

Effect of Nano-Encapsulated Peptide on Some Blood Parameters of Broiler Exposed to Oxidative Stress

Nareman Badri Arkan¹, Fadhil Rasool Abbas Al-Khafaji², Hashim Hadi Al-Jebory³

^{1,2,3} Department of Animal Production- college of agriculture-Al-Qasim Green University- Babylon province, Iraq

ABSTRACT

The study was conducted on the Al-Anwar poultry farm, 360 broiler chicks (Ross 308) divided into six treatments (T1, T2, T3, T4, T5, T6). The treatments were as follows: T1 was the control treatment, T2 was the control treatment with 0.5% H₂O₂, and T3, T4, T5, T6 were the treatments that were supplemented with 1, 2, 3, and 4 ml of synthetic peptides with 0.5% H₂O₂. The results shown: significant increase for T2 in cholesterol, triglyceride, LDL, VLDL, glucose, uric acid, AST, ALT, significant increase for T2 and T3 in MDA level, significant increase for T4, T5, T6 in total protein, while significant increase for T4 in albumin, significant increase for T6 in globulin, as significant increase for T5 and T6 in SOD, while glutathione peroxidase increase in T1, T3, T4, T5, T6, compared to T2.

Published Online:
May 05, 2025

KEYWORDS: encapsulated peptide, oxidative stress, broiler, iron, methionine, tryptophan.

Corresponding Author:
Hashim Hadi Al-Jebory

INTRODUCTION

Oxidative stress can be defined as an imbalance between the oxidation and antioxidant systems in the body, which in turn causes lipid peroxidation, protein peroxidation and dysregulation of neurotransmitter signaling, and DNA damage within cells (Surai et al., 2019; Al-Jebory et al., 2024). Therefore, oxidative stress is one of the main factors that negatively affect productive performance (Vandana and Sejan, 2018; Ajafar et al., 2024 a,b). Oxidative damage to poultry disrupts the normal metabolism of birds due to damage occurring within cells (Carvalho et al., 2016; Al-Saeedi et al., 2021; Al-Saeedi et al., 2023). Nano-encapsulation of peptides or amino acids is a promising method to reduce protein utilization while maintaining the benefits of slower absorption. Various desired complementary nutrients are encapsulated in lipids, lipid-like substances, or polymers, resulting in more efficient utilization of the encapsulated nutrient (Collins, 2021). Nano-peptides of natural and synthetic origin have gained interest in recent years due to their biological activities as potential alternatives to conventional antimicrobial agents that can combat pathogenic and drug-resistant microorganisms as well as their antioxidant properties (Davies and Davies, 2010). Iron is an essential mineral that is routinely added to broiler diets and is an essential component of many enzymes and proteins that play a role in oxygen transport, maintaining health, and regulating cell growth and differentiation, especially red blood cells (Mendel and Hansch, 2009). Iron deficiency leads to a breakdown of the immune system and antioxidants, with severe effects on the health of poultry (Sahin et al., 2001). Methionine is an essential amino acid in poultry because it cannot be synthesized in the body. It is a sulfur-containing amino acid (Burley et al. 2016). Methionine is required for building the immune system, improving production performance, feed utilization efficiency, and muscle growth (Hicklig et al. 1990). Methionine is an important methyl group donor during protein metabolism. It is essential for feather growth and immune system function and is the first specific amino acid in poultry (Lai et al. 2018). Tryptophan helps increase levels of growth hormone and serotonin and is a component of melatonin, so it plays a major role in stimulating growth and immunity. Tryptophan also contributes to the production of niacin, which plays a major role in regulating cholesterol metabolism in the body; tryptophan is the third most important specific amino acid in chicken feed after methionine and lysine (Yao et al., 2011), the importance of tryptophan is directly related to its role in protein synthesis and indirectly related to its receptors such as serotonin and melatonin, tryptophan also affects hormone secretion, immune system development, and meat production, tryptophan is also an essential amino acid for chickens and has many metabolic roles (Fouad, 2021). Therefore, the current study aims to study the effect of nano-encapsulated peptides composed of iron, methionine and tryptophan on some biochemical indicators of broiler exposed to oxidative stress.

