IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 14, Issue 1 Ser. III (January 2021), PP 50-54
www.iosrjournals.org

Semen Characteristics of Monosodium Glutamate Diabetes Induced-Rabbits

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Abstract
Background*: Mono Sodium Glutamate is used in the food industry as a flavor enhancer. Its' umami taste intensifies the meaty, savory flavor of food, as naturally occurring glutamate does in foods such as stews and meat soups. Its effect on the reproductive system however is poorly studied. This study evaluated the semen characteristics of rabbits induced with diabetes mellitus using monosodium glutamate (MSG).*
Materials and Methods*: The study utilized three groups of 5 rabbits each weighing between 1.2-1.7kg. Group 1 (T1) served as control and received no MSG while groups 2 (T2) and 3 (T3) received MSG at a dose rate of 3.33 mg/L and 6.66 mg/L, respectively. The rabbits were given feed and water ad libitum. The rabbits in T2 and T3 were confirmed to have significantly high glucose levels at two weeks of MSG administration following tests using a glucometer(Accu-check and Accu-check test strips) on a weekly basis. The semen samples were collected at the end of diabetes induction by means of an artificial vagina. The data obtained were analyzed using SPSS version 20.0. Significance of differences between treatment means were determined at $P \leq 0.05$ using one-way analysis of variance (ANOVA).*
Results*: Diabetes induced rabbits treated with 6.66 mg/L of monosodium glutamate for diabetes induction recorded semen with least gross motility (70.00 ± 0.00 %) and volume (0.27 ± 0.03 mL), live spermatozoa (70.67 ± 1.76 %) and had the most morphological defects compared with other groups.*
Conclusion: *It was concluded that diabetes has detrimental effects on semen characteristics of monosodium glutamate induced diabetic rabbits especially on the tail pieces of individual sperm cells. Further studies should be carried out to determine the similarities/differences in semen characteristics of rabbits induced with diabetes using other compounds/techniques as well as effects on reproduction in female rabbits.*
Key Words: *Monosodium glutamate, diabetes mellitus, semen*

Date of Submission: 17-01-2021 Date of Acceptance: 02-02-2021

I. Introduction

Monosodium Glutamate (MSG) is one of the world's most extensively used food additives which are ingested as part of commercially processed foods (Veronika and Daniela, 2013; Kamal et al., 2018).In the late 1960s numerous case reports appeared in the scientific literature describing a complex of symptoms which came to be known as the Chinese restaurant syndrome (CRS) because they typically followed ingestion of a Chinese meal. Investigations have mainly focussed on MSG as the causative agent in CRS (Food Standards Australia, 2003). At the onset of symptoms patients experience complaints such as a burning sensation at the back of the neck, blistering on both arms and occasionally on the anterior thorax, general weakness, fatigue and palpitations. These symptoms occur 20 minutes after consumption of a meal rich in MSG (Bawaskar et al., 2017).MSG has a characteristic taste called unami ("savoury deliciousness"), which is considered distinct from the four other basic tastes (sweet, sour, salty, and bitter). The optimal palatability concentration for MSG is between 0.2 – 0.8% with the largest palatable dose for humans being about 60mg/kg body weight (Food Standards Australia, 2003).MSG gives a special aroma to processed foods which is known as umami in Japanese. This taste sensation is also called "savoury" (Xiong et al., 2009). Umami is one of the five basic tastes (together with sweetness, sourness, bitterness, and saltiness). A loanword from the Japanese, umami can be translated as "pleasant savory taste" (Kaushalya and Jagath, 2017).

Beside its flavour enhancing effects, MSG has been associated with various forms of toxicity. MSG has been linked with obesity, metabolic disorders, Chinese Restaurant Syndrome, neurotoxic effects and detrimental effects on the reproductive organs (Kamal et al., 2018).Results from both animal and human studies have demonstrated that administration of even the lowest dose of MSG has toxic effects (Kamal et al., 2018).Animal studies have demonstrated that neonatal MSG consumption sets a precedent for the development of obesity later on (Araujo et al., 2017).Insulin resistance and reduced glucose tolerance in rodents due to MSG consumption raise concerns about the development of obesity in MSG consuming humans (Araujo et al., 2017).In a study into

