



Inclusion of Monosodium Glutamate in Poultry Feed Did Not Alter Semen Characteristics and Certain Biochemical Parameters in Cock Testes

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Abstract

Monosodium glutamate (MSG) is a food flavor enhancer but its intake has adverse effects including oxidative stress and inflammation. Recently, there is a growing interest regarding the inclusion of MSG in poultry feed to enhance feed palatability and intake but the safe dose for poultry is yet to be determined. Given the crucial role of reproductive efficiency in poultry production, this study was conducted to assess semen characteristics, lipid profile, inflammatory status and redox balance in the testes of cocks fed with diet containing varying inclusion level of MSG. A total of thirty-two (32) 28-weeks old cocks were randomly allotted into four groups (ten/group): control (group A) and MSG-diet groups- B, C and D. The groups were placed on feed of the same composition but feeds of MSG diet groups-B, C and D were supplemented with 0.5 %, 1 %, 1.5 % MSG (Ajinomoto®) respectively for eight weeks. On day 57, semen and testicular samples were collected for evaluation of semen characteristics, and analysis of lipid profile, oxidative stress and inflammation biomarkers respectively. Mean $\pm$ SD values were calculated for each group and compared for significant difference with ANOVA at $p < 0.05$. Semen volume, sperm count, viability and motility of both and MSG-diet groups were not statistically different. Level of oxidative stress biomarkers, SOD, catalase, GSH and MDA, as well inflammation mediators- COX-2, PGE2, IL-1 β , IL-6 were similar in the control and MSG-diet groups. Likewise, total cholesterol, triglyceride, HDL and LDL level in the testes of MSG-diet groups were not significantly affected. Findings of this study showed that intake of feed containing MSG up to 1.5 % inclusion had no detectable effect on the semen characteristics as well as lipid profile, inflammatory and redox states of cock testes.

Keywords: Monosodium Glutamate, Reproductive Hormones, Semen Characteristics, Cocks

Introduction

Overall reproductive capacity of poultry flocks depends on the fertility of both males and females, with males playing more important role. Like other male animals, the fertility of male birds is determined by the quality of semen, which is often assessed using sperm count, motility and viability (Peter *et al.*, 2004). Reproduction being an energy-demanding physiological process is associated with high metabolic rate and generation of ROS (Blount *et al.*, 2016). Other studies have also reported high vulnerability of testes and sperm cells to oxidative stress due to inefficient antioxidant system and abundance of poly unsaturated fatty acid in sperm membrane (Khaled *et al.*, 2016; Pintus and Ros-Santaella 2021). Reproductive dysfunction in males is linked to several factors such as abnormalities in lipid profile (Sheriff, 1982; Funes *et al.*, 2019), poor nutrition, diseases, oxidative stress and testicular inflammation (Harchegani *et al.*, 2019). Under normal condition, testicular lipids

contribute to optimal function of testes. However, abnormalities in lipid profiles involving derangement in HDL level or function correlated with impaired fertility (Helena *et al.*, 2001). Also, available data show that oxidative stress has negative effects on sperm quality and fertility (Tvrdá *et al.*, 2011) and could trigger peroxidation of PUFAs on sperm membrane with consequent loss of membrane functionality (Pagl *et al.*, 2006). Inflammatory response in testes could impair male fertility by triggering oxidative stress (Hasan *et al.*, 2022), testicular damage and generation of inflammatory mediators, which could adversely affect semen quality (Gruschwitz *et al.*, 1996; Fraczek and Kurpysz, 2015). Conversely, several feed-related factors like good nutrition and high feed palatability are known to improve male reproduction. High palatability boosts feed intake (Olarotimi *et al.*, 2020) and ultimately improves accessibility of birds to good nutritional requirements needed for optimal reproduction. However, feed palatability is



adversely affected by a couple of factors, particularly long storage of feed, protein putrefaction and rancidity of fats in feed. Deterioration of these feed nutrients results into generation of off flavors and odors capable of rendering feed less palatable. Efforts to mitigate this feed-related problem have led to recommendation of several natural and inorganic flavor enhancers such as garlic, ginger (Utsav *et al.*, 2018) and monosodium glutamate (MSG) in feed (Saad *et al.*, 2009).

