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Original article

Physiologic changes in acetylcholinesterase, specific acetylcholinesterase and total protein concentrations in the brain of domestic fowl (*Gallus domesticus*) cocks fed dietary monosodium glutamate

Olumuyiwa Joseph Olarotimi[a,*], Oluwasegun Emmanuel Ibidiran[a], Imoleayo Sarah Oladeji[a], Olufemi Adesanya Adu[a], Francis Ayodeji Gbore[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: olarotimioj@futa.edu.ng

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ABSTRACT

The objective of this study was to determine the effect of varied levels of monosodium glutamate (MSG) on acetylcholinesterase, specific acetylcholinesterase and total protein concentrations in the brain regions of chicken cocks. Three hundred cocks of 5 months old were used for the trial and they were randomly allotted to six dietary treatments: A, B, C, D, E and F containing 0.00 (control), 0.25, 0.50, 0.75, 1.00 and 1.25 g MSG/kg diet respectively. Two birds were housed per cell in the cage for the feeding trial in a completely randomised designed and the experiment lasted 12 weeks. At the end of the feeding trial, three cocks/replicate were sacrificed and brain dissected into different regions. The different regions of the brain studied were medulla oblongata, olfactory lobe, optic lobe and cerebellum. Samples were collected from these regions and homogenised to determine acetylcholinesterase, specific acetylcholinesterase and total protein concentrations. Result showed that the acetylcholinesterase activity in the olfactory lobe and pineal gland brain regions were not significantly (P≥0.05) influenced by the dietary MSG among the treatments when compared with the control diet. The cerebellum and medulla oblongata were only significantly

($p<0.05$) influenced when fed above 0.50 g MSG/kg diet while optic lobe was significantly ($p<0.05$) influenced at an inclusion level above 0.75 g MSG/kg diet. The total protein concentrations were significantly ($p<0.05$) higher in olfactory lobe and cerebellum of the brain regions of cocks fed 0.25 and 0.50 g MSG/kg and control diet than those fed other diets. Total protein concentration in the pineal gland and optic lobe regions of the brain in cocks fed the control diet were not significantly ($P\geq0.05$) different among the treatment diets. This study suggests that dietary MSG above 0.50 g/kg diet increased the activities of acetylcholinesterase concentration in the optic lobe, cerebellum, pineal gland, medulla oblongata and reduced the total protein in the olfactory lobe, cerebellum and medulla oblongata regions of the brain with tendency to impair brain function.

1. Introduction

The use of phytogenic additives as possible alternatives to replace growth promoting antibiotics (GPAs) in poultry production has gained a general acceptance (Windisch et al., 2008). Phytogenics are natural growth promoters used as feed additives (Yitbarek, 2015). Despite the great potentials of phytogenic additives in poultry production, Windisch et al. (2008) had reported that some of these beneficial plant additives adversely affect feed palatability due to their bitter taste. Hence, it reduces feed intake of the birds and therefore leading to feed rejection or wastage. Any additive that could be incorporated in feed to enhance feed intake will definitely be useful in improving the palatability of the phytogenic additives. Monosodium Glutamate (MSG), one of the world's most used flavor enhancer, has been reported to increase the palatability of food (Khalil and Khedr, 2016). 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 implicated as a potent neurotoxin especially when consumed in excessive amounts. MSG is believed to act through ionotropic and metabotropic glutamate receptors thereby increasing the excitability and activating the proteolytic enzymes (Weil and Norman, 2008) and causes neurotoxic effects when consumed in large amount (Fernandez and Hernnandez, 2005). Excessive dosage of MSG administration had also been implicated to confer a negative effect on the cerebellum of adult wistar rats, thus, resulting in tremor, ataxia and uncontrollable movement of the rats (Eweka and Om'Iniabohs, 2007). Despite documented reports on negative consequences of MSG on laboratory animals, International bodies and organizations such as the Food and Drug Administration (FDA) as well as National Agency for Food and Drug Administration Control in Nigeria (NAFDAC) considered MSG as a safe additive.

Since the human population is on increase, especially in the sub-saharan Africa, meeting the protein needs of this growing population is a concern to the Animal Scientists. Cocks have the potential of converting the readily available phytogenic additives as well as non-conventional protein sources into meat and this could be sold at affordable prices to the populace. Broiler chicken meat has increased in cost in the recent years and several reports have reviewed the potency of cocks providing the animal protein need of the populace. Improving the feed intake and weight gain of cocks in the poultry industry will be beneficial to both the farmers and the consumers. Since the avian brain controls all body functions, and as a result, it is easily affected by the feed, enzymes and hormones secreted in the body of an animal (Adejumo and Egbunike, 2004).

