFE (%)	50.17	50.15	49.98	50.35	49.84	49.84
Crude ash (%)	11.54	11.45	11.49	11.29	11.76	11.23

NFE = Nitrogen Free Extract; ME = Metabolizable Energy * Composition of premix (Nutrivitas®): 2.5 kg of premix contains: Vit. A (10,000,000 iu), Vit. D3 (2,500,000 iu), Vit. E (12,000 iu), Vit. B1 (2000 mg), Niacin (25000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2500 mg), Biotin (75 mg), Folic Acid (2000 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (50 %) (200000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10,000 mg), Zinc (60000mg), Selenium (200 mg), Iodine (1500 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Cobalt (250 mg)

in a unit. At the end of the feeding trial, four (4) cocks per replicate were selected and fasted overnight for semen evaluation.

Semen Collection

Semen was harvested from the already trained cocks selected before sacrificing for evaluation. The collection of semen was done between 6 to 8 am. The cocks were handled with care to avoid fright and undue physical stress. Semen was collected by the manual massage technique, as described by Udeh *et al.* (2011). Labeled plain sample tubes were used for the collection of the semen.

Semen Evaluation

Each semen sample was evaluated for volume, gross motility, live-dead count and concentration within 6 min after collection as described by Ewuola and Akinyemi (2017). Semen volume was determined by drawing the semen with a graduated tuberculin syringe of 1.0 ml capacity and reading directly to the nearest 0.01 ml. Progressive motility was evaluated by diluting a drop of semen with two drops of normal saline on a clean pre-warmed (37 °C) glass slide and covered with a clean coverslip. The observation was done under a microscope at x 40 magnification and scored 0 – 100 % with 0 representing no progressive motility.

A dilution of 0.1 ml of the ejaculate with 5 ml normal saline was prepared in a clean test tube for the evaluation of the sperm concentration. Sperm concentration was evaluated using a Neubauer Haemocytometer and a binocular microscope (Olympus CH-2 CHS Binocular Microscope, Olympus

Corporation, Japan) at 100 x magnification, as described by Ewuola and Egbunike (2010). The mass activity was estimated as described by Ogunlade (2015). A drop of raw undiluted semen was examined on pre-warmed slides under a microscope at 10 x magnification. The mass activity was scored subjectively according to the intensity of the wave motion, from the absence of wave motion (0) to slow motion (+), rapid motion (+ +) or turbulent motion (+ + +) characterized by the appearance of dark prominent wave in a rapid motion. The ratio of live sperm cells to dead sperm cells was evaluated by eosin-nigrosin vital staining technique. A drop of the ejaculate was placed onto a clean pre-warmed glass slide and two drops of eosin-nigrosin stain were added. This was mixed and a smear was prepared from the mixture and then viewed under a microscope at × 40 magnification. Dead sperm cells absorbed the stain while live sperm cells repelled it. The dead sperm percentage was obtained by dividing the number of dead cells in a field by the total number of sperm cells counted in the same field multiplied by 100. Percentages of live sperm cells (Liveability/Viability) were obtained by subtracting the value of the dead cell percentage from 100. From the values obtained above, the following characteristics were determined:

Sperm Concentration (Sperm Cells.ml^{-1}) = N x C x D (Maina *et al.*, 2006).

Where:
N = number of sperm cells counted; C = constant = 52000 (Ewuola *et al.*, 2014); D = dilution factor = vol. normal saline/volume of semen

$$\text{Liveability} = \frac{\text{Total number of counted cells} - \text{Dead cells} \times 100}{\text{Total number of counted cells}}$$

$$\text{Total sperm cells/Ejaculate} = \text{Sperm concentration/ml} \times \text{Volume of ejaculate}$$

$$\text{Total live sperm/ml} = \frac{\text{Liveability} \times \text{Sperm concentration/ml}}{100}$$

$$\text{Total motile sperm cells/ml} = \frac{\text{Motility} \times \text{Sperm concentration/ml}}{100}$$