Monosodium glutamate is the sodium salt of glutamic acid found in many proteins. Apart from its flavor and palatability-enhancing properties in human food, MSG-supplemented feed also displayed improved palatability following evidence of high-feed intake and weight gain in chickens fed MSG-enriched diet (Pascaline *et al.*, 2019). Compared with antibiotics which could be used to promote birds' growth but has public health implications, growth-promoting activity of MSG was not associated with disturbance in chicken intestinal microflora (Pascaline *et al.*, 2019). Several studies have reported that under normal dietary condition MSG is completely metabolized in the gut but showed toxic effects at high dose. Moderate and high intake of MSG by rodents led to impaired hypothalamic-pituitary-gonadal axis (Igwebuike *et al.*, 2011) and also caused a significant decrease in sperm count, motility, viability (Jubaidi *et al.*, 2019; Oyeniran *et al.*, 2022). A report showed that ingestion of MSG correlated with significant dyslipidemia (Alwaleedi, 2016; Banerjee *et al.*, 2021). Also, there is evidence that rodents fed MSG-diet displayed oxidative stress in tissues as evaluated by intense lipid peroxidation (Onaolapo *et al.*, 2016) and reduction in antioxidant enzymes (Farombi and Onyema, 2006; Kumar and Bhandari, 2013).

Although there is a growing call for usage of MSG as a growth enhancer and alternative to antibiotics in poultry industry, there is dearth of data on the dose of MSG that is safe for birds' health, particularly reproductive system. Therefore, the goal of this study was to determine the effects of feed containing graded inclusion level of MSG on semen characteristics as well as lipid profile, oxidative stress and inflammation biomarkers in cock testes.

Materials and methods

Source of birds and MSG

Forty mature noiler cocks were bought at a commercial farm in Abeokuta and monosodium

glutamate (Ajinomoto®) was sourced locally in Igboora.

Study location and housing

The study was conducted at experimental pen of Oyo State College of Agriculture and Technology, Igboora. The birds were housed in a spacious, well partitioned and ventilated pen made from blocks. The pen floor was covered with wood shavings to provide beddings for the birds.

Acclimatization of birds and study design

Before the commencement of feeding trial, the birds were acclimatized for two weeks with provision of water and basal feed to the birds *ad libitum*. After the acclimatization, the birds were randomly allotted to four treatment groups of ten (10) birds per group as follow: Control group (basal feed) and MSG (Ajinomoto®) groups- 0.5 % MSG (5 g of MSG/kg of feed), 1 % MSG (10 g of MSG/kg of feed) and 1.5 % MSG (15 g of MSG/kg of feed). Both the control and MSG-diet groups were fed *ad libitum* throughout the duration of the feeding trial (eight weeks).

Sample collection

On day 56, semen were collected manually by massaging the cloaca of the birds to achieve phallic tumescence and cloacal stroke (Shinde *et al.*, 2012). Thereafter, the birds were euthanized and dissected with scapel blade to access the abdominal cavity. The testis were carefully removed and put in organ bottles containing PBS for homogenization. The homogenized testicular tissues were cold centrifuged at 10000 rpm for 15 minutes and supernatant was collected for biochemical analysis of lipid profile, inflammation and oxidative stress biomarkers.

Semen analysis

Semen was put unto slides and sperm count was determined using the improved Neuber counting chamber in the haemocytometer (Vibuntita *et al.*, 2022). To assess sperm motility, a 10 L diluted sample (1:20 in 2.9% sodium citrate) was placed on warm slide (37⁰ C) and then covered with a cover slid. In ten microscopic fields, the percentage of sperm motility was determined using light microscope (Agbaje *et al.*, 2020). The viability was assessed by staining of sperm smear with eosin-nigrosin staining technique. The resulting stain was then studied under the microscope using x 40 objective lens magnification of light



microscope. The motile (live) sperm cells were unstained while the non-motile sperm (dead) sperm cells were stained. The stained and unstained sperm were counted and average was taken for the percentage.

Lipid profile analysis

Serum and testicular lipid profile was assessed with commercial kits specific for each analyte using a diagnostic analyzer. Total cholesterol, triglycerides and HDL were measured, whereas LDL were calculated using the Friedewald equation.

Analysis of biomarkers of oxidative stress and inflammation

The activities of catalase and glutathione peroxidase (GPx) were determined by commercial assay kits-Fortress diagnostic kit and Elabsience, USA respectively based on protocol recommended by the manufacturers. The catalase assay technique involved measurement of complex formed from reaction of H_2O_2 with ammonium molybdate to determine the enzyme activity. Protocol described by Marklund and Marklund (1974) was used to determine activity of SOD. MDA levels in the testis were measured using thiobarbituric acid reactive substance assay (TBARS) method following the reaction of MDA with thiobarbituric acid as described by a previous study (Aguilar Diaz De Leon and Borges, 2016). Concentrations of H_2O_2 in prostate tissues homogenate were assessed by measuring absorbance value of yellow complex formed from reaction of H_2O_2 with ammonium molybdate at 405nm. Griess assay was used to examine NO level in the testicular tissue (Oktem *et al.*, 2012). ELISA kits (Elabsience®) were used for assay of IL-1 β , IL-6 and PGE2. The

analysis was done following manufacturer's procedure. The activity of COX-2 was determined as described in a previous study (Petrovic and Murray, 2010).

