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# High level dietary inclusion of monosodium glutamate lowers daily sperm production and efficiency in cocks

Olumuyiwa Joseph Olarotimi<sup>1,2\*</sup>, Olufemi Adesanya Adu<sup>2</sup>, Abosede Oluwakemi Olarotimi<sup>3</sup>

<sup>1</sup> Animal Physiology Unit, Department of Animal Science, Faculty of Agriculture, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Nigeria

<sup>2</sup> Animal Physiology Laboratory, Department of Animal Production and Health, School of Agriculture and Agricultural Technology, The Federal University of Technology Akure, P.M.B. 704, Akure, Nigeria

<sup>3</sup> Chemistry Laboratory, Department of Research and Development, Engineering Materials Development Institute (EMDI), KM 4, P.M.B. 611, Akure, Nigeria

\*Correspondence: Phone: +234 803 565 0055; E-mail: [olarotimioj@futa.edu.ng](mailto:olarotimioj@futa.edu.ng)

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**ABSTRACT:** In a 16-week feeding trial, an investigation was carried out with 240 sexually matured cocks of twenty 24 weeks of age to assess the daily sperm production (DSP) and sperm production efficiency (SPE) 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 diet designated diets A, B, C, D, E and F, respectively). The cocks were weighed ( $1888.33 \pm 44.10$  kg) and allotted to the 6 treatment diets. Each treatment was replicated 5 times with 8 cocks/ replicate in a completely randomized design. At the end of the feeding trial, 2 cocks per replicate (i.e. 4 cocks per treatment) were humanely sacrificed and their reproductive tracts dissected. The testes were carefully sampled, weighed and processed for estimation of DSP and SPE using both histological and homogenate methods of analyses. Results showed that the inclusion of MSG at 1.25 g/kg significantly reduced the DSP under the two estimation methods ( $P < 0.05$ ). The SPE was equally significantly lowered at 0.75 g MSG/kg diet and above when determined using the homogenate method. It was also observed that MSG at 1.00 g/kg diet and above lowered the DSP and SPE when determined histometrically. A high positive correlation was established between the DSP and the testicular volume of the cocks. However, the paired testicular sperm reserves were not significantly influenced ( $P \geq 0.05$ ). Sperm reserves in both testicles of the cocks fed diets B and C were similar to the control. This study therefore, suggests that MSG has a potential to significantly reduce the reproductive potentials of cocks when administered in excess of 0.75 g/kg diet.

**Keywords:** Cocks; Monosodium glutamate; Sperm production; Testes.

## 1. INTRODUCTION

Feed additives are included in diets for the purpose of enhancing palatability and providing enhanced digestibility of the feed materials to achieve improved performance such as weight gain (WG), increased laying performance, enhancing the sperm production capacity for breeding purposes, improved hatchability and prevention of diseases [1]. Monosodium glutamate (MSG) is reputable for enhanced food palatability, and

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 has been stressed [2]. The meat palatability of chickens fed diet containing up to 0.75 g MSG/kg diet was reportedly enhanced with fat content significantly reduced [3]. Gbore [4] equally reported enhanced WG, feed intake (FI) and feed conversion ratio (FCR) in rabbit does orally administered low to medium dosage of MSG. In another development, Olateju [5] reported an increase in WG among broiler chickens fed dietary MSG at 0.25 to 0.50 g/kg diet with corresponding better FCR as opposed to the birds fed higher dosage of MSG.

Despite the potential of MSG as feed flavour enhancer, there have been concerns about the attendant risks of using it as feed additive. Various conflicting reports about the safety of MSG as flavour enhancer have been documented by several authors. The testicular toxicity effect of MSG was reported by [6] who observed a significant reduction in sperm production and an increase in abnormal sperm morphology in a dose-dependent manner in male Wistar rats. Igwebuike et al. [7] also reported that MSG administration lowered serum testosterone levels and reduced caudal epididymal sperm reserves of male Sprague- Dawley rats, without any overt pathological lesions in testis. Olarotimi and Adu [8] equally reported that dietary MSG did not significantly have deleterious effects on the sperm characteristics and sperm reserves of cocks when administered between 0.25 to 0.50 g/kg diet. The overuse of MSG has been posited to trigger toxicity, thereby, preventing palatability, productivity and reproductive potentials [8]. Though, a general agreement had been reached scientifically, based on numerous biochemical, toxicological and medical studies, that MSG is safe for the general population [9], comprehensive studies on the effects of MSG reproductive potentials such as daily sperm production and efficiency of cocks are scarce. This study, therefore, sought to quantitatively evaluate the possible effects of varied inclusion levels of MSG on daily sperm production (DSP) and sperm production efficiency (SPE) of domestic fowl cocks using both the histological and homogenate methods of estimation.

