Effect of Nano Methionine- zinc in Productive Performance of Broiler Exposed to Heat Stress

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Abstract

Nano amino acids manufactured by green synthesis are a promising method in the poultry industry to achieve more significant benefits from minerals and amino acids. From 25-7-2023 to 29-8-2023, 300-day-old (Ross 308) broiler chicks were allocated into five random treatments, each being replicated three replicate with 15 chicks in each replication. Methionine-zinc nanoparticles were supplemented to the diet at 0.0, 1, 2, 3, and 4 mg/kg for treatments T1 (control treatment), T2, T3, T4, and T5, respectively, all treatments exposed to heat stress of (35±1 ºC) during the experiment period. Our findings showed that the treatments T2 and T5 had significant ($P<0.05$) increases in live body weight, well the T3, T4, and T5 treatments had a significant advantage in total weight gain, in feed intake, the T5 treatment was a more significant significantly in a fifth week overall, as well significant ($P<0.05$) improvement for T2, T3, T4, and T5 treatments in total feed conversation ratio, packed cell volume (PCV), and H/L ratio compared to T1 treatment, at the same time, the control treatment had the highest percentage of mortality and heterophil cell, significant ($P<0.05$) increase for T3 treatment in lymphocyte cell. It is concluded that nano methionine-zinc improved broilers' productive and physiological performance under heat-stress conditions.

Keywords: Green synthesis, Heat stress, Methionine, Nano amino acid, zinc.

Introduction

Heat stress causes substantial economic losses in broiler production (1), as it causes a reduction in the production rate of birds and an imbalance in their physiological performance. This rate depends on the length of the period and intensity of exposure to heat stress (2). It also causes many physiological changes related to the immune system, oxidative stress, intestinal integrity, and many other disorders, culminating in decreased bird growth performance and high mortality rates (3). It has also been proven that heat stress can affect intestinal integrity due to a deficiency of oxygenation of the intestinal epithelium (4). Poor epithelial integrity during heat stress can cause intestinal inflammation, increased permeability to pathogens, and an imbalance in the gut microbial community (5), reflected in the digestibility of feed materials consumed and thus reduced availability of nutrients to the bird. (6, 7). Nanotechnology is a new technology in scientific research, as material particles whose size ranges from 1 to 100 nanometers are transformed, which increases the surface area, which increases their chemical interactions; these characteristics make nanomaterials and elements highly efficient in transport and absorption (8). Studies have indicated that adding Methionine to the diet of broilers can alleviate the effects of stress by working to increase liver activity to produce antioxidant compounds (9). It has also been found that Methionine has a role in protecting membrane cells from stress damage because, in addition to being involved in the synthesis of glutathione peroxidase, Methionine, along with the compound thioredoxin, also has a direct effect on the scavenging of reactive oxygen species (ROS), high environmental temperature reduces feed intake to regulate body temperature and reduces subsequent weight gain, digestibility, and feed efficiency (10). Diets with an amino acid imbalance or methionine deficiency increase heat production. When the environmental temperature is high, the body has a more negative effect of heat stress, nano zinc is considered necessary in cell proliferation, increasing growth, improving fertility, immunity, and gene expression, and is an essential part of the activity of many enzymes.

It participates directly in metabolic pathways and is one of the essential components of cellular defense against oxidative stress as an essential part of the superoxide dismutase enzyme, zinc positively affects the utilization of food through its participation in the metabolism of carbohydrates, proteins, and fats (11) . As a result of the above, after converting the amino acid methionine into nanoparticles and loading it on nano-zinc, the current study aims to evaluate the effectiveness of nano-zinc-methionine as a feed additive when raising broilers under heat stress. Studying the effect of nano-zinc-methionine on improving the productive and physiological performance of broilers raised under heat-stress conditions determines the best level of addition that can be recommended.

Materials and methods

Sampling

This research was conducted in the Al-Anwar company farm and Department of Animal Production - Faculty of Agriculture / Al Qasim Green University's poultry laboratory.

The chicks were fed (table 1) the starter ration (the crud protein was 23.04% and the metabolic energy was 3027.1 kilocalories/kg of feed) from one day old until the third week of the birds' life, after which it was replaced with the growth diet (the crud protein was 20.06% and the metabolic energy was 923195.3 kilocalories/kg of feed), until the end of the fifth week, feed and water were provided ad libitum.

