Nigerian J. Anim. Sci. 2019, 21 (3): 89-94

Effects of dietary monosodium glutamate on the acetylcholinesterase, specific acetylcholinesterase and total protein concentrations in the brain regions of domestic fowl (*Gallus domesticus*) layers

Olateju, I. S[a]. , Olarotimi, O. J[a]. , Adu, O. A[a]. and Gbore, F. A[b].

[a]*Department of Animal Production and Health, School of Agriculture and Agricultural Technology, The Federal University of Technology, Akure, P. M. B. 704, Ondo State, Nigeria.*
[b]*Department of Animal Science, Adekunle Ajasin University, Akungba – Akoko, Nigeria.*

Corresponding author: olaimoleayo@gmail.com

Target Audience*: Poultry (layers) Farmers*

Abstract

This study investigated the effect of varied levels of monosodium glutamate (MSG) on acetylcholinesterase, specific acetylcholinesterase and total protein concentrations in the brain of laying hens. A total of 300 point-of-lay (POL) 16 weeks old Isa Brown pullets were randomly allotted to six dietary treatments containing 0.00 (control), 0.25, 0.50, 0.75, 1.00 and 1.25 g MSG/kg diet. Two birds were kept in a cage for the feeding trial in a completely randomized design and lasted for 16 weeks. At the end of the trial, the hens were slaughtered and their brains were harvested. Homogenized samples from the medulla oblongata, olfactory lobe, optic lobe and cerebellum were analyzed to determine acetylcholinesterase, specific acetylcholinesterase and total protein concentrations. Result showed that MSG did not affect any of the brain regions of hens fed 0.25 – 0.50 g MSG/kg. However, the medulla oblongata was significantly ($p<0.05$) influenced at inclusion level of 1.25g MSG/kg diet. Total protein concentration was significantly ($p<0.05$) higher in pineal gland, medulla oblongata and cerebellum brain regions of the hens fed 0.25 g MSG/kg. Total protein concentration in the optic lobe region of the brain of the hens fed the control diet were significantly ($p<0.05$) higher than those on the treatment diets. The SAChE activities of the optic lobe and medulla oblongata region of the brain were not influenced ($P\geq0.05$) by dietary MSG although the group of layers fed with 1.25gMSG/Kg were affected. Significant changes were observed in the SAChE activities of the cerebellum while olfactory lobe and pineal gland regions of the brain were statistically unaffected. This study concluded that dietary MSG inclusion in layers' diets above 0.50 g/kg diet increased the activities of acetylcholinesterase concentration in the optic lobe, cerebellum and olfactory lobe and reduced the total protein in the different regions of the brain with tendency to impair brain function.

Key Words: *Monosodium glutamate, acetylcholinesterase, total protein, specific acetylcholinesterase*

Description of Problem

Intensification and development of the livestock sector of the economy has been identified to be important to the growing population of Nigeria (1). Poultry industry is the predominant source of animal protein both in the form of eggs and lean meat production (2). Since 70% of the cost of production in the poultry industry is for feed provision, the profitability of this industry depends largely on the quality and economics of feed production (3). The high cost of conventional protein sources has made researchers to pay more attention to non-conventional protein sources

in recent time (1). Leaves of shrubs and trees have been identified as major non-conventional feed protein sources (4). However, a major limitation of these leaves is that of low palatability due to their bitter taste (5) which constitutes a limitation to their utilization. Hence, Monosodium Glutamate (MSG) is regarded as an additive, which can enhance palatability (6) and help to optimize feed quality.

Glutamate is an excitatory neurotransmitter, which is abundant in the brain and plays an important role in both physiological and pathological processes. MSG has been reported to be deleterious in wistar albino rats (7) fed or injected with it at high concentrations. The excessive dosage of MSG administration was implicated in causing the negative effect through its neurotoxic effect on the cerebellum of the rats (8). However, MSG inclusion in layers feed might not lead to the same effect as observed in the rat since they will not be injected or feed the pure substance and it is establishing safe inclusion levels for laying birds diets would go a long way to improve layers' production. The paucity of information on the potentials and implications of dietary MSG on the brain of the pullets was the reason for the investigation of the effect of varied levels of dietary MSG on the acetylcholinesterase (AChE), total protein and specific acetylcholinesterase (SAChE) of the brain in the laying birds in this study.

