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The effects of monosodium L-glutamate administration on the reproduction and serum biochemistry of adult male rabbits

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ABSTRACT: In this study the effects of monosodium L-glutamate (MSG) administration on the reproductive parameters and serum biochemistry of male rabbits were investigated. Sixteen mature male New Zealand mixed-breed of rabbits (*Oryctolagus species*) weighing 1.1–1.65 kg were used for this study. They were randomised into four groups of four rabbits each. Group A which served as the untreated control, received only distilled water while Groups B, C and D which were the treated groups, received 0.25 g/kg, 0.5 g/kg and 1 g/kg body weight of MSG from a 40% MSG stock solution. The MSG was administered to the rabbits by oral gavage every forty-eight hours for a period of eight weeks. Serum levels of luteinising hormone (LH), testosterone, total cholesterol and total protein, and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated on Days 14, 28 and 56 of MSG administration. Results showed that when compared to the untreated group (Group A), on Day 56 of MSG administration the mean serum levels of both LH and testosterone were significantly ($P < 0.05$) lower in Group D that received 1 g/kg body weight MSG, while the serum cholesterol levels of Group C rabbits that received 0.5 g/kg body weight MSG was significantly ($P < 0.05$) lower on Day 28 of MSG administration. The mean serum ALT activity of Groups B and C rabbits were significantly ($P < 0.05$) lower on Day 56 of MSG administration, while the mean serum AST activity of the Group D rabbits was significantly ($P < 0.05$) higher on Day 14 of MSG administration. There were no significant ($P > 0.05$) variations in the mean serum total protein between the groups. Testicular histomorphology revealed that MSG administration did not affect the testes of the rabbits as there was no obvious testicular histopathology. It was concluded that administration of MSG to male rabbits significantly lowered serum LH, testosterone and cholesterol levels and serum ALT activity without affecting testicular histomorphology.

Keywords: monosodium L-glutamate, testosterone, testes, cholesterol, *Oryctolagus species*

Monosodium L-glutamate (MSG) also called sodium glutamate, is the sodium salt of naturally occurring glutamic acid. Molecules of MSG can exist in two different stereo-isomers with the chemical formula of $C_5H_8NNaO_4.H_2O$. MSG isomers have divergent physiological effects and only the levo-isomer has flavour-enhancing properties (Othmer 1978). MSG is naturally produced by organic synthesis, microbial fermentation or vegetable protein hydrolysis and synthetically by an organic reaction on acrylate. At room temperature MSG typically exists as a white crystalline salt that is soluble in water and alcohol. Under acidic conditions (pH 2.2–2.4) and at high temperatures MSG is partially dehydrated and converted into 5-pyrolidone-2-carboxylate (Meister 1979).

Kikunae Ikeda was the first person to produce MSG from the seaweed *Lamina japonica* by aqueous extraction and crystallisation (Lindermann 2002). He observed that it has a peculiar taste different from the four classical tastes (sweet, salty, sour and bitter) that had not been scientifically described at that time, which he called *umami* (Ninomiya 1998). MSG is used most commonly as a flavour enhancer because it balances, blends and rounds the total perception of other tastes (Loliger 2000). It is manufactured and marketed as a flavouring agent under various trade names such as Ac'cent®, Aji-No-Moto®, Vestin®, Vedan® and A-one®. There are anecdotal reports that MSG is used to 'knock out' libido in male goats (Igwebuike et al. 2011; Ochiogu et al. 2014; Ochiogu et al. 2015a;

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Ochiogu et al. 2015b). Olney (1993) also reported the use of MSG as a bleaching agent in Nigeria.

Apart from the earlier reported "Chinese restaurant syndrome" (Kwok 1968) and other reports of the adverse effects of MSG (such as neurotoxic, neuro-excitatory, psychotic, hepatotoxic, diarrhoeic and reproductive-endocrine dysfunction) on different organs and systems in humans and other animals (Schaumberg et al. 1969; Egbuonu et al. 2009), there is growing concern about the safety of ingesting MSG (Olney and Prince 1978).

