



Impact of Nano-Encapsulated of Anti-Bacterial Peptide on Broiler Growth Performance under Oxidative Stress Condition

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Abstract | This study carried out in two experiments. The first was a laboratory experiment to study the effect of nano-peptides made from methionine, tryptophan and iron on the growth of *E. coli* and *Salmonella* cultured from chicken intestines (concentrations of 50, 100 and 150 $\mu\text{L}/\text{plate}$). The results showed a positive effect of the manufactured nano-peptides (NPe) on both types of bacteria. There was significant increase in the diameter of decrease for concentrations of 100 and 150 microliters compared to concentration 50. The second experiment was conducted in the field, using 360 broiler (Ross 308), divided into six treatments (C1: Control treatment, C2: Control treatment with 0.5% H_2O_2 , and C3, C4, C5, C6 adding 1,2,3,4 ml of synthetic peptides with 0.5% H_2O_2). Significant increase in live body weight and weight gain for treatments C4 and C6. All supplementation treatments improved in the feed conversion ratio compared with the control treatments. Packed cell volume (PCV) and hemoglobin (Hb) levels increased in treatment C5. Improvement in treatments C4, C5 and C6 appeared in the percentage of lymphocytes and heterophil. All supplementation treatments improved in the H/L ratio compared with the control treatment C2.

Keywords | Nano, Encapsulated, Anti-bacterial peptide, Broiler, Growth oxidative stress

Received | March 16, 2025; **Accepted** | April 12, 2025; **Published** | May 03, 2025

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Citation | Arkan NB, Al-Jebory HH, Al-Khafaji FRA (2025). Impact of nano-encapsulated of anti-bacterial peptide on broiler growth performance under oxidative stress condition. *Adv. Anim. Vet. Sci.* 13(6): 1169-1179.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2025/13.6.1169.1179>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331



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INTRODUCTION

The poultry industry has garnered a lot of interest from researchers and farmers who want to develop this sector because of its high feed conversion efficiency and rapid growth (Marangoni *et al.*, 2015; Donma and Donma, 2017). This may make birds susceptible to different types of stress that lower immunity and productivity, such as oxidative stress, which is defined as an imbalance between the body's antioxidant and oxidative systems, which leads to DNA damage, lipid peroxidation, protein peroxidation, and dysregulation of intracellular signal transduction

(Surai and Yoshikawa, 2019). One promising way to reduce protein utilization is by nano-encapsulating peptides or amino acids, which maintains the advantages of slower absorption. Lipids, lipid-like compounds, or polymers are used to encapsulate a variety of desired nutritional supplements, increasing the nutrient's utilization efficiency (Collins, 2021). Gheisar *et al.* (2015) reported that supplements including organic acids and essential oils in nanocapsule form, it enhances intestinal health and growth, indicating that the capsule shields the mixture from accelerated and premature absorption. In one study, compared to a similar diet containing unencapsulated glycine, nano-encapsulated

MATERIALS USED IN THE EXPERIMENT

Preparation of nano-peptides from Methionine, Tryptophan, and Iron The preparation of nano-peptides was conducted in the Nano Technologies Laboratory, Babylon Governorate, following the method of *Ruhul et al. (2017)* for preparing nano-peptides (Figure 1).

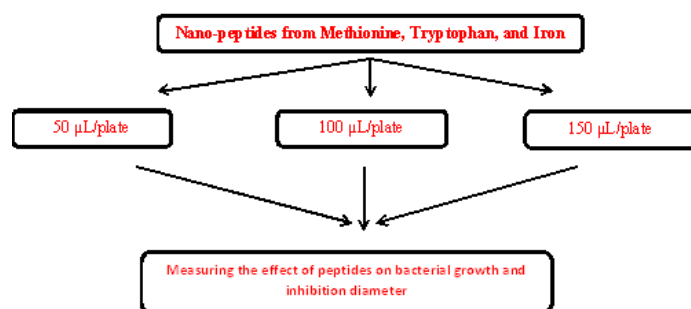


Figure 1: The first experiment.

SEM TEST

SEM analysis results showed spherical particles with nano-sized sizes ranging between (9.66, 10.25, 14.50, 17.09, 17.43) nm (*Pirtarighat et al., 2018*) (Figure 2).

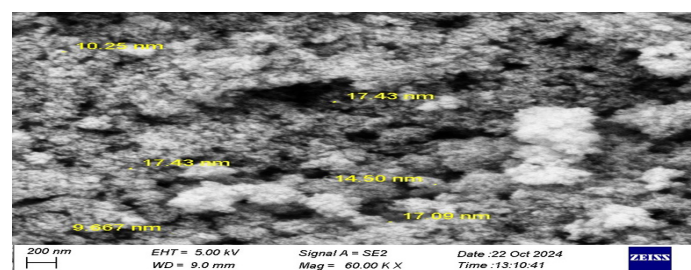


Figure 2: SEM test of nano-peptides.

