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Effects of oral administration of Monosodium glutamate (msg) on semen characteristics and sperm morphology of the West African Dwarf bucks (*Capra hircus*)

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ABSTRACT: Monosodium glutamate (MSG) is a sodium salt of glutamic acid, popularly known as (Ajinomoto) and indiscriminately consumed as a flavour enhancer in various foods. The consequences of Monosodium glutamate consumption on semen characteristics and sperm morphology remain unknown. This study aimed to investigate the effects of MSG on semen characteristics and sperm morphology. Sixteen (16) mature bucks weighing between 10 and 14 kilograms were randomly assigned to four groups (A-D) of four bucks per group. Group A served as the control, while B, C and D served as the treatment groups and received graded doses of 0.25, 0.50 and 1.0 g/kg of the MSG salt at 48 hours intervals for 28 days respectively. Semen was collected from all the groups at the second- and fourth weeks post-treatment using the electro-ejaculation method. The semen was evaluated using light microscopy. The mean semen volume and percentage of progressively motile sperm cells significantly reduced ($p<0.05$) at four weeks post-treatment in groups B, C and D compared to the control group. Mean sperm count significantly reduced ($p<0.05$) in group D two- and four weeks post-treatment while the mean percentage liveability only reduced in group D four weeks post-treatment. The significantly observed abnormal sperm morphology was bent mid-piece and curved tail in groups B, C and D, two- and four weeks post-treatment. The total proportion of abnormal cells was less than the acceptable 20%. The sperm volume, sperm motility and sperm morphology reduced across the groups as the dosages of MSG increased. Hence, monosodium glutamate adversely affects semen characteristics; impairs sperm fertilizing ability and may reduce bucks' fertility. Thus, its continuous application as a condiment in various foods and administration to breeding bucks should be used with caution.

Keywords: Food, Monosodium glutamate, semen characteristics, West African Dwarf goat.

Running title: Effects of oral administration of *Monosodium glutamate* on West African Dwarf bucks.

INTRODUCTION

Monosodium glutamate (MSG) is a sodium salt of glutamic acid that contains 78% of glutamic acid, 22% of sodium, and water. MSG is commonly marketed as a flavour enhancer and a food additive particularly in West African and Asian dishes (Othmer, 1978). It is available in the open market (Nayanatara *et al*., 2008). MSG became an important industrial chemical when its sodium salt was found to enhance the flavour of certain foods (Filter *et al.,* 1979).

Glutamate is commonly found in food primarily from protein sources. Foods and ingredients that contain glutamate as an inherent component are not required to list glutamate on the label. However, when synthetic MSG is added to food the FDA requires "monosodium glutamate" to be listed on the label. Other glutamate salts such as mono-potassium glutamate and mono-ammonium glutamate also have to be declared on labels and cannot be lumped together under spices or other general terms

(Meadows, 2003). MSG appears as a white crystalline powder in water and it rapidly dissociates into sodium and glutamate ions (Samuels, 1999). It has a unique taste called umami or meaty taste that falls outside the recognized taste of sweet, salty, sour and bitter (Vivek and Deshmukh., 2015).

Recently, the Pakistan Food Authority (PFA) banned Ajinomoto after the authority's scientific panel found it hazardous to health. Headaches, fatigue, palpitations, nausea and vomiting, sweating, flushing and numbness of the face were listed as the adverse effects associated with the salt (Bilal, 2018).

The West African Dwarf goat (WAD) also known as Congo dwarf and Nigerian dwarf is very important in the economy and nutrition of small-scale farmers due to its high fertility rates and relative resistance to adverse climatic conditions (Gall, 1996; Oppong and Yebuah, 1981; Oyeyemi *et al.,* 2000). Domestic animals on an intensive system of management in the tropics especially goats have access to all foods consumed by humans in form of kitchen waste offered to them by the owners and through scavenging and such food mostly contains MSG as a condiment (Ochiogu *et al.,* 2015). In addition, salt has been used by farmers in the Northern part of Nigeria to reduce the libido in bucks (Igwebuike *et al*., 2011)

Studies on the reproductive effects of MSG, various plant extracts, drugs and chemicals in animals have been reported (Raiten *et al.,* 1995; FSANZ, 2003; Nayantara *et al.,* 2008; Oyeyemi *et al.,* 2008; Das and Ghosh, 2010; Igwebuike *et al.*, 2011; Kadir *et al.,* 2011; Barry-Jester, 2016).