MATERIALS

The preparation of nanopeptides was carried out in the Nano Technology Laboratory, Babylon Governorate, and the method of Ruhul et al. (2017) was followed to prepare nanopeptides and nano-encapsulate them from iron, methionine, and tryptophan.

This experiment was conducted in the fields of Al-Anwar Poultry Company in Babil Governorate for a period of 35 days from 10/7/2024 to 11/10/2024. During this period, the effect of adding synthetic peptides to the drinking water of broiler chickens raised under conditions of oxidative stress induced by hydrogen peroxide on some productive and physiological traits and oxidation indicators was studied. The chicks, housing, nutrition and preventive program were managed under the same conditions as the first experiment. In addition, hydrogen peroxide (H₂O₂) was added to the drinking water at a concentration of (0.5%) to induce oxidative stress as shown in the treatments used in the second experiment. Hydrogen peroxide was added from the first day of the experiment and the water was replaced twice a day, at 9 am and 9 pm, to ensure that the effect of hydrogen peroxide continued effectively. The chicks were randomly distributed into 18 cages, with 6 treatments (60 birds per treatment). Each treatment contained 3 replicates, each containing 20 birds, totaling 360 Ross-308 broiler chicks for the entire experiment. Synthetic peptides were added at different concentrations, along with hydrogen peroxide (0.5%), from the first day of the experiment, as follows:

T1: Control treatment without any additions.

T2: Control treatment exposed to oxidative stress induced by adding 0.5% hydrogen peroxide to the drinking water.

T3: Add 1 ml of the synthetic peptides / liter / exposed to oxidative stress induced by adding 0.5% hydrogen peroxide to the drinking water.

T4: Add 2 ml of the synthetic peptides / liter / exposed to oxidative stress induced by adding 0.5% hydrogen peroxide to the drinking water. T5: Add 3 ml of synthetic peptides / liter / exposed to oxidative stress induced by adding 0.5 L of hydrogen peroxide to drinking water.

T6: Add 4 ml of synthetic peptides / liter / exposed to oxidative stress induced by adding 0.5 L of hydrogen peroxide to drinking water.

Lipid profile, liver enzymes and antioxidant status were studied and statistical analysis was performed using SAS (2012) and Duncan's multiple range tests (1955).

RESULTS AND DISCUSSION

Lipid Profile

Table (1) shows the effect of the studied treatments on the lipid profile of broiler chickens exposed to oxidative stress. A significant increase ($P \leq 0.01$) in cholesterol concentration was observed compared to the remaining treatments. Cholesterol concentration also increased in treatments T3, T4, and T6 compared to treatment T5, which did not significantly differ from treatment T1. Triglyceride concentration increased significantly ($P \leq 0.05$) in treatment T2 compared to treatments T3, T4, T5, and T6. No significant difference was observed between treatments T1 and T2. There was no significant difference between the study treatments in the concentration of high-density lipoproteins (HDL), and in the concentration of low-density lipoproteins (LDL), a significant increase ($P \leq 0.05$) was found in the blood of birds in treatment T2 compared to treatment T4. There was no significant difference between treatments T1, T3, T4, T5, T6. The concentration of very low-density lipoproteins (VLDL) increased significantly ($P \leq 0.05$) in treatment T2 compared to treatment T3. No significant difference was found between treatments T1, T3, T4, T5, T6. As for the concentration of malondialdehyde, it increased significantly ($P \leq 0.01$) in treatments T1, T2, T3 compared to treatments T5 and T6. Treatment T4 also increased compared to treatment T5, and no significant difference was found between treatments T1, T2, T3, T4.