Assessment of Gonadal and Extragonadal Morphometric Indices

After semen collection, the cocks were humanely sacrificed through cervical dislocation and eviscerated for a gross examination of organs *in situ*. The reproductive tracts of the slaughtered cocks were carefully harvested. Testicular weights were recorded using a highly sensitive weighing balance in the laboratory. The testes and epididymides were separated free of adhering connective tissues and fats. The left and right testes and epididymides were measured separately and their weights recorded. The volumes of the testes were measured volumetrically using Archimede's principle of water displacement in a measuring cylinder, as described by Olarotimi *et al.* (2015) and the result were recorded. The testes densities were calculated from the testicular weights and volumes and expressed as g.ml^{-1} (Olarotimi *et al.*, 2015).

$$\text{Testis density} = \frac{\text{Testis weight (g)}}{\text{Testis volume (ml)}}$$

Estimation of gonadal sperm reserves

The reproductive organs were carefully dissected; the testes and epididymides were carefully collected, trimmed off adhering tissues and weighed using a sensitive electronic balance. Testicular and epididymal morphometric characteristics were determined. Gonadal/testicular spermatozoa reserves (GSR/TSR) were determined haemocytometrically as described by Orlu and Egbunike (2009). A sample of each testis was sectioned and weighed. The samples were homogenized separately with a pair of sharp scissors in 0.9 % NaCl (normal/physiological saline) at the rate of 5 ml.g^{-1} testis. The testicular homogenate sample was stored overnight at 4 °C to allow the spermatozoa ooze out of the organ. The suspensions were mixed and filtered through a double layer of sterile gauze into clean glass test tubes and the filtrate diluted with distilled water to the ratio 1:10 (Ewuola, 2013; Amao and Akanbi, 2017). Some drops of the homogenate were placed into an improved Neubauer haemocytometer counting chamber. All the elongated spermatids and mature sperm cells in the four diagonal and the centre squares of the haemocytometer were counted in each diluted homogenate. The concentration of the sperm cells

per gram of testis parenchyma was calculated as follows:

RTSR/LTSR = N x C x D

Where:
N = number of sperm cells count
D = dilution factor = d_1 x d_2

$$d_1 = \frac{\text{Volume of normal saline for homogenization}}{\text{Weight of sample homogenized}}$$

$$d_2 = \frac{\text{Volume of normal saline for filtrate dilution}}{\text{The volume of filtrate diluted}}$$

C = constant = 52,000
RTSR = right testicular sperm reserve; LTSR = left testicular sperm reserve
Right/Left Testis Sperm Reserve (RTSR/LTSR)/testis =

$$= \frac{\text{RTSR/LTSR x Total weight of right/left testis}}{\text{Weight of homogenated right/left testis}}$$

Therefore, Paired TSR (PTSR)/testis = RTSR/testis + LTSR/testis

$$\text{RTSR/LTSR per gram testis} = \frac{\text{RTSR/testis}}{\text{Total weight of right testis}} \text{ OR } \frac{\text{LTSR/testis}}{\text{Total weight of left testis}}$$

Therefore, Gonadal Sperm Reserve (GSR) or paired TSR (TSR)/gram testis = RTSR/gram testis + LTSR/gram testis

Estimation of extragonadal (epididymal) sperm reserves (ESR)

The left and right epididymides were removed from the testes and separated into the caput, corpus and cauda epididymides. They were separately homogenized in normal saline at 5ml.g^{-1}. The homogenate was kept refrigerated overnight. It was then filtered through double layers of gauze into graduated test tubes. Extragonadal sperm reserves were determined by direct counting of sperm cells in a haemocytometer after 1:1(v/v) dilutions as described by Ewuola *et al.* (2014). The extragonadal sperm reserve was the total number of spermatozoa in all the sections of epididymal tissues expressed in millions.