However, there is a dearth of information in the available literature on the effects of MSG on semen characteristics and sperm morphology of WAD bucks. Therefore, this study was designed to study the effects of graded doses of MSG on semen characteristics (volume, sperm motility, count, live/dead ratio) and morphology of the WAD buck.

MATERIALS AND METHODS

Study location

The study was conducted at the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria between latitude 15°N and 30°S with relative humidity varying from 50-80%. Rainfall was about 7.0 inches per annum and the temperature is between 28-34°C (Oyeyemi and Fayomi, 2011).

Experimental animals and ethics

Sixteen (16) sexually matured WAD bucks weighing between 10 and 14 kilograms body weight, were acquired from the local market in Ibadan, Oyo state and housed at the Goat and Sheep unit, Faculty of Veterinary Medicine, University of Ibadan.

The protocols were approved by the University of Ibadan Animal Care and Use Research Ethics Committee with the number: UI-ACUREC/0053.

MSG preparation

The test chemical used was Vedan ® 99% MSG, ($C_5H_8NO_4Na.H_2O$) which was purchased in Ibadan city, and 28% aqueous solution (454g in 1620 ml of distilled water of MSG was prepared before use (Nayanatara *et al.,* 2008; Ochiogu *et al.,* 2015). Doses were selected to correspond to human intake of MSG which is directly related to the quantity exposed by the animals (Ochiogu *et al.,* 2014).

Experimental design

The bucks were allowed fourteen days before the commencement of the study for acclimatization to the new environment and the duration of MSG administration was 28 days, following the acclimatization period, the bucks (n=16) were randomly assigned into four groups each containing four animals. Group A served as the control and was not given MSG while groups B, C, and D received 0.25, 0.50, and 1.00 g/kg respectively. MSG was administered at 48 hours intervals for 28 days. Doses of MSG were selected according to the graded percentage of LD50 of 15 and 18 g/kg in rats and mice, respectively (Joint Expert Committee on Food Additives, 1988).

Procedure for administration to the experimental groups

Bucks in the experimental groups were given oral doses of Monosodium Glutamate (MSG) through a drenching gun for small ruminants based on their respective body weight.

Semen collection

A semen sample was collected from all four groups on days 28th and 42nd of the study, using the electroejaculation method (Zemjanis, 1970).

Sperm motility

A drop of semen was placed on a warm microscopic slide mixed with a drop of 2.9% sodium citrate and covered with a coverslip. The sample was observed under a microscope at X10 magnification and the percentages were recorded; only sperm cells moving in a unidirectional motion were included in the count, while cells moving in circles, backward direction or pendulous movements were excluded (Oyeyemi *et al.,* 2008).

Percentage liveability

A drop of semen was placed on a microscopic slide in 1% eosin Nigrosin stain solution cells were distinguished by adding one drop of the stain to one drop of the semen at room temperature and smearing the mixture on a microscopic slide, membrane permeability was used as a basis for differentiation (Oyeyemi *et al*., 2008).

Sperm morphology

A drop of semen was placed on a warm microscopic slide mixed with a warm drop of Wells and Awa stain, the smear was made, air-dried, and observed under a light microscope from low power to high power magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total was recorded (Oyeyemi *et al*., 2008). The spermatozoa abnormalities observed were according to Sekoni and Gustafsson (1981) classified into the head, mid-piece, and tail abnormalities. Types of abnormality observed included headless tail (tail without head), tailless head (head without tail), bent midpiece, bent tail, rudimentary tail, looped tail, and coiled tail (Sekoni and Gustafsson, 1981; Oyeyemi *et al.*, 2011).

Morphometry

Sperm cells were captured using light microscopy connected to a digital camera and image analysis software (×1000 objective lens @ 200% magnification) (Maree *et al.,* 2010; Wibowo *et al.*, 2013). Cells were subjected to detailed measurements. The measurements of head length, width, mid-piece length, tail length, end-piece length, and sperm length, all the measurements were recorded and compared with the control group (Maree *et al.,* 2010).