The major regions that make up the avian brain are the forebrain which consists mainly of the cerebral hemispheres and the olfactory lobes, the midbrain which mainly consists of the optic lobes and the hindbrain which consists mainly of the cerebellum and the medulla oblongata. The dearth of knowledge on the potentials and implications of dietary MSG inclusion on the brain parts of the cocks, therefore, motivated the investigation of effect of varied levels of dietary MSG on the acetylcholinesterase (AChE) activities, total protein and specific acetylcholinesterase (SAChE) of the brain of the cocks in this present study.

2. Materials and methods

The study was carried out at the poultry unit of the Teaching and Research Farm, The Federal University of Technology, Akure. Three hundred birds of twenty weeks old were procured from a reliable source for the experiment. The birds were housed in an open-sided building in a thoroughly cleaned, washed and disinfected three tier cage system of 32 x 38 x 42 cm dimension, two birds were caged per cell. They were weighed on arrival and randomly assigned to six (6) treatments (diets): A, B, C, D, E and F containing 0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 g MSG/kg diet respectively. Each treatment was replicated five times with 10 birds per replicate. The birds were placed on experimental cock's diet (Table 1) for a period of twelve weeks the study lasted. At the end of the experiment, two birds per replicate were humanely slaughtered through cervical dislocation and the brains were removed immediately, freed of all adhering meninges and blood vessels. The brains obtained were dissected on ice-cold porcelain tile into the cerebellum, optic lobe, olfactory lobe and medulla oblongata. The brain acetylcholinesterase (AChE) activities and total protein concentrations were determined by colorimetic method according to Ellmann et al. (1961) and Biuret method of Reinhold (1953) using Randox commercial kit respectively. 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 AChE activity of each sample was divided by its total protein concentration to give the specific acetylcholinesterase (SAChE) activity in µmole/g protein /min. Completely randomized design was used for the experiment and the data collected were subjected to one way analysis of variance (ANOVA) using SAS (2008, version 9.2). Duncan Multiple Range Test of the same software was used for means comparison where significant differences existed.

Table 1
Ingredient composition of the experimental cock diets (g/kg).

		Inclusion level of MSG (g)				
Ingredients	**A (0.00)**	**B (0.25)**	**C (0.50)**	**D (0.75)**	**E (1.00)**	**F (1.25)**
Maize	330.00	330.00	330.00	330.00	330.00	330.00
Groundnut cake	80.00	80.00	80.00	80.00	80.00	80.00
Bone Meal	16.00	16.00	16.00	16.00	16.00	16.00
Limestone	20.00	19.75	19.50	19.25	19.00	18.75
Salt	2.40	2.40	2.40	2.40	2.40	2.40
MSG	0.00	0.25	0.50	0.75	1.00	1.25
Corn Bran	100.00	100.00	100.00	100.00	100.00	100.00
BDG	248.10	248.10	248.10	248.10	248.10	248.10
Palm Kernel Cake	200.00	200.00	200.00	200.00	200.00	200.00
Methionine	1.00	1.00	1.00	1.00	1.00	1.00
Broiler Premix	2.50	2.50	2.50	2.50	2.50	2.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients						
ME (Kcal/Kg)	2520.66	2520.66	2520.66	2520.66	2520.66	2520.66
Crude Protein (%)	16.07	16.07	16.07	16.07	16.07	16.07
Calcium (%)	1.13	1.12	1.12	1.11	1.10	1.09
Phosphorus (%)	0.59	0.59	0.59	0.59	0.59	0.59
Lysine (%)	0.65	0.65	0.65	0.65	0.65	0.65
Methionine (%)	0.27	0.27	0.27	0.27	0.27	0.27
Crude Fibre (%)	6.28	6.28	6.27	6.26	6.26	6.26

*Composition of premix (Nutrivitas®): 2.5 kg of premix contains: Vit. A (10,000,000 iu), Vit. D3 (2,500,000 iu), Vit. E (12,000 iu), Vit. B1 (2000 mg), Niacin (25000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2500 mg), Biotin (75 mg), Folic Acid (2000 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (50%) (200000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10,000 mg), Zinc (60000 mg), Selenium (200 mg), Iodine (1500 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Cobalt (250 mg). BDG = Brewer Dry Grain, MSG = monosodium glutamate.