Adding hydrogen peroxide to birds' drinking water negatively affects them because it causes oxidative stress and the formation of free radicals, which disrupts lipid metabolism pathways in liver cells. Furthermore, oxidative stress damages cell membranes, disrupting fat digestion in the intestines, causing problems with the excretion of harmful steroid compounds and a decrease in bile salts (Lee et al., 2006). Low-density lipoprotein (LDL) is the main carrier of cholesterol, triglycerides, and phospholipids. Therefore, damage to the LDL molecule by free radicals resulting from oxidative stress causes it to lose its effectiveness in transporting lipids, change its shape, and decrease its metabolism in the liver due to the receptors not recognizing it, which causes an accumulation of cholesterol, triglycerides, LDL, and VLDL in the blood serum (Lewington et al., 2007). This explains the high values of the lipid profile in the control treatment T2, while the improvement of the lipid profile, especially in the treatment T5, may be due to the role of methionine, which has a major role in the antioxidant system. In the body (Mishra and Jha, 2019), it protects lipids, polyunsaturated fatty acids, proteins, and DNA by activating several antioxidant enzymes and activating iron and copper to bind to proteins to reduce the activity of enzymes involved in reactions that produce free radicals, such as NADPH oxidase and xanthine oxidase, or by reducing free radicals and forming methionine sulfoxide, or by activating thioredoxin, which reduces the severity of oxidative stress within the body and thus reduces lipid oxidation and its levels in blood serum (Lugata et al., 2022). The improvement may also be due to the role of tryptophan, which reduces the secretion of hormones that cause stress in chickens, such as corticosterone. Tryptophan also maintains the integrity of cell membranes from oxidation and reduces the oxidation of lipids present in the cell membrane (Fouad et al., 2021).

Impact of nano-encapsulated peptide on lipid profile of broiler exposed to oxidative stress						
	Cholestero l (mg/ 100 ml)	Triglyceride (mg/ 100 ml)	HDL (mg/ 100 ml)	LDL (mg/ 100 ml)	VLDL (mg/ 100 ml)	MDA μmol/mol
T1	127.16± 6.90bc	122.62 ±9.58ab	70.88± 1.43	31.75 ±6.41ab	24.52 ±1.92ab	17.12± 0.23a
T2	152.86± 3.55a	139.75 ±1.56a	72.30± 3.85	52.75± 6.41a	27.94 ±0.32a	20.15± 1.17a
T3	134.98± 4.57b	108.10± 9.34b	74.85 ±1.44	38.51 ±4.15ab	21.62± 1.87b	17.66± 0.45a
T4	139.24 ±1.83b	117.91 ±2.44ab	79.88± 0.25	35.78± 1.09ab	23.58± 0.49ab	16.77 ±0.24ab
T5	119.82 ±1.53c	118.12± 12.76ab	78.67± 4.26	17.52± 0.18b	23.62± 2.55ab	10.09± 0.27c
T6	133.51 ±1.85 b	118.36± 6.00ab	78.16 ±8.58	31.67 ±11.63ab	23.67± 1.20ab	13.49± 2.10bc
	**	*	N.S	*	*	**