FTIR TEST

The FTIR analysis used in these images shows the changes in chemical bonds before and after loading methionine and tryptophan onto iron oxide. The images show the chemical bonds between methionine, tryptophan, and iron (Figure 3).

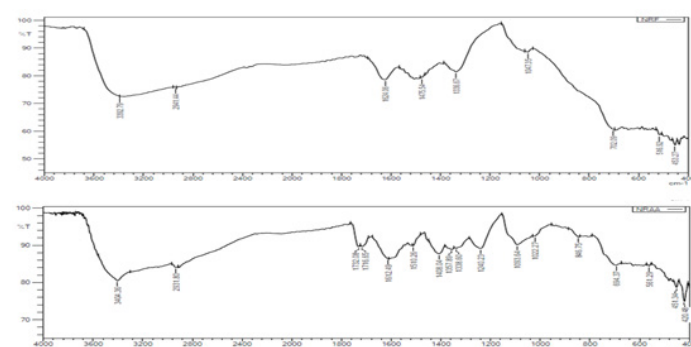


Figure 3: FT-IR test of nano-peptides.

glycine was detected in higher amounts throughout the gastrointestinal tract (*Dahiya et al., 2007*). Antibacterial peptides also have significant advantages and broad application prospects in supporting bird immunity and combating oxidative stress. Antibacterial peptides can penetrate the cell plasma membrane through various channels to kill bacteria (*Nikaido, 1989*). A common mechanism of bacterial inhibition by antibacterial peptides is perforation of the bacterial cell plasma membrane to form ion channels through charge attraction, which disrupts the cell membrane structure, causing the release of large amounts of intracellular substances and bacterial death. Therefore, resistant strains against antimicrobial peptides cannot be easily generated (*Bernd et al., 1988; Xuan et al., 2023*). Methionine is a non-enzymatic antioxidant that directly scavenges reactive oxygen species and activates antioxidant thioredoxin proteins, preventing oxidative damage to lipids and proteins (*Del Vesco et al., 2013*). Methionine is essential for energy generation and improves mortality, growth performance, and feed utilization efficiency in poultry (*Daryoush and Simab, 2022*). Tryptophan is the third most important amino acid found in chicken feed, following methionine and lysine (*Harms and Russell, 2000*). It also contributes to protein synthesis in the body (*Fouad, 2021*). Nanomaterials, for instance, take up very little space yet have quite large surface areas. As a result, when big materials are reduced to the nanoscale, their surface chemical properties become more effective, and the material's physical qualities change while its chemical properties remain unchanged, Iron is an important mineral that is required for the function and production of numerous enzymes and proteins involved in cell growth and differentiation, oxygen transport, and avian immunity (*Hänsch and Mendel, 2009*). *Abdel-Rahman et al. (2022)* contributes considerably to the Krebs cycle as a coenzyme, and aids in the elimination of toxic oxidative chemicals via catalase and peroxidase (*Nikonov et al., 2011*). Stress lowers iron levels in serum and tissues (*Combs and Combs, 1984*). Low iron levels affect immunological and antioxidant capabilities, which is harmful to bird health (*Sahin et al., 2001*). Iron is primarily linked to phytate in grains and oilseeds (*Yu et al., 2000*), reducing its availability in poultry feeds when phytase is not provided (*Gibson et al., 2010*). Therefore, the current study aims to: Study the synthesis and effects of nanopptides of the amino acids methionine and tryptophan loaded on to nano- iron nano-particles on the growth and productive, physiological, and immunological performance of Rose 308 broiler chickens exposed to oxidative stress.

MATERIALS AND METHODS

FIRST EXPERIMENT (LABORATORY)

This investigation examined the impact of synthetic peptides on laboratory-grown *Salmonella* and *E. coli* bacteria

The result was around 25 nm (Wu *et al.*, 2010; Saravanan *et al.*, 2013).

BACTERIAL COUNT

Concentrations of 50, 100, and 150 $\mu\text{L}/\text{plate}$ were added using an automatic pipette after bacteria culture with specific aggar, then bacteria count according to (Rai, 2016) and the diameter of inhibition according to Theivasanthi and Alagar (2011).

