

Serum luteinising hormone, testosterone and total cholesterol levels, libido and testicular histomorphology of male West African Dwarf goats orally or subcutaneously treated with monosodium L-glutamate

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ABSTRACT: This study investigated the effects of administration of monosodium L-glutamate (MSG) on serum luteinising hormone, testosterone and cholesterol levels, libido and testicular histomorphology of male West African Dwarf (WAD) goats. Thirty-two WAD goats (28 males and four females) were used for the study. The 28 males were randomly assigned to seven groups (A, B^O, B^S, C^O, C^S, D^O and D^S) of four goats each. Goats in groups B^O, C^O, and D^O were given MSG orally at doses of 0.25, 0.50 and 1 g/kg body weight respectively for 28 days, while groups B^S, C^S and D^S goats were given MSG subcutaneously at doses of 0.25, 0.50 and 1 g/kg body weight, respectively, for 28 days. Group A goats served as untreated control. The four female goats did not receive MSG, but were used to assess the levels of expression of libido by all the males. Serum luteinising hormone and testosterone were assayed prior to MSG administration (Day 0) and on Days 2, 14 and 28 of MSG administration, while serum cholesterol was assayed on Days 0, 14 and 28 of MSG administration. Libido scores and testicular histomorphology were evaluated on Days 26 and 28 of MSG administration, respectively. Results showed that on Days 14 and 28 of MSG administration the mean serum luteinising hormone, testosterone and cholesterol levels of the treated groups were significantly ($P < 0.05$) lower than those of the control group. The mean libido scores of all the treated groups were significantly ($P < 0.05$) lower than that of the control. Sections of the testes of the male WAD goats that received varying doses of MSG orally or subcutaneously showed no obvious lesions; the seminiferous tubules and interstices were normal and comparable to those of the untreated control. It was concluded that MSG administration for up to 14 and 28 days led to a significant lowering of serum luteinising hormone, testosterone and cholesterol, as well as libido scores.

Keywords: dietary supplements; flavouring agents; traditional means of castrating bucks; reproductive toxicity

List of abbreviations

LH = luteinising hormone, MSG = monosodium L-glutamate, PPR = peste des petites ruminants, WAD = West African Dwarf

There has been increasing concern regarding the safety of individual amino acids used as dietary supplements for enhancing health and/or as flavouring agents (Garlick 2004). Of all the amino acids used by brain cells, none is more prevalent in cerebral tissues, or more controversial, than glutamic acid (Tafelski and Lamperti 1977). Adverse effects at-

tributed to glutamic acid have been extensively reported (Lemkey-Johnston and Reynolds 1974; Raiten et al. 1995; Das and Gosh 2010; Nosseir et al. 2012).

Goats became extremely important to humans upon their domestication some 9000 years ago, and in the recent past, awareness of the merits of

keeping them has increased, stimulated by their ability to adapt to adverse climates and to survive extended periods of drought (Gall 1996). Goats can subsist on lands unusable to farmers and provide many valuable products including milk, skin and meat. Goats have been extensively used in experimental research, probably owing to the convenience of their use (Linzell 1972). Dwarf goats can be found from the southern Sudan to the west coast of Africa. In the 15 countries of the West African humid zone, 38% of the total population of approximately 38 million goats belongs to the West African Dwarf (WAD) breed (Gall 1996). The WAD goat is therefore an economically important breed in the humid tropics of South Eastern Nigeria and its continual existence and survival has to be protected through healthy reproductive life. These animals mostly roam around scavenging for food. They thus have access to leftover foods and leaves used in wrapping such foods cooked with monosodium l-glutamate (MSG) as an additive. Even their owners feed them with leftovers and supplements that commonly contain MSG. More importantly, the *Fulani* animal growers in Nigeria traditionally use MSG in “knocking out” libido in bucks (Igwebuiké et al. 2011). They traditionally administer MSG as a means of castrating bucks.

