Research Article

Effects of Monosodium Glutamate (MSG) on Nutritional and Biochemical Parameters in Wistar Rats

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ABSTRACT

The use of seasonings in soups, sauces and institutional cooking is well documented. This study assessed the effects of Monosodium Glutamate (MSG) commonly referred to as "AJI-NO-MOTO" on some nutritional indices, serum micronutrient status and the blood lipid profile of Wistar strain male albino rats. Twenty-five (25) rats distributed into 5 groups were used in this experimental study. The MSG crystals were ground into a powdery form and added to the formulated diet. The first group (control) contained 0% MSG, while we fed the experimental groups with formulated diets containing 5%, 10%, 15% and 20% MSG. We fed all the groups ad libitum for 28 days, weighed and sacrificed thereafter. We determined the nutritional indices, serum concentrations of calcium, zinc, ferritin, retinol, and lipid profiles using standard methods. We analyzed data using ANOVA ($p<0.05$). Weight gain in the control group (40.35 g) was significantly higher than experimental groups (26.93-36.22g) ($p<0.05$). There was a significant difference in serum calcium (7.70±1.04-13.38±0.91ppm), zinc (0.13±0.01-0.33±0.22ppm), and retinol (50±5.01-77±2.83µg/ dL) compared with the control: (6.85±0.49 ppm), (0.10±0.01ppm), and (52±1.41 µg /dL) respectively ($p<0.05$). Serum ferritin in the experimental groups (0.09±0.02-0.79±0.20 ppm) was not different from that of control (0.71±0.01ppm) ($p>0.05$). Low density Lipoprotein (LDL) concentration in the experimental groups was higher (15.76±2.78-30.94±20.81mg/dL) compared with the control (21.51±4.50mg/ dL). The use of MSG influence some important serum parameters such calcium, zinc, retinol and low density lipoprotein.

Keywords: Monosodium Glutamate, Nutritional indices, Biochemical profile, Rats.

INTRODUCTION

Seasonings are ingredients added to food to preserve its qualities such as safety, freshness, taste, texture or appeareance [1]. Numerous types of seasonings such as Star Maggi, Knorr, Royco, Doyin, Jumbo (cubes), Onga, Mixpy, Benny, Alubashrimp seasoning (powdered), A-one, Vedan, Aji-no-moto, Salsa and Tasty (monosodium glutamate) are available in the open markets, in-street shops and supermarkets. Studies have shown that the chief components in flavour enhancers are salt (NaCl) and monosodium glutamate (MSG). Monosodium glutamate (MSG) is one of the most common amino acids. It is present in various foods as a flavour enhancer and as a food additive (E621) in the form of hydrolyzed protein or as purified monosodium salt [1]. Consumer and institutional food service providers in animal feed, food processing industry, and restaurants used MSG for various purposes. China is one of the top producers (65%), consumer (55%), and exporter (44%) of MSG worldwide.

Indonesia is the second largest (16%) exporter of MSG. The Middle East and Africa consumed 4%, Europe 3%, North America 2%, and central and South America 2% MSG [2]. Change in dietary patterns, increased urbanization, improved living standards, and continuous development in food processing industry account for the increased consumption of MSG. In West Africa, it is highly demanded for use in foods like potatoes, noodles, soup, and rice. Also, more involvement of women at workplaces, increase in middle class, and hectic lifestyles are reasons for the increased consumption of MSG in many countries. Conversely, countries like the United States, Mexico, and Canada forbid MSG because of growing concerns about obesity. Even though, MSG stimulates taste and improves appetite, some studies have shown that it is toxic to humans and experimental animals [3]. Sodium content of seasonings has, however, been a source of fear because of the relationship between dietary sodium and hypertension [4]. Chinese restaurant syndrome is associated with MSG consumption [5]. Also, MSG intake could induce an increase in energy intake [6], which could lead to obesity [7], or alter the levels of carbohydrates, lipids and proteins in rats [8].

Toxic effects of MSG showed that excessive or continuous intake may affect metabolism, disturbing the absorption or functioning of certain substances including nutrients and electrolytes. The effects of MSG in many animal species and its toxic effects on some organs have been studied, but there is a dearth of information on the consequences of MSG on certain nutritional parameters. Hence, this study investigated the effects of monosodium glutamate on some nutritional and biochemical parameters in male Wistar rats.