Total protein, albumin, globulin, uric acid, and glucose

Table (2) indicates the effect of the studied treatments on some biochemical characteristics. It is noted that the total protein concentration increased significantly ($P \leq 0.05$) in treatments T4, T5, and T6 compared to treatments T1 and T2. Albumin concentration increased significantly ($P \leq 0.05$) in treatment T4 compared to treatments T1, T2, T3, and T6. Treatment T5 also increased significantly compared to treatment T2. Treatment T6 had the highest significant ($P \leq 0.05$) globulin concentration compared to treatments T1 and T2. No significant difference was observed between treatments T3, T4, T5, and T6. In terms of uric acid concentration, treatment T2 increased significantly ($P \leq 0.05$) compared to treatments T5 and T6, and no significant difference was found between treatments T1, T2, T3, and T4. As for glucose concentration, a highly significant increase ($P \leq 0.01$) was found in treatment T2 compared to the rest of the treatments. Treatments T1 and T3 also increased compared to treatment T6, and no significant difference was found between treatments T4, T5, and T6. The increased total protein levels in treatments T4, T5, and T6 may be due to the role of peptides added to the drinking water and the role of methionine and tryptophan in increasing protein levels for the formation of body tissues and cells. Tryptophan contributes to protein synthesis as an essential amino acid (Ghazaghi et al., 2024). Methionine also plays a fundamental role in protein synthesis, as it replaces the amino acid cysteine. Methionine is involved in the transsulfuration pathway and is a sulfur donor (Partlin, 2005). Methionine also stimulates insulin secretion from the pancreas by accumulating it in the plasma, which in turn releases amino acids and fatty acids from stored body sources, leading to increased protein synthesis in the body (Vieira et al., 2004). At the same time, these important roles of methionine and tryptophan explain the increased concentrations of albumin and globulin in the blood serum of birds treated T4 and T6, respectively. The reason for the decrease in blood uric acid levels in our current study in treatments T5 and T6 may be due to the effectiveness of tryptophan and methionine, which improves the body's use of the nitrogen source (uric acid) and reduces its waste, which reduces the percentage of uric acid in the blood serum. It is noted in Table (1) that the level of triglycerides decreased in the addition treatments, indicating that the blood energy properties coincided with the use of reused amino groups in protein synthesis (Abou-Elkhair et al., 2020). The addition of methionine and tryptophan acids to broiler chickens also enhances the release of insulin-like growth factor 1 (IGF-I), which enhances the use of amino acids and glucose. This process enhances protein synthesis, reduces protein breakdown, and maintains glucose levels (Fouad et al., 2021). Tryptophan regulates the process of gluconeogenesis during stress by balancing amino acids and reducing their breakdown, as stress leads to the breakdown of amino acids and proteins, which is accompanied by an increase in Blood glucose levels (El-Kholy et al., 2025) may explain the lower glucose concentration in the T6 treatment. Stress deteriorates the bird's physiological state, causing increased secretion of catecholamines from the adrenal medulla to increase blood glucose secretion (Naga and Narendra, 2018). Stress also activates the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. Corticotrophin-releasing hormone (CRH) is secreted from the hypothalamus, leading to the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. ACTH increases the production and secretion of corticosterone by the adrenal glands (Smith and Vale, 2006). Corticosterone stimulates gluconeogenesis to increase plasma glucose levels (Naga and Narendra, 2018). This explains the significant increase in glucose levels in the control treatment. T2, Methionine stimulates the secretion of insulin from the pancreas, which works to introduce glucose into the cells, thus reducing its concentration in the blood serum (Vieira et al., 2004).

Impact of nano-encapsulated peptide on biochemical traits of broiler exposed to oxidative stress					
	Total protein (g/ 100 ml)	Albumin (g/ 100 ml)	Globulin (g/ 100 ml)	Uric acid (mg/ 100 ml)	Glucose (mg/ 100 ml)
T1	3.23±0.02b	1.19±0.01bc	2.04±0.03b	3.48±0.07ab	193.93±3.30b
T2	3.26±0.02b	1.13±0.01c	2.13±0.01b	3.95±0.06a	239.41±0.72a
T3	3.51±0.12ab	1.21±0.01bc	2.30±0.12ab	3.60±0.09ab	192.40±5.90b
T4	3.76±0.24a	1.39±0.02a	2.37±0.23ab	3.48±0.27ab	185.25±1.05bc
T5	3.71±0.06a	1.27±0.08ab	2.44±0.15ab	3.28±0.17b	186.80±10.40bc
T6	3.95±0.14a	1.21±0.04bc	2.74±0.11a	3.42±0.06b	172.57±3.32c
	*	*	*	*	**