ESR = ESR (caudal) + ESR (corpus) + ESR (caput)
ESR (caudal/corpus/caput) = N x C x D

Statistical analysis

All data obtained were subjected to a One-Way Analysis of Variance (ANOVA) of the GraphPad Prism, software version 6.01 (2012). Significant differences between the treatment means were revealed using the Tukey's Honestly Significant Difference (α 0.05) option of the same software.

RESULTS

The semen characteristics of the cocks fed diets supplemented with varying inclusion levels of MSG are shown in Table 2. It was observed that cocks on the diet containing 1.25 g MSG.kg^{-1} did not produce any ejaculate at all; therefore, no semen characteristics could be studied on the cocks placed on this diet. The cocks on the diets containing 0.25 and 0.50 g MSG.kg^{-1} did not show a significant (P > 0.05) effects of MSG inclusions when compared with one another and

Table 2. Semen characteristics 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
Ejaculate Volume (ml)	0.60 ± 0.01^{a}	0.48 ± 0.18^{ab}	0.43 ± 0.12^{ab}	0.32 ± 0.09^{b}	0.22 ± 0.06^{bc}	0.00 ± 0.00^{c}	0.0003*
Sperm Motility (%)	94.30 ± 1.17^{a}	81.00 ± 4.09^{ab}	89.30 ± 1.17^{a}	67.70 ± 4.80^{b}	23.30 ± 4.41^{c}	0.00 ± 0.00^{d}	< 0.0001*
Sperm Viability (%)	97.44 ± 0.71^{a}	96.94 ± 0.86^{a}	91.95 ± 1.62^{a}	85.78 ± 2.11^{ab}	72.57 ± 7.15^{b}	0.00 ± 0.00^{c}	< 0.0001*
Sperm Conc. (x 10^8 ml^{-1})	7.32 ± 0.72^{a}	5.53 ± 0.29^{ab}	5.47 ± 0.27^{ab}	5.56 ± 1.53^{ab}	4.53 ± 0.56^{b}	0.00 ± 0.00^{c}	0.0122*
TSC/ejacualte (x 10^8 ml^{-1})	4.39 ± 0.56^{a}	2.65 ± 0.39^{ab}	2.35 ± 0.41^{ab}	1.78 ± 0.15^{b}	1.21 ± 0.22^{c}	0.00 ± 0.00^{d}	< 0.0001*
Total Live Cells.ml^{-1} (x 10^8)	7.13 ± 1.67^{a}	5.34 ± 1.02^{ab}	5.00 ± 1.80^{ab}	4.73 ± 1.84^{ab}	3.28 ± 0.09^{b}	0.00 ± 0.00^{c}	0.0325*
Total Motile Cells.ml^{-1} (x 10^8)	6.90 ± 1.54^{a}	4.48 ± 1.02^{ab}	4.88 ± 1.92^{ab}	3.76 ± 1.15^{b}	1.29 ± 0.42^{c}	0.00 ± 0.00^{d}	0.0269*
Mass Activity Grade	+++	+++	+++	++	+	0.00	

Values are means ± SEM; Means in a row without common superscripts are significantly (P < 0.05) different. Level of significance = ns (non significant) = P > 0.05; * = P < 0.05, Conc. = Concentration; TSC = Total Sperm Cells; MSG levels in g.kg^{-1} diet. +++: Very turbulent motion; ++: Rapid wave motion; +: Slow wave motion.

those on the control diet for semen characteristics such as ejaculate volume, sperm motility, testicular sperm cells/ejaculate and total motile cells.ml^{-1} but recorded a significantly (P < 0.05) higher means than those on the diets containing above 0.50 g MSG.kg^{-1} diet for the same parameters. However, the sperm viability, concentration and total live cells.ml^{-1} among the cocks fed diets containing up to 0.75 g MSG.kg^{-1} did not show any significant (P > 0.05) difference when compared with one another and those on the control diet but significant reductions in these parameters were observed among the cocks fed diet containing 1.00 g MSG.kg^{-1}. The mass activity ranged from very turbulent motion for the cocks on the control diet to slow motion for those on the diet containing 1.00 g MSG.kg^{-1}.

