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# Monosodium Glutamate Adversely Affects Serum Electrolytes and Antioxidant Status of Laying Hens

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**Abstract**

This research assessed the effect of varying inclusions of monosodium glutamate (MSG) on serum electrolytes and antioxidative enzymes of laying hens. A total of 300 Isa Brown point-of-lay pullets, aged 16 weeks, were evenly allocated to six distinct experimental lots, each containing different levels of MSG (0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 g/kg). Throughout the study, the experimental hens were granted unrestricted freedom to diets, and clean water was consistently provided. In the twelfth week, five hens per replicate were chosen randomly after an overnight fast for blood collection through the wing veins. Standard procedures were employed to analyze serum electrolytes, including Na<sup>+</sup> (sodium), K<sup>+</sup> (potassium), and Cl<sup>-</sup> (chloride), together with antioxidant enzymes such as T-OAC (total antioxidant capacity), SOD (superoxide dismutase), and GSH-Px (glutathione peroxidase). Additionally, an assessment of the oxidative stress indicator malondialdehyde (MDA) was undertaken. The findings revealed that the addition of MSG at 1.00 and 1.25 g/kg in the feed significantly increased the serum Na<sup>+</sup> concentration of the pullets, while the serum K<sup>+</sup> level decreased significantly at the 1.25 g/kg MSG administration level, compared to the hens in the control group. In contrast, the blood Cl<sup>-</sup> level significantly decreased with MSG inclusion at the 0.50 g/kg level. Moreover, MSG inclusion levels at or above 0.75 g/kg led to an increase in MDA concentration, accompanied by a significant reduction in serum SOD, GSH-Px, and T-AOC levels. Thus, including MSG at levels exceeding 0.50 g/kg in the diet may potentially predispose laying hens to oxidative stress and other physiological imbalances.



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## Introduction

The deterioration in the quality of raw materials, particularly by-products like corn and rice bran, during extended storage can lead to the development of undesirable odors that avian species find unappealing [1]. This, in turn, significantly affects feed quality and ultimately the performance of laying hens. The components of their diet have a profound impact on the health and performance of laying hens, with particular emphasis on their electrolyte balance and antioxidant status [2]. Monosodium glutamate (MSG), a commonly used flavor enhancer in the food industry, has gained considerable attention in recent years due to its potential effects on avian physiology [3]. While MSG is well-known for enhancing umami flavors in human cuisine, its impact on animals, especially laying hens, remains a relatively unexplored aspect of poultry nutrition. As the poultry industry continues to expand to meet global protein demands, the health and productivity of laying hens become paramount concerns [4]. MSG, comprising sodium ions and glutamate, can influence the levels of electrolytes in the bloodstream and the balance of oxidative processes in avian species, thereby affecting hydration levels, cardiovascular performance and metabolic equilibrium [5].

Serum electrolytes, including crucial ions such as sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), and calcium ( $\text{Ca}^{2+}$ ), play a vital role in avian homeostasis. Maintaining proper electrolyte balance is essential for nerve conduction, muscle function and regulation of osmotic pressure, all of which are critical for the overall health and reproductive performance of laying hens. Fluctuations in serum electrolyte levels can lead to adverse physiological consequences, such as dehydration and impaired cardiovascular function [6]. Furthermore, there is a growing interest in understanding the interaction between dietary components and the antioxidant status of laying hens. Oxidative stress, arising from the production of reactive oxygen species (ROS), can impact the reproductive health and egg quality of laying hens [7].

Recent studies have started to shed light on the dietary influence of MSG on serum electrolytes and antioxidant status in avian species. For instance, Olarotimi revealed significant alterations in serum electrolyte concentrations in broilers exposed to dietary MSG supplementation [1]. Similarly, another research investigated the oxidative stress induced by MSG consumption in rats, providing

insights into its potential impact on antioxidant status [8]. Given the limited research on the specific effects of MSG on laying hens, this study aims to contribute to our understanding of how dietary MSG influences serum electrolyte balance and antioxidant defense mechanisms. It seeks to elucidate the impact of dietary MSG on the serum electrolyte concentrations and antioxidant status of laying hens, addressing crucial aspects of poultry nutrition and health. To achieve these objectives, the study integrates rigorous methodologies in poultry nutrition, electrolyte analysis and antioxidant assessment. The findings not only hold implications for poultry nutrition and welfare but also contribute to discussions regarding the safe and responsible use of MSG as a dietary component in laying hen diets.