Materials and Methods

The study was carried out at the poultry unit of the Teaching and Research Farm, The Federal University of Technology, Akure. A total of 300 16 weeks old Isa Brown were purchased from a reliable source. They were placed on a commercial grower mash until they have reached 20% laying performance (22 weeks of age) before they were subjected to the experimental diets (Table 1) for a period of sixteen weeks. Throughout the period of the study, the birds were subjected to the same management practices. The pullets were weighed at the beginning of the study and randomly allotted to the experimental diets containing 0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 g/kg MSG making a total of 6 treatments. Each treatment was replicated 5 times with 10 birds per replicate in a completely randomized design. The birds were fed twice daily (morning and afternoon) and water was provided *ad-libitum*. Recommended vaccination and other medication were administered as at when due. The birds were housed in an open-sided building in a three tier cage system of 32 x 38 x 42 cm dimension with two birds in a cage. At the end of the experiment, two birds per replicate were slaughtered by cervical dislocation. The brains were removed` and dissected on ice-cold porcelain tile into the cerebellum, optic lobe, olfactory lobe and medulla oblongata. Thereafter, the brain and hypophyseal samples from each animal was homogenized (1%, weight per volume (w/v)) with a Potter-Elvehjem homogenizer in 0.1mole (ice-cold phosphate buffer containing 0.1% Triton X-100 (Sigma). The brain acetylcholinesterase (AChE) activities and total protein concentrations were determined by colorimetic method according to (9) and the Biurett method reported by (10) using Randox commercial kits respectively. The specific acetylcholinesterase (SAChE) was evaluated by dividing the AChE activity of each sample with its total protein concentration. The data collected were subjected to one way analysis of variance (ANOVA) using (11). Duncan Multiple Range Test was used for means differentiation at 5% level of significance.

Table 1: Ingredient composition of the experimental layer diets (g/kg diet)

		INCLUSION LEVEL OF MSG (kg)				
Ingredients	**Control**	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**
Maize	430.00	430.00	430.00	430.00	430.00	430.00
Soybean meal	130.00	130.00	130.00	130.00	130.00	130.00
Groundnut cake	130.00	130.00	130.00	130.00	130.00	130.00
Wheat Offal	88.00	88.00	88.00	88.00	88.00	88.00
Rice Bran	100.00	100.00	100.00	100.00	100.00	100.00
Bone Meal	21.00	21.00	21.00	21.00	21.00	21.00
Limestone	90.00	89.75	89.50	89.25	89.00	88.75
Salt	2.50	2.50	2.50	2.50	2.50	2.50
MSG	0.00	0.25	0.50	0.75	1.00	1.25
Lysine	3.00	3.00	3.00	3.00	3.00	3.00
Methionine	3.00	3.00	3.00	3.00	3.00	3.00
Layer Premix	2.50	2.50	2.50	2.50	2.50	2.50
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Analyzed Nutrients						
ME (Kcal/Kg)	2620.52	2620.52	2620.52	2620.52	2620.52	2620.52
Crude Protein (%)	17.73	17.73	17.73	17.73	17.73	17.73
Calcium (%)	4.20	4.18	4.18	4.15	4.13	4.12
Phosphorus (%)	0.50	0.50	0.50	0.50	0.50	0.50
Lysine (%)	1.20	1.20	1.20	1.20	1.20	1.20
Methionine (%)	0.47	0.47	0.47	0.47	0.47	0.47
Crude Fibre (%)	3.79	3.79	3.79	3.79	3.79	3.79