SECOND EXPERIMENT (FARM)

This experiment was carried out in the farms of Al-Anwar Poultry Company in Babil Governorate for 35 days, from 10/7/2024 to 11/10/2024. During this time, the effect of adding synthetic peptides to the drinking water of broiler chickens raised under oxidative stress caused by hydrogen peroxide was investigated in terms of some productive and physiological aspects, as well as oxidation indicators. The chicks, housing, nutrition, and preventive program were managed in the same way as the first experiment. Furthermore, as shown by the treatments employed in the second experiment, oxidative stress was induced by adding hydrogen peroxide (H_2O_2) to the drinking water at a concentration of 0.5%. Starting on the first day of the experiment according to Chen *et al.* (2021), Merzah and Ali (2023), hydrogen peroxide was introduced, and to make sure the hydrogen peroxide's effect persisted, the water was changed twice a day, at 9 am and 9 pm, oxidative stress indicators were monitored through blood parameters and growth performance. Eighteen cages containing six treatments (each with sixty birds) were randomly assigned to the chicks. For the duration of the trial, there were 360 Ross-308 broiler chicks total—three replicates of each treatment, each with 15 birds. Beginning on the first day of the experiment, hydrogen peroxide (0.5%) and synthetic peptides were administered in varying amounts as follows:

- C1: Control treatment (no additives).
- C2: Control treatment adding 0.5% $\text{H}_2\text{O}_2/\text{L}$.
- C3: Add 1 ml NPe + 0.5% $\text{H}_2\text{O}_2/\text{L}$.
- C4: Add 2 ml NPe + 0.5% $\text{H}_2\text{O}_2/\text{L}$.
- C5: Add 3 ml NPe + 0.5% $\text{H}_2\text{O}_2/\text{L}$.
- C6: Add 4 ml NPe + 0.5% $\text{H}_2\text{O}_2/\text{L}$.

These were fed a beginning meal that contained 23% protein and 3027 kcal/kg of feed. Until the end of the fifth week, they were substituted with a growth diet that contained 20% protein and 3195.3 kcal/kg feed. They have unlimited access to food and drink (Figure 4).

TRAITS STUDY

Productive traits of LBW, WG, FI, FCR, and mortality rate were studied according to (Ibrahim and Al-jebory, 2020; AL-saeedi *et al.*, 2023; Al-Jebory *et al.*, 2024).

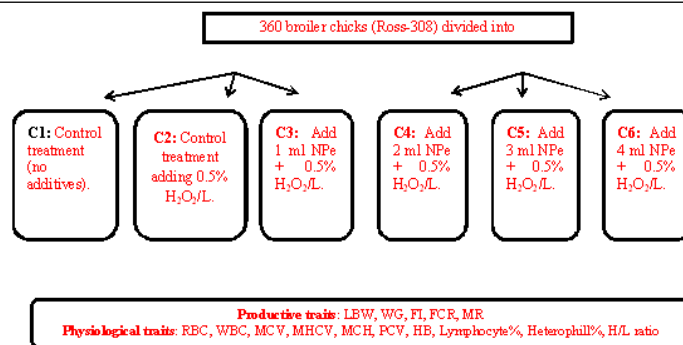


Figure 4: Second experiment.

Physiological traits RBC, WBC, MCV, MCHC, MCH, PCV, HB, Lymphocyte, Heterophil, H/L ratio according to (Salman *et al.*, 2024 a,b; Al-Saeedi *et al.*, 2024; Ajafar *et al.*, 2024a,b).

Data was analyzed using (CRD) Duncan's (1955) The SAS (2012).

RESULTS AND DISCUSSIONS

EFFECT OF NANO-PEPTIDES IN BACTERIA

Table 1 shows the effect of the synthesized peptides as antibacterial peptides and their ability to affect *E. coli* and *Salmonella* bacteria. A positive effect was observed for all concentrations studied (50, 100, and 150 microliters).

Table 1: Effect of nanopeptides of methionine, tryptophan, and iron on *E. coli* and *Salmonella* bacteria in vitro.

Concentration	<i>E. coli</i>	<i>Salmonella</i>
50 μm	+	+
100 μm	+	+
150 μm	+	+

Note: +: positive impact.

Table 2: Effect of synthesized nano-peptides on bacterial inhibition zones in *E. coli* and *Salmonella* cultures (mm for inhibition zone).

Concentration	<i>E. coli</i>	<i>Salmonella</i>
50 μm	2.33± 1.20 b	3.00± 0.57 b
100 μm	4.73± 0.37 a	5.33± 1.76 a
150 μm	4.67± 0.88 a	8.00± 2.08 a
Significant	*	*

Note: *($P < 0.05$).

Regarding the diameter of bacterial growth inhibition in cultured dishes (Table 2), a significant ($P \leq 0.05$) superiority was observed for the 100 and 150 microliter concentrations compared to the 50 microliter concentration for *E. coli* and *Salmonella* bacteria.