the inflammatory profile of MSG induced obesity, it has been shown that MSG triggers micro-RNA (mRNA) expression of interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), resistin and leptin in visceral adipose tissue. This in turn leads to enhanced insulin, resistin and leptin concentrations in the circulation and ultimately an impaired glucose tolerance (Roman-Ramos et al., 2011).The effects of MSG on the reproductive system are documented in a smaller number than other effects are limited only to animal studies (Veronika and Daniela, 2013). It has been reported to decrease the weight of pituitary glands and testes and lower testosterone level in 4 month old sexually mature male rats (Miskowiak et al. 1993). In adult female Wistar rats fed by MSG at the doses of 0.04 mg/kg or 0.08 mg/kg on a daily basis the pathological changes have been observed in ovaries (Eweka and Om'iniabohs 2011) and fallopian tube (Eweka et al. 2010).Although these studies have shown the effects of MSG on female reproductive organs in relevant doses after peroral intake, further studies must be done to corroborate these results and to explore the ingested MSG effects also on male sex organs (Veronika and Daniela, 2013). This is the basis for this study bearing in mind that rabbits serve as excellent models for human studies.

II. Materials and Methods

The study was conducted at Teaching and Research Farm Department of Animal Science, Federal University Dutsinma, Katsina State, Nigeria. Dutsin-Ma lies on latitude 11º22'48.684 N and longitude 7º33'21.0024 E. The town is located in the central part of Katsina state, bounded to the west with Safana Local Government to the south by Danmusa Local Government, to the north by Kurfi and to the east by Charanchi Local Governments (Wikipedia, 2019).

Experimental Animals and management

Fifteen (15) adult male rabbits of mixed breeds weighing 1.2-1.7kg sourced from the National Animal Production Research Institute, Ahmadu Bello University Zaria (NAPRI) were used for the study. The rabbits were housed intensively in well-constructed hutches measuring 60x60x65. Each group of five randomly selected rabbits was kept in a different hutch. All management protocols were adhered to. The rabbits were conditioned for two weeks before the experiment commenced.

Experimental Layout

A complete randomized design (CRD) was used for the study. Fifteen adult male rabbits of mixed breeds were randomly divided into three treatment groups of five each. The feed used for this experiment and the monosodium glutamate (Ajinomoto®) were purchased locally. The diet consisted of grower mash, groundnut hay and vegetables and water was provided *ad libitum.* Before the treatments with MSG started blood samples were collected via ear vein randomly from three rabbits in each treatment to check the glucose level.

Treatment 1 which served as control was given feed and water ad libitum without MSG. Treatment 2 were given feed and water containing MSG at dose of 3.33mg/ml of drinking water *ad libitum* for two weeks. Treatment 3 received feed and water containing MSG at a dose of 6.6mg/ml of drinking water for two weeks. Blood samples were collected on a weekly basis to monitor blood glucose levels.

Semen Collection and Evaluation

Semen samples were collected using an artificial vagina filled with a warm liquid (about 45°C) and a teaser doe from 3 rabbits in each group and evaluated. The does were put on tops of the bucks cages for some minutes prior to presentation and each male was allowed three false mounts of the female before collecting semen (Jacqueline *et al.*, 1990) since sexual preparation enhances the sperm concentration and quantity of semen produced (International Rabbit Reproduction Group, 2005).A different artificial vagina was used for each collection and was collected under hygienic conditions to prevent bacterial and environmental contamination (George *et al*., 2017). Semen samples were collected at the end of two weeks following observation of spike in blood glucose levels in treatment groups. The volume, consistency and colour of the ejaculate were noted. The ejaculated semen was placed in a flask with warm water at 37ºC. Semen samples collected were evaluated as described by International Rabbit Reproduction Group (2005). This included the visual or gross evaluation of the ejaculate soon after collection for volume and color as well as microscopically for motility, concentration, live-dead ratio and morphology.

Data Analysis

Data collected were expressed as means (and standard errors of mean, SEM) and were analysed using the software SPSS 20. Significance of differences between treatments means were determined at $P \leq 0.05$ using one-way analysis of variance (ANOVA) and Students T test.