## **2. MATERIALS AND METHOD**

### **2.1. Experimental site**

The study was carried at the Poultry Unit, Teaching and Research Farm, the Federal University of Technology Akure, Nigeria. The geographical coordinates of the location is between 7°17' North and 5°9' East [10]. 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% [11]. It was conducted in accordance to the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750).

### **2.2. Experimental cocks, diets and management**

A total of 240 sexually matured barred Plymouth Rock of twenty four (24) weeks of age were used for the study. The cocks were weighed and divided into six (6) experimental groups. They were fed with commercially prepared grower mash containing 15.13% crude protein, 2.19% fat, 8.19% crude fiber, 1.13% calcium, 0.42% phosphorus, 1.07% lysine, 0.41% methionine and 2512.06 Kcal/kg metabolizable energy which was in consonance with NRC nutritional requirements for cocks [12]. The six (6) experimental diets A, B, C, D, E and F were constituted by including 0.25, 0.50, 0.75, 1.00 and 1.25 g MSG respectively per kg of the grower mash while the control diet was without MSG inclusion. Each experimental treatment was

replicated 5 times with 8 cocks ( $1888.33 \pm 44.10$  kg) per replicate in a completely randomized design. The experimental diets were given according to body weight (BW) twice daily while fresh and cool 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 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 end of the feeding trial, ten (10) cocks per treatment (2 birds per replicate) were randomly selected, humanely sacrificed through cervical dislocation and the reproductive tracts were carefully harvested. The testes were separated and freed of adhering connective tissues and fats. The left and right testes were weighed separately using a highly sensitive weighing balance in the laboratory and their weights recorded. The volumes of the testes were measured volumetrically using the Archimede's principle of water displacement in a measuring cylinder as described by Olarotimi et al. [13] and the result recorded. The testes densities were calculated from the testicular weights and volumes and expressed as g/ml [13].

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

### 2.3. Estimation of daily sperm production (DSP) and daily sperm efficiency (SPE)

#### 2.3.1. Homogenate method of estimation

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 5ml/g testis. The testicular homogenate sample was stored overnight at 4°C to allow the spermatozoa ooze out of the organ [14]. The suspensions was mixed and filtered through a double layer of sterile gauze into clean glass test tubes and the filtrate diluted with distil water to ratio 1:10 [14]. Some drops of the homogenate were introduced 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 testicular sperm reserve (TSR) which is the concentration of the sperm cells per gram of testis parenchyma was calculated as described by Olarotimi and Adu [8]. The DSP for each cock was therefore calculated by dividing the TSR by the time divisor for chicken. The time divisor was obtained by multiplying the fraction of the cycle of seminiferous epithelium occupied by these cells by the duration of a cycle. Orlu and Egbunike [15] reported that 48.25% of the cycle of 4 days was documented by researchers to be occupied by these cells.

$$\text{DSP} = \text{Testicular Sperm Reserve (TSR)} / \text{Time divisor (1.93)}$$

The efficiency of sperm production also known as daily sperm production per gram (DSP/g) parenchyma (testis) was estimated as [15]:

$$\text{DSP/g} = [\text{Paired TSR/g}] / [\text{Time divisor (1.93)}]$$

#### 2.3.2. Histological method of estimation

A portion of the right testis of each cock was taken for histological processing. Testicular tissues were fixed in Bouins' fixative solution for 24 h; it was serially dehydrated in ascending grades of alcohol. Histological sections of 7 µm thick were stained with haematoxylin and eosin. The slides were observed at 800x magnification as described by Bitto and Egbunike [16] in boars. The volume percent of round spermatids and seminiferous tubules were determined as previously described [17]. The diameter of round spermatids and seminiferous tubules were determined as well. Daily sperm production (DSP) was estimated

by histometric method using formula [15].

$$\text{DSP} = [\text{CTV} \times \text{Volume \% of round spermatid nuclei in testis}] / [\text{Average Vol. per round spermatid nucleus} \times \text{Lifespan of round spermatid in days}]$$

CTV (corrected testicular volume) was determined using the modified formula [18].

$$\text{CTV} = [(\text{Gross testicular weight} - \text{Tunica albuginea weight}) / \text{Testis density}] \times \text{shrinkage correction}$$

Life span of round spermatids = 2.23 days [15].