Liver Enzymes and Antioxidant Enzymes

Table (3) shows the effect of experimental treatments on some liver enzymes. AST enzyme concentrations showed a significant increase ($P \leq 0.01$) in the serum of birds in treatment T2 compared to the rest of the treatments. Treatment T4 also increased compared to treatments T1, T5, and T6, while treatment T3 increased compared to treatment T5. ALT concentrations showed a significant increase ($P \leq 0.05$) in treatment T2 compared to the rest of the experimental treatments. SOD enzyme concentrations showed a significant increase ($P \leq 0.01$) in treatments T5 and T6 compared to treatments T1 and T2. Treatments T1, T3, and T4 also significantly increased compared to treatment T2. Glutathione peroxidase concentrations significantly increased ($P \leq 0.05$) in treatments T1, T3, T4, T5, and T6 compared to treatment T2. Changes in blood AST and ALT values are used as indicators to assess the health status of the organism. Evaluation of ALT and AST levels in chickens is considered an indicator of poor liver function in the body (El-Kholy et al., 2022; El-Kholy et al., 2024). Oxidative stress causes damage to the membranes of liver cells by free radicals, as they attack unsaturated fatty acids present in the membranes of liver cells, which leads to the accumulation of lipid peroxide in the liver tissue and an increase in free fatty acids. These changes subsequently lead to a change in the permeability of cell membranes, which allows the liver enzymes AST and ALT to exit and accumulate in the blood, in addition to their participation in protein decomposition when exposed to stress (Li et al., 2015). By reviewing Table (2), we see an increase in the level of glucose in the T2 treatment and a decrease in the protein level in it, leading to an increase in the levels of AST and ALT enzymes in the blood. This is because these enzymes work to encourage the process of manufacturing glucose from non-carbohydrate sources, especially proteins, as they work to increase the process of protein decomposition. To provide amino acids and convert them into ketone acids to enter the reaction chain to form glucose (Nelson and Cox, 2004). The improvement in the levels of AST and ALT enzymes in the nanopptide treatments may be due to the improvement of the antioxidant status of birds in the same treatments, through the increased concentration of SOD and glutathione peroxidase enzymes in the blood serum of birds treated with nanopptides (Table 3). It may also be due to the antioxidant role of methionine (Lu, 2013), in addition to its essential role in balancing amino acids in the feed (Bunchasak et al., 2009). Tryptophan also plays a pivotal role in reducing oxidative damage in broiler chickens, as it is a precursor to melatonin and serotonin (Patil et al., 2013), and iron in activating the catalase enzyme, thus protecting body tissues from oxidative stress (Aljebory et al., 2021). Total antioxidant status reflects the activity of enzymatic reactions and non-enzymatic processes. Glutathione peroxidase and SOD enzyme measures are commonly used to assess oxidative stress and antioxidant defense mechanisms (Shuai et al., 2023). The improvement in SOD levels in treatments T5 and T6 and in glutathione peroxidase levels in treatments T3, T4, T5, and T6 may be due to tryptophan, as a precursor to melatonin and serotonin, playing a pivotal role in reducing oxidative damage in broiler chickens (Patil et al., 2013). It may also be due to methionine, a non-enzymatic antioxidant, directly scavenging reactive oxygen species, thus preventing oxidative damage to lipids, proteins, and nucleic acids (Luo and Levine, 2009). Methionine can also act as a precursor to glutathione (Lu, 2013). The decrease in GSH activity is due to the fact that tryptophan, as a precursor to melatonin and serotonin, plays a pivotal role in reducing oxidative damage in broiler chickens (Patil et al., 2013). SOD and glutathione are evidence of oxidative stress in T2 treatment birds due to the addition of hydrogen peroxide to drinking water, which stimulates the initiation of a series of chemical reactions that lead to oxidative stress by increasing the production of active oxygen compounds and their entry into the blood, thus increasing the oxygen pressure in the cells, in addition to causing an excessive increase in these compounds. In contrast, an imbalance occurs between the oxidation process and the antioxidant system due to the decreased efficiency of this system due to the free radicals formed as a result of hydrogen peroxide, which has a direct inhibitory effect on the activity of antioxidant systems, including SOD and glutathione, which are responsible for capturing and removing free radicals (Shehata and Yousef, 2010; Zaki and Al-Jebory, 2021; Khalil et al., 2022).