Nano methionine-zinc was prepared in the Iraqi Ministry of Science and Technology laboratories according to the method (12).

All tests were also conducted at the Ministry of Science and Technology. When examining a sample of nano-zinc with acid methionine, the results of the SEM analysis showed spherical-shaped particles within a nanometer size ranging between (23.89, 30.34, 41.79).

Three hundred one-day-old unsexed broiler chicks (Ross 308) were randomly divided into five treatments of 60 chicks. Each treatment was divided into three replicates with 20 chicks per each one. Birds were reared in a 1x1.5 m cage under similar management conditions until the end of the study (35 days of age).

Experimental design

Methionine-zinc nanoparticles were added to the experimental treatments' diet at concentrations of 0.0, 1, 2, 3, and 4 mg/kg for treatments T1 (control group), T2, T3, T4, and T5, respectively. Diets (The composition and nutrient content) were designed to meet the nutrient requirements for the birds during stages of growth (starter, grower, and finisher). For the first three days, the temperature was kept at 32°C.

Table 1. Shows the percentages of diet ingredients used in the study and their chemical composition

Ingredients	Starter diet % 1-21 day	Finisher diet % 22-35 day
Yalow corn	30	40
White	28.25	24
Soybean meal (48% protein)	31.75	24.8
Protein concentration*	5	5
Sun flower oil	2.9	4.4
Limestone	0.9	0.6
DCP	0.7	0.9
Salt	0.3	0.1
vitamins and minerals mixture	0.2	0.2
Total	100	100
Crud protein %	23.04	20.06
Metabolic energy (kcal/ kg)	3027.1	3194.92
Lysine %	1.16	1.07
Methionine %	0.41	0.38
Cysteine %	0.36	0.32
Methionine + cysteine %	0.81	0.71
Available phosphor %	0.47	0.48
C/P %	131.14	159.77

*BROCON-5 SPECIAL W protein concentrate: Chinese origin. Each kg contains: 40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorus, 3.25% lysine, 3.90% methionine + cysteine , 2.2% sodium, 2100 kilocalories/kg representative energy, 20,000 IU Vitamin A, 40,000 IU Vitamin D3, 500 mg Vitamin E, 30 mg Vitamin K3, 15 mg Vitamin B1+B2, 150 mg B3, 20 mg B6, 300 B12 mg, 10 mg folic acid, 100 mcg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidants (BHT).

Performance Traits Measurement

Live body weight and weight gain

The body weight, weight gain, feed intake, and feed conversation ratio were calculated according to (13).

Mortality %

The mortality was indicated throughout the whole study and calculated as follows.

Total mortality percent = (The number of mortality birds during the experiment period)/(the total number of birds) X100

Physiological traits

The volume of packed blood cells (PCV) and the number of red and white blood cells (RBC & WBS) were calculated by taking blood samples in tubes containing an anticoagulant that included one male and one female from each replicate at the age of 14 and 35 days from the wing vein. The PCV was calculated according to the method of Archer (15) and the number of (RBC & WBS) according to Pierson (16). Heterophil (H) cells and lymphocyte (L) cells were counted by taking blood smears on glass slides and stained with Giemsa-Wright stains according to the method (17), then examined under a microscope as stated in Campbell (18), and the (H/L) ratio was extracted by dividing Heterophil (H) cells on (L) lymphocytes ×100.

Statistical analysis

The Statistical Analysis System -SAS (19) was used in data analysis with Completely Randomized Design (CRD), and the mean differences between the averages were compared to the Duncan (20) polynomial test.