#### 2.4. Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) of the GraphPad Prism [19]. Correlation analysis was used to establish the relationships between the studied parameters. Significant differences between the treatment means were separated using the Tukey's Honestly Significant Difference ( $\alpha 0.05$ ) option of the same software.

### 3. RESULTS

The effects of MSG on BW and the studied testicular parameters of the cocks fed different levels of MSG are as shown on Table 1. The body weight (BW) of cocks on the diet containing 0.75 g MSG/kg was significantly ( $P < 0.05$ ) higher when compared with those on the control and other experimental diets. It was also observed there was a progressive increase in the BW of the cocks from 0.00 to 0.75 g MSG/kg diet inclusion level whereas a progressively declining trend was noted from 1.00 to 1.25 g MSG/kg diet inclusion level. Furthermore, it was observed that the varied inclusion levels of MSG used in the present study did not significantly ( $p > 0.05$ ) influence the paired testicular volume (PTV), paired parenchymal weight (PPW), paired tunica albuginea weight (PTAW) and paired whole testicular weight (PWTW) of the cocks. However, the cocks fed diet containing 1.00 g MSG/kg recorded the highest significant ( $P < 0.05$ ) values for the paired testicular density.

**Table 1.** Effects of MSG on BW and some testicular parameters 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
BW (g)	2350±14.40 <sup>bc</sup>	2370±30.00 <sup>bc</sup>	2480±30.00 <sup>b</sup>	2670±44.10 <sup>a</sup>	2470±68.20 <sup>bc</sup>	2300±38.20 <sup>c</sup>	< 0.0001*
PWTW (g)	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 <sup>ns</sup>
PPW (g)	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 <sup>ns</sup>
PTAW (g)	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 <sup>ns</sup>
PTV (ml)	31.30±4.76	34.00±0.50	36.30±0.33	33.00±1.50	25.30±2.73	23.00±4.19	0.1632 <sup>ns</sup>
PTD (g/ml)	0.96±0.06 <sup>bc</sup>	0.78±0.01 <sup>c</sup>	0.91±0.05 <sup>bc</sup>	0.94±0.12 <sup>bc</sup>	1.20±0.04 <sup>a</sup>	1.21±0.24 <sup>a</sup>	< 0.0001*

Values are means ± SEM; Means in a row without common superscripts are significantly ( $P < 0.05$ ) different. Level of significance = ns (not significant) =  $P > 0.05$ ; \* =  $P < 0.05$ . BW= Body weight, PTV = Paired testicular volume, PWTW = Paired whole testicular weight, PPW = Paired parenchymal weight, PTAW = Paired testicular albuginea weight, PTD = Paired testicular density. MSG levels units in g/kg diet.

The volumetric proportion of the round spermatids (Table 2) was significantly ( $p < 0.05$ ) higher among the cocks fed diet containing 0.75 g MSG/kg when compared with those on other diets while the birds on 1.25 g MSG/kg recorded the significantly ( $p < 0.05$ ) lowest means for this parameter. The cocks on the diets containing MSG at 0.75 and 1.25 g/kg levels significantly ( $p < 0.05$ ) recorded the highest values for total length and diameter of the seminiferous tubules respectively.