III. Results and Discussion

Table 1: Blood glucose levels of monosodium glutamate induced rabbits

Groups	Initial Glucose levels mg/dl (mean)	1st week Glucose levels mg/dl (mean)	2nd week Glucose levels mg/dl (mean)
Treatments	37.66[a]	71.66 [a]	95.66[a]
Control	36.66[a]	37.00[b]	37.33[b]
SEM	2.23	4.27	5.95

*Values with same superscripts are not statistically significant.

Blood Glucose levels

The normal glucose levels of the experimented rabbits ranged from 30-45 mg/dl at first week of acclimatization (Table 1). Following administration of monosodium glutamate, it ranged from 50-100mg/dl in the first week and from 120-150mg/dl at second week of monosodium glutamate administration. This finding agrees with the work ofBahadoran *et al.* (2009) who induced mice with diabetes using monosodium glutamate and achieved increase glucose level.

The mean values (±Standard Error of Mean, SEM) of semen characteristics, abnormal to normal cell ratio and semen abnormalities are presented in Tables 2-4.

Table 2: Semen Characteristics of monosodium glutamate induced rabbits

Parameter	Treatment 1 (control) N=3	Treatment 2 N=3	Treatment 3 N=3	P value
Ejaculate Volume (mL)	0.57 ± 0.03	0.37 ± 0.03	0.27 ± 0.03	0.002
pH	6.67 ± 0.17	7.00 ± 0.00	7.17 ± 0.17	0.098
Gross Motility (%)	84.00 ± 1.00	76.67 ± 4.41	70.00 ± 0.00	0.025
Semen Concentration ($x10^6$)	221.67 ± 3.53	84.67 ± 29.99	127.33 ± 4.48	0.004

Ejaculate/Semen Volume

Treatment 1 recorded the highest mean ejaculate volume of the experimental rabbits(0.57 ± 0.03) while Treatment 3 had the lowest mean ejaculate volume (0.27 ± 0.03 ml) (Table 2). There was statistically significant difference ($p > 0.05$) as well as an observable decrease in volume between the control group (T1) and diabetic groups (T2 MSG 3.33mg/l and T3 MSG 6.66mg/ml). The normal ejaculate volume for rabbit bucks as reported by Campos *et al*. (2014) ranges between 0.3-0.6 ml. This shows that diabetic state lowered the ejaculate volume in T3 (MSG 6.66mg/ml) below normal levels. T2 (MSG 3.33mg/ml) however had an ejaculate volume within normal range. This could be attributed to the fact that T3 recorded lower blood glucose levels than T2 and this possibly had a more detrimental effect of the semen volumes. According to Campos *et al*. (2014), Semen composition and volume are influenced by the size of the accessory gland which is in turn influenced by testicular testosterone production among other factors. Also according to Hafez (1995), the accessory glands contribute to the greater part of the volume of ejaculate. Thus if these organs are small in size as a result of poor development during the animal's growth period (Chikaodiri, 2020) or adversely affected due to disease such as diabetes, the composition and volume of sperm that will be secreted in the epididymis will be influenced when compared with the production of sperm from a normal reproductive organ.

Gross Semen Motility

There was a difference in mean semen gross motility with a decrease observed in T2 and T3 as compared to T1. No statistical significance was observed for this finding ($p < 0.05$). The lowest gross motility was observed in T3 (70.00 ± 0.00 %) while the highest was in the control group T1 (84.00 ± 1.00 %) as shown in Table 2.

The study showed that the mean percentage gross motility of the semen for rabbits obtained in the pre-experimental period and in the control group was within the normal range.However, rams in T2 and T3that received MSG had significantly lower mean percentage gross motility compared to those in the control group.

Semen Concentration

The lowest mean semen concentration in millions/ml was observed in T2 ($84.67 \times 10^6 \pm 29.99$) followed by T3 ($127.33 \times 10^6 \pm 4.48$) (Table 2). T1 had the highest mean semen concentration. There was a statistical significant difference observed for semen concentration ($p > 0.05$) between the control group (T1) and T2 and T3. Sperm concentration in rabbit ranges from $150 - 500 \times 10^6$ sperm/ml (Lebas*et al.*, 1997). The mean

semen concentration obtained for the rabbits in T1 was within the normal range reported for rabbits. However T2 and T3 had lower concentration counts than the reported normal levels. This indicates that diabetic state had a deleterious effect on semen concentration. There is a strong correlation between animal nutrition and spermatogenesis, sperm maturation and male reproductive system development (Cheah and Yang, 2011). Thus it is expected that a disease affecting dietary uptake such as diabetes would adversely affect semen concentration.