Statistical analysis

Data obtained were analysed by one-way analysis of variance (ANOVA) using statistical package for social science (SPSS) version 15.0 for determination of variation across treatment groups and control. The package determined any significant difference at a 95% confidence interval and a p-value with less than 0.05 was considered significant. The results were presented as Mean ± SEM.

RESULTS

Semen volume

The mean volume of semen in millilitres observed throughout the study is presented in Figure 1. For two- and four weeks post-MSG administration, semen volume was from 0.63 to 0.48 and 0.65 to 0.35 ml for the two treatments, respectively. There was a significant decrease ($p<0.05$) in semen volume at two- and four weeks post-treatment compared to the control group. The semen volume decreased as the dosage of MSG increased across the groups.

Sperm motility

The mean percentages of progressive sperm motility observed are as presented in Table 1. The percentage of sperm motility reduced as the concentration of MSG increased across the group. These changes were significant ($p<0.05$) compared to the control group.

Sperm count

The mean sperm count values are presented in Figure 2. They showed a significant difference ($p<0.05$) at a higher dose of 1.0 g/kg (Group D), two- and four weeks post-treatment compared with the control group. The sperm count is reduced as the MSG concentration increases.

Sperm liveability

The mean percentage of spermatozoa liveability as presented in Table 2 showed a significant difference compared to the control in Group D four weeks post-treatment ($p<0.05$). The sperm liveability is reduced as the concentration of MSG increases.

Sperm morphometry

Sperm morphometry measurements observed in both control and treatment groups are presented in Tables 3 and 4. Morphometry indices measured were; sperm head length and width, midpiece length, principal piece length, and sperm length. There was no significant difference ($p>0.05$) observed between the control group and all the treatment groups for both two- and four weeks post-treatment.

Sperm morphology

Morphological studies observed in this study for two- and four weeks post-treatment were presented in Tables 5, 6, 7, and 8 respectively. Abnormalities observed include; a rudimentary tail, coiled tail, bent tail, twin head, curved mid-piece, tailless head, headless tail, bent midpiece and looped tail. In addition, Tables 7 and 8 gave the summary of total sperm cells counted in percentages.