**Table 2.** Some histometric characteristics of the testes of the 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
Vol. %	45.40±3.140 <sup>c</sup>	78.60±4.603 <sup>b</sup>	75.50±4.700 <sup>b</sup>	130.00±1.920 <sup>a</sup>	56.90±0.482 <sup>c</sup>	22.30±0.415 <sup>d</sup>	<0.0001*
DST (µm)	2000±139.00 <sup>c</sup>	1980±117.00 <sup>c</sup>	2860±203.00 <sup>ab</sup>	2440±36.00 <sup>bc</sup>	2140±18.100 <sup>c</sup>	2980±55.400 <sup>a</sup>	<0.0001*
LST (µm)	345±23.900 <sup>b</sup>	403±23.700 <sup>b</sup>	552±39.300 <sup>a</sup>	574±8.460 <sup>a</sup>	383±3.240 <sup>b</sup>	397±7.370 <sup>b</sup>	<0.0001*

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. Vol. % = Volume percentage of seminiferous tubule, DST = Diameter of seminiferous tubule, LST = Length of seminiferous MSG levels units in g/kg diet.

The daily sperm production (DSP) was observed to progressively increase with increasing level of MSG inclusion with the highest significant (P<0.05) value recorded among the cocks fed diet containing 0.50 g MSG/kg for both the homogenate and histological methods (Table 3). The sperm production efficiency (SPE) remained significantly (P<0.05) highest among the cocks on the control diet for the homogenate method while birds on the diet containing 0.50 g MSG/kg recorded the highest significant (P<0.05) SPE for the histological method of estimation. However, inclusion of MSG at 1.25 g/kg diet significantly (P<0.05) lowered the DSP and SPE of the cocks in both methods of estimation when compared with those on all other diets. Among the positively correlated parameters (PWTW, PPW, PTAW and PTV) with the DSP (Table 4), only the PTV showed a significant (P<0.05) positive correlation with the DSP while the bodyweight showed a non-significant (P>0.05) positive correlation with the SPE.

**Table 3.** Daily sperm production and sperm production efficiency 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
DSP (x10 <sup>9</sup> )*	1.09±0.08 <sup>ab</sup>	1.16±0.08 <sup>a</sup>	1.19±0.02 <sup>a</sup>	1.05±0.10 <sup>ab</sup>	1.04±0.06 <sup>ab</sup>	0.93±0.02 <sup>c</sup>	0.0119*
SPE (x10 <sup>6</sup> )*	88.70±9.41 <sup>a</sup>	84.60±5.58 <sup>ab</sup>	85.10±2.67 <sup>ab</sup>	76.20±7.04 <sup>b</sup>	77.70±5.73 <sup>b</sup>	66.50±7.54 <sup>c</sup>	0.0476*
DSP (x10 <sup>9</sup> )**	1.14±0.03 <sup>ab</sup>	1.18±0.15 <sup>ab</sup>	1.33±0.10 <sup>a</sup>	0.88±0.02 <sup>b</sup>	0.83±0.13 <sup>bc</sup>	0.36±0.04 <sup>d</sup>	0.0002*
SPE (x10 <sup>6</sup> )**	71.80±1.59 <sup>ab</sup>	77.60±2.25 <sup>ab</sup>	92.00±5.29 <sup>a</sup>	68.33±5.06 <sup>b</sup>	58.30±1.59 <sup>c</sup>	20.70±0.74 <sup>d</sup>	0.0002*

Values are means ± SEM; Means in a row without common superscripts are significantly (P<0.05) different. Level of significance = \* = P< 0.05; DSP = Daily Sperm Production, SPE = Sperm Production Efficiency, DSP\*/SPE\* = homogenate method of estimation, DSP\*\*/SPE\*\* = histological method of estimation, MSG levels units in g/kg diet.

**Table 4.** Relationship between BW, testicular parameters and reproductive potentials of the cocks.

Parameters	BW	PWTW	PPW	PTAW	PTV	PTD	DSP	SPE
BW	1.000	-0.142	-0.142	-0.145	-0.117	-0.048	-0.071	0.218
PWTW		1.000	1.000*	0.999*	0.800*	0.040	0.256	-0.751*
PPW			1.000	0.999*	0.801*	0.040	0.256	-0.751*
PTAW				1.000	0.804*	0.035	0.257	-0.747*
PTV					1.000	-0.549*	0.422*	-0.547*
PTD						1.000	-0.325*	-0.152
DSP							1.000	0.047
SPE								1.000