The results of the extragonadal lengths and weights for left and right epididymides, as well as *vas deferens* of the cocks, fed different levels of MSG are shown in Table 3. It was observed that all of the epididymal and *vas deferens* lengths of the cocks across all the dietary treatments were progressively reduced with increasing inclusion levels of MSG. The cocks on the diets containing up to 0.50 g MSG.kg^{-1} diet showed statistical (P > 0.05) similarities among themselves and those on the control diet which were significantly (P < 0.05) higher than those on the diets containing 0.75 to 1.25 g MSG.kg^{-1} for left, right and paired epididymides and vas deference lengths. Similarly, the cocks on the control diets recorded the highest significant (P < 0.05) left, right and paired epididymal and *vas deferens* weights when compared with cocks on other diets. The cocks on the diets containing 0.25 to 0.75 g MSG.kg^{-1} did not differ significantly (P > 0.05) when the weights recorded by them for right, left and paired epididymides as well as right, left and paired *vas deferens* were compared with those on the control diet. However, increasing levels of MSG above 0.75 g.kg^{-1} diet significantly (P < 0.05) reduced the weights of the right, left and paired epididymides and *vas deferens* of the cocks. For gonadal characteristics (Table 4), it also was observed that the cocks on the diet containing 0.50 g MSG.kg^{-1} had the higher non-significant (P > 0.05) values for the right testicular volume; left testicular

Table 3. Extra gonadal lengths and weights of cocks fed diets MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Extra Gonadal Length (cm)							
Epididymis							
Left	2.83 ± 0.12^{a}	2.80 ± 0.14^{a}	2.70 ± 0.14^{a}	2.07 ± 0.06^{b}	2.07 ± 0.02^{b}	2.17 ± 0.02^{b}	< 0.0001*
Right	3.47 ± 0.12^{a}	3.00 ± 0.18^{ab}	2.87 ± 0.07^{abc}	2.63 ± 0.27^{bc}	2.53 ± 0.17^{bc}	2.27 ± 0.06^{c}	< 0.0001*
Paired	6.30 ± 0.21^{a}	5.80 ± 0.29^{ab}	5.57 ± 0.11^{abc}	4.70 ± 0.08^{bc}	4.60 ± 0.18^{c}	4.44 ± 0.27^{c}	< 0.0001*
Vas deferens							
Left	12.53 ± 0.19^{a}	8.37 ± 0.19^{ab}	8.37 ± 0.35^{ab}	8.77 ± 0.71^{b}	7.63 ± 0.19^{bc}	5.67 ± 0.03^{c}	< 0.0001*
Right	10.93 ± 1.26^{a}	10.40 ± 0.46^{ab}	8.33 ± 0.18^{abc}	7.74 ± 0.36^{bc}	7.46 ± 0.82^{c}	7.97 ± 0.21^{bc}	< 0.0001*
Paired	23.46 ± 0.48^{a}	18.77 ± 1.15^{ab}	16.67 ± 0.57^{ab}	16.51 ± 0.49^{b}	15.09 ± 0.79^{bc}	13.64 ± 0.21^{c}	< 0.0001*
Extra Gonadal Weight (g)							
Epididymis							
Left	0.63 ± 0.10^{a}	0.60 ± 0.06^{ab}	0.53 ± 0.06^{ab}	0.50 ± 0.02^{ab}	0.40 ± 0.03^{bc}	0.37 ± 0.02^{c}	0.0063*
Right	0.73 ± 0.11^{a}	0.53 ± 0.12^{ab}	0.60 ± 0.06^{ab}	0.63 ± 0.09^{ab}	0.43 ± 0.06^{b}	0.30 ± 0.00^{b}	0.0094*
Paired	1.37 ± 0.20^{a}	1.13 ± 0.07^{ab}	1.13 ± 0.05^{ab}	1.13 ± 0.08^{ab}	0.83 ± 0.03^{bc}	0.67 ± 0.02^{c}	0.0002*
Vas deferens							
Left	0.70 ± 0.02^{a}	0.57 ± 0.07^{ab}	0.56 ± 0.08^{ab}	0.53 ± 0.15^{ab}	0.47 ± 0.07^{b}	0.30 ± 0.02^{c}	< 0.0001*
Right	0.66 ± 0.14^{a}	0.53 ± 0.03^{a}	0.56 ± 0.09^{a}	0.52 ± 0.07^{ab}	0.46 ± 0.15^{b}	0.30 ± 0.08^{b}	0.0006*
Paired	1.36 ± 0.24^{a}	1.10 ± 0.08^{ab}	1.13 ± 0.14^{ab}	1.05 ± 0.09^{ab}	0.93 ± 0.10^{bc}	0.61 ± 0.03^{c}	< 0.0003*