Table 3: Normal/Abnormal Cells of monosodium glutamate induced rabbits

PARAMETER	TREATMENT 1 (Control) n=3	TREATMENT 2 n=3	TREATMENT 3 N=3	P value
Live Spermatozoa (%)	82.00 ± 2.31	88.00 ± 0.00	70.67 ± 1.76	0.001
Abnormal Spermatozoa (%)	18.00 ± 2.31	12.00 ± 0.00	29.33 ± 1.76	0.001

Live Spermatozoa

Table 3 shows the mean values of normal to abnormal spermatozoa of rabbits administered MSG at 3.33mg/ml and 6.66mg/ml. Group T2 recorded the highest percentage live/normal spermatozoa mean values while T3 recorded the lowest vales. There was a significant decrease in percentage live spermatozoa in T3 compared to the control ($p < 0.05$).The results indicated that the diabetic state could have led to the release of immature spermatozoa and hence the high abnormality rates observed in T3.

Table 4: Sperm Abnormalities of Monosodium Glutamate Diabetes Induced-Rabbits

PARAMETER	TREATMENT 1 (Control) n=3	TREATMENT 2 n=3	TREATMENT 3 N=3	P value
Dead Cells (%)	16.00 ± 3.06	25.00 ± 2.89	30.00 ± 1.15	0.021
Bent Tail(%)	1.33 ± 0.33	3.00 ± 0.00	5.00 ± 0.00	0.000
Curled Tail(%)	4.00 ± 0.58	0.00 ± 0.00	4.67 ± 0.88	0.003
Detached Tail(%)	6.67 ± 1.76	7.00 ± 0.00	3.67 ± 0.88	0.154
Free tail (%)	6.00 ± 0.00	2.00 ± 0.00	6.00 ± 0.00	

Sperm Abnormalities

The mean total sperm abnormalities increased in the treatment groups T2 and T3 and were significantly higher ($p < 0.05$) than that of the control group for dead cells, bent tail and curled tail sperm. No statistically significant difference was observed between the mean detached tail and free tail values between groups ($p < 0.05$).

IV. Conclusions and Recommendations

The study concluded that diabetic/hyperglycemic state in rabbits significantly altered their semen percentage gross motility, ejaculate volume, semen concentration and percentage live spermatozoa. Only the semen ph was not significantly affected by hyperglycemia/diabetes. Of the sperm abnormalities evaluated, bent tail (BT) and coiled tail (CT) were significantly higher in rabbits administered MSG than the control. Thus, MSG induced hyperglycemia/diabetes caused increased abnormality of the tails, which could be responsible for decreased gross motility in the semen of MSG treated rabbits. Further studies should be carried out to determine the effects of MSG induced hyperglycemia/diabetes on the reproduction of female rabbits as well as similarities/differences in semen characteristics of male rabbits induced with diabetes using other compounds/techniques.

Ethical Clearance

Ethical clearance was sought from the Animal Ethics committee of the Ahmadu Bello University Zaria prior to the start of research work.

References

[1]. Araujo T.R., Freitas I.N., Vettorazzi J.F., Batista T.M., Santos-Silva J.C., Bonfleur M.L. (2017) Benefits of L- alanine or L-arginine supplementation against adiposity and glucose intolerance in monosodium glutamate-induced obesity. *Eur J Nutr*. 2017; 56:2069-80

[2]. Bawaskar H.S., Bawaskar P.H., Bawaskar P.H. (2017) Chinese restaurant syndrome. *Indian J Crit Care Med*. 2017; 21(1):49

[3]. Campos, A.C.N., Gadelha, C.R.F., Guerrre, M.E.F., Pereira, E.S., Lima, I.C.S., Linard, M.A.B., Meneses, H.M., Castelo-Branco, K.F. and Estevam, F.N.L. (2014) Male Rabbit Reproductive Physiology. *Standard Research Journal of Agricultural Sciences*, 2(8): 120-128.