Effect of nanopeptides of methionine, tryptophan and iron on liver enzymes and antioxidants of broiler chickens exposed to oxidative stress				
Treatments	AST (U/L)	ALT (U/L)	SOD (μmol/mol)	Glutathione peroxidase (μmol/mol)
T1	144.11±1.98cd	19.74±2.38b	136.42±7.83b	285.21±25.37a
T2	183.93±5.28a	28.36±1.80a	112.54±2.70c	222.91±17.45b
T3	151.23±4.45bc	16.84±2.52b	142.07±5.82ab	305.60±5.04a
T4	157.31±4.10b	19.53±1.17b	152.25±1.01ab	334.03±6.64a
T5	133.08±2.39d	17.12±0.23b	154.59±1.18a	321.94±5.69a
T6	141.10±0.86cd	14.98±3.34b	154.59±5.62a	338.72±23.44a
Significant	**	*	**	*

CONCLUSION

It is concluded from the results of our study that nano-peptides of iron, methionine and tryptophan improved the lipid profile and antioxidant status and improved some indicators that suggest a reduction in the severity of oxidative stress in reared broilers.

REFERENCES

1. Abou-Elkhair, R., Ahmed, H., Ketkat, S., Selim, S., 2020. Supplementation of a low- protein diet with tryptophan, threonine, and valine and its impact on growth performance, blood biochemical constituents, immune parameters, and carcass traits in broiler chickens. *Vet Worl.* 13 (6), 1234–1244.
2. Ajafar M, Al-Jebory HH, Al-Saeedi MKI (2024 a). Effect of in Ova injection of lysophospholipid in hatching traits, chick's quality, and chicks physical traits of broiler (Ross 308). *Adv. Anim. Vet. Sci.*, 12(7):1206-1213. <https://dx.doi.org/10.17582/journal.aavs/2024/12.7.1206.1213>.
3. Ajafar, M., H.H. Al-Jebory and M.K.I. Al-Saeedi. 2024 b. Effect of Feed Restriction and Manganese Supplementation on the Immunological and Physiological Traits and Glycogen Level of Broiler Ross 308. *Kufa Journal For Agricultural Sciences* . 16(4): 141-150.
4. Al-Jebory HH, Al-Saeedi MKI, Ajafar M, Ali NAL (2024). Impact of melatonin on improving productive traits of broiler exposed to environmental stress. *Adv. Anim. Vet. Sci.* 12(4): 775-781. <https://dx.doi.org/10.17582/journal.aavs/2024/12.4.775.781>.
5. Aljebory, H.H.D., S.A.H. Naji. 2021. Effect of Pelleted Fermented Feed-in Egg Quality of Laying Hens. *Diyala Agricultural Sciences Journal* 13 (1): 41-57. <https://dx.doi.org/10.52951/dasj.21130105>.
6. AL-SAEEDI, M.K.I., H.H. AL-JEBORY AND M. AJAFAR. 2023. Effect of in Ova Injection with Nano-copper in Productive Performance of Japanese Quail Exposed to Pathological and Environmental Challenges. *Annals of Agri-Bio Research* 28 (2): 361-366.
7. Al-Saeedi, M.K.I., H.H. Dakhil, and F.R.A. Al-Khafaji. 2021. Effect of adding Silver Nanoparticles with drinking Water on some Lymphatic Organs and Microflora in the intestinal for broiler Chickens (ROSS 308). 1st INTERNATIONAL VIRTUAL CONFERENCE OF ENVIRONMENTAL SCIENCES IOP Conf. Series: Earth and Environmental Science 722 (2021) 012004 IOP Publishing doi:10.1088/1755-1315/722/1/012004.
8. Bunchasak, C. 2009. Role of Dietary Met in Poultry Production. *J Poult Sci.* 2009; 46: 169-179.
9. Carvalho R., shimokomaki M. and Estevez M., poultry meat color and oxidation, Pages 133-157 in *Poultry Quality Evaluation*, 2016,
10. Collins, E.A. 2021. Influence of Encapsulation of Supplemental Amino Acids on their Utilization in Broilers. *Poultry science Undergraduate Honors Theses* Retrieved from <https://scholarworks.uark.edu/poscuht/9>.
11. Davies, J.; Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 2010, 74, 417–433.
12. Duncan, D.B. 1955. Multiple Rang and Multiple F-test. *Biometrics.* 11: 4-42.
13. El-Kholy, K.H., T.A. Ebeid, M.E. Sokariaa, I.T. El-Ratel, M.F. El Basuini , M.M. Azzam, S.F. Fouda, A.C.A.A. Elolimy , M. Madkour, M. Alagawany. 2025. Tryptophan and/or canthaxanthin in quail diets: effects on performance, carcass traits, hematology, blood chemistry and hepatic antioxidant capacity. *Poultry Science.* 104: 104703. <https://doi.org/10.1016/j.psj.2024.104703>.
14. El-Kholy, K.H., El-Din, H.T., Tawfeek, F.A., Tufarelli, V., Losacco, C., Alhotan, R.A., Shafi, M.E., Attia, Y.A., Hassab, S.H.M., 2024. Performance, blood and antioxidant status in dual-purpose laying hens supplemented with aqueous extract of