The significant effect of the peptides synthesized in *E. coli*

and Salmonella bacteria in the laboratory may be due to the antibacterial properties of iron nanoparticles. Studies have shown that the antioxidant properties of iron nanoparticles are the same as those that give them antibacterial properties against gram-negative bacteria, nanocapsules are nano-carrier materials with molecular dimensions ranging from 1-100 nm and are considered as encapsulating and attracting materials for encapsulating hydrophobic drugs. The active drug molecule is encapsulated inside the carrier or nano-spheres where the drug molecules are absorbed and activated. These are in the form of polymeric nanocapsules (polymers) that allow the materials loaded inside them to be slowly decomposed, thus allowing active and effective compounds to remain for a longer period (Mishra *et al.*, 2010). Iron nanoparticles attract free radicals and enter the gram-negative bacteria. The free radicals then destroy the bacteria (Aisida *et al.*, 2020). This is due to the type of bacterial membrane of gram-negative bacteria, which contains a layer of phospholipids, thus allowing these compounds to enter the bacterial cell (Touati, 2000; Taylor and Webster, 2009). The small size of iron nanoparticles also allows them to penetrate the bacterial cell membrane, thus halting the functions cell membrane (Ezealigoa *et al.*, 2021). Lee *et al.* (2008) indicated that iron nanoparticles enter E. coli bacteria by diffusion. Iron ions are released, which damage E. coli bacteria by destroying the cell membrane. Furthermore, the method of manufacturing nano-iron by linking it to proteins (amino acids) or encapsulating it with polymers reduces its toxicity to living cells and increases its antibacterial properties (Aisida *et al.*, 2019). Linking nano-iron with methionine enhances its antibacterial and antioxidant peptide properties, as amino acids act as a mediator for green synthesis nano-iron to produce peptides that are antibacterial and less toxic to living cells (Yeganeh *et al.*, 2020). The way nanopeptides interact with microbial membranes is either by destroying the functions of the bacterial cell membrane, directly killing the bacteria, or supporting the body's immunity against pathogens due to the amino acids they contain that support the immune system, or by shrinking or exploding the bacterial cell, or damaging the nuclear material and stopping bacterial reproduction (Raheem and Straus, 2019; Seyfi *et al.*, 2020). Moreover, the antioxidant capacity of nanopeptides also depends on their amino acid components and their ability to stimulate the antioxidant system (Fadaka *et al.*, 2021).

PRODUCTIVE TRAITS

LIVE BODY WEIGHT: Table 3 compares the impact of nanopeptide levels on the average live body weight of broiler chickens. Treatment C6 outperformed treatment C3 in the third week ($P \leq 0.05$), while treatments C1, C2, C4, C5, and C6 did not differ significantly in the initial weight or first and second weeks. In the fourth week, treatments C1 and C4 increased significantly ($P \leq 0.05$) compared with treatment C3, and no significant difference was found

between treatments C1, C2, C4, C5, and C6. In the fifth week, a highly significant superiority ($P \leq 0.01$) was shown for treatment C6 compared with treatments C1, C2, C3, and C5. Treatments C4 and C5 also outperformed treatments C1, C2, and C3. Treatments C1 and C3 also increased significantly over treatment C2 (Figure 5).

Table 3: Growth performance trends in broiler chickens under different dietary treatments.

Treat-ments	0	1	2	3	4	5
C1	42.41 ±1.08	184.41 ±7.29	464.75 ±27.02	999.75 ±8.46ab	1743.33 ±12.02 a	2154.67 ±4.48C
C2	43.17 ±0.33	185.67 ±7.89	452.83 ±12.91	987.64± 16.91ab	1681.67 ±10.13ab	2102.33 ±7.33d
C3	42.75 ±0.63	184.91 ±9.39	425.50 ±7.76	908.83± 14.93 b	1602.67 ±19.32b	2158.67 ±14.89c
C4	42.75 ±0.52	182.25 ±3.39	468.33 ±25.54	1005.41 ±10.01ab	1747.33 ±18.97a	2278.00 ±14.01ab
C5	41.33 ±0.68	187.17 ±2.62	442.83 ±22.61	981.58± 42.20ab	1690.66 ±15.01ab	2216.67 ±13.33b
C6	42.08 ±1.10	177.67 ±4.84	427.25 ±27.79	1066.50 ±14.59a	1732.33 ±13.99ab	2281.67 ±14.19a
Significant	N.S	N.S	N.S	*	*	**

Note: * ($P < 0.05$), ** ($P < 0.01$), N.S: not significant.

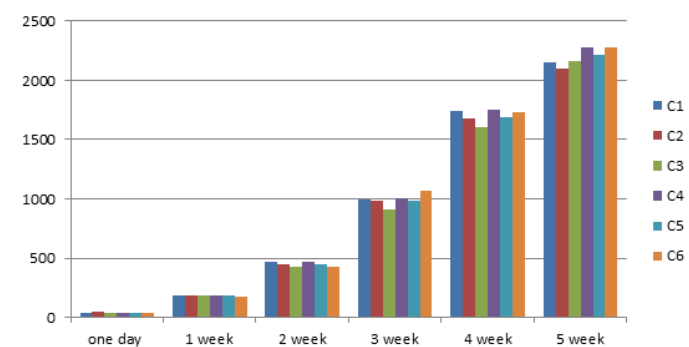


Figure 5: Weekly live body weight variations in broiler chickens.