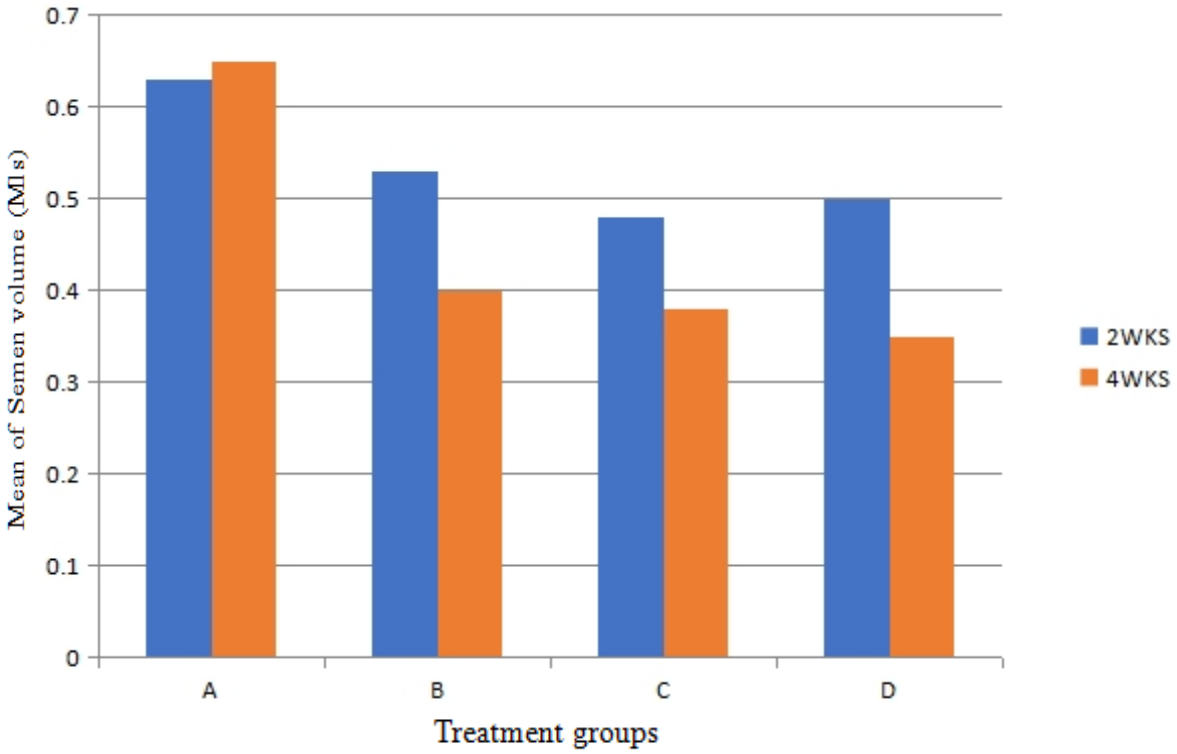

Figure 1. Compound bar chart showing semen volume in milliters (mls), mean values versus treatment groups, two- and four-weeks post msg treatment. **Key:** A= Control, B = 0.25 g/kg, C = 0.50 g/kg D = 1.00 g/kg, 2wks = 2 weeks post treatment, 4wks = 4 weeks post treatment.

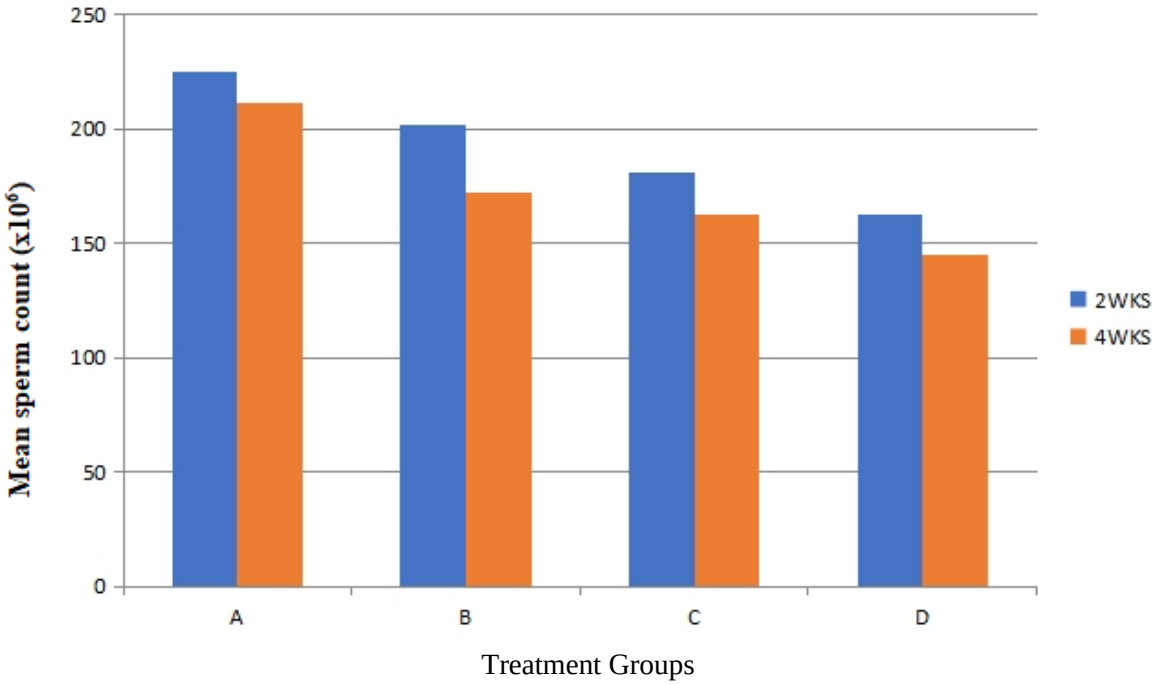

Figure 2. Compound bar chart showing sperm count ($\times 10^6$), mean values versus treatment groups, two and four weeks post MSG treatment. **Key:** A = Control, B = 0.25 g/kg, C = 0.50 g/kg, D = 1.00 g/kg, 2 wks. = 2 weeks post treatment, 4wks = 4 weeks post treatment.

Table 1. Mean sperm motility percentages following treatment with *Monosodium glutamate*.