Rabbits (*Oryctolagus species*), a small domesticated animal species, has not only assumed importance world-wide as a laboratory and research animal (Harkness et al. 2013), but is also commercially exploited for meat production (Harkness et al. 2013). The reproductive life and general health of this important animal species need to be protected especially as they are usually fed with household food items, the flavour of which may be enhanced with MSG.

Since there are virtually no reported studies on MSG using rabbits as animal model, the objective of this study was to evaluate the effects of MSG on the serum levels of testosterone, luteinising hormone, cholesterol and total protein, serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the testicular histomorphology of adult male rabbits.

MATERIAL AND METHODS

Sixteen mature male New Zealand mixed-breed of rabbits (*Oryctolagus species*) weighing 1.1–1.65 kg were used for this study. They were procured from a commercial rabbitry in Emene, Enugu, Enugu State, Nigeria. The rabbits were housed in clean iron-mesh cages in the Animal House Unit of the Department of Veterinary Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Nsukka (ambient temperature: 27–32 °C; approximately 12 h natural light per day; humidity: 50–60%). The rabbits were humanely handled throughout the duration of the study in compliance with the principles of laboratory animal care (NAS 2011). Commercially prepared pelleted feed (Vital® Feeds, Grand Cereals and Oil Mill Limited, Jos, Nigeria) containing 14.5% crude protein and 2500 kcal/kg metabolisable energy, and potable water were provided *ad libitum* for the rabbits.

The rabbits were acclimatised for two weeks before the commencement of the study. After acclimatisation, they were randomly assigned into four groups of four rabbits each. Group A which served as the untreated control, received only distilled water while Groups B, C and D which were the treated groups, received 0.25 g/kg, 0.5 g/kg and 1 g/kg body weight from a 40% MSG stock solution, respectively. The rabbits were dosed MSG by oral gavage between 09:00 h and 10:00 h every forty-eight hours for a period of eight weeks. The brand of MSG used for the study was Vedan® (99% MSG) manufactured by Vedan Enterprise Corporation, Taiwan. It was dissolved in distilled water before use (Nayanatara et al. 2008).

Serum samples were harvested by centrifugation of blood samples collected aseptically from the rabbits' ear vein on Days 14, 28 and 56 of MSG administration. The serum samples were then used for evaluation of the levels of luteinising hormone (LH), testosterone, total cholesterol and total protein, and activities of ALT and AST. The enzyme-linked immunosorbent assay (ELISA) technique (Ekins 1998) was used to assay both LH and testosterone using Accu-Bind® LH and testosterone test kits (Monobind Inc., Lake Forest, CA 92630, USA). The absorbance was read using a microplate reader (Mindray® microplate reader MR-96A, Shanghai, China).

Total cholesterol levels were evaluated using the Quimica Clinica Applicada (QCA) serum total cholesterol test kit (QCA, S.A. Spain) based on the enzymatic colorimetric method (Allain et al. 1974). Serum activities of ALT and AST were determined according to the colorimetric method of Reitman and Frankel (Reitman and Frankel 1957) using Quimica Clinica Applicada (QCA, Spain) test kits. Total protein levels were determined by the direct biuret method (Lubran 1978) using the QCA test kit.

On Day 56 of MSG administration, the rabbits were humanely sacrificed and the testes carefully dissected out and prepared for histomorphological examination (Drury and Wellington 1976). Briefly, the testes were fixed by immersion in Bouin's solution for 48 h. Subsequently, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Five micrometre-thick sections were cut, mounted on glass slides, and stained with haematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd. 1999–2004).

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Ethics. The housing, handling and welfare of the rabbits used for the study were in accordance with the Ethics and Regulations guiding the use of animals for research in the University of Nigeria, Nsukka.

Data analysis. Generated data were subjected to one-way analysis of variance (ANOVA). Variant means were separated using the least significant difference (LSD) method. Significance was accepted at a probability level of less than 0.05 ($P < 0.05$). Data are presented as mean ± standard error of mean (SEM).

RESULTS

On Days 14 and 28 of MSG administration, there was no significant ($P > 0.05$) difference between the mean serum LH concentrations of the treated groups when compared to the control group (Table 1). However on Day 56 of MSG administration the mean serum LH concentration of the treated Group D was significantly ($P < 0.05$) lower when compared to the untreated control (Table 1).