WEIGHT GAIN: Table 4 depicts the influence of nanopeptides on the weekly weight increase rate. There were no significant differences between the experimental treatments in the first, second, third, and fourth weeks. In the fifth week, treatments C3, C4, C5, and C6 showed a significant increase ($P \leq 0.05$) compared to C1 and C2. C4 and C6 had considerably greater overall rates ($P \leq 0.01$) than the other treatments. Treatment C5 also outperformed treatment C2, with no significant difference between treatments C1, C2, and C3 (Figure 6).

FEED INTAKE: The impact of the investigated treatments on the average weekly feed weight consumed is displayed in Table 5. It is observed that, in comparison to treatments C4 and C6, there was a significant increase ($P \leq 0.05$)

for treatment C2 during the first week. Additionally, treatment C5 performed better than treatment C6. In the second, third, and fifth weeks, there was no significant difference between the treatments under study and the average amount of feed consumed overall. However, when comparing treatment C1 to treatment C3, there was a significant increase ($P \leq 0.05$) in the fourth week. Treatment C1 did not differ significantly from treatments C2, C4, C5, and C6.

Table 4: Effect of nano-peptides of methionine, tryptophan, and iron on the weekly WG of broiler chickens exposed to oxidative stress.

Treatments	1	2	3	4	5	Total
C1	142.00 ±8.31	280.34 ±22.41	535.00 ±34.46	743.58 ±3.79	411.34± 12.91 b	2112.26 ±3.45bc
C2	142.50 ±8.21	267.16 ±20.78	534.81 ±27.20	694.03 ±14.65	420.66± 4.70 b	2059.10 ±10.66 c
C3	142.16 ±8.95	240.59 ±15.66	483.33 ±21.01	693.84 ±11.63	556.00± 58.02 a	2115.92 ±14.31bc
C4	139.50 ±3.04	286.08 ±27.82	537.08 ±13.47	741.92 ±9.82	530.67± 17.88 a	2235.26 ±24.30a
C5	145.84 ±2.02	255.66 ±22.68	538.75 ±64.78	709.08 ±4.35	526.01± 11.08 a	2175.34 ±12.72 b
C6	135.59 ±4.33	249.58 ±27.84	589.25 ±19.10	715.83 ±9.83	549.34± 17.02 a	2239.59 ±14.54a
Significant	N.S	N.S	N.S	N.S	*	**

Note: *($P < 0.05$), ** ($P < 0.01$), N.S: not significant.

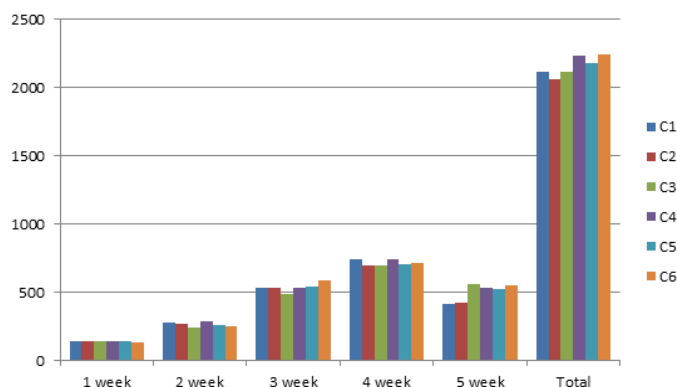


Figure 6: Cumulative weight gain across dietary treatments.

FEED CONVERSION RATIO: The impact of adding varying concentrations of nano-peptides to the investigated therapies is displayed in Table 6. Treatments C1, C3, C4, C5, and C6 showed a substantial improvement ($P \leq 0.05$) over treatment C2 during the first week. There was no significant difference between treatments C1, C3, C4, C5, and C6. However, there was no significant difference between the examined treatments in the second, third, or fourth weeks. When compared to treatments C1 and C2, treatments C3, C4, C5, and C6 showed a substantial improvement ($P \leq 0.05$) in the fifth week. Compared to treatments C1, C2, and C3, treatments C4 and C6 showed

a substantial improvement ($P \leq 0.05$) in the overall rate of the feed conversion factor. In comparison to treatment C2, treatment C5 also showed improvement.

Table 5: Feed intake comparison among treatment groups across the study period.