Values are means ± SEM; Means in a row without common superscripts are significantly (P < 0.05) different. Level of significance = ns (non significant) = P > 0.05; * = P < 0.05, MSG levels in g.kg^{-1} diet.

Table 4. Gonadal weights, volume and density 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
Parameters Right Testicle (g)							
Whole Weight	15.16 ± 2.40	18.28 ± 1.742	17.96 ± 0.58	15.59 ± 0.86	16.02 ± 1.60	13.12 ± 0.39	0.1404^{ns}
Parenchymal Weight	14.10 ± 2.23	17.00 ± 1.62	16.70 ± 0.54	14.50 ± 0.78	14.90 ± 1.49	12.20 ± 0.37	0.1404^{ns}
Albuginea Weight	1.06 ± 0.18	1.28 ± 0.12	1.26 ± 0.04	1.09 ± 0.06	1.12 ± 0.11	0.92 ± 0.03	0.1404^{ns}
Volume (ml)	16.00 ± 2.57	18.00 ± 1.89	18.70 ± 0.17	16.70 ± 1.20	13.30 ± 1.67	16.70 ± 0.44	0.2350^{ns}
Density (g.ml^{-1})	0.97 ± 0.03^{b}	1.03 ± 0.04^{b}	0.96 ± 0.03^{b}	0.96 ± 0.06^{b}	1.23 ± 0.04^{a}	0.79 ± 0.01^{c}	< 0.0001*
Left Testicle (g)							
Whole Weight	14.89 ± 2.03	13.40 ± 0.29	15.64 ± 0.63	15.42 ± 0.69	14.15 ± 1.36	14.89 ± 2.17	0.8806^{ns}
Parenchymal Weight	14.00 ± 1.91	12.60 ± 0.27	14.70 ± 0.59	14.50 ± 0.65	13.30 ± 1.28	14.00 ± 2.04	0.8806^{ns}
Albuginea Weight	0.89 ± 0.12	0.80 ± 0.02	0.94 ± 0.04	0.92 ± 0.04	0.85 ± 0.08	0.89 ± 0.13	0.8806^{ns}
Volume (ml)	15.30 ± 2.20	17.30 ± 0.17	17.70 ± 0.17	16.30 ± 0.44	12.00 ± 1.15	15.00 ± 2.31	0.0742^{ns}
Density (g.ml^{-1})	0.99 ± 0.03^{ab}	0.77 ± 0.01^{b}	0.88 ± 0.03^{ab}	0.96 ± 0.07^{ab}	1.19 ± 0.01^{a}	1.16 ± 0.21^{a}	0.0156*
Paired Testicles (g)							
Whole Weight	30.06 ± 4.42	26.52 ± 0.48	33.12 ± 1.06	31.01 ± 1.09	30.28 ± 2.84	33.18 ± 3.794	0.4977^{ns}
Parenchymal Weight	28.10 ± 4.13	24.80 ± 0.44	31.40 ± 1.03	29.00 ± 1.02	28.30 ± 2.65	31.00 ± 3.55	0.5003^{ns}
Albuginea Weight	1.96 ± 0.29	1.72 ± 0.03	1.72 ± 0.03	2.01 ± 0.07	1.98 ± 0.19	2.18 ± 0.24	0.4599^{ns}
Volume (ml)	31.30 ± 4.76	34.00 ± 0.50	36.30 ± 0.33	33.00 ± 1.50	25.30 ± 2.73	33.00 ± 4.19	0.1632^{ns}
Density (g.ml^{-1})	1.96 ± 0.06^{ac}	1.56 ± 0.01^{c}	1.85 ± 0.05^{bc}	1.91 ± 0.12^{bc}	2.42 ± 0.04^{a}	2.19 ± 0.24^{ab}	< 0.0001*