Statistical analysis

The data were analyzed with SPSS version 23.0 for windows. The means of control and MSG diet groups were tested for significant difference with one-way ANOVA followed by post hoc analysis using Duncan's Multiple Range Test. A value of $P < 0.05$ was considered statistically significant.

Results

Semen volume increased in groups fed MSG diet with highest value recorded in 1 % MSG group but it was not significant ($P > 0.05$) when compared with the value recorded in the control. Compared with the control, sperm count was higher in 1 % and 1.5 % MSG groups and lower in 0.5 % MSG group but was not statistically significant ($P > 0.05$). Sperm viability increased in 0.5 % MSG group and decreased 1 % and 1.5 % MSG groups when compared with the control but it was not significant ($P > 0.05$). Sperm motility was highest in 1 % MSG group but it was statistically similar to values recorded in other groups ($P > 0.05$). Total cholesterol increased in 1 % and 1.5 % MSG groups and decreased in 0.5 % MSG group when compared with the control but it was not significant ($P > 0.05$). Triglyceride and HDL levels in the testis were higher in MSG-diet groups but not statistically significant when compared with the control. The concentration of LDL parameters in MSG-diet groups was not significantly different from the value recorded in the control.

Table 1: Testicular Lipid Profile and Semen Characteristics

Groups	Control	0.5% MSG	1 % MSG	1.5% MSG
cholesterol (mmol/l)	2.95 $\pm$ 0.23	2.75 $\pm$ 1.11	3.29 $\pm$ 0.17	3.17 $\pm$ 0.12
Triglyceride(mmol/l)	0.90 $\pm$ 0.10	1.08 $\pm$ 0.48	1.19 $\pm$ 0.43	1.19 $\pm$ 0.14
HDL (mmol/l)	0.67 $\pm$ 0.05	0.76 $\pm$ 0.21	0.78 $\pm$ 0.10	0.84 $\pm$ 0.05
LDL (mmol/l)	2.10 $\pm$ 0.17	1.81 $\pm$ 0.80	2.28 $\pm$ 0.16	2.10 $\pm$ 0.18
Sperm motility (%)	65.65 $\pm$ 12.05	68.40 $\pm$ 7.64	70.66 $\pm$ 11.64	65.60 $\pm$ 16.43
Sperm viability (%)	88.80 $\pm$ 9.63	93.80 $\pm$ 5.63	84.20 $\pm$ 10.91	84.40 $\pm$ 5.03
Sperm count ($\times 10^6$)	64.72 $\pm$ 5.70	64.48 $\pm$ 8.19	65.96 $\pm$ 6.11	66.00 $\pm$ 16.52
Semen volume (ul)	460.00 $\pm$ 200.94	470.00 $\pm$ 198.75	494.00 $\pm$ 193.47	485.00 $\pm$ 193.57



Biomarkers of Oxidative Stress and Inflammation in the Testis

While the activity of superoxide dismutase, GSH and MDA increased in MSG-diet groups, catalase activity decreased when compared with the control but it was not statistically significant ($P>0.05$). Also, there was no significant difference ($P>0.05$) in the testicular levels of these biomarkers in MSG-diet groups

when compared. Though not significantly different ($P>0.05$) from the values recorded in the control, IL-6, PGE₂ levels as well as COX-2 activity were higher in all MSG-diet groups, and IL-1 β level increased only in 0.5 % and 1.5 % MSG groups. Similarly, the levels of inflammation biomarkers in MSG-diet groups were statistically similar.