Treatments	1	2	3	4	5	Total
C1	147.08 ±4.69abc	386.50 ± 5.39	718.00 ± 15.64	1009.75 ± 5.87 a	1210.90 ± 26.43	3472.23 ± 74.62
C2	157.75 ±3.90 a	385.67 ± 8.10	718.33 ± 11.57	978.00± 9.95 ab	1203.82 ± 37.32	3443.57 ± 64.57
C3	150.58± 4.20 abc	378.50 ± 4.12	695.75 ± 14.99	948.09± 6.48 b	1243.14 ± 42.31	3416.07 ± 41.22
C4	144.25± 1.89 bc	380.91 ± 11.96	710.09 ± 2.85	995.44± 12.51ab	1164.44 ± 29.39	3395.14 ± 41.21
C5	154.25± 4.99 ab	385.16 ± 6.92	696.83 ± 8.06	985.15± 15.64ab	1203.73 ± 29.41	3445.13 ± 18.75
C6	140.41± 1.08 c	384.02 ± 2.76	696.92 ± 19.35	993.18 ±1.08ab	1193.61 ± 36.69	3408.16 ± 45.01
Signifi- cant	*	N.S	N.S	*	N.S	N.S

Note: *($P < 0.05$), N.S: not significant.

Table 6: Changes in feed conversion ratio (FCR) across experimental treatments.

Treatments	1	2	3	4	5	Total
C1	1.035 ±0.12b	1.378± 0.01	1.342± 0.07	1.357 ±0.11	2.943± 0.04a	1.643± 0.03ab
C2	1.107± 0.11a	1.443± 0.10	1.343± 0.03	1.409± 0.14	2.861± 0.03b	1.672± 0.04a
C3	1.059± 0.29b	1.573± 0.10	1.439± 0.07	1.366± 0.08	2.235 ±0.03c	1.614 ±0.05b
C4	1.036± 0.189b	1.331± 0.02	1.322± 0.03	1.341 ±0.13	2.194± 0.01c	1.518 ±0.02c
C5	1.057± 0.15b	1.506± 0.13	1.293± 0.19	1.389± 0.13	2.288± 0.03c	1.583± 0.02bc
C6	1.035± 0.15b	1.538± 0.09	1.182± 0.03	1.387± 0.19	2.172± 0.04c	1.521 ±0.01c
Significant	*	N.S	N.S	N.S	*	*

Note: *($P < 0.05$), N.S: not significant.

MORTALITY: Table 7 shows that the overall mortality rate for each therapy did not significantly differ during the course of the investigation.

Polymeric nanocapsules (polymers) are chemically linked fragments of hydrophobic and hydrophilic polymer units to generate amphoteric molecules (Warriner *et al.*, 1996). Vesicles formed from polymers are commonly referred to as polymersomes and are among the best characterized polymeric vesicles. By controlling the formula weight of the blocks, polymers with different properties are created, including those that vary in elasticity, permeability, and

mechanical stability. By their nature, polymeric building blocks allow for greater lipid uptake (Meng *et al.*, 2009). Due to their higher molecular weights, polymers are thicker, stiffer, and more stable than other nanocapsules. Polymeric membranes have been shown to withstand an expansion upon digestion in the gastrointestinal tract of up to 40–50% compared to 5% or less for lipid membranes (Needham and Zhelev, 2000). In addition, polymers can be designed so that it responds to environmental factors during digestion such as pH, temperature, redox potential, magnetic field, light and ultrasound (Meng *et al.*, 2009). The addition of nanopeptides (containing methionine, tryptophan, and iron) to broiler chickens' drinking water may have contributed to the improvement in production performance of the treatments with nanopeptides, particularly treatments C4 and C6. This is because methionine plays a crucial role in improving the effectiveness of nutrient utilization in addition to its primary function of balancing amino acids in the feed (Bunchasak, 2009). In addition to methionine's role in the production of hormones and enzymes, this amino acid balance is demonstrated by improved muscle mass and body growth (Lee *et al.*, 2023).

Table 7: Effect of nanopeptides of methionine, tryptophan, and iron on the total mortality rate of broiler chickens exposed to oxidative stress.

Treatments	Mortality ratio %
C1	0.133±0.03
C2	0.117±0.02
C3	0.100±0.05
C4	0.117±0.06
C5	0.067±0.03
C6	0.050±0.00
Significant	N.S

Note: N.S: not significant.

Additionally, El-Shobokshy *et al.* (2022) found that nano-methionine increased the gene expression of related to the hormone tryptophan, which plays a major role in protein synthesis, enhances the antioxidant system by entering into the production of melatonin, and enhances feed intake by entering into the production of serotonin. These findings suggest that nanomethionine may have a higher ability to stimulate insulin-like growth factor-1 (IGF-1) and growth hormone, which work to stimulate growth and may improve the growth performance of birds. Tryptophan also plays a role in the production of niacin in the body, which supports growth and metabolism and enhances digestive health (Le Floch *et al.*, 2011). Tryptophan is considered an essential amino acid in regulating metabolic processes and transmitting nerve signals (Michael *et al.*, 2009). Moreover, tryptophan is involved in regulating humoral immunity in birds and increasing the level of immunoglobulins, which