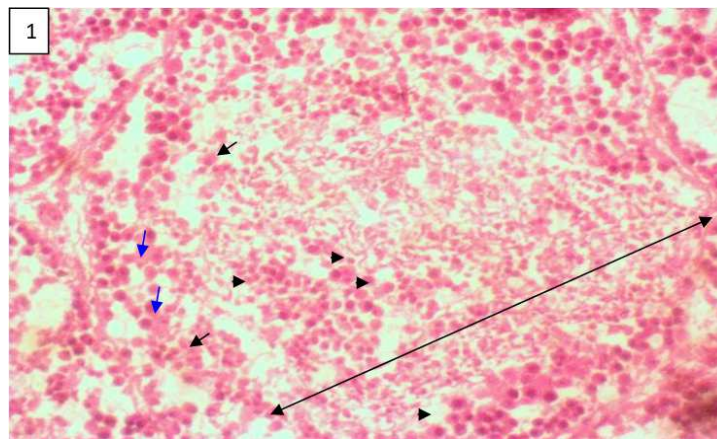
PTV = Paired testicular volume, PWTW = Paired whole testicular weight, PPW = Paired parenchymal weight, PTAW = Paired testicular albuginea weight, PTD = Paired testicular density, BW = Body weight, DSP = Daily sperm production, SPE = Sperm production efficiency. Significance level at 5% (\*) (p<0.05), values not superscripted are not significant (p>0.05).

Furthermore, the histometric parameters of the seminiferous tubules (Table 5) indicated that the length of the seminiferous tubules and volume percentage of the round spermatids had a significant ( $P < 0.05$ ) positive correlation with both the DSP and SPE.

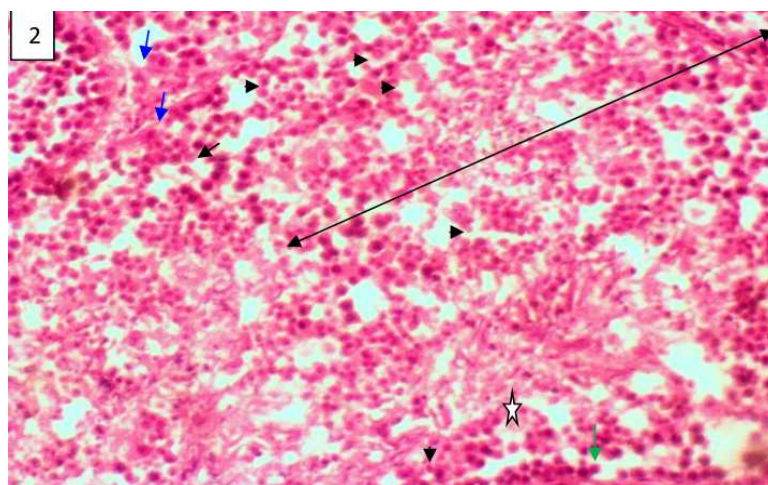
**Table 5.** Relationship between histometric parameters and reproductive potentials of the cocks.

Parameters	DST	LST	Vol.	DSP	SPE
DST	1.000	0.665*	0.029	-0.060	-0.234
LST		1.000	0.748*	0.325*	0.344*
Vol.			1.000	0.376*	0.529*
DSP				1.000	0.826*
SPE					1.000

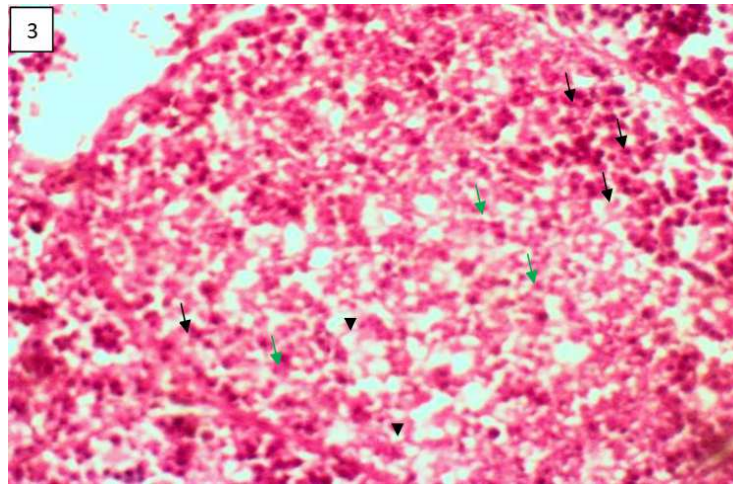
Vol. % = Volume percentage of seminiferous tubule, DST = Diameter of seminiferous tubule, LST = Length of seminiferous tubule. DSP = Daily sperm production, SPE = Sperm production efficiency. Significance level at 5% ( $p < 0.05$ ), values not superscripted are not significant ( $p > 0.05$ ).