[4]. Cheah Y. and Yang W. (2011) Functions of essential nutrition for high quality spermatogenesis. *Adv. Biosci. Biotechnol*., 2: 182-197. doi:10.4236/abb.2011.24029

[5]. Chikaodiri H.O. (2020) Reproductive Physiology of Male Rabbits: A Key Factor in Buck Selection for Breeding (Paper Review). *Advances in Reproductive Sciences*, 8: 97-112

[6]. Eweka, A.O. & Om'iniabohs, F.A.E. (2011). "Histological Studies of the Effects of Monosodium Glutamate on the Ovaries of Adult Wistar Rats," *Annal of Medical & Health Sciences Research*, 1(1), 37-43.
[7]. Eweka, A. O., Eweka, A. &Om'iniabohs, F. A. E. (2010) "Histological Studies of the Effects of Monosodium Glutamate of the Fallopian Tubes of Adult Female Wistar Rats," *North American Journal of Medical Sciences*, 2(3), 146-9.
[8]. George O.S., Ologbose F.I, Akintola O.A.I (2017) Sperm Characteristics Of Rabbit Bucks Fed Graded Levels Of Moringa (MoringaOleifera) Leaf Meal. *Scientia Agriculturae* 20 (3), 2017: 67-70
[9]. Hafez, E.S.E. (1995) 'Anatomia da reprodução masculina',*Reprodução animal*. 6th Edition, Manole, São Paulo.
[10]. International Rabbit Reproduction Group (2005) Guidelines for the Handling of Rabbit Bucks and Semen. *World Rabbit Sci*. 13: 71 – 91
[11]. Jacqueline W., Beth C.G., Steven M.S., Terry W.T., Jerry L.P. and Robert E.C. (1990) Semen Analysis and Fertility Assessment in Rabbits: Statistical Power and Design Considerations for Toxicology Studies. *Fundamental and applied toxicology* 15, 651-665
[12]. Johnson LA. (2002) Successful gender pre-selection in swine. *J. Reprod. Fertil. Suppl*. 52: 255-266.
[13]. Kamal N., Elizabeta Z., Jonathan S. (2018) Extensive use of monosodium glutamate: a threat to public health? *EXCLI Journal* 2018; 17:273-278
[14]. Kaushalya W. and Jagath W. (2017) Uses, effects and properties of monosodium glutamate (MSG) on food & nutrition. *International Journal of Food Science and Nutrition*. 2(3): 132-143
[15]. Lebas, F., Coudert, P., Rochambeau, H., Thébault, R.G. and Rouvier, R. (1997) Reproduction. In: The Rabbit: Husbandry, Health and Production, *New Revised Version, FAO, Roma, Chap*. 3, 45-55.
[16]. Miskowiak, B., Limanowski, A. & Partyka, M. (1993) "[Effect of Perinatal Administration of Monosodium Glutamate (MSG) on the Reproductive System of the Male Rat]," *EndokrynologiaPolska*, 44 (4), 497-505.
[17]. Roman-Ramos R, Almanza-Perez JC, Garcia-Macedo R, Blancas-Flores G, Fortis-Barrera A, Jasso E.I. (2011) Monosodium glutamate neonatal intoxication associated with obesity in adult stage is characterized by chronic inflammation and increased mrna expression of peroxisome proliferator-activated receptors in mice. *Basic Clin Pharmacol Toxicol*. 2011; 108: 406-13
[18]. Veronika H. and Daniela O. (2013) Monosodium Glutamate Toxic Effects and Their Implications for Human Intake: A Review. *JMED Research*, Vol. 2013 (2013), Article ID 608765, DOI: 10.5171/2013.608765
[19]. Xiong JS, Branigan D, Li M. (2009) Deciphering the MSG controversy. *Int J Clin Exp Med*. 2009; 2:329-36

Yahaya M.A, et. al. "Semen Characteristics of Monosodium Glutamate Diabetes Induced-Rabbits." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 14(1), 2021, pp. 50-54.