Table 2. Biomarkers of Oxidative Stress and Inflammation in the Testis

Groups	Control	0.5% MSG	1 % MSG	1.5% MSG
SOD (u/ml)	1.37 $\pm$ 0.02	1.41 $\pm$ 0.12	1.44 $\pm$ 0.07	1.40 $\pm$ 0.07
Catalase (u/mg)	13.22 $\pm$ 0.67	12.69 $\pm$ 4.09	11.04 $\pm$ 1.28	10.20 $\pm$ 1.23
GSH (mM)	0.72 $\pm$ 0.04	1.00 $\pm$ 0.40	0.96 $\pm$ 0.12	1.14 $\pm$ 0.23
MDA (μ M)	0.85 $\pm$ 0.04	1.03 $\pm$ 0.18	1.36 $\pm$ 0.43	0.85 $\pm$ 0.07
IL-1 β (pg/ml)	44.86 $\pm$ 4.68	87.40 $\pm$ 55.91	44.39 $\pm$ 8.06	72.29 $\pm$ 33.69
COX-2 (U/L)	1.87 $\pm$ 0.46	2.02 $\pm$ 1.54	1.35 $\pm$ 1.22	2.38 $\pm$ 0.98
IL-6 (pg/ml)	7.39 $\pm$ 0.42	7.42 $\pm$ 0.68	7.60 $\pm$ 0.74	7.80 $\pm$ 0.66
PGE ₂	22.24 $\pm$ 2.18	23.44 $\pm$ 2.98	23.58 $\pm$ 2.76	23.78 $\pm$ 2.70

SOD-Superoxide dismutase, GSH-Glutathione, MDA-Malondialdehyde, IL-Interleukin, COX-2-Cyclooxygenase-2, PGE₂-Prostaglandin E₂

Discussion

The present findings indicate that poultry feed containing MSG up to 1.5 % inclusion level cannot affect semen quality and certain biological processes in the testes of poultry birds. Semen volume, sperm concentration, motility and viability in cocks fed MSG diet were not appreciably altered, suggesting non-toxic effect of MSG on semen quality. Following ingestion, MSG dissociates in the body into sodium ions and glutamate. Results of past findings revealed that glutamate accounts for most MSG-related toxicities, particularly when its plasma level increases exceedingly after intake high quantity of MSG (Hermanussen *et al.*, 2006; Kianifard *et al.*, 2015). Therefore, the present finding could be related to the dose of MSG used. The result of the present study is also corroborated by the findings of past studies which revealed that rats fed low-dose and high-dose of MSG for long and short durations respectively had no detectable alteration in their sperm parameters (Kianifard, 2016).

Testicular lipids is considered as one of the important indices of testicular function (Sherif, 1982) and alterations in testicular lipid content and metabolism caused damage to sperm quality (Crisóstomo *et al.*, 2020). The present study did not find significant changes in the lipid

fractions such as triglyceride, total cholesterol and lipoproteins in the testes of cocks fed MSG-diet, suggesting absence of abnormalities in the testicular lipid metabolism. Similar to the result of the present study, findings of Maluly *et al.* (2013) also demonstrated that rats fed MSG-containing diet had no considerable derangements in their lipid parameters.

Inflammation is a physiological response to tissue injury resulting from toxic substances, trauma and foreign agents. Inflammatory responses in the testis were found to correlate with reduced sperm quality and consequently infertility (Oghbaei *et al.*, 2020). In the present study, testicular concentrations of IL-6, IL-1, COX-2 and PGE₂ in cocks fed MSG diet were not significantly affected, indicating poor pro-inflammatory role of MSG in the birds' testes. Non-detectable changes in COX-2 concentration may conceivably account for this finding given the modulatory effect of COX-2 on cytokines production, particularly IL-6, by facilitating synthesis of PGE₂ (Hinson *et al.*, 1996). Contrary to the present finding, there are reports of inflammatory reactions in rodents administered with both high and low doses of MSG (Abd El Hady Mousa *et al.*, 2021; Kesharwani *et al.*, 2022). Similarly, MSG was reported to promote inflammation in organs such as spleen, leading to elevation in IL-6



level. Plausibly, the conflicting results of the present and past studies could be linked to the species of animal used and mode of MSG administration. Previous studies have shown that MSG-induced toxicity is influenced by the type of animal species, mode of administration (gavage versus feed added) and dose of MSG used (Walker and Lupien, 2000, Fernstrom, 2018).

Oxidative stress develops as a result of accumulation of free radicals and serves as a contributory factor for various health challenges including reproductive dysfunction. In the present results, feeding of birds with MSG-supplemented diet was not associated with significant changes in the concentration of anti-oxidants, SOD, catalase, GSH, and lipid peroxidation marker-MDA, highlighting weak pro-oxidative activity of MSG at studied doses following extensive glutamate oxidation and utilization by the enterocytes (He *et al.* 2018). Similar to the present finding is a report that shows that SOD and catalase activities in rodents administered with MSG were not considerably affected (Maluly *et al.*, 2013).

Conclusion

Feed containing MSG up to 1.5 % had no effect on semen characteristics, testicular lipid profile as well as inflammation/oxidative stress biomarkers of cocks. Therefore, the use of MSG to improve feed palatability, feed intake and ultimately performance of birds is recommended. Future studies can investigate the effects of higher and chronic intake of MSG on poultry health.

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