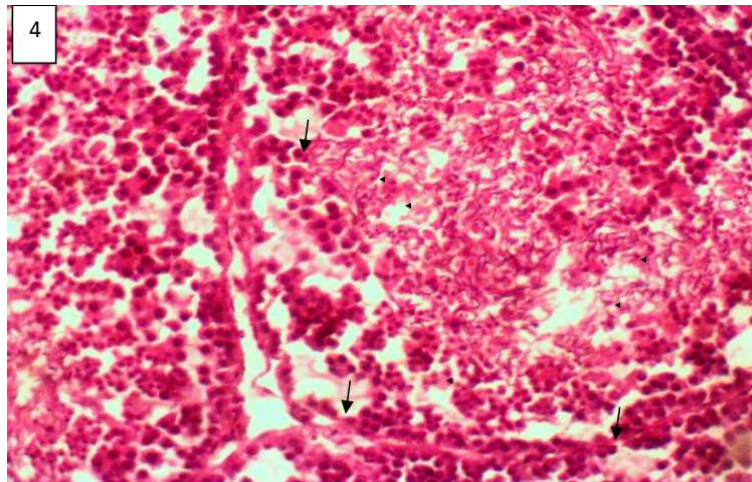
**Figure 1.** A photomicrograph of a section in the testis of cock on Diet A showing diameter of a seminiferous tubule with germinal epithelium comprising spermatogonia (blue arrow), spermatocytes (black arrow) and elongated spermatids (arrow head) in the lumen. HE x400.



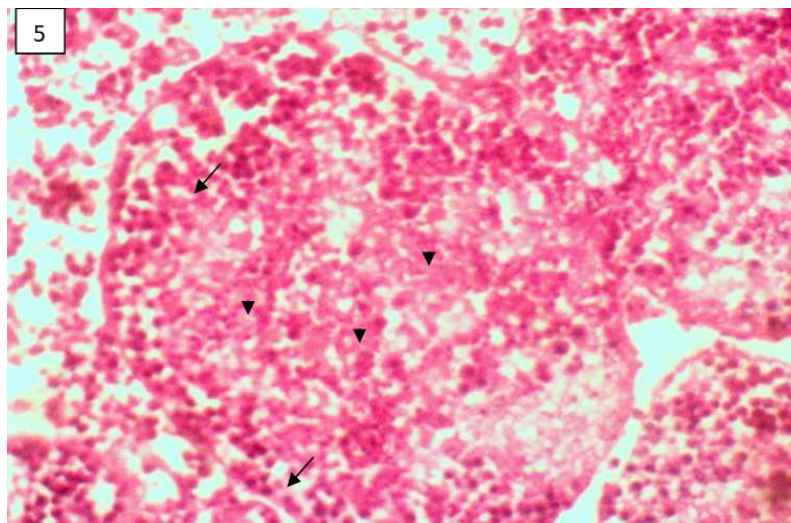
**Figure 2.** A photomicrograph of a section in the testis of cock on Diet B showing seminiferous tubule with spermatocytes (black arrow) and round spermatids (green head), elongated spermatids (arrow head) and spermatozoa (asterick) in the lumen. HE x400.