is positively reflected in the growth of treated birds (Wei *et al.*, 2011). Tryptophan may improve body growth and feed conversion ratio because it is closely linked to maintaining insulin secretion from pancreatic beta cells in animals (Kim *et al.*, 2014; Mund *et al.*, 2020). Thus, insulin is a growth hormone that increases the body's growth rate by increasing metabolism. Stress is closely related to the production performance of broiler chickens. Tryptophan is a major precursor to serotonin, which has an important role in modifying biological functions in the body to reduce stress (Bai *et al.*, 2017). Tryptophan has the ability to reduce oxidative stress and enhance antioxidants in broiler chickens (Mund *et al.*, 2020). Liu *et al.* (2015) reported that tryptophan increased the levels of glutathione peroxidase and catalase in serum, liver, and breast muscles. Patil *et al.* (2013) showed that tryptophan is a precursor to serotonin and melatonin, which prevent oxidative damage in broiler chickens and improve the enzymatic activity of catalase and SOD. Studies have indicated that a 1.5% increase in tryptophan levels over the recommended level can reduce oxidative stress and improve the efficiency of utilization of Feeds in broiler chickens under stress conditions (Wang *et al.*, 2014). Tryptophan also increases the activity of the paraoxonase-1 (PON1) gene, which has a major role in protecting lipoproteins from oxidation in the blood and tissues (De la Iglesia *et al.*, 2014). The improvement is also attributed to the role of nanoiron, which increases the metabolism of amino acids and increases the level of arginine in the liver (Miroshnikov *et al.*, 2017). This may be reflected in the growth of birds. Iron is also important in the oxidation and reduction processes in many enzymes (Andreini *et al.*, 2008). It also participates in several different physiological reactions. It is necessary in the synthesis of hemoglobin, myoglobin, and red blood cells (Andrews *et al.*, 2003). It has multiple roles in the Krebs cycle and facilitates the removal of harmful free radicals by assisting enzymes such as catalase (Nikonov *et al.*, 2011). The green preparation of nanoiron using amino acids makes it less toxic and more available to the bird, as it is absorbed in its organic form through the intestine more than free iron in the intestinal lumen (Saif *et al.*, 2016). Moreover, the nano-encapsulation of peptides or acids Amino acids are considered an optimal method due to the slower absorption of amino acids (Collins, 2021). The improvement in feed conversion factor may be due to the role of nano-encapsulation, which allows the nano-encapsulated active compounds (methionine, tryptophan, and iron) to remain for a longer period and improve their absorption (Needham and Zhelev, 2000). This may make nano-encapsulation more beneficial than synthetic peptides, thus reflecting on the growth performance of birds. Alternatively, the improvement in the productive performance of birds in supplementation treatments may be due to the antibacterial role of synthetic peptides, which may improve the health of birds (first experiment, Tables

1 and 2), which is reflected in growth performance. The decreased productive performance of the control treatment C2 may be due to exposure to oxidative stress, which in turn causes lipid oxidation, oxidation, and disruption of the regulation of neuronal signal transmission, protein, and DNA molecule degradation within cells (Surai *et al.*, 2019). Therefore, oxidative stress is one of the main factors that negatively affect productive performance (Oke *et al.*, 2014; Vandana and Sejan, 2018). Although there are no statistically significant differences in the mortality rate, the resulting rate may be due to oxidative stress and its harmful effects (Hamzah Merzah and Abdul-Lateef, 2022).

Table 8: Hematological parameters response to nano-peptide supplementation.

	RBC	PCV	Hb	MCH	MCV	MCHC	WBC
C1	3.95 ±0.45	27.50± 1.50ab	9.16 ±0.50b	70.97 ±11.88	23.64 ±3.96	33.30± 0.01	32.50 ±0.50
C2	4.07± 0.63	00.26± 4.00b	8.66± 1.33b	63.88± 0.06	21.28 ±0.02	33.32± 0.01	29.50± 0.50
C3	4.57 ±0.37	30.50± 0.50ab	10.16± 0.17ab	67.02 ±4.40	22.33 ±1.47	33.32± 0.01	31.50± 0.50
C4	4.05± 0.53	31.00± 2.00ab	10.67 ±0.67ab	81.07± 12.47	27.01 ±4.15	33.32± 0.01	32.00± 1.00
C5	3.95 ±0.86	34.00± 2.00a	11.33± 0.67a	86.06± 16.20	28.68 ±5.41	33.32 ±0.01	32.00± 0.04
C6	4.39± 0.45	33.50± 1.50ab	11.16± 0.50ab	76.68 ±4.52	25.54 ±1.51	33.31± 0.07	32.50± 3.50
Signif- icant	N.S	*	*	N.S	N.S	N.S	N.S

Note: *($P < 0.05$), N.S: not significant.