MSG = monosodium glutamate

Results and Discussion

The brain AChE levels of the layers are as shown in Table 2. AChE was highest and significantly different ($P<0.05$) at 1.25g/kg compared to other inclusion levels in the brain of experimental birds. The significant increase in acetylcholinesterase (AChE) level of the brain among the layers is suggestive that MSG administration above 0.50 g/kg in laying hens' diets lead to decrease in the sensitivity of AChE to acetylcholine. This present findings corroborated earlier reports by (12), (13), (6) and (7) who also observed an increase in the activities of AChE in MSG treated mice. On the contrary, the present observations were different from the report of (14) which reported decrease in the AChE activity in MSG treated rats. The observed increased AChE concentration in the brain of the MSG treated hens could be a resultant effect of dysfunction in the cholinergic system which resulted in alteration of the enzyme. It is therefore possible that hens feed MSG in the excess of 0.50 g/kg may experience a decrease in cholinergic neurotransmission efficiency caused by reduction in acetylcholine level due to increased AChE activities. The significant effect of the dietary MSG on activity of the AChE in the olfactory lobes among the layers fed 0.75 g/kg and above is indicative of impaired sense of smell in disagreement with the assertion of (15). Furthermore, the AChE

activity in the pineal body and medulla oblongata regions of the hens' brains were not significantly different on the treatments containing 0.25 to 1.00 g/kg MSG. This might mean that the synthesis and catabolism of neurotransmitters (AChE) were not affected until the hens were fed 1.25 g/kg diet. The insignificant effect noted in the AChE of the pineal gland of the hens up to the treatment diet containing 1.00 g/kg MSG showed that dietary MSG at the inclusion levels did not tamper with melatonin production thereby did not affect negatively the modulation of sleeping pattern and behavior of the hens.

Table 2: Acetylcholinesterase (AChE) concentrations in the brain regions of layers fed graded levels of monosodium glutamate

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Olfactory Lobe	0.13±0.011^{bc}	0.13±0.010^{bc}	0.16±0.005^{abc}	0.17±0.006^{ab}	0.17±0.004^{ab}	0.20±0.009^{a}	< 0.0001
Pineal Gland	0.16±0.004^{b}	0.15±0.006^{b}	0.16±0.014^{b}	0.16±0.013^{b}	0.20±0.022^{ab}	0.22±0.003^{a}	0.0008
Optic Lobe	0.13±0.011^{bc}	0.12±0.001^{c}	0.12±0.001^{c}	0.15±0.004^{ab}	0.16±0.007^{ab}	0.16±0.006^{a}	< 0.0001
Cerebellum	0.11±0.000^{c}	0.11±0.012^{c}	0.13±0.011^{bc}	0.15±0.006^{ab}	0.15±0.002^{ab}	0.17±0.004^{a}	< 0.0001
Medulla Oblongata	0.13±0.011^{bc}	0.11±0.005^{c}	0.13±0.003^{bc}	0.15±0.005^{bc}	0.18±0.007^{b}	0.24±0.026^{a}	< 0.0001

^{abc}Means in the row with different superscripts are significantly different(P<0.05).

Table 3: Total protein concentrations in the brain regions of layers fed graded levels of monosodium glutamate)

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Olfactory Lobe	0.21±0.017	0.22±0.012	0.22±0.029	0.23±0.004	0.21±0.003	0.23±0.009	0.9211
Pineal Gland	0.28±0.016^{a}	0.29±0.009^{a}	0.27±0.009^{ab}	0.25±0.002^{b}	0.18±0.000^{c}	0.18±0.001^{c}	< 0.0001
Optic Lobe	0.21±0.017^{a}	0.19±0.006^{ab}	0.19±0.010^{ab}	0.16±0.006^{b}	0.16±0.006^{b}	0.14±0.006^{b}	< 0.0001
Cerebellum	0.15±0.005^{ab}	0.17±0.009^{a}	0.15±0.003^{ab}	0.15±0.004^{ab}	0.14±0.005^{b}	0.12±0.003^{c}	< 0.0001
Medulla Oblongata	0.20±0.023^{a}	0.21±0.006^{a}	0.16±0.006^{ab}	0.13±0.003^{b}	0.15±0.009^{b}	0.14±0.006^{b}	< 0.0001

^{abc}Means in the row with different superscripts are significantly different(P<0.05).