Numerous investigative studies on the health effects of MSG have been carried out using mostly rodents, humans and non-human primates (Raiten et al. 1995; FSANZ 2003). There is no information in the available literature on studies using caprine species, let alone the WAD breed of goats. Further, no reported studies have compared different routes of administration of MSG either within or between animal species (Raiten et al. 1995). Finally, it was of interest to investigate the traditional use of MSG in “knocking out libido” and in castration. The objectives of this study were to evaluate the effects of oral or subcutaneous administration of MSG on serum luteinising hormone, testosterone and total cholesterol levels, libido and testicular histomorphology in male WAD goats.

MATERIAL AND METHODS

Experimental animals. Thirty-two young sexually mature WAD goats (28 males and four females) were used for the study. They were raised in Nsukka, Enugu State, Nigeria. The goats were 12

to 15 months of age, and weighed between 8 and 12 kg. They were vaccinated during acclimatisation against *peste des petites ruminants* (PPR) using the PPR vaccine obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The animals were also dewormed with mebendazole (Wormin[®], Cadila Pharmaceuticals, Dholka, India) at a recommended dose of 15 mg/kg *per os* (Bishop 2005). After a two-week period of acclimatisation, they were randomly assigned to groups and properly tagged. The goats were housed within the Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and were fed *ad libitum* on common edible grasses and shrubs (*Panicum maximum*, *Stylosanthes gracilis*, and *Penisetum purpureum*) in addition to routine supplements. They had free access to drinking water throughout the whole period of the study.

Monosodium L-glutamate. The test compound used was Vedan[®] (99% MSG) brand of monosodium L-glutamate (MSG) ($C_5H_8NNaO_4 \cdot H_2O$), manufactured by Vedan Enterprise Corporation, Taiwan. It was dissolved in distilled water before use (Nayanatara et al. 2008). A stock solution was prepared by dissolving a 454 g sachet of MSG crystals in distilled water up to a volume of 1620 ml. The dose schedule was so adjusted that the amount of MSG administered per animal corresponded to their body weight.

Experimental design. The 28 male WAD goats were randomly assigned into seven groups (A, B^O, B^S, C^O, C^S, D^O and D^S) of four each. Group A served as a control that was not given MSG. Group B^O was given 0.25 g of MSG per kg body weight orally, while group B^S was given the same dose subcutaneously. Group C^O was given 0.5 g of MSG per kg body weight orally, while group C^S was given the same dose subcutaneously. Group D^O was given 1 g of MSG per kg body weight orally, while group D^S was given the same dose subcutaneously. The MSG was administered at 48 h intervals during the 28 days of the experimental treatment. The 4 females did not receive MSG, but were only used to assess the levels of expression of libido by all the males.

Assay of serum luteinising hormone and testosterone. The serum luteinising hormone (LH) and testosterone levels were assayed before the commencement of MSG administration (Day 0) and on Days 2, 14 and 28 of MSG administration using the Accu-Bind LH and testosterone test kits (Monobind Inc., Lake Forest, USA) based on the

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enzyme-linked immunosorbent assay (ELISA) technique (Ekins 1998).

Evaluation of serum total cholesterol. The serum levels of total cholesterol were evaluated on Days 0, 14 and 28 of MSG administration 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).

Determination of libido levels. The libido score method (Chenoweth and Osborne 1975) was used to assess for libido levels in the male goats on Day 26 of MSG administration.

Testicular histomorphology. Testicular histomorphology was evaluated on Day 28 of MSG administration. The testes were dissected from each buck under local anaesthesia on Day 28 of MSG administration. The testes were fixed by immersion in Bouin's fluid for 48 h. Later, 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).

Ethics. The housing, handling and welfare of the goats 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.

Statistical analyses. Data generated from the study 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.

RESULTS

There were no significant ($P > 0.05$) variations between the serum LH levels of all the groups at the commencement of the study (Day 0) and on Day 2 of MSG administration (Table 1). However, on Days 14 and 28, the mean serum LH levels of all the treatment groups were significantly ($P < 0.05$) lower than that of the control group (Table 1). Also on Day 14 within the treatment group, groups C^S, D^O and D^S had significantly ($P < 0.05$) lower serum LH levels when compared with group B^O. On Day 28 of MSG administration, there were, however, no significant ($P > 0.05$) variations within the MSG-treated groups though all of them had serum LH levels significantly ($P < 0.05$) lower than that of the control (Table 1).