Values are means ± SEM; Means in a row without common superscripts are significantly (P < 0.05) different. Level of significance = ns (non significant) = P > 0.05; * = P < 0.05, MSG levels in g.kg^{-1} diet.

parenchymal weight, albuginea weight, volume, the whole left testicular weight and paired testicular volume. The right testicular parenchymal weight, albuginea weight and whole right testicular weight as well as the paired testicular parenchymal weight, albuginea weight and whole testicular weight on the diet containing 1.25 g MSG.kg^{-1} were higher but statistically similar (P > 0.05) when compared to those recorded on other diets. However, cocks on the diet containing 1.00 g MSG.kg^{-1} showed the highest significant (P < 0.05) values for the right testicular, left testicular and paired testicular densities.

The epididymal and testicular sperm reserves of cocks on each of the experimental diets are shown in Table 5. A common trend was noticed for each of the studied parameters as there were elevations in epididymal sperm reserves as the inclusion rates of MSG in the diets were increased from 0.00 to 0.50 g MSG.kg^{-1} diet with the cocks on 0.50 g MSG.kg^{-1} diet recording the highest significant (P < 0.05) means for all the parameters. Also, progressive decreases were observed in the epididymal sperm reserves of the cocks fed 0.75 to 1.25 g MSG.kg^{-1} diet with the cocks on 1.25 g MSG.kg^{-1} diet recording the least significant (P < 0.05) values for the studied parameters. The highest significant (P < 0.05) value for the testicular sperm reserve per testis (TSR/testis) for the right testis was recorded for birds on the control diet, while those of the left and paired testes were found to be significantly (P < 0.05) highest among the cocks on the diet containing 0.50 g MSG.kg^{-1}. TSR.g^{-1} testis (Testicular Sperm Reserve per gram testis) for the right (P < 0.05) and paired testes (P > 0.05) were also found to be highest on the control diet, while cocks on the diets containing 0.50 g MSG.kg^{-1} had the highest significant (P < 0.05) value for that of the left testis.

DISCUSSION

A high dose of monosodium glutamate has been implicated to be responsible for various abnormalities in the micro-architecture of the testis as well as semen