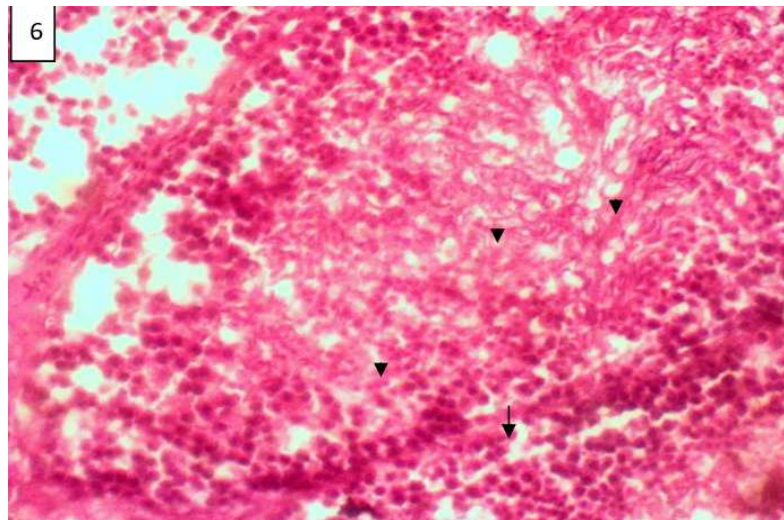
**Figure 3.** A photomicrograph of a section in the testis of cock on Diet C showing seminiferous tubule with preponderance of pachytene spermatocytes (black arrows), a few round spermatids (green arrows) and elongated spermatids (arrow heads). HE x400.



**Figure 4.** A photomicrograph of a section in the testis of cock on Diet D showing a seminiferous tubule with a few pachytene spermatocytes (black arrows), and preponderance of elongated spermatids (arrow heads). HE x400.



**Figure 5.** A photomicrograph of a section in the testis of cock on Diet E showing a seminiferous tubule with a few pachytene spermatocytes (black arrows), and preponderance of elongated spermatids (arrow heads). HE x400.



**Figure 6.** A photomicrograph of a section in the testis of cock on Diet F showing a seminiferous tubule with a few pachytene spermatocytes (black arrows), and preponderance of elongated spermatids (arrow heads). HE x400.

Cross-sections of the seminiferous epithelia of the cocks fed different inclusion levels of MSG are shown in Figures 1-6. The photomicrograph of the transverse section of the testes of the cocks on the control diet showed the seminiferous tubules with germinal epithelium comprising spermatogonia, spermatocytes and elongated spermatids in the lumen. The microarchitectural organization of cells is well preserved. The tubules were regular and densely populated with spermatogonia (Fig. 1). The cocks that received 0.25 g MSG/kg diet (Fig. 2) showed normal seminiferous tubule with spermatocytes and round spermatids, elongated spermatids and spermatozoa in the lumen. There was no observable distortion of tissue structures. The cells of the seminiferous tubules were not disorganized. The seminiferous tubule of the cocks on diet containing 0.50 g MSG/kg (Fig. 3) showed preponderance of pachytene spermatocytes, a few round spermatids and elongated spermatids. The cocks on diets containing 0.75 and 1.00 g MSG/kg (Figs. 4 and 5) showed seminiferous tubules with a few pachytene spermatocytes and preponderance of elongated spermatids (arrowheads). They displayed mild reduction in spermatogenic cells, stroma of the interstitial cells, and the luminal cells. The Leydig cells were also sparsely populated when compared to those on the diets containing 0.00, 0.25 and 0.50 g MSG/kg. The photomicrograph of the cross-section of the testes of the cocks on diet containing 1.25 g MSG/kg (Fig. 6) showed seminiferous tubules with very few pachytene spermatocytes, and limited elongated spermatids. The cocks showed distortions in their testicular tissue also as there was disorganization of cells of the seminiferous tubules, with closer adherence to each other compared to the cocks on diets containing 0.00, 0.25 and 0.50 g MSG/kg. The seminiferous tubules also revealed a moderate decrease in the number of spermatogonia and matured spermatozoa when compared to other groups.

#### 4. DISCUSSION

Comparison of the gross testicular weights, volume and density as well as testicular parenchymal and tunica albuginea weights revealed that MSG inclusion up to 0.75 g/kg diet did not have significant adverse effects on the studied parameters. The result of this finding upheld the report [20], which found very similar results for most of the testis parameters evaluated in MSG-treated and control rats. The significant increase observed in the testicular densities above 0.75 g/kg level of inclusion may be indicative of oligozoospermia or increased abnormal sperm morphology which resulted in lowered testicular volume as observed in those fed MSG above 0.75 g/kg diet MSG. Alterations in the testicular tissue function such as hemorrhage induced at