On Days 0 and 2 of MSG administration, there were no significant ($P > 0.05$) variations in the serum testosterone levels of all the experimental groups (Table 2). The mean serum testosterone levels of all the groups treated with MSG were,

Table 1. Serum luteinising hormone (LH) levels of male West African Dwarf goats given different doses of monosodium L-glutamate (MSG) orally or via the subcutaneous (*s.c.*) route for 28 days

Groups, with treatments and route where applicable	Means \pm standard error (mIU/ml)			
	Day 0	Day 2	Day 14	Day 28
Group A (untreated control)	3.23 \pm 1.06 ^a	2.57 \pm 0.93 ^a	3.24 \pm 0.44 ^a	2.29 \pm 0.78 ^a
Group B ^O (0.25g/kg bw MSG, oral)	2.89 \pm 1.39 ^a	2.65 \pm 0.73 ^a	1.39 \pm 0.29 ^b	0.35 \pm 0.28 ^b
Group B ^S (0.25g/kg bw MSG, <i>s.c.</i>)	4.21 \pm 3.02 ^a	2.14 \pm 0.72 ^a	0.60 \pm 0.30 ^{bc}	0.53 \pm 0.25 ^b
Group C ^O (0.5g/kg bw MSG, oral)	2.46 \pm 1.58 ^a	2.19 \pm 0.77 ^a	0.83 \pm 0.47 ^{bc}	0.17 \pm 0.12 ^b
Group C ^S (0.5g/kg bw MSG, <i>s.c.</i>)	2.27 \pm 0.74 ^a	2.27 \pm 0.61 ^a	0.30 \pm 0.24 ^c	0.27 \pm 0.21 ^b
Group D ^O (1.0g/kg bw MSG, oral)	3.27 \pm 0.93 ^a	2.01 \pm 1.04 ^a	0.30 \pm 0.24 ^c	0.43 \pm 0.24 ^b
Group D ^S (1.0g/kg bw MSG, <i>s.c.</i>)	3.48 \pm 1.69 ^a	1.68 \pm 0.82 ^a	0.29 \pm 0.27 ^c	0.28 \pm 0.14 ^b

^{abc}different superscripts in a column indicate significant difference between the means, ($P < 0.05$)

Table 2. Serum testosterone levels of male West African Dwarf goats given different doses of monosodium L-glutamate (MSG) orally or via the subcutaneous (*s.c.*) route for 28 days

Groups, with treatments and route where applicable	Means \pm standard error (ng/ml)			
	Day 0	Day 2	Day 14	Day 28
Group A (untreated control)	8.85 \pm 0.85 ^a	8.47 \pm 0.73 ^a	8.68 \pm 0.62 ^a	9.06 \pm 1.84 ^a
Group B ^O (0.25g/kg bw MSG, oral)	8.44 \pm 2.44 ^a	8.29 \pm 1.86 ^a	2.59 \pm 0.75 ^b	1.39 \pm 0.39 ^b
Group B ^S (0.25g/kg bw MSG, <i>s.c.</i>)	7.98 \pm 1.75 ^a	8.12 \pm 2.24 ^a	2.46 \pm 0.80 ^b	1.40 \pm 0.65 ^b
Group C ^O (0.5g/kg bw MSG, oral)	8.55 \pm 2.08 ^a	8.07 \pm 1.63 ^a	2.43 \pm 1.60 ^b	2.39 \pm 1.11 ^b
Group C ^S (0.5g/kg bw MSG, <i>s.c.</i>)	8.50 \pm 1.83 ^a	7.37 \pm 1.51 ^a	1.92 \pm 1.03 ^b	1.35 \pm 0.72 ^b
Group D ^O (1.0g/kg bw MSG, oral)	7.88 \pm 1.51 ^a	7.21 \pm 1.77 ^a	1.34 \pm 0.59 ^b	1.35 \pm 0.38 ^b
Group D ^S (1.0g/kg bw MSG, <i>s.c.</i>)	8.92 \pm 1.38 ^a	7.42 \pm 1.43 ^a	1.23 \pm 0.70 ^b	0.96 \pm 0.47 ^b

^{ab}different superscripts in a column indicate significant difference between the means, ($P < 0.05$)

however, significantly ($P < 0.05$) lower than that of the untreated control group on Days 14 and 28 with no significant ($P > 0.05$) differences between the MSG-treated groups (Table 2).