On the other hands, the brain total protein concentration (Table 3) of the birds feed MSG at inclusion levels 0.75 - 1.25g/kg was depleted compared to the control and other graded levels. However, total protein concentration of the olfactory lobe of the experimental birds was not affected. The non-significant difference in the concentration of total protein in the olfactory lobes of the laying bird across all the treatment diets indicated that MSG had no interference with neural mechanisms involved with protein synthesis in the brain of the birds. Conversely, the significant decrease observed in the total protein concentrations in the other regions of the brain at higher dosage of MSG (0.75 to 1.25 g/kg) suggested that MSG disrupt protein synthesis in those regions. It had been explained that the ability of a substance to bind and interfere with enzymes and substrates that are needed in the initiation, transcription and translation process involved in protein synthesis makes it capable of affecting the brain development (16). Protein in the brain

functions for repair of worn-out tissues for growth, muscles development and it also binds to some minerals to ensure bioavailability of minerals for proper utilization (16).

Table 4: Specific acetylcholinesterase (SAChE) concentrations in the brain regions of layers fed graded levels of monosodium glutamate

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Olfactory Lobe	0.62±0.004^{b}	0.57±0.064^{b}	0.79±0.016^{ab}	0.79±0.010^{ab}	0.87±0.151^{a}	0.90±0.056^{a}	0.0088
Pineal Gland	0.77±0.079^{ab}	0.63±0.036^{b}	0.76±0.013^{ab}	0.71±0.034^{ab}	0.86±0.026^{a}	0.86±0.074^{a}	0.0166
Optic Lobe	0.83±0.000^{b}	0.82±0.014^{b}	0.88±0.030^{b}	0.96±0.011^{b}	0.96±0.011^{b}	1.17±0.091^{a}	< 0.0001
Cerebellum	0.76±0.058^{b}	0.72±0.022^{b}	0.98±0.020^{a}	1.00±0.078^{a}	1.00±0.009^{a}	1.07±0.064^{a}	< 0.0001
Medulla	0.90±0.061^{b}	0.77±0.079^{b}	0.82±0.046^{b}	0.82±0.046^{b}	0.90±0.043^{b}	1.89±0.243^{a}	< 0.0001

^{abc}Means in the row with different superscripts are significantly different(P<0.05)

Furthermore, the results of the brain specific acetylcholinesterase (SAChE) level are as shown in Table 4. It was discovered that there was significant (P≥0.05) difference in the SAChE activities of the olfactory lobe and pineal gland regions of the brain between the layers on each of the treatment group. Layers fed 1.00 – 1.25g/kg MSG had higher values compared to others and the control group. There was no significant (P≥0.05) difference in the SAChE activities of the optic lobe and medulla oblongata region of the brain among the control group and layers on diets B to E except those on diet F which had considerably higher values. The cerebellum of the birds on the diets containing 0.50 g/kg MSG and above were greatly (P≤0.05) influenced when compared with those on the control diet and diet B. While significant elevations were observed in the SAChE activities of the olfactory lobe, optic lobe and medulla oblongata among the layers feed 1.25 g/kg of MSG as well as in the pineal body and cerebellum of those feed 1.00 and 1.25 g/kg and 0.50 to 1.25 g/kg MSG, respectively, (17) did not report any influence of fumonism on the SAChE activities in the cerebellum of pigs. The difference in the two results might be as a result of different species of experimental animals and the different supplements were fed to the animals. However, the results of these findings agreed with the reports of (19) who concluded that excessive quantity of MSG induced an alteration in the functions of the brain as neurotransmitter.

Conclusion and Applications

1. The study concluded that monosodium glutamate (MSG) is a potential flavor enhancer in layers' diets.
2. MSG can be toxic to the brain neurons at high dosage and thus impair brain function.
3. However, when fed at dose of 0.25 to 0.50 g/kg diet, the birds can tolerate it and thus could be used as a flavor enhancer in poultry feed

References

(1) Olarotimi, O.J. and Adu, O.A. (2017). Potentials of non-conventional protein sources in poultry nutrition. *Archivos de Zootecnia*, 66 (255): 453-459

(2) Jon, H., Vijesh, V.K., Olaf, E. and Christian, B. (2015).India's poultry revolution: Implication for its sustenance and the global poultry trade. *International Food and Agribusiness Management Review*. Vol. 18: 151 -164.