MATERIALS AND METHODS

Experimental Animals and Treatment

Twenty-five Wister rats were purchased from the Physiology Department, Ekiti State University, and were housed in metabolic cages throughout the feeding experiment in a room maintained at a 12 h light–dark cycle and a constant temperature of 20±3°C and relative humidity of 65±15% at the animal house of Afe Babalola University. The animals were allowed to acclimatize for seven days in the metabolic cages and fed *ad libitum* with commercial rat pellets and clean tap water before randomisation into diet groups. The rats were weighed and then randomly distributed to five diets' groups (i.e., experimental, and control) of five rats each. Each rat was fed with 10 g of the formulated diet daily and their drinking water was changed every other day for 28 days. The unused foods were pooled together and weighed daily. We followed the standard principles of Laboratory Animal care of the United State National Institute of Health in handling the rats. All animals were weighed before the commencement of the feeding trial and at the end of the experiment. The change in body weight was noted.

Measurement of Nutritional Indices

Mean weight gain

This was determined as the difference in mean final weight and the mean initial weight of rats in each diet group.

Animal sacrifice

The animals were sacrificed by cervical dislocation following the intracardial perfusion fixation with 10% formal saline, and the rats were exposed in order to remove the organs of choice.

Blood collection

Heart puncture was used to collect blood samples and collected in glass tubes. Centrifugation separated serum at 3000 rpm for 10 min and stored at -80^{0}C pending biochemical analysis.

Determination of Serum Zinc, Calcium and Ferritin

One millilitre (1ml) of the blood sample was pipetted using a 3 mL micropipette into 30 mL digestion tube, 5 mL of concentrated HNO_3 (Optima grade), 2 mL of concentrated H_2O_2 (Hydrogen peroxide), and we added a 13 mL of deionized water to the digestion tube. The peroxides permitted higher digestion temperatures by reducing the nitric acid vapours and removing the complex matrix and blood biohazards. The digestion tube was placed in the Digestion's hole Block Heater (TECATOR BD20) and allowed to digest to a clear colourless solution. The clear colourless solution after cooling down was transferred to a 50 mL volumetric flask and made up to the mark with de-ionised water. This diluent was used to evaluate metals such as Fe, Ca, Zn and others on a BUCK 211 VGP Atomic Absorption Spectrophotometer (AAS) at the respective wavelength of each metal using each metal respective hollow cathode lamp to atomise.

Determination of Serum Retinol in Blood

Homogenized sample of 0.5 mL was poured into a 250 mL Quartz round flask (QRF). 25 mL of Methanol and 10 mL of 50% KOH were added for stability. The mixture above was placed in a water bath set at 100°C connected to a condenser (cold finger type) for 30 min to reflux. The QRF mixture was then cooled down in ice and kept in the dark for 1 hour. The whole mixture in QRF was transferred to a 250ml volumetric flask and washed with 3:1 methanol/H_2O mixture and made up to 250ml mark. The flask was rotated up and down to ensure uniform mixing. The volumetric flask was put in the dark overnight. 20ml supernatant of the above was pipetted into a centrifuge tube and 20mL petroleum ether added and shakes for 1min. This mixture was centrifuge for 30 min in a Gallen kamp centrifuge. 2mL of the supernatant from the centrifuge tube was pipette into 20 mL tube and 1mL of chloroform added. 10mL of carr-price reagent (20% antimony chloride dissolved in chloroform with acetone). USP reference standard solution of transretinyl acetate which is equivalent to 30mg retinol was used as stock and working standard of range 0-5ug/mL was prepared from the stock. The working standard was treated like sample above. The absorbance of standard as well as sample was read on a cecil 2483 spectrophotometer at a wavelength of 430nm.

Vitamin A unit/100mL as we calculate retinol using the formula:

$$\frac{\text{Absorbance of standard}}{\text{Absorbance of sample}} \times \frac{\text{Conc. of standard}}{1}$$

Serum retinol was determined using the Association of Official Analytical Chemists (AOAC) (2005), whilst serum ferritin and zinc were determined using AOAC International (2006) methods 983.24 and 991.11. All experiments were performed under the guidelines of the Committee for Animal Experimentation.