Treatment	2 weeks post treatment	4 weeks post treatment
A (Control)	83.75±5.54[a]	80.00±4.08[a]
B (0.25mg/kg)	83.75±4.27[a]	60.00±4.08[d]
C (0.5mg/kg)	77.50±11.27[a]	57.50±2.50[c]
D (1.0mg/kg)	52.50±7.50[b]	55.00±2.88[b]

Values are reported as Mean ±SEM. Means with the same superscripts are not significantly different compared with the control group at (p<0.05) level of significance across the columns.

Table 2. Percentage sperm liveability following *Monosodium glutamate* treatment

Treatment	2 weeks post treatment	4 weeks post treatment
A (Control)	87.50±4.33[a]	80.00±7.07[a]
B (0.25 mg/kg)	86.25±2.39[a]	63.75±2.39[a]
C (0.50 mg/kg)	73.75±8.98[a]	62.50±2.50[a]
D (1.00 mg/kg)	63.75±6.57[a]	60.00±4.08[d]

Values are reported as Mean ±SEM. Means with the same superscripts are not significantly different compared with the control group at (p<0.05) level of significance across the columns.

Table 3. Buck sperm morphometry values two weeks (2) post treatment with *Monosodium glutamate.*

Treatment	Head length(um)	Head width(um)	Mid piece length(um)	Principal piece length(um)	Sperm length(um)
A (control)	6.56±0.11[a]	3.90±0.60[a]	13.58±1.37[a]	36.99±4.02[a]	60.50±4.03[a]
B (0,25 mg)	6.20±1.91[a]	3.60±0.40[a]	12.89±1.93[a]	37.64±4.28[a]	58.00±5.40[a]
C (0.50 mg)	6.56±0.15[a]	3.64±0.25[a]	12.77±1.54[a]	38.59±0.96[a]	59.00±3.28[a]
D (1.00 mg)	6.46±0.31[a]	3.90±0.12[a]	12.63±2.70[a]	37.81±4.04[a]	60.00±5.91[a]

Values are reported as Mean ±SEM. Means with the same superscripts are not significantly different compared with the control group at (p<0.05) level of significance across the columns. **Key: µm** = Micrometre, all measurements were made in µm. There was no significant difference observed throughout.

Table 4. Buck sperm morphometry four weeks (4) post treatment with MSG.

Treatment	Head length (um)	Head width (um)	Mid piece length (um)	Principal piece length(um)	Sperm length(um)
A (control)	6.34±1.13[a]	3.72±0.19[a]	12.67±0.40[a]	37.95±4.09[a]	58.66±4.03[a]
B (0.25 mg)	6.35±1.12[a]	3.72±0.39[a]	12.95±0.21[a]	38.55±3.15[a]	59.31±3.10[a]
C (0.50 mg)	6.58±1.90[a]	3.79±0.39[a]	12.81±2.21[a]	36.83±3.11[a]	58.12±3.15[a]
D (1.0 mg)	6.66±2.20[a]	3.93±1.18[a]	12.64±3.20[a]	38.63±3.32[a]	59.19±3.30[a]

Values are reported as Mean ±SEM. Means with the same superscripts are not significantly different compared with the control group at (p<0.05) level of significance across the columns. Key: **µm** = Micrometre, all measurements were made in µm. There was no significant difference observed throughout.

DISCUSSION

The semen volume observed in this study fell within the range of values reported for goat species 0.3 to 1.6 ml per ejaculate (Onakpa *et al.*, 2010; Oyeyemi *et al.*, 2002; Hafez and Hafez, 2000). However, there was a significant difference (p<0.05) in semen volume at four weeks post-treatment with MSG (in all the three Groups B, C, and D) compared to the control group. The result corroborates an earlier report of a significant (p<0.05) reduction in epididymal sperm of rats following oral treatment with graded doses of MSG by Igwebuike *et al*. (2011). Reduced semen volume could be associated with MSG-induced cytotoxicity (Bakare and Adeyemo, 2004), resulting in a reduction of testicular weight. This reduction had been reported following intra-peritoneal administration of MSG

Table 5. Sperm morphological studies two weeks post treatment with *Monosodium glutamate*.