- Christ's thorn jujube (*Ziziphus spina-christi* L.) leaves as phytogetic agent in subtropical conditions. *Anim. Biosci.* 37 (5), 896–907.
15. El-Kholy, K.H., Tag El-Din, H., Tawfeek, F.A., Hassab, S.H.M., 2022b. Climate change mitigation by dietary phytogetic addition in layer hens: some re-productive traits and endocrine hormones responses. *J. Anim. Health Prod.* 10, 81–87.
16. Fouad, A. M. 2021. Tryptophan in poultry nutrition: Impacts and mechanisms of action, *Journal of Animal Physiology and Animal Nutrition* /Volume 105, Issue 6/ p.1146-1153.
17. Fouad, A.M., El-Senousey, H.K., Ruan, D., Wang, S., Xia, W., Zheng, C., 2021. Tryptophan in poultry nutrition: impacts and mechanisms of action. *J. Anim. Physiol. Anim. Nutr.* 105, 1146–1153.
18. Ghazaghi, M., Asghari-Moghadam, M., Mehri, M., 2024. Immunoregulatory and antioxidant properties of tryptophan in quail chick. *Heliyon* 10, e29115.
19. Hansch, R., Mendel, R.R., 2009 physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), *Current Opinion in Plant Biology*, 12, 259-266.
20. Ibrahim, M.K., and H.H.D Al-Jebory. 2020. Study of the effect of Bee Propolis on some biochemical, immunological traits and intestinal microflora of broiler chickens (Ross 308). 1st SCIENTIFIC INTERNATIONAL VIRTUAL AGRICULTURAL CONFERENCE IOP Conf. Series: Earth and Environmental Science 553 (2020) 012022 IOP Publishing doi:10.1088/1755-1315/553/1/012022.
21. Khalil Ibrahim Al-Saedi, M., Hadi Al-Jebory, H., Hameed Abood, M. 2022. Progress Phenotypic Traits of Hatched Chicks and Growth Indicators of Broiler Chicks Fed Embryonically with Zinc Methionine. *Archives of Razi Institute.* 77(6): 2139-2145.
22. Lee, D. H., B. S. Lim, Y. K. Lee and H. C. Yang. 2006. Effects of hydrogen peroxide (H₂O₂) on alkaline phosphatase activity and matrix mineralization of odontoblast cell lines. *Cell Biol. and Toxicol.*, 22 (1) : 39 – 46.
23. Lewington, S., G. Whitlock, R. Clarke, P. Sherliker, J. Emberson, J. Halsey, N. Qizilbash, R. Peto and R. Collins. 2007. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 370 (9602) : 1892 – 1839.
24. Li, S., H. Tan, N. Wang, Z. Zhang, L. Lao, C. Wong and Y. Feng. 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int. J. Mol. Sci.* 16: 26087–26124. doi:10.3390/ijms161125942
25. Lu, S.C., 2013. Glutathione synthesis. *Biochimica et Biophysica Acta* 1830, 3143–3153.
26. Lugata, J.K., A.D.S.V. Ortega and C. Szabó. 2022. The Role of Methionine Supplementation on Oxidative Stress and Antioxidant Status of Poultry-A Review. *Agriculture*. 12, 1701. <https://doi.org/10.3390/agriculture12101701>.
27. Luo, S.; Levine, R.L. Methionine in Proteins Defends against Oxidative Stress. *FASEB J.* 2009, 23, 464–472.