high inclusion levels of MSG could also explain the decrease in testicular densities observed. The quality and quantity of testicular sperm production as well as storage capacity played a key role in selection for breeding purposes [21]. Important indicators of accessing male fertility potential are the number of spermatids present in the testis, sperm production efficiency (SPE) and the total daily sperm production (DSP). Fernandes et al. [21], in their study, reported that the reduction in daily sperm production in MSG-treated rats was caused by reduced testicular weight, seminiferous tubular diameter, and testicular seminiferous and epithelium height. The present study absolutely agreed with this position as significant reductions were observed in the testicular weight, length of seminiferous tubules and the volume percent of round spermatids at inclusion level above 0.75 g MSG/kg diet. Direct correlation was also established between the DSP and parameters such as length of seminiferous tubules and the volume percent of round spermatids. However, there testicular weight was not directly correlated with DSP. This study showed that inclusion of MSG up to 0.50 g/kg diet was tolerable for DSP and SPE in cocks. The least DSP and SPE were observed among the cocks on the diet containing 1.25 g MSG/kg in both homogenate and histological method of estimations. This could possibly have happened due to the imbalance in the hypothalamic-pituitary-testis regulatory (HPG) axis resulting from higher level of MSG inclusion which compromises the potential of daily sperm production and sperm production efficiency of the cocks placed on this diet. Also, the reduction if DSP and SPE may possibly be the resultant effects of high MSG administration on degeneration of cells of sertoli that provides nourishment for the growth and survival of sperm cells within the seminiferous tubules. This agreed with the report [27] who affirmed the negative effect of MSG on spermatogenesis resulting from disruption of the HPG axis. This study agrees with Igwebuikwe et al. [7] who reported a dose-response relationship between seminiferous tubule fluid (STF) testosterone concentration and the number of advanced spermatids produced by the testis. It is, hence, worthy of note that the most acute effect of high level of MSG inclusion in cocks diets has to do with sperm cells maturation or morphology than the quantity of spermatids present. However, the variances obtained between the values of DSP and SPE from the two methods of estimation employed in this study was earlier explained [14]. He suggested that the discrepancy was due to the attrition between round spermatids and maturation phases of spermatids development. It was further explained [15] that these differences were due to the time divisors obtained, and also, the degeneration of spermatids between round types used for the histological method and elongated type used for the homogenate method.

The results of the histological investigations revealed that MSG inclusion up to 0.50 g/kg diet was safe in cocks' diet. The changes recorded among the cocks fed 0.75 g MSG/kg diet and above leading to the increase in the number of the pachytene stage of primary spermatocyte are in accordance to the histological finding carried out by Alalwani [22] on the testes of Wistar rats treated with 30 and 60 g MSG/kg body weight and found that this affected both the germinal epithelium and Leydig cells which also agreed with the report of Das and Ghosh [23]. The hypospermatogenesis and degenerated spermatogenic cells recorded among the cocks on diet containing 1.25 g MSG/kg in the present study agreed with the investigation of Mohamed [24]. In the current investigation, the histo-architecture of the testes of the cocks on the diets containing lesser than 1.25 g MSG/kg did not differ; the testicular sections did not show any pathological lesions. This is indicative that the inclusion of MSG in cocks' diet up to 1.25 g/kg may have negatively impacted on spermatogenesis by disrupting the hypothalamic-pituitary-testis regulatory axis, and not inducing direct toxic effect on the testis and this agreed with the report of Igwebuikwe et al. [7]. The present findings suggests that MSG inclusion level above 0.75 g/kg diet in cocks' diet may have a deleterious effect on the Sertoli cells and Leydig cells of the testis and may adversely affect spermatogenesis, spermiogenesis and testosterone in poultry cocks and this validated the results of previous studies [25, 26].

## 5. CONCLUSION

This study has demonstrated that high inclusion level of MSG, above 0.75 g/kg, in poultry cocks diets is a potential toxicant that has pathophysiological effects on the reproductive potential of cocks as it significantly reduced the daily sperm production and sperm production efficiency of cocks. Therefore, it could be recommended that the diets for cocks to be used for breeding purpose should not be fortified with MSG, as a taste enhancer, above 0.75 g/kg diet.

**Authors' Contributions:** OOO conceived and designed the experiment, analysed and interpreted the data and prepared the manuscript, AOA and OAO were involved in the laboratory analyses and proof-reading of the manuscript. The final manuscript has been read and approved by all authors.

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