Treatment	TH	HT	BM	CM	RT	BT	CT	LT
A (control)	6.75±0.85^{a}	5.25±0.63^{a}	7.05±0.41^{a}	8.25±0.48^{a}	1.75±0.25^{a}	7.50±0.65^{a}	6.75±0.85^{a}	1.25±0.25^{a}
B (0.25mg/kg)	8.75±0.41^{a}	7.50±0.50^{a}	11.00±0.41^{b}	9.25±0.85^{a}	3.25±0.25^{a}	10.75±0.48^{a}	9.25±0.85^{b}	2.28±0.25^{a}
C (0.5mg/kg)	12.00±1.75^{a}	9.25±0.75^{a}	10.75±0.85^{c}	11.00±0.71^{a}	2.00±0.41^{a}	10.00±1.08^{a}	9.75±0.85^{d}	2.00±0.41^{a}
D (1.0mg/kg)	6.75±0.85^{a}	6.75±0.85^{a}	10.75±0.85^{d}	10.75±085^{a}	2.25±0.48^{a}	9.75±0.75^{a}	11.00±0.58^{c}	2.25±0.48^{a}

Values are reported as Mean ±SEM. Means with the same superscripts are not significantly different compared with the control group at (P<0.05) level of significance across the columns. **Key:** A = Control, B = 0.25 g/kg, C = 0.5 g/kg D = 1.0 g/kg, 2 wks. = 2 weeks post-treatment, 4wks = 4 weeks post-treatment, TH = tail without head, HT = head without tail, BM = bent mid-piece, CM = curved mid-piece, RT = rudimentary tail, BT = Bent Tail, CT = curved tail, LT = looped.

Table 6. Summary of sperm morphological studies expressed in percentages, two weeks post-treatment with *Monosodium glutamate*.

Treatment	TNC (%)	TAC (%)	TCC
A (control)	1450(89.00)	180(11.00)	1630(100)
B (0.25mg/kg)	1366(84.60)	249(15.40)	1615(100)
C (0.5mg/kg)	1374(84.30)	256(15.70)	1630(100)
D (1.0mg/kg)	1373(84.80)	247(15.20)	1620(100)

Key: TAC = total normal cells counted, TNC = total abnormal cells counted, TCC = total cells counted.

Table 7. Sperm morphological studies four weeks post treatment with *Monosodium glutamate.*

Treatment	TH	HT	BM	CM	RT	BT	CT	LT
A (control)	7.00±0.71^{a}	6.50±0.29^{a}	9.25±0.85^{a}	8.00±0.41^{a}	2.25±0.25^{a}	8.50±0.65^{a}	7.75±0.63^{a}	1.75±0.25^{a}
B(0.25mg/kg)	9.00±0.41^{a}	7.75±0.48^{a}	11.00±0.41^{bc}	10.25±0.63^{ab}	1.75±0.25^{a}	10.25±0.48^{a}	9.25±0.85^{ab}	1.75±0.25^{a}
C (0.5mg/kg)	10.25±1.75^{a}	9.25±0.75^{a}	12.25±0.48^{c}	10.75±0.85^{ac}	1.75±0.25^{a}	10.25±1.31^{a}	10.75±0.48^{ad}	2.50±0.29^{a}
D (1.0mg/kg)	9.25±1.44^{a}	9.00±1.58^{a}	11.75±0.48^{d}	11.00±0.41^{ad}	2.50±0.50^{a}	11.75±0.48^{a}	11.75±0.23^{ac}	2.25±0.25^{a}

Values are reported as Mean ±SEM. Means with the same superscripts are not significantly different compared with the control group at (P<0.05) level of significance across the columns. **Key:** TH = tail without head, HT = head without tail, BM = bent mid-piece, CM = curved mid-piece, RT = rudimentary tail, BT = bent tail, CT = curved tail, LT = looped tail.

to Wistar rats (Nayanatara *et al.*, 2008) and extended ejaculation time caused by plant extract, chemical, or drug administration (Oyeyemi *et al.*, 2008).

A significant decrease (p<0.05) in mean percentage sperm motility was observed as the mean percentage of sperm motility decreased (p<0.05) in group D two weeks and groups B, C, and D four weeks post-treatment compared to the control group. This finding agrees with an earlier report of decreased sperm motility by Kadir *et al*. (2011) as one of the effects of MSG on semen quality in Wistar rats. Kadir *et al.* (2011) reported a significant dose-dependent reduction effect at two weeks post-treatment. The significantly decreased sperm motility in this study suggests that MSG consumption adversely affects sperm motility in a non-dose dependent fashion in group D two

Table 8. Summary of sperm morphological studies expressed in percentages, four weeks post treatment with *Monosodium glutamate*.