There were no significant ($P > 0.05$) variations in the mean serum total cholesterol levels of all the experimental groups at the commencement of the study (Day 0), but on Days 14 and 28 of MSG administration, the mean serum total cholesterol levels of all the MSG-treated groups were significantly ($P < 0.05$) lower than that of the untreated

control group (Table 3). Within the MSG-treated groups, there were no significant ($P > 0.05$) variations in serum total cholesterol levels on Days 14 and 28 (Table 3).

The mean libido scores of all the MSG-treated groups were significantly ($P < 0.05$) lower than that of the untreated control group on Day 26 of MSG administration when libido scores were evaluated (Figure 1). There were, however, no significant ($P > 0.05$) differences in the mean libido scores between the MSG-treated groups (Figure 1).

Table 3. Serum total cholesterol levels of male West African Dwarf goats given different doses of monosodium L-glutamate (MSG) orally or via the subcutaneous (*s.c.*) route for 28 days

Groups, with treatments and route where applicable	Means \pm standard error (mg/dl)		
	Day 0	Day 14	Day 28
Group A (untreated control)	105.00 \pm 5.00 ^a	104.58 \pm 2.67 ^a	106.82 \pm 4.35 ^a
Group B ^O (0.25g/kg bw MSG, oral)	105.00 \pm 9.57 ^a	83.33 \pm 6.80 ^b	73.87 \pm 8.19 ^b
Group B ^S (0.25g/kg bw MSG, <i>s.c.</i>)	100.00 \pm 0.17 ^a	75.00 \pm 4.81 ^{bc}	74.25 \pm 5.74 ^b
Group C ^O (0.5g/kg bw MSG, oral)	102.50 \pm 10.31 ^a	70.84 \pm 0.47 ^{bc}	66.67 \pm 6.80 ^{bc}
Group C ^S (0.5g/kg bw MSG, <i>s.c.</i>)	107.50 \pm 0.26 ^a	75.00 \pm 10.76 ^{bc}	62.22 \pm 4.50 ^{bc}
Group D ^O (1.0g/kg bw MSG, oral)	95.00 \pm 9.57 ^a	70.83 \pm 7.98 ^{bc}	61.03 \pm 5.24 ^{bc}
Group D ^S (1.0g/kg bw MSG, <i>s.c.</i>)	107.50 \pm 9.47 ^a	62.50 \pm 4.17 ^c	55.35 \pm 3.94 ^c

^{abc}different superscripts in a column indicate significant difference between the means, ($P < 0.05$)

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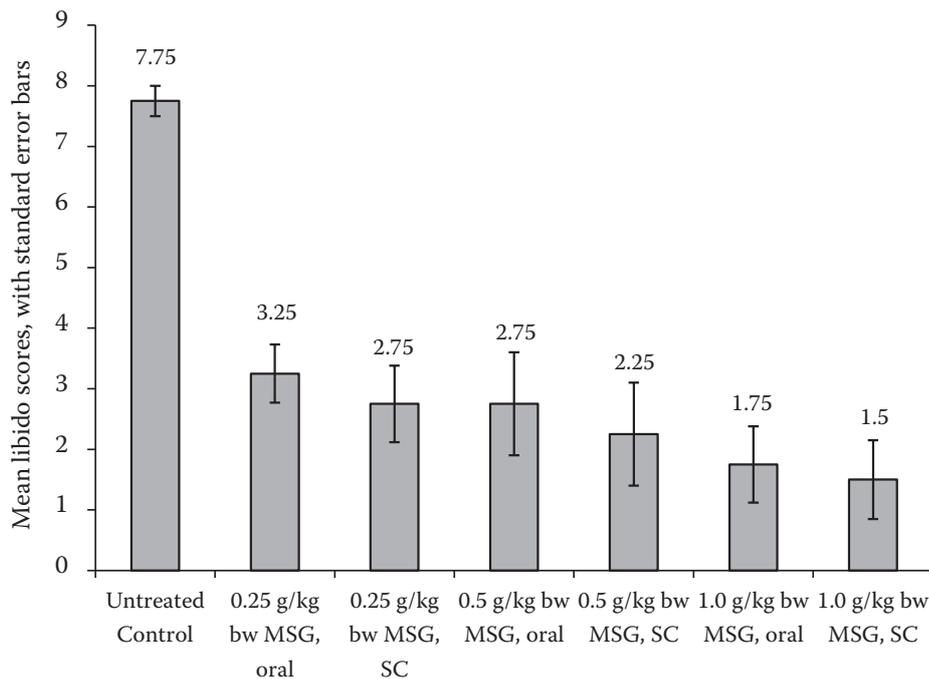