3. Results and discussion

The brain AChE levels of the cocks are as shown in Table 2. From the table, it was observed that the olfactory lobe of the cocks on diet F had the highest non-significant (P≥0.05) AChE value compared with those on other treatment diets. For pineal gland, cocks on diets A to D showed statistical similarities (P≥0.05) while cocks on diets E and F showed the highest significant (P<0.05) means. The highest significant (P<0.05) value for the optic lobe was also recorded on diet F while those on diets A to D were not significantly (P≥0.05) different. For the cerebellum, diets E and F also showed similar values which were statistically significant (P<0.05) as compared with cocks on diet son diets A to C. For medulla oblongata, highest significant (P<0.05) value was recorded on diet F but this was statistically similar (P≥0.05) to what was obtainable on diets D and E but significantly different when compared with the means of the cocks on diets A and B. Considering the total protein of the cocks' brain, Table 3 shows the results. It was observed that the cocks on diet A (control) recorded the highest significant (P<0.05) value of total protein for the olfactory lobe though statistically similar (P≥0.05) to what is obtainable from those on diets B and C. However, there was no significant difference (P≥0.05) in the values of those placed on the other experimental diets. For optic lobe and pineal gland, cocks on A and B diets recorded the highest but non-significant (P≥0.05) values while those on other diets were similar. Cerebellum values obtainable on diets A, B and C were significantly (P<0.05) highest while those on other diets were similar. The highest significant (P<0.05) value was recorded on A for medulla oblongata. The results of the brain SAChE of the cocks are displayed in Table 4. There were no significant (P≥0.05) differences among the SAChE activities of the olfactory and optic lobes regions of the brain among hens on diets B to E when compared with the on the control diet. It was only the pullets on diet F showed significant (P<0.05) influence in the studied parameters when compared with the control. However, pineal gland, cerebellum and medulla oblongata means across all the treatment diets and the control was statistically (P≥0.05) similar.

Table 2

Brain Acetylcholinesterase (AChE) Activities of Cocks Fed Different Levels of MSG.

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±0.018	0.11±0.005	0.16±0.007	0.18±0.014	0.18±0.038	0.19±0.035	0.6213^{ns}
Pineal gland	0.14±0.010^{ab}	0.14±0.001^{ab}	0.13±0.012^{b}	0.15±0.004^{ab}	0.16±0.001^{ab}	0.16±0.006^{a}	0.0190*
Optic lobe	0.15±0.006^{c}	0.13±0.001^{c}	0.16±0.004^{c}	0.19±0.011^{c}	0.30±0.074^{b}	0.47±0.009^{a}	< 0.0001*
Cerebellum	0.14± 0.006^{c}	0.15±0.006^{bc}	0.13±0.006^{c}	0.17±0.001^{ab}	0.18±0.003^{a}	0.18±0.005^{a}	< 0.0001*
Medulla oblongata	0.15±0.004^{b}	0.15±0.004^{b}	0.20±0.006^{ab}	0.22±0.032^{a}	0.23±0.011^{a}	0.24±0.003^{a}	< 0.0001*

Values are means ± SEM; Means in a row without a common superscript are significantly (P<0.05) different. Level of significance = ns (not significant) = P≥0.05; * = P< 0.05

Table 3

Brain Total Protein of Cocks Fed Different Levels of MSG (g/kg).

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Olfactory lobe	0.23±0.003^{a}	0.22±0.001^{a}	0.20±0.003^{ab}	0.18±0.001^{b}	0.17±0.017^{b}	0.17±0.012^{b}	< 0.0001*
Pineal gland	0.28±0.023	0.28±0.001	0.24±0.025	0.23±0.023	0.25±0.002	0.26±0.007	0.1324^{ns}
Optic lobe	0.28±0.027	0.28±0.027	0.23±0.007	0.21±0.048	0.21±0.002	0.21±0.019	0.1727^{ns}
Cerebellum	0.17±0.001^{a}	0.17±0.001^{a}	0.17±0.001^{a}	0.12±0.009^{b}	0.12±0.002^{b}	0.12±0.001^{b}	< 0.0001*
Medulla Oblongata	0.19±0.003^{a}	0.15±0.003^{ab}	0.15±0.003^{ab}	0.12±0.007^{b}	0.13±0.013^{b}	0.12±0.007^{b}	< 0.0001*

Values are means ± SEM; Means in a row without a common superscript are significantly (P<0.05) different. Level of significance = ns (not significant) = P≥0.05; * = P< 0.05.

Table 4

Brain Specific Acetylcholinesterase (SAChE) Activities of Cocks Fed Different Levels of MSG.

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Olfactory lobe	0.73±0.032^{bc}	0.67±0.015^{c}	0.78±0.047^{bc}	0.66± 0.078^{c}	1.11±0.167^{b}	2.12±0.124^{a}	< 0.0001*
Pineal body	0.68±0.119	0.53±0.009	0.56±0.010	0.45±0.045	0.63±0.011	0.63±0.038	0.0590^{ns}
Optic lobe	0.72±0.020^{b}	0.65±0.055^{b}	0.66±0.002^{b}	0.71±0.155^{b}	0.93±0.186^{b}	1.9±0.236^{a}	< 0.0001*
Cerebellum	1.19±0.039	1.19±0.027	1.87±0.037	1.34±0.021	1.55±0.060	1.54±0.022	0.0679^{ns}
Medulla oblongata	1.24±0.101	1.32±0.124	1.33±0.013	1.32±0.124	1.28±0.037	1.48±0.253	0.7903^{ns}

Values are means ± SEM; Means in a row without a common superscript are significantly (P<0.05) different. Level of significance = ns (not significant) = P≥0.05; * = P< 0.05.