On Day 14 of MSG administration, the mean testosterone concentration of the treated Group C was significantly ($P < 0.05$) lower when compared to the untreated control (Table 2). On Day 28 of MSG administration, the treated Group D had a mean testosterone concentration that was significantly ($P < 0.05$) higher when compared to the untreated control group (Table 2). On Day 56 of MSG administration, the mean serum testosterone concentration of the treated Group C was significantly ($P < 0.05$) higher, while that of treated Group D was significantly ($P < 0.05$) lower when compared to that of the control group (Table 2).

There was a significant ($P < 0.05$) lowering of the mean serum total cholesterol concentration of the

Table1. Mean serum luteinizing hormone concentration (IU/ml) of male rabbits administered various doses of MSG orally (± SEM)

Groups	A 0.0 g/kg	B 0.25 g/kg	C 0.5 g/kg	D 1.0 g/kg
Day 14	0.06 ± 0.02[a]	0.08 ± 0.01[a]	0.07 ± 0.02[a]	0.06 ± 0.02[a]
Day 28	0.10 ± 0.06[a]	0.03 ± 0.01[a]	0.14 ± 0.10[a]	0.05 ± 0.02[a]
Day 56	0.06 ± 0.04[a]	0.08 ± 0.01[a]	0.10 ± 0.02[a]	0.01 ± 0.02[b]

[ab]different superscripts within a row indicate significant differences between the means ($P < 0.05$)

Table 2. Mean serum testosterone concentration (nmol/l) of male rabbits administered various doses of MSG orally (± S.E.M.)

Groups	A 0.0 g/kg	B 0.25 g/kg	C 0.5 g/kg	D 1.0 g/kg
Day 14	30.47 ± 13.21[a]	37.17 ± 3.57[a]	2.08 ± 0.35[b]	25.41 ± 10.05[ab]
Day 28	7.56 ± 4.13[a]	22.12 ± 10.82[a]	6.80 ± 4.58[a]	43.68 ± 0.00[b]
Day 56	3.50 ± 0.49[a]	4.20 ± 0.97[a]	24.79 ± 15.43[b]	0.55 ± 0.52[c]

[abc]different superscripts within a row indicate significant differences between the means ($P < 0.05$)

Table 3. Mean serum total cholesterol concentration (mmol/l) of male rabbits administered various doses of MSG orally (± S.E.M.)

Groups	A 0.0 g/kg	B 0.25 g/kg	C 0.5 g/kg	D 1.0 g/kg
Day 14	2.43 ± 0.60[a]	2.72 ± 0.51[a]	2.22 ± 0.53[a]	2.63 ± 0.42[a]
Day 28	1.82 ± 0.13[a]	2.01 ± 0.19[a]	1.21 ± 0.11[b]	1.86 ± 0.20[a]
Day 56	1.47 ± 0.07[a]	1.42 ± 0.24[a]	1.28 ± 0.34[a]	1.46 ± 0.07[a]

[ab]different superscripts within a row indicate significant differences between the means ($P < 0.05$)

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Table 4. Mean serum ALT activities (nkat/l) of male rabbits administered various doses of MSG orally (± S.E.M.)

Groups	A 0.0 g/kg	B 0.25 g/kg	C 0.5 g/kg	D 1.0 g/kg
Day 14	334.40 ± 48.0[a]	419.75 ± 39.67[a]	402.75 ± 39.51[a]	441.76 ± 81.18[a]
Day 28	357.74 ± 17.34[a]	362.74 ± 6.50[a]	377.58 ± 29.01[a]	322.40 ± 23.67[a]
Day 56	497.77 ± 42.34[a]	370.91 ± 33.01[b]	377.08 ± 9.50[b]	407.58 ± 49.18[ab]

[ab]different superscripts within a row indicate significant differences between the means ($P < 0.05$)

Table 5. Mean serum AST activities (nkat/l) of male rabbits administered various doses of MSG orally (± S.E.M.)