BLOOD PARAMETERS: The impact of the investigated therapies on a few physical traits of broiler chickens subjected to oxidative stress is displayed in Table 8. It was discovered that the research treatments did not significantly differ in RBC, MCH, MCV, MCHC, or WBC. Regarding the PCV, there was no significant difference between treatments C1, C3, C4, and C6, and treatment C5 was much better than treatment C2 ($P \leq 0.05$). There was no significant difference in hemoglobin concentration between treatments C1, C2, C3, C5, and C6, however treatment C5 showed a substantial increase ($P \leq 0.05$) in comparison to treatments C1 and C2.

There was no significant difference between treatments C1, C3, C4, C5, and C6, and the percentage of heterophil (Table 9) increased significantly ($P \leq 0.05$) in treatment C2 compared with treatments C4, C5, and C6, while the percentage of lymphocytes increased significantly ($P \leq 0.05$) for treatments C4 and C6 compared with treatment C2, and no significant difference was observed between treatments C1, C3, C4, C5, and C6. There was no significant difference between treatments C1, C3, C5, and C6, or between

treatments C4, C5, and C6, but the percentage of H/L in the blood of birds in treatment C2 increased significantly ($P \leq 0.01$) when compared to the other treatments, and this percentage also increased in treatments C1 and C2 when compared with treatment C4.

Table 9: Immunological markers in broilers fed different experimental diets.

	Lymphocytes %	Heterophil %	H/L %
C1	60.50±0.50ab	25.00±1.00ab	0.410±0.01b
C2	56.50±1.50b	29.50±0.50a	0.515±0.01a
C3	61.50±1.50ab	24.50±2.50ab	0.390±0.03b
C4	65.50±0.50a	20.00±2.00b	0.300±0.03c
C5	62.00±2.00ab	23.50±0.50b	0.375±0.01bc
C6	63.00±3.00a	22.50±1.50b	0.355±0.04bc
Significant	*	*	**

Note: *($P < 0.05$), ** ($P < 0.01$).

The significant improvement in hemoglobin and hemoglobin concentration in the blood of birds treated with C5 compared to C2 may be due to the role of iron, which is involved in the formation of Hb and stimulates the formation of red blood cells (erythropoiesis) (Adamson, 1996). Blood cells are also susceptible to oxidative stress caused by free radicals, especially red blood cells, due to the thin cell membrane that transports oxygen inside the body (Oke *et al.*, 2024). This explains the decrease in hemoglobin and hemoglobin in treatment C2, which is a result of the exposure of birds in that treatment to oxidative stress. The reason for the improvement in the addition treatments of nanopeptides, especially treatments C5 and C6, in the percentage of lymphocytes and heterophil cells and their ratio may be due to the role of tryptophan in reducing the effect of oxidative stress through its role in regulating the secretion of corticosterone, cortisol, heat shock protein HSP 70, and serotonin (Bello *et al.*, 2018; Wen *et al.*, 2019) which has a fundamental role in the occurrence of stress in poultry, as the increase in the hormone corticosterone during stress reduces the percentage of lymphocytes due to its receptor on the surface of lymphocytes, causing their decomposition (Kazim *et al.*, 2002), which increases the H/L ratio, which is considered a physiological indicator of the occurrence of stress (Kassab *et al.*, 1992). The improvement in blood cellular characteristics may also be due to the addition of methionine's antioxidant role, as methionine protects cells from the harmful effects of free radicals through two pathways: the first is by indirectly producing antioxidants (cysteine and glutathione precursors), and the second is by entering into protein formation and protecting proteins from damage by free radicals (Santana *et al.*, 2021). Methionine enters into the formation of methyl sulfoxide reductases, which are enzymes that work to reduce reactive oxygen species and protect cells. From the harmful action

of oxidation, thioredoxin plays a major role in these anti-oxidant pathways (Luo and Levine, 2009; Del Vesco *et al.*, 2015; Al-Saeedi *et al.*, 2021).

CONCLUSIONS AND RECOMMENDATIONS

It is concluded from the results of the two experiments that the peptides manufactured from methionine, tryptophan and iron and nano-encapsulated have proven their effectiveness against bacteria in the laboratory and in improving the productive performance of broiler chickens under conditions of oxidative stress. We recommend, based on the study results, the possibility of applying the use of nano-peptides or oxidative stress supplements to other types of poultry.

ACKNOWLEDGEMENTS

The authors would like to thank Al-Anwar Poultry Company and Department of Animal Production, College of Agriculture, Al-Qasim Green University.

NOVELTY STATEMENT

The novelty in this study is the manufactured nano peptide from methionine, tryptophan, and iron and nano-encapsulated, and used in broiler.

AUTHOR'S CONTRIBUTIONS

Nareman Badri Arkan: Contributed to the data collection, data analysis and manuscript preparation.

Hashim Hadi Al-Jebory: Had the role of designing and generating the concept, supervising, monitoring and controlling the research, data analysis and interpretation, and manuscript preparation.

Fadhil Rasool Abbas Al-Khafaji: Contributed to supervise the research.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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