Table 5. Gonadal and extra-gonadal sperm reserves of cocks fed MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
TSR/g testis (x 10^8)							
Left	0.65 ± 0.08^{ab}	0.81 ± 0.07^{ab}	1.02 ± 0.08^{a}	0.91 ± 0.10^{ab}	0.59 ± 0.02^{b}	0.71 ± 0.14^{ab}	0.0122*
Right	1.07 ± 0.10^{a}	0.83 ± 0.07^{ac}	0.62 ± 0.07^{bc}	0.56 ± 0.06^{c}	0.91 ± 0.10^{ab}	0.57 ± 0.08^{c}	< 0.0001*
Paired	1.72 ± 0.18	1.64 ± 0.11	1.64 ± 0.05	1.47 ± 0.14	1.50 ± 0.11	1.28 ± 0.15	0.2079^{ns}
TSR/testis (x 10^9)							
Left	0.78 ± 0.03^{c}	1.01 ± 0.08^{bc}	1.45 ± 0.10^{a}	1.32 ± 0.15^{ab}	0.78 ± 0.06^{c}	0.82 ± 0.15^{c}	< 0.0001*
Right	1.32 ± 0.12^{a}	1.00 ± 0.07^{ab}	0.85 ± 0.07^{b}	0.92 ± 0.09^{ab}	1.25 ± 0.05^{ab}	0.98 ± 0.14^{ab}	0.0053*
Paired	2.11 ± 0.15^{ab}	2.01 ± 0.12^{ab}	2.30 ± 0.03^{a}	2.25 ± 0.16^{a}	2.03 ± 0.02^{ab}	1.79 ± 0.03^{b}	0.0149*
ESR (x 10^7)							
Left	1.72 ± 0.09	1.73 ± 0.26	1.85 ± 0.43	1.76 ± 0.19	1.65 ± 0.23	1.49 ± 0.23	0.4850^{ns}
Right	3.05 ± 0.10^{ab}	3.06 ± 0.12^{ab}	3.32 ± 0.16^{a}	2.46 ± 0.27^{bc}	2.14 ± 0.25^{c}	1.64 ± 0.25^{c}	< 0.0001*
Paired	4.77 ± 0.42^{a}	4.79 ± 0.46^{a}	5.17 ± 0.17^{a}	4.22 ± 0.17^{ab}	3.79 ± 0.30^{b}	3.13 ± 0.34^{b}	0.0033*

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; TSR/testis (Testicular Sperm Reserve per testis); TSR/g testis (Testicular Sperm Reserve per gram testis), ESR (Epididymal/Extra-gonadal Sperm Reserve) MSG levels in g.kg^{-1} diet.

characteristics (Eweka and Om'Iniabohs, 2007). In the present study, the inclusion of MSG in the cocks' diets at 0.25 to 0.50 g.kg^{-1} did not adversely affect the ejaculate volume significantly. While cocks on 0.75 and 1.00 g MSG.kg^{-1} diet were hypospermic, 1.25 g MSG.kg^{-1} diet inclusion level was observed to have caused aspermia among the cocks on this diet. This has supported the reports of earlier findings that high level of MSG consumption causes oligozoospermia, increased abnormal sperm morphology and various degenerative changes, and also, causing deleterious effects on the Sertoli cells and Leydig cells of the testis, thereby, adversely impacting on spermatogenesis, spermiogenesis and testosterone production in adult Wistar rat males (Oforofuo *et al.*, 1997; Onakewhor *et al.*, 1998).

Furthermore, while MSG inclusion levels of 0.25 and 0.50 g.kg^{-1} did not significantly affect semen characteristics such as sperm motility, total live cells, sperm concentration and viability, no adverse effects were documented for total live cells.ml^{-1} and total motile cells.ml^{-1} in the present study, though a slight decrease was observed in certain parameters as MSG inclusion increases. However, a significant effect occurred at an inclusion rate above 0.50 g MSG.kg^{-1} diet. This agreed with the report of Kianifard (2016) that high administration of MSG (60 mg.kg^{-1} body weight) altered sperm parameters in preadolescent rats, thereby, leading to a significant reduction in the sperm count, sperm viability and sperm motility. Dong and Robbins (2015) also supported this finding by reporting that high dose intake of MSG negatively impacted sperm count and concentrations in adult rats fed 4 g.kg^{-1} MSG. The significant decrease observed for all the sperm characteristics in the group fed 1.25 g MSG.kg^{-1} diet could be attributed, probably, to the anti-fertility effect of MSG applied at higher doses.

The quality and quantity of testicular sperm production, as well as storage capacity, played a key role in the selection for breeding purposes (Ewuola and Akinyemi, 2017). Testicular and epididymal parameters, such as weight and length, are usually used in assessing their normality, thus, improving the detection of any deviation from normal that might result during the experimental process (Franca and Russel, 1998). These parameters are usually positively correlated with the spermatogenic activity of the testis (Nosseir *et al.*, 2012). The significant gradual decrease observed in the paired epididymal and *vas deferens* lengths and weights in the present study suggests that MSG might have a structural toxic effect on the epididymides and *vas deferens* at an inclusion level above 0.75 g MSG.kg^{-1} diet. This