Groups	A 0.0 g/kg	B 0.25 g/kg	C 0.5 g/kg	D 1.0 g/kg
Day 14	510.77 ± 39.5[a]	589.45 ± 45.34[a]	609.46 ± 177.04[a]	825.00 ± 182.04[b]
Day 28	368.57 ± 45.18[a]	374.24 ± 36.84[a]	431.59 ± 103.35[a]	315.23 ± 27.5[a]
Day 56	449.09 ± 75.18[a]	362.08 ± 26.84[a]	429.25 ± 11.84[a]	498.10 ± 100.85[a]

[ab]different superscripts within a row indicate significant differences between the means ($P < 0.05$)

Table 6. Mean serum total protein (g/l) of male rabbits administered various doses of MSG orally (± S.E.M.)

Groups	A 0.0 g/kg	B 0.25 g/kg	C 0.5 g/kg	D 1.0 g/kg
Day 14	53.30 ± 1.60[a]	54.80 ± 0.70[a]	52.50 ± 1.90[a]	55.50 ± 3.40[a]
Day 28	59.70 ± 2.20[a]	58.60 ± 0.70[a]	60.40 ± 0.30[a]	56.80 ± 1.70[a]
Day 56	53.60 ± 1.90[a]	54.20 ± 0.60[a]	51.90 ± 2.30[a]	50.60 ± 2.80[a]

[a]superscript indicates no significant difference between the means within a row ($P > 0.05$).

treated Group C when compared to the untreated control on Day 28, but not on Days 14 and 56 of MSG administration. However, there was no significant ($P > 0.05$) difference in the mean serum total cholesterol concentrations of the other treated Groups (B and D) on Days 14, 28 and 56 of MSG administration when compared with the untreated control group (Table 3).

The mean serum ALT activities of the treated Groups B and C were significantly ($P < 0.05$) lower when compared to the untreated control on Day 56 of MSG administration. However, on Days 14 and 28 of MSG administration, there was no significant ($P > 0.05$) difference in the mean ALT serum activities between any of the groups (Table 4).

Apart from the treated Group D in which the mean serum AST activity was significantly ($P < 0.05$) higher when compared to the untreated control on Day 14 of MSG administration, there were no significant ($P > 0.05$) differences in the mean serum AST activities between any of the groups on Days 14, 28 and 56 MSG administration (Table 5).

There were no significant ($P > 0.05$) variations in the mean serum total protein between the groups on Days 14, 28 and 56 of MSG administration (Table 6).

Analysis of testicular histomorphology revealed that MSG administration did adversely (between

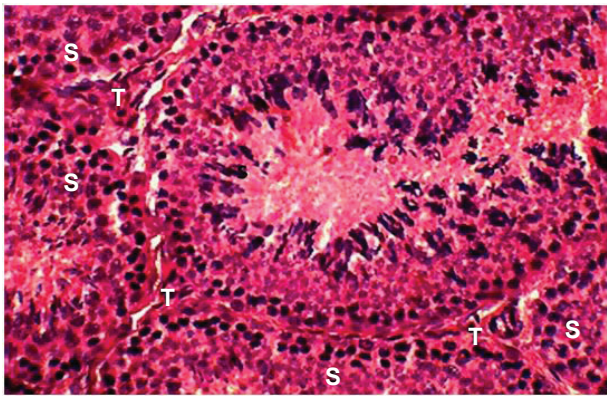

Figure 1. Photomicrograph of the testis of a rabbit in Group C on Day 56 of MSG administration, showing no obvious pathology. Note active seminiferous tubules (S) and interstitial spaces (T).Stained with H and E; × 400

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not and affect) the testes of the rabbits as there was no obvious testicular histopathology. In fact, the testes showed active seminiferous tubules and interstitial spaces (Figure 1).