Determination of Blood Lipid Profile

Blood plasma was obtained and the plasma lipoproteins were separated into density fractions by ultra centrifugal flotation at various densities between 1.006 and 1.21 g/mL [9], using a Beckman 50-Ti rotor (Spinco Div., Palo Alto, CA) at 45,000 rpm in an L5-50 ultracentrifuge. After which various density fractions were subjected to zonal ultracentrifugation as previously described. [10, 11]

Statistical analysis

We calculated the means and standard deviations for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with least significant difference (LSD).

Statistical Package for Social Sciences (SPSS) for Windows, version 20.0 was used.

RESULTS

Table 1: Composition of formulated diets for feeding trial (g/1000 g diet)

Nutrient	1	2	3	4	5
Corn starch	588	546	504	462	420
Cellulose (5%)	42	42	42	42	42
Vegetable oil (8%)	67.2	67.2	67.2	67.2	67.2
Mineral mix (4%)	33.6	33.6	33.6	33.6	33.6
Vitamin mix (1%)	8.4	8.4	8.4	8.4	8.4
Casein (12%)	100.8	100.8	100.8	100.8	100.8
5% MSG	-	42		-	-
10% MSG	-	-	84	-	-
15% MSG	-	-	-	126	-
20% MSG	-	-	-	-	168
TOTAL	840	840	840	840	840

The groups are: 1(Control, 0% MSG), 2(5% MSG), 3(10% MSG), 4(15% MSG) and 5(20% MSG). The percentages of composition of formulated (nutrient) diets were indicated in table 1 with different doses of MSG in experimental rats.

Table 2: Initial, final and weight gain in control and experimental Animals

Group	Initial Body Weight (g)	Final Body Weight (g)	Weight gain (g)
Control	81.27±26.12^{a}	122.60±31.79^{a}	40.35±44.08^{a}
5% MSG	78.22±18.15^{a}	107.50±25.42^{a}	29.28±8.99^{b}
10% MSG	88.45±11.52^{a}	115.38±14.71^{a}	26.93±35.37^{c}
15% MSG	90.26±17.59^{a}	126.48±29.47^{ab}	36.22±39.38^{d}
20% MSG	73.27±49.84^{a}	100.62±49.84^{ab}	27.35±26.36^{e}

Values are mean ± standard deviation of duplicate determinations. Means with different superscript in the same column are significantly different ($p < 0.05$).

The results of weight gain or loss in different groups at the end of the experiment is shown in Table 2. Control group had the highest weight gain, followed by the group that received 15% MSG in their diet. At 5%, and 10% inclusion of MSG, the weight gain was significant ($P < 0.05$), but loss of weight was observed as the percentage of MSG in the diet was increased to 20%.

Table 3: Serum micronutrient status in animals on control and MSG diets

Parameters	Control	5%	10%	15%	20%
Ca+ (ppm)	6.85±0.49^{ab}	11.13±1.02^{b}	7.70±1.04^{ab}	13.38±0.91^{b}	9.20±1.13^{ab}
Fe+ (ppm)	0.71±0.01^{ab}	0.09±0.02^{a}	0.14±0.61^{ab}	0.77±0.20^{ab}	0.14±0.01^{ab}
Zn+ (ppm)	0.10±0.01^{a}	0.33±0.22^{a}	0.18±0.01^{a}	0.13±0.01^{a}	0.21±0.02^{a}
Retinol (µg/dL)	52±1.41^{ab}	50±5.01^{ab}	68±12.17^{ab}	50.5± 8.62^{ab}	77±2.83^{ab}

Values are mean ± standard deviation of duplicate determinations. Means with different superscript in the same row are significantly different ($p < 0.05$). Ca= calcium, Fe= Iron, Zn=zinc.

Effects of MSG at different levels of addition to the diets on serum micronutrients status are shown in Table 3. The concentration of Calcium increased in the experimental groups compared to the control group. The rat group that consumed the diet containing 15% MSG had the highest serum calcium compared to the control and other experimental groups. The concentration of serum Ferritin levels increased across the experimental groups when compared to the control group. The concentration of zinc increased in all the MSG diets compared to the control with a 5% MSG diet having the highest zinc concentration. Likewise, the serum retinol increased in all the MSG diet groups respectively.