28. Mishra, B.; Jha, R. Oxidative Stress in the Poultry Gut: Potential Challenges and Interventions. *Front. Vet. Sci.* 2019, 6, 60.
29. Naga, R.K., and K.N. Narendra. 2018. Ameliorative measures to counter heat stress in poultry. *World's Poult. Sci. J.* 2018, 74, 117–130.
30. Nelson, D. L. and M. M. Cox. 2004. *Lehninger principles of biochemistry*. Fourth ed. Copyright by: W. H. Freeman and Company.
31. Partlin, J. 2005. In *Encyclopedia of Human Nutrition* Sec ed. Available at: <https://www.elsevier.com/books/encyclopedia-of-human-nutrition/allen/978-0-08-045428-3>.
32. Patil, R., Tyagi, J., Sirajudeen, M., Singh, R., Moudgal, R., & Mohan, J. (2013). Effect of dietary melatonin and L-tryptophan on growth performance and immune responses of broiler chicken under experimental aflatoxicosis. *Iranian Journal of Applied. Animal Science*, 3, 139–144.
33. Ruhul A. Bepari, Palash Bharali, Birinchi K. Das. 2017. Controlled synthesis of α- and γ-Fe₂O₃ nanoparticles via thermolysis of PVA gels and studies on α-Fe₂O₃ catalyzed styrene epoxidation, *Journal of Saudi Chemical Society*, **Volume 21, Supplement 1**, January, Pages S170-S178. <https://doi.org/10.1016/j.jscs.2013.12.010>.
34. Sahin, K., Sahin, N., Onderci, M., Yarlioglu, S., Kucuk, O., 2001. Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress, *Veterinarni medicina-Praha*, 46, 140-144.
35. SAS, 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
36. Shehata, A. M. and O. M. Yousef. 2010. Physiological studies on the risk factors responsible for atherosclerosis in Rats. *Nature and Sci.*, 8(5): 144-151.
37. Smith, S.M., W.W. Vale. 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin. Neurosci.* 2006, 8, 383.
38. Surai, P. F., I.I. Kochish, V. I. Fisinin, and M. T. Kidd. 2019. Antioxidant defence systems and oxidative stress in poultry biology: an update. *Antioxidants (Basel)* 8:235.
39. Vandana, G.D., and V. Sejian. 2018. Towards identifying climate-resilient poultry birds. *J. Dairy Vet. Anim. Res.* 73:84-85.

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40. Vieira, S.L., A. Lemme, D.B. Goldenberg, and I. Brugalli. 2004. Responses of growing broilers to diets with increased sulfur amino acids to lysine ratios at two dietary protein levels. *Poult Sci.* 2004; 83: 1307-1313.
41. Yao, K., J. Fang, Y. Yin, Z. Feng, Z. Tang, G. Wu. 2011. Tryptophan metabolism in animals: important roles in nutrition and health. *Frontiers in Bioscience S.* 3(1): 286-297.
42. Zaki, A.N., and H.H.D. Al-jebory. 2021. effect of early feeding with zinc-methionine on improving growth performance and some biochemical characteristics of broilers. 1st INTERNATIONAL VIRTUAL CONFERENCE OF ENVIRONMENTAL SCIENCES IOP Conf. Series: Earth and Environmental Science 722 (2021) 012035 IOP Publishing doi:10.1088/1755-1315/722/1/012035.