Figure 1. The mean libido scores of West African Dwarf goats given different doses of MSG either orally or via the subcutaneous (*s.c.*) route ($P < 0.05$)

Sections of the testes of the WAD bucks that received varying doses of MSG orally or subcutaneously showed no obvious lesions; the seminiferous tubules and interstices were normal and comparable to those of the untreated control (Figures 2–4).

DISCUSSION

The lack of significant change in serum LH and testosterone on Day 2 of MSG administration is

an indication that the doses of MSG used in this study (whether administered orally or subcutaneously) did not exert an immediate effect on the serum LH and testosterone levels of the treated bucks. This finding is in agreement with the reports of Adamo and Ratner (1970) and Fernstrom (2000) who administered a 4 g/kg body weight single treatment to rats, but differs from that of Tafelski and Lamperti (1977) who administered 8 g/kg body weight to hamsters. It is thought that the possible reasons why Tafelski and Lamperti

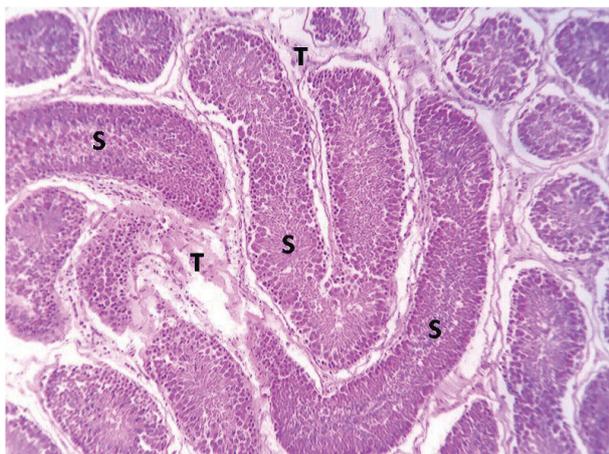


Figure 2. Histological appearance of testis of male WAD goat given 0.5 g/kg body weight of MSG (mid-dose) orally for 28 days, showing no obvious lesions. Note active seminiferous tubules (S) and interstitial spaces (T); H&E, $\times 100$

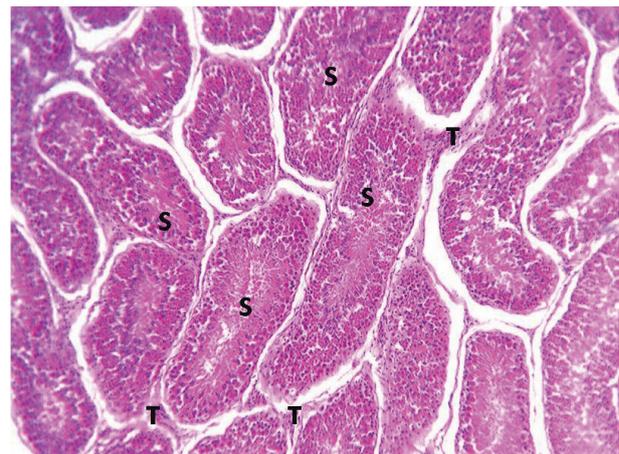


Figure 3. Histological appearance of testis of male WAD goat given 1.0 g/kg body weight of MSG (high-dose) orally for 28 days, showing no obvious lesions. Note active seminiferous tubules (S) and interstitial spaces (T); H&E, $\times 100$