agreed with the finding of Fernandes *et al.* (2012), who reported significant reductions in the absolute and relative weight of epididymis and testis in MSG-treated Wistar rats at the rate of 4 mg.kg^{-1} bodyweight, but disagreed in the case of testis weight, as no significant difference was observed in this experiment. They assumed that the reduction in the epididymal weight and length resulted in reduced sperm count in the epididymis and it may be responsible for the acceleration of the sperm transit time through the epididymis. Acceleration in sperm transit time has been reported to have an impairment tendency on sperm maturation and cause a reduction in the number of sperm cells available for ejaculation and fertility (Kempinas and Klinefelter, 2010).

Comparison of the gross testicular weights, volume, density, parenchymal and albuginea weights of the left, right and paired testes revealed that MSG inclusion up to 0.75 g.kg^{-1} diet did not have significant adverse effects on the studied parameters. The result of this finding upheld the report of Franca et al (2006), who found very similar results for most of the testis parameters evaluated in MSG-treated and control rats. Extragonadal sperm reserves (ESR), which are reflected in the amount of sperm storage in the epididymis, has been known to be correlated to sperm production by the testes (Azubuike *et al.*, 2016). In this study, the paired total epididymal sperm reserves of the cocks fed varying levels of MSG significantly reduced with increasing inclusion levels in the diets. This trend is suggestive of the dietary influence of MSG, since all the cocks were fed isocaloric and isonitrogenous diets with only the inclusion levels of MSG being the varying factor. The result of the study corroborates with the findings of Igwebuike *et al.* (2011) who reported a significant decrease in the mean caudal epididymal sperm reserves of the rats that were given medium- to-high doses of monosodium glutamate relative to the control group and the low dose group. The dose-related significant reduction in the ESR of the cocks on diets 0.75, 1.00 and 1.25 g MSG.kg^{-1} could be that the normal process of spermatogenesis was adversely affected in the cocks that consumed the diets containing MSG above 0.50 g.kg^{-1} diet. Since testosterone plays a major role in the spermatogenesis (Oforofuo *et al.*, 1997; Nayataraet *et al.*, 2008) the dose-related reduction in the ESR might be an indication that there could have been a considerable decline in the influence of testosterone on spermatogenesis in the significantly affected cocks (Dong and Robbins, 2015). The paired testicular sperm reserves (TSR) of the cocks decreased, though not significantly, with an inclusion level of 0.50 g MSG.kg^{-1} diet and above despite higher testes weight. The decline may possibly be the resultant effects of MSG on the degeneration of Sertoli cells that provide nourishment for the growth and survival of sperm cells within the seminiferous tubules. This corroborates with the opinion of Igwebuike *et al.* (2011) that monosodium glutamate may have a negative impact on spermatogenesis through its disruption of the hypothalamic-pituitary-testis regulatory (HPG) axis and not through any direct toxic effect on the testis. The results of our findings in the present study may be limited due to the length of exposure of the cocks to the diets treated with MSG, since adult birds were used and the exposure time to MSG was sixteen weeks. However, further research is required to assess the impact of dietary MSG on cocks fed treated diets from day old to the maturity stage.

CONCLUSION

Supplementation of cocks' diets with MSG up to 0.5 g.kg^{-1} did not compromise the semen characteristics, gonadal and extragonadal sperm reserves in the treated birds. Enhancing feed palatability for optimum feed utilization could be achieved with MSG in cocks' diet if the tolerable limit is not exceeded. This study reveals the possible taste-enhancing effect of monosodium glutamate in cocks' diet as a feed flavour additive. This study will help the farmers and feed millers to enhance the palatability of poultry feeds for optimum feed utilization. This will also add value to the use of phytogenic feed additives and non-conventional feedstuffs, which are reported to possess great potentials in poultry production but poor palatability might be a limiting factor. Thus, a new theory on the combined use of monosodium glutamate and non-conventional feedstuffs may be developed.

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