Treatment	TNC (%)	TAC (%)	TCC
A (control)	1421(87.40)	204(12.60)	1625(100)
B (0.25 mg/kg)	1386(85.00)	244(15.00)	1630(100)
C (0.50 mg/kg)	1351(83.40)	269(16.60)	1620(100)
D (1.00 mg/kg)	1372(83.40)	273(16.20)	1645(100)

Key: TNC = total normal cells counted, TAC = total abnormal cells counted, TCC = total cells counted.

weeks post-treatment; and all groups four weeks post-treatment (Nass *et al*.,1990).

There was significant ($p<0.05$) oligozoospermia (reduced sperm count below the normal range for a given species) in groups D (1.00 g/kg) of both two- and four weeks post-treatment compared to the control group. The finding suggests the dose-dependent effect of MSG on sperm count and this also validates earlier reports of the dose-dependent related reduction effect of MSG on sperm count in rats (Oforofuo *et al*., 1997; Onakewhor *et al.,* 1998; Igwebuike *et al.*, 2011; Iamsaard *et al.*, 2014). The observed significant reduction in sperm count could be due to the cytotoxic effect of MSG on mitotic cell division through an effect on mitotic index and the chemical that binds to tubulin and prevent the formation of the mitotic spindle (Bakare and Adeyemo, 2004) and testicular degeneration (Onakewhor *et al*., 1998). It can also be attributed to reduced serum testosterone levels observed following MSG administration in rats (Igwebuike *et al.*, 2011). Dosh *et al.* (1994) observed and reported a positive correlation between testosterone concentration and total sperm count in buffalo bulls treated with clomiphene citrate, the report established that a low level of testosterone was always associated with low values of sperm parameters (Onakpa *et al*.,2010).

The mean percentage of buck sperm live/dead ratio in this study showed a significant reduction ($p<0.05$) in liveability four weeks post-treatment at group D (1.00 g/kg) 60.00±4.08 compared to control group 80.00±7.07. This finding corroborates the earlier report of Alalwani (2003) and Kadir *et al.* (2011) who reported that MSG administration to rats led to increasing sperm cell dead and necrosis of spermatogonia. They suggest that administering MSG at higher doses may adversely reduce fertility in WAD bucks by increasing the percentage of dead sperm cells.

Investigation on sperm morphology in this study was consistent with the previous report of Moss *et al. (*1979) that a certain number of abnormal spermatozoa were found in all ejaculates regardless of specie and method of collection, thus only when in large numbers prejudice fertility (Oyeyemi *et al.,* 2008). There was no significant difference ($p<0.05$) observed in the abnormal sperm cells recorded for the tailless head (head without tail), headless tail (tail without head), curved midpiece, rudimentary tail, bent tail, and looped tail compared to the control group. However, there was a significant difference ($p<0.05$) in the bent midpiece (10.7±0.85) and curved tail (11.75±0.23) compared to control groups (7.05±0.41) and (6.75±0.85) for the two treatments respectively. This finding corroborates earlier work reported by Nayanatara *et al. (*2008) and Kadir *et al.* (2011), that MSG treatment was associated with an increase in abnormal sperm cell morphology. In addition, mid piece abnormality was reported to occur during epididymal storage (Oyeyemi and Babalola, 2006).

Zinc deficiency has also been incriminated as a cause of sperm cell abnormality due to its role in DNA and RNA synthesis. However, the exact mechanism is unknown (Wong *et al.,* 2000). The percentage of total percentages of abnormalities observed (<20%) was in the range that could not impair male animal fertility according to an earlier previous report by Hafez and Hafez (2000) who stated that all semen samples are bound to contain a proportion of abnormal cells, it may however not be associated with infertility except when their proportion exceeds 20%.

In conclusion, the sperm volume, sperm motility and sperm morphology reduced across the groups as the dosages of MSG increased. Hence, monosodium glutamate adversely affects semen characteristics; impairs sperm fertilizing ability and may reduce bucks' fertility. Thus, its continuous application as a condiment in various foods and administration to breeding bucks should be used with caution.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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