Figure 4. Histological appearance of testis of male WAD goat given 1.0 g/kg body weight of MSG (high-dose) subcutaneously for 28 days, showing no obvious lesions. Note active seminiferous tubules (S), interstitial spaces (T) and testicular blood vessel (V); H&E, $\times 400$

(1977) were able to observe an immediate effect of MSG in hamsters in the form of lesions of the arcuate nucleus of the hypothalamus within 6–12 h of treatment were because of the high dose of MSG (8 g/kg body weight) and the immature nature of the hamsters used in their study. The significantly lower serum LH and testosterone levels recorded in this present study on Days 14 and 28 of MSG administration are in agreement with the reports of Redding et al. (1971) who recorded a decrease in the pituitary content of LH in rats treated with MSG. It is thought that the significantly lower serum LH recorded in the MSG-treated groups at Days 14 and 28 of MSG administration may be as a result of reduced gonadotrophin-releasing hormone (GnRH) associated with the lesions on the arcuate nucleus of the hypothalamus that occurs in animals given MSG (Palkovits et al. 1974; Pizzi et al. 1977; Hawkins et al. 1995; Raiten et al. 1995; Igwebuike et al. 2010; Igwebuike et al. 2011). The significantly lower levels of serum testosterone, however, may be attributed to the low serum LH and total cholesterol recorded in the study as the main action of LH is the conversion of cholesterol to pregnenolone – a rate-limiting step in the biosynthesis of steroid hormones, of which testosterone is one (Hinshelwood 1998). It is possible that the effects of MSG administration on LH and testosterone on Days 14 and 28 may be as a result of accumulated toxic effects (Samuels 1999), that would not manifest immediately on commence-

ment of administration of the MSG. The absence of significant differences in the serum LH of the different groups with respect to dose levels and routes of administration used in this study implies that the effect of MSG on LH was neither dose-dependent nor route of administration-dependent.

The significantly lower serum levels of cholesterol on Days 14 and 28 of MSG administration is worthy of note as cholesterol is a precursor of steroid hormones (Hinshelwood 1998). We propose that this lowered serum cholesterol in conjunction with the lowered serum LH was responsible for the lowered serum testosterone levels recorded in this study. The significantly lower serum cholesterol recorded for goats given MSG in this present study is in agreement with the reports of Bazzano et al. (1970) in humans and gerbils. However, Ahluwalia and Malik (1989) reported no effects on serum cholesterol in mice given MSG for six days. The lowering of serum cholesterol by MSG administration in this study may be attributable to the reported destructive effects of MSG on the arcuate nucleus of the hypothalamus, which is known to partly function in regulation of fat metabolism (Bazzano et al. 1970; Ahluwalia and Malik 1989; Dieguez et al. 2011).

The reduced libido scores recorded for the goats treated with MSG in this study may have been due to their lowered serum levels of testosterone (Chenoweth 1981), as all other parameters and factors apart from MSG doses and routes of administration were equally applicable to all. The finding of reduced libido in the WAD bucks treated with MSG in this study tends to validate the traditional or folkloric use of MSG by the Nigerian *Fulani* animal growers in “knocking out libido” and castration of bucks (Igwebuike et al. 2011). This reduced libido recorded in this present study is in agreement with the reports of Pizzi et al. (1977) on male mice treated with MSG.

The absence of any obvious pathological lesions in the histomorphology of the testes of the bucks treated with MSG in this study suggests that MSG treatment did not have any direct effect on the structure of the testes. This observation is in agreement with what had been reported in rats following oral administration of MSG (Igwebuike et al. 2011). The inference is that MSG treatment may have impacted on libido through its disruption of the hypothalamic-pituitary-testis regulatory axis, and not through any direct effect on testicular

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structure. However, we intend to carry out a more detailed endocrine and reproductive study on MSG using other animal models.

Based on the results of this study, we conclude that administration of MSG to male WAD goats leads to a significant lowering of their serum levels of LH, testosterone and total cholesterol, and libido scores after 14 and 28 days of administration.

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