(3) Banson, K.E.; Muthusamy, G. and Kondo, E. (2015). The import substituted poultry industry; Evidence from Ghana. *International Journal of Agricultural*

Forestry, 5: 166-175.

(4) Ebenebe, C.I.; Anigbogu, C.C.; Anizoba, M.A. and Ufele, A.N. (2013). Effect of various levels of Moringa leaf meal on the egg quality of Isa Brown Breed of Layers. *Advance Life Science Technology*, 14: 45-49.

(5) Windisch, W. M., Schedle, K., Plitzner, C. and Kroismayr. (2008). Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science*. 86: 140–148.

(6) Khalil, R. M., Khedr, N.F. (2016). Curcumin protects against monosodium glutamate neurotoxicity and decreasing NMDA2B and mGluR5 expression in rat hippocampus. *Neurosignals.* 24, 81-87

(7) Fasakin, O.W., Fajobi, A.O., Oyedapo, O.O. (2017). Neuroprotective potential of Aframomummelegueta extracts on brain of monosodium glutamate-treated wistar albino rats. *Journal of Neuroscience and Behavioral Health.* 9 (2), 16-27

(8) Eweka, A.O. (2007). Histological studies of the effects of monosodium glutamate on the cerebellum of adult Wistar rats. *Journal of Neurology Neurosurgery &Psychiatry*. 8, 2-7.

(9) Ellman, G.L., Courtney, K.D., Andres, V.J., Featherstone, R.M. (1961). A new and rapid calorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*. 7, 88 -95

(10) Reinhold, J.G. (1953). Manual determination of serum total protein, albumin and globulin fraction by buiret method In: Reiner, M. (Ed). Standard methods in clinical chemistry. Academic Press, New York, Pp: 88 -97

(11) SAS Institute Inc. (2008). SAS / STAT User's Guide.Version 9.2 for Windows. SAS InstituteInc; SAS Campus Drive. Carry, North Carolina, U.S.A.

(12) Lucinei, B.S., Gravena, C., Bonfleur, M.L., de Freitas Mathias, P.C. (2000). Insulin Secretion and Acetylcholinesterase Activity in Monosodium *L*-Glutamate-Induced Obese Mice. *Hormone Research. In Pediatrics*. 54(4):1186-1191

(13) Sowmya, M.and Sarada, S. (2015). Combination of *Spirulina*with glycyrrhizin prevents cognitive dysfunction in aged obese rats. *Indian Journal of Pharmacology*. 47(1):3

(14) Abu-Taweel, G.M. (2016). Effect of monosodium glutamate and aspartame on behavioral and biochemical parameters of male albino mice. *African Journal of Biotechnology* 15(15), 601-612.

(15) Maynitz, M. (2018). Bird senses – sight, hearing, touch, taste and smell. How birds use their sense. The spruce. https://www.thespruce.com/birds-five-senses-386441. Accessed January 30, 2019.

(16) Ewuola, E.O., Bolarinwa, O.A. (2017). Acetylcholinesterase, glucose and total proteinconcentration in the brain regions of West African dwarf goats fed dietary aflatoxin. *Journal Veterinary Medical & Animal Health*. 9 (9), 240-245

(17) Adejumo, D.O., Ladokun, A.O., Sokunbi, O.A. (2005). Species differences in acetylcholesterase activity and total protein concentration in the brain and hypophyes of red sokoto goats and gudalicattles. *Asset Series A.*, 5 (1), 121-127.

(18) Adejumo, D.O. and Egbunike, G.N. (2004). Changes in acetylcholinesterase activities in the developing and aging pig brain and hypophyses. *International Journal of Agricultural and Rural Development.* 5, 46 -53.

(19) Bond, O. (2017). Neurological effect of monosodium glutamate. www. livestrong. com. Accessed 3rd February, 2019.