## Materials and Methods

### Management and application of treatments

For this research, a reputable farm was carefully selected for the sourcing of 300 point-of-lay (POL) Isa Brown pullets, all aged sixteen weeks. These pullets were initially nourished with a commercial grower mash until they reached a laying performance of 20% at 24 weeks of age. Subsequently, a transition was made to the treatment diets detailed in Table 1 for 16 weeks. Consistent management procedures were applied to the hens throughout the experimental period, with the primary variation being the different levels of dietary MSG inclusion. We initiated the study by measuring and recording the initial weights of the pullets. The birds were then randomly assigned to various experimental groups, each of which was provided with feed containing different amounts of MSG, specifically 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25 g/kg. Each group consisted of 50 pullets and was replicated five times, resulting in a total of 10 birds per replicate. Throughout the experiment's duration, the birds received two daily feedings, one in the morning and one in the afternoon. They also had unrestricted access to water. Furthermore, the administration of recommended vaccinations and other necessary medications was carried out in a timely manner, adhering to the prescribed schedule.

### Collection of blood

At the expiration of the 12-week experiment, a total of 5 birds per replicate were selected randomly for blood sampling. These birds underwent an

overnight fast, after which blood samples were collected from their wing veins. The blood was collected into clean glass tubes without any anticoagulant to allow for the subsequent separation of serum, which would be used for determining serum electrolytes and indicators of antioxidant status. The collected blood samples were left to stand at room temperature for 15 minutes. Subsequently, the tubes were subjected to centrifugation at 3000 rpm for duration of 10 minutes, enabling the separation of a clean supernatant serum. These serum samples were then frozen and stored at -20°C until the time of analysis for serum T-OAC (total antioxidant capacity), SOD (superoxide dismutase), GSH-Px (glutathione peroxidase) and malondialdehyde (MDA) levels and Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> concentrations.

**Quantification of electrolytes**

Serum electrolyte levels, including sodium anion, potassium anion and chloride cation were evaluated using an automated analyzer called the Kodak Ektachem, developed by the Eastman Kodak Company in Rochester, New York. The determination of blood K<sup>+</sup> and Na<sup>+</sup> concentrations was done by following the protocols of Terri and Sesin [9], while the assessment of serum Cl<sup>-</sup>

concentration adhered to the method outlined by Skeggs and Hochstrasser [10].

**Measurement of antioxidant status indicators**

The quantification of serum malondialdehyde (MDA) was carried out through the thiobarbituric acid (TBA) assay method, following the procedure outlined by Baliga et al. [11]. The measurement of serum glutathione peroxidase enzyme (GSH-Px) activity adhered to the methodology established by Flohe and Gunzler [12]. The assessment of serum superoxide dismutase (SOD) activity was performed using the procedure outlined by Oyanagui [13]. To determine the serum total antioxidant content (T-AOC), we employed the colorimetric approach established by Lusignoli et al. [14].

**Statistical analysis**

In this study, statistical analysis techniques were employed to assess and interpret the gathered data. The experimental data were subjected to analysis through the utilization of ANOVA, with the support of GraphPad Prism software, specifically version 6.01. To compare treatment means and identify significant differences at a 5% level of significance, Tukey's HSD feature within the same software was employed.

**Table 1** Ingredient composition of the experimental layer diets.

Ingredients	Control	Inclusion level of monosodium glutamate				
		0.25%	0.50%	0.75%	1.00%	1.25%
Maize	43	42.75	42.5	42.25	42	41.75
Soybean meal	13	13	13	13	13	13
Groundnut cake	13	13	13	13	13	13
Fish meal	0	0	0	0	0	0
Wheat offal	8.8	8.8	8.8	8.8	8.8	8.8
Rice bran	10	10	10	10	10	10
Corn bran	0	0	0	0	0	0
Bone meal	2.1	2.1	2.1	2.1	2.1	2.1
Limestone	9	9	9	9	9	9
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Monosodium glutamate	0	0.25	0.5	0.75	1	1.25
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Layer premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated nutrients						
ME (Kcal/Kg)	2620.52	2611.94	2603.35	2594.77	2586.18	2577.6
Crude protein (%)	17.73	17.71	17.68	17.66	17.63	17.59
Calcium (%)	4.2	4.2	4.2	4.2	4.2	4.2
Phosphorus (%)	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	1.2	1.2	1.2	1.2	1.2	1.2
Methionine	0.47	0.47	0.47	0.47	0.47	0.47
Crude fiber (%)	3.79	3.78	3.78	3.77	3.76	3.76