Acetylcholinesterase (AChE) is the primary enzyme responsible for the hydrolyticmetabolism of the neurotransmitter acetylcholine (ACh) into choline and acetate. It has an important role in the CNS and is implicated in behavioral as well as learning and memory and neurodegenerative diseases. The significant increase in the acetylcholinesterase (AChE) activities of the optic lobe, cerebellum and medulla oblangata sections of the brain of the cocks fed 1.00 g/kg MSG and above in the present study could be attributed to the fact that high dose of MSG administration could cause a decrease in the sensitivity of AChE to acetylcholine. This result was in disagreement with the report of Abu-Taweel (2016) which reported decrease in the AChE activity in MSG treated rats but validated the findings of Lucinei et al. (2000), Sowmya and Sarada (2015), Khalil and Khedr (2016) and Fasakin et al. (2017) which reported an increase the activities of AChE in MSG treated mice. They further opined that the increased AChE activity in the hippocampus of MSG treated mice was as a result of dysfunction in the cholinergic system, which resulted in alteration of the enzyme. It is, therefore, speculative in this study that significant increased AChE activity in cocks fed diet above 0.75 g/kg MSG may lead to a decrease in cholinergic neurotransmission efficiency due to a reduction in acetylcholine level in the synaptic cleft, thus inducing progressive cognitive impairment in the cocks. This further strengthened the claim of Sreejesh and Sreekumaran (2018) that reported a significant increase in the enzyme AChE in hippocampal region of the brain of the rats treated 4 g MSG/ kg body weight when compared with those on the control and low diet rats. Furthermore, the AChE activity that was not significantly different in the brain regions of among the cocks on the treatments containing 0.75 g/kg MSG and below indicated that normal synthesis and catabolism of neurotransmitters (AChE) were presumably only affected when the cocks were fed above a tolerable level of 0.50 g/kg diet. This could also probably be that cocks have higher resistance to dietary MSG inclusion than rats. The insignificant effect noted in the AChE of the pineal gland of the cocks across the treatment diets in comparison with the control 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 bird. The insignificant effect of the dietary MSG on activity of the AChE of the olfactory lobes of the cocks revealed that their sense of smell was not negatively affected by the treatment diets which is attributed to the fact that birds have the least sense of smell and do not use smell extensively to make most of their decisions (Maynitz, 2018). The significant elevations observed in the SAChE activities of the olfactory lobe and optic lobe among the cocks on the diet containing 1.00 and 1.25 g/kg MSG in this study agreed with the reports of Bond (2017) who stated that excessive quantity of MSG induced an alteration in the functions of the SAChE activity of the brain as neurotransmitter. Adejumo and Egbunike (2004) also agreed with the non-significant influence observed in the pineal gland, cerebellum and medulla oblongata SAChE activities of the brain regions.

The significantly lower concentration of total protein in the olfactory lobe, cerebellum and medulla oblongata of the cocks on diets containing above 0.50 g MSG/kg diet is indicative of the interference of dietary MSG with neural mechanisms involved with protein synthesis. The insignificant decrease in the total protein concentration in the optic lobe of the cocks further confirms the possibility of the interference of MSG with protein synthesis in some brain sections. Ewuola and Bolarinwa (2017) explained that the ability of aflatoxin 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. MSG may have similar effects aflatoxin had in their report on the brain of the cocks. Protein in the brain is important for several functions such as repair of worn-out tissues for growth, muscles development and it also binds to some minerals to ensure bioavailability of minerals for proper utilization (Adejumo et al., 2005). The total protein levels in the brain regions in the present study were generally lower than the values reported for the brain regions of male West African dwarf goats Ewuola and Bolarinwa (2017) and Bitto (2008) for rabbit bucks. This disparity may be due to species differences in biochemical characteristics of brain regions. Furthermore, the progressive decline in the concentration of total protein in the optic lobe as MSG inclusion increases might eventually lead to retina degeneration if the cocks were consistently fed above 0.50 g/kg tolerable level. This agreed with the report of Ohguro et al. (2002) that rats fed 10 grams MSG/100 gram daily diet had a significant increase in amount of glutamic acid in vitreous, had damage to the retina, and had deficits in retinal function.

4. Conclusion

This study posited that monosodium glutamate (MSG) could be used as taste enhancing additive in cocks' diets when the inclusion level does not exceed 0.50 g/kg diet. Excessive dosage may predispose the cocks to glutamate-induced neurotoxicity and suppress protein synthesis, thereby, affecting protein-lipid complexes in some brain regions.

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