Table 4: Effects of MSG on lipid profile in experimental animals

Parameters	Control	5%	10%	15%	20%
LDL (mg/dL)	21.51±4.50^{a}	27.32±17.36^{a}	15.76±2.78^{a}	20.50±8.1^{a}	30.94±20.81^{a}
VLDL (mg/dL)	55.26±1.47^{a}	55.37±16.77^{a}	46.37±4.45^{a}	50.93±8.74^{a}	36.67±0.73^{a}
TC (mg/dL)	137.40±39.04^{a}	192.30±47.32^{a}	168.97±49.16^{a}	175.20±45.50^{a}	142.10±26.18^{a}
TRG (mg/dL)	276.30±7.33^{a}	276.85±83.84^{a}	231.85±22.259^{a}	254.63±43.72^{a}	183.33±3.67^{a}

Values are mean ± standard deviation of duplicate determinations. Means with different superscript in the same row are significantly different (p <0.05). LDL= Low density Lipoprotein, VLDL= Very low density Lipoprotein, TC= Total Cholesterol and TRG= Triglyceride.

Lipid profiles of the different groups are shown in Table 4. As seen on the table, the serum Low density Lipoprotein (LDL) balloons in all the experimental groups compared to the control group (p <0.05), except for 10% MSG group which shows a decrease. Serum total cholesterol (TC) showed a significant increase for all experimental groups compared to the control group. The concentration of very-low-density lipoprotein (VLDL) decreased across all experimental groups compared to the control group. The level of triglycerides only nudge up in the 5% MSG group and reduces in the other groups.

DISCUSSION

Many studies have reported increased food intake by adding MSG as a flavouring agent. This study showed that the addition of MSG to the diet increased food intake and weight gain in animals up to 15% of including MSG. Likewise, human subjects given soups with different concentrations of MSG and no MSG revealed that soup with MSG increased food intake [12]. Study conducted among the Chinese showed a positive correlation between MSG users and increased body mass index (BMI) [13]. Therefore, the potential link between MSG and obesity includes the MSG effect on energy balance by increasing palatability of food and by disrupting the hypothalamic signaling cascade of leptin action [13, 14]. The results are consistent with several other similar experiments carried out on rodents [13, 15, 16]. The mean serum calcium, ferritin and retinol concentrations in rats fed with experimental diets were significantly higher than the control groups (p<0.05). The concentration of zinc was not different compared to the control group. According to a certain study conducted among 100 French men, increased intake of calcium, magnesium, and fat was thought to be related to MSG-added food [17]. Because of the bioavailability of these micronutrients in this present study, MSG has the potential to serve as a vehicle for calcium, iron and vitamin A fortifications if used in moderation. For instance, as with an intervention study in Chinese preschool children, multiple-micronutrient fortification with three seasoning powders containing either vitamin A acetate (500 µg), vitamin A and ferric sodium acetate (12mg), or vitamin A and iron with additional folic acid (0.2 mg); zinc oxide (12 mg); Thiamine; Riboflavin; niacinamide and calcium were added to foods served at the children's nursery [18]. The mobilization and metabolism of lipoproteins of the liver may be compromised in the presence of MSG [19, 20]. The elevations in concentrations of LDL, Cholesterol and Triglycerides in the experimental group conforms to the findings of Collison [19], the study noted that combinatorial administration of trans-fatty acid and MSG resulted in raised levels of serum Total Cholesterol. The administered dose of MSG and duration of exposure of the rats to MSG were critical factors that may influence the level of alteration of LDL, VLDL, Triglyceride and Cholesterol respectively in the other experimental groups compared to the control group. Also, the method of administration of MSG may have resulted in dissimilarities in the results of previous studies. However, there is no significant difference recorded between all the parameters in the experimental and control groups (P> 0.05). By extension, previous reports showed that MSG may significantly alter adiposity, glucose homeostasis and hepatic and adipose tissue gene expression [19]. Similar results concerning fat content/body weight ratio have been observed in 30 days old rats injected with 4g/kg of MSG within the first 10 days of life. Higher adipocyte lipid content, cell diameter, surface area and volume despite lower body weight which results in arrested growth and obesity after MSG administration compared to control rats have been found [21].

CONCLUSION

This study shows that MSG can influence body weight by increasing appetite. Increase bioavailability of some micronutrients, even in small amounts (5% MSG). However, an increase in concentrations of LDL, VLDL, and Triglycerides could further result in clinical complications such as cardiovascular diseases, hence, increasing the prevalence of morbidity and mortality from non-communicable diseases. Prolonged usage in high doses may cause kidney and liver dysfunctions, hence the need for moderation in its usage.

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