## Results and Discussion

### Serum electrolytes

The serum electrolyte results for the layers provided rations containing varying MSG levels are displayed in Table 2. It is noted that MSG inclusion levels of up to 0.75 g/kg diet did not produce a significant ( $P>0.05$ ) impact on the serum  $\text{Na}^+$  concentration in the pullets when compared to the control group. However, a notable ( $P<0.05$ ) increase was observed when the pullets were fed diets containing 1.00 and 1.25 g MSG/kg. Regarding serum  $\text{K}^+$  concentration, a substantial ( $P<0.05$ ) reduction was only observed in the birds on a diet containing 1.25 g MSG/kg when compared to those on the control diet. A similar trend was observed for serum  $\text{Cl}^-$  concentration, with MSG inclusion levels above 0.50 g/kg diet causing a significant ( $P<0.05$ ) progressive decrease in the mean value. The findings unveil distinct patterns of response in serum  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  concentrations, shedding light on the potential physiological implications of MSG inclusion in poultry diets. The absence of a significant effect on serum  $\text{Na}^+$  concentration at MSG inclusion levels up to 0.75 g/kg diet when compared to the control group aligns with previous research conducted by Olarotimi [1]. This prior study also reported minimal changes in serum  $\text{Na}^+$  levels in broilers exposed to dietary MSG supplementation within this threshold. These results suggest that lower levels of MSG incorporation in poultry diets may have a limited impact on serum  $\text{Na}^+$  concentrations. However, a distinct departure from this trend becomes evident when MSG inclusion levels reached 1.00 and 1.25 g/kg diets, resulting in significant increases in serum  $\text{Na}^+$  concentrations. This phenomenon may be attributed to the sodium component of MSG, indicating that higher dietary sodium intake could potentially lead to increased serum  $\text{Na}^+$  levels. This observation aligns with the established understanding that dietary sodium levels can influence serum  $\text{Na}^+$  concentrations in birds [15, 16]. Furthermore, the noteworthy decrease in serum

$\text{K}^+$  concentration among birds fed a diet containing 1.25 g MSG/kg compared to those on the control diet deserves attention. Potassium is an essential electrolyte involved in various physiological processes, including nerve function and muscle contraction. The observed decrease in serum  $\text{K}^+$  concentration may reflect potential disturbances in electrolyte balance that warrant further investigation. Similar to the trend observed in serum  $\text{Na}^+$  concentrations, the study revealed a progressive decrease in serum  $\text{Cl}^-$  levels with MSG inclusion levels above 0.50 g/kg diet. This finding is in line with the established relationship between dietary salt intake and chloride concentration in poultry [16, 17]. Elevated dietary sodium levels, as provided by MSG, could potentially disrupt the  $\text{Cl}^-$  balance in the birds.

### Serum antioxidant enzymes

The outcomes related to the antioxidant status of the layers fed various MSG levels in their diets are summarized in Table 3. In terms of GSH-Px, SOD, and T-AOC, the pullets receiving control diets exhibited the most substantial and statistically significant means ( $P<0.05$ ) for these parameters. In contrast, those on diets containing 0.25 and 0.50 g MSG/kg did not display any significant differences ( $P>0.05$ ) when compared to the control group. However, it's noteworthy that the inclusion levels of 0.75 g MSG/kg diet and above led to a significant ( $P<0.05$ ) reduction in the mean values of these examined parameters. Notably, there appeared to be an inverse relationship between the mean values of MDA and the other three antioxidant status parameters, as a significant ( $P<0.05$ ) increase was observed when the pullets were fed 0.75 g MSG/kg diet and above, with the highest significant ( $P<0.05$ ) value recorded for those on the 1.25 g MSG/kg diet. These findings imply that the basal diet adequately provided antioxidant capacity and supported the birds' defense mechanisms against oxidative stress. The antioxidant enzymes, GSH-Px and SOD, play vital roles in neutralizing harmful reactive oxygen species and maintaining the cellular redox balance. Additionally, the absence of

**Table 2** Serum electrolytes of layers-fed diets with different inclusion levels of monosodium glutamate (g/kg).

Parameters	0.00	0.25	0.50	0.75	1.00	1.25	P-value
$\text{Na}^+$ (mEq/L)	140±0.06 <sup>b</sup>	140±0.01 <sup>b</sup>	140±0.02 <sup>b</sup>	144.87±0.01 <sup>ab</sup>	149±0.31 <sup>a</sup>	150±0.14 <sup>a</sup>	0.01
$\text{K}^+$ (mEq/L)	3.60±0.01 <sup>ab</sup>	3.70±0.00 <sup>ab</sup>	4.90±0.00 <sup>a</sup>	3.60±0.00 <sup>ab</sup>	3.00±0.00 <sup>b</sup>	2.90±0.00 <sup>c</sup>	0.04
$\text{Cl}^-$ (mEq/L)	102±0.06 <sup>ab</sup>	105±0.08 <sup>a</sup>	98±0.06 <sup>b</sup>	90±0.21 <sup>c</sup>	84±0.03 <sup>d</sup>	76±0.05 <sup>c</sup>	0.01

Values are means ± SEM. Means in a row without common superscripts are significantly ( $P<0.05$ ) different. Sodium ( $\text{Na}^+$ ); Potassium ( $\text{K}^+$ ); Chloride ( $\text{Cl}^-$ ), monosodium glutamate levels are in g/kg diet.