DISCUSSION

In this study, the effect of oral administration of MSG on the concentration of serum LH at the highest dose administered was delayed and significant only on Day 56 of MSG administration. This is at variance with the reports of Tafelski and Lamperti (1977) and Ochiogu et al. (2015a) who recorded immediate and earlier effects, respectively. Blaylock (1994) reported that different individuals have different tolerance thresholds to MSG. It is therefore conceivable that in rabbits this threshold was overcome only by a relatively high dose and long duration of MSG administration and that subsequently the effects became evident. This lowering effect on the LH concentration could be attributed to the reported lesions MSG produces in the arcuate nucleus of the hypothalamus which secretes gonadotrophin-releasing hormone (GnRH) (Tafelski and Lamperti 1997; Ochiogu et al. 2015a). The GnRH controls the biosynthesis and secretion of LH by the anterior pituitary (Norris 1997; Ochiogu et al. 2015a; Ochiogu et al. 2015b).

Following MSG administration, there was an earlier drop in the serum testosterone concentration in the intermediate dose group (Group C) on Day 14. However, the effect on testosterone concentration varied depending on the duration of MSG administration and dose. For instance there was a lowering in the intermediate dose group on Day 14, and on Day 28 there was a significantly higher serum testosterone concentration in Group D rabbits, but by Day 56 Group C had significantly higher testosterone and Group D exhibited lower testosterone levels. However, there was no observed effect in the low dose group, showing that the effect may be dose-dependent but not time-dependent. These observed effects may also have other causes apart from LH bio-stimulation of testosterone production by interstitial (Leydig) cells (McLachlan et al. 1996) of the rabbits' testes.

The significant lowering of the serum total cholesterol by MSG administration on Day 28 in this present study is corroborated by the reports of Bazzano et al. (1970), Ochiogu et al. (2015a) and Ochiogu et al. (2015b) on studies with gerbils, rats and goats, respectively. However, it is not clear what effect this lowering of serum cholesterol exerted on testosterone concentrations in this study, as it is known that cholesterol is a precursor for testosterone biosynthesis (Hinshelwood 1998).

The absence of any obvious testicular histological lesions in all the groups in this study is in agreement with the reports of Igwebuike et al. (2011) and Ochiogu et al. (2015a) in rats, and Ochiogu et al. (2015b) in goats. They used doses similar to the ones used in this study and they opined that MSG treatment may have impacted on testosterone production through its disruption of the hypothalamic-pituitary-testis regulatory axis, and not through any direct effect on testicular structure. However, the absence of lesions in the testes of rabbits given MSG in this study contradicts the results of Oforofuo (1997), Ismail (2012) and Nosseir et al. (2012) who administered higher doses of MSG at more regular intervals or longer duration.

The reduction in the activity of serum ALT in this study is in agreement with the report of Ochiogu et al. (2014) that showed a decrease in serum ALT activity in male goats given MSG. However, Egbuonu et al. (2009) reported an elevation of the serum ALT activity in rats. Determination of serum AST levels in this study showed an immediate elevation of serum AST in the Group D rabbits. An elevation in serum liver enzymatic activity may indicate hepatotoxic effects of MSG (Egbuonu et al. 2009), whereas a decrease indicates hepato-protection (Ochiogu et al. 2014). The observed immediate elevation of serum AST activity may indicate a hepatotoxic effect of MSG at the 1 mg/kg body weight dose in this study. We propose that the high dose (1 mg/kg) initially overwhelmed the threshold of the liver to biotransform the MSG, but following establishment of tolerance a reduced activity (hepato-protective effect) of the MSG was then observed. This subsequent lowering of serum AST activity may also have arisen from the ability of enterocytes to "mop up" MSG by using it as oxidative fuel (Young and Ajami 2000), and thereby reducing its bioavailability.

Administration of MSG to rabbits in this study neither increased nor decreased serum total protein throughout the period of the study, and this is contrary to the increased serum total protein reported by Ochiogu et al. (2014). The differences in the animal models used (goats and rabbits) may have been responsible for these different observa-

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tions. This gives credence to the report of Blaylock (1994) in which it is described that individuals differ in their tolerance to MSG, thereby resulting in dramatic variations in the way they react to the substance.

Based on the results of this present study, it was concluded that administration of MSG to male rabbits led to a significant lowering of serum LH, testosterone and cholesterol levels and serum ALT activity, but did not cause any overt pathological lesions in their testes. Also, no obvious effect was observed on serum total protein.

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