**Table 3** Serum antioxidant status of layers-fed diets with different inclusion levels of monosodium glutamate.

Parameters	0.00	0.25	0.50	0.75	1.00	1.25	P-value
GSH-Px ( $\mu\text{mol/ml}$ )	280 $\pm$ 0.29 <sup>a</sup>	250 $\pm$ 0.30 <sup>ab</sup>	240 $\pm$ 0.29 <sup>ab</sup>	220 $\pm$ 0.44 <sup>b</sup>	160 $\pm$ 0.30 <sup>c</sup>	160 $\pm$ 0.29 <sup>c</sup>	0.03
SOD ( $\mu\text{mol/ml}$ )	160 $\pm$ 0.15 <sup>a</sup>	150 $\pm$ 0.15 <sup>ab</sup>	140 $\pm$ 0.31 <sup>ab</sup>	130 $\pm$ 0.06 <sup>b</sup>	97 $\pm$ 0.30 <sup>c</sup>	96 $\pm$ 0.15 <sup>c</sup>	0.02
T-AOC ( $\mu\text{mol/ml}$ )	7.30 $\pm$ 0.03 <sup>a</sup>	6.90 $\pm$ 0.03 <sup>ab</sup>	6.50 $\pm$ 0.02 <sup>ab</sup>	6.10 $\pm$ 0.07 <sup>b</sup>	3.20 $\pm$ 0.03 <sup>c</sup>	2.30 $\pm$ 0.02 <sup>d</sup>	0.01
MDA (nmol/ml)	2.58 $\pm$ 0.014 <sup>c</sup>	2.60 $\pm$ 0.00 <sup>c</sup>	3.50 $\pm$ 0.02 <sup>bc</sup>	4.40 $\pm$ 0.01 <sup>b</sup>	5.20 $\pm$ 0.00 <sup>ab</sup>	6.20 $\pm$ 0.03 <sup>a</sup>	0.01

Values are means  $\pm$  SEM. Means in a row without common superscripts are significantly ( $P < 0.05$ ) different; glutathione peroxidase (GSH-Px); total antioxidant Activity (T-AOC); malondialdehyde (MDA); superoxide dismutase (SOD); monosodium glutamate levels are in g/kg diet.

significant differences between the groups fed diets containing 0.25 and 0.50 g MSG/kg in comparison to the control diets suggests that these lower MSG inclusion levels did not significantly impair the birds' antioxidant defense systems. This observation aligns with prior research indicating that moderate MSG consumption may not significantly disrupt antioxidant status [1]. However, the reduction observed in the mean values of GSH-Px, SOD and T-AOC among birds fed MSG inclusion levels of 0.75 g/kg diet and above implies that higher dietary MSG levels might have an inhibitory impact on these antioxidant enzymes and the overall antioxidant capacity. Such reductions in antioxidant defense mechanisms could potentially make the birds more susceptible to oxidative stress and its associated harmful effects on cellular health. The elevation of MDA levels among the pullets fed diets with 0.75 g/kg MSG and above suggests an increase in lipid peroxidation and oxidative damage to cellular membranes, indicating heightened oxidative stress due to higher MSG concentrations. These observations are consistent with studies linking excessive dietary MSG intake to oxidative stress and lipid peroxidation in other animal models [2, 18, 19].

## Conclusion

In conclusion, the results indicate that incorporating MSG into the diet at levels up to 0.50 g/kg is safe for laying hens in terms of its impact on their serum electrolytes and antioxidant status. However, MSG inclusion levels at 0.75 g/kg and higher, led to a significant rise in serum  $\text{Na}^+$  concentration. This is accompanied by decreased serum  $\text{K}^+$  and  $\text{Cl}^-$  levels, reduced antioxidant enzyme activity and an increase in oxidative stress, as reflected by elevated MDA levels. These findings underscore the significance of carefully considering the appropriate MSG concentration in poultry diets to maintain optimal electrolyte levels and antioxidant defense mechanisms while minimizing oxidative stress. Further research is imperative to unveil the underlying mechanisms behind these alterations

and assess their potential consequences for poultry health and performance.

## Conflict of interest

The authors declare no conflict of interest.

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