Results and discussion

Table 2 shows the effect of the study on live body weight, with no significant difference in initial weight in the first, second, and third weeks. In contrast, in the fourth week, there was a significant (P<0.05) increase for T4 treatment compared to T1 and T5 treatments, and in the fifth week, there was a significant (P<0.05) increase for

Table 2. Effect of nano methionine- zinc in live body weight of broiler exposed to heat stress

Treatments	Mean± standard error					
	Initial weight	First week	Second week	Third week	Fourth week	Fifth week
T1	40.41± 1.12	179.67± 3.90	507.50± 6.11	982.50± 33.63	1576.17 ±33.79 b	2210.00± 58.20 b
T2	39.58± 1.36	179.50± 5.02	522.97± 12.31	1010.50± 26.75	1624.17± 19.83 ab	2390.67± 29.46 a
T3	39.58 ±1.21	183.00± 0.90	525.08 ±2.08	1002.17± 20.93	1659.83± 36.47 ab	2344.00± 56.62 ab
T4	40.00 ±1.04	184.50± 1.42	538.00± 9.09	1008.83 ±34.74	1708.17± 68.48 a	2366.33± 31.63 ab
T5	41.00± 0.62	185.83± 6.28	526.50± 8.02	970.00 ±6.82	1568.83± 13.37 b	2423.00± 63.21 a
Significant	N.S	N.S	N.S	N.S	*	*

Means with different letters differ significantly with * (P<0.05), and NS: insignificant.
Treatments: T1 is a control treatment (without addition); T2, T3, T4, and T5 add 1, 2, 3, and 4 mg nano methionine-zinc/kg feed.

T2 and T5 treatments compared to T1 treatment.

Table 3: shows the effect of the experiment's weekly weight gain, noted no significant difference in the first, third and fourth weeks, as well as in second week, with a significant (P<0.01) increase for T4 compared to other treatments and a significant increase for T2 treatment compared T1 treatment, in fifth week the T5 treatment had a significant superiority level (P<0.05) above the T1 and T4 treatments, while in total weekly weight gain noting a significant superiority (P<0.05) for T2, T4, and T5 treatments over T1 treatment.

The effect of feeding nano methionine- -zinc in the feed intake of chicks is shown in Table (4) with no significant differences between treatments in the first, second, third, or fourth weeks and total feed intake, as well in the fifth week significant (P<0.01) increase for T5 treatment compared other treatments.

Table (5) shows the effect of combining nano methionine- zinc on feed conversion ratio. In the first and fourth weeks, there was no significant

Table 3. Effect of nano methionine- zinc in weight gain of broiler exposed to heat stress

Treatments	Mean± standard error					
	First week	Second week	Third week	Fourth week	Fifth week	Total
T1	139.25± 2.78	317.83± 2.29 c	475.00± 27.88	593.67 ±58.19	633.83± 8.67 b	2169.58± 5.08 b
T2	139.91± 3.82	343.47 ±7.71 b	487.52± 37.20	613.67± 34.34	766.50± 4.53 ab	2351.08± 8.81 a
T3	143.41± 0.44	342.08± 1.21 bc	477.08± 19.37	657.66± 35.08	684.16± 6.59 ab	2304.42± 5.41 ab
T4	144.50± 2.37	365.50± 4.61 a	470.83± 42.73	699.33± 34.06	658.17± 4.87 b	2338.33± 6.91 a
T5	144.83± 5.70	340.50 ±2.78 bc	443.50± 1.32	598.83 ±7.72	854.16± 6.53 a	2381.83± 6.81 a
Significant	N.S	**	NS	N.S	*	*

Means with different letters differ significantly with ** (P<0.01) * (P<0.05), NS: not significant. Treatments: T1 is a control treatment (without addition); T2, T3, T4, and T5 add 1, 2, 3, and 4 mg nano methionine-zinc/kg feed.

Table 4. Effect of nano methionine- zinc in feed intake of broiler exposed to heat stress

Treatments	Mean± standard error					
	First	Second	Third	Fourth	Fifth week	Total

	week	week	week	week		
T1	138.58 ±2.47	380.00± 10.41	720.00± 10.00	913.75± 30.31	1174.16± 5.93 b	3326.49± 7.20 b
T2	132.08 ±5.75	365.00± 2.88	718.42± 10.87	935.12 ±8.81	1133.94± 8.81 b	3284.56± 5.74 c
T3	130.25 ±6.33	371.67 ±6.01	728.33± 7.26	956.08± 32.58	1152.02± 2.21 b	3338.35± 5.40 b
T4	135.00 ±3.36	385.83± 10.80	720.96± 26.32	921.70± 57.76	1126.12± 6.44 b	3289.61± 8.93 c
T5	137.33 ± 3.85	370.00± 10.00	731.67± 10.92	912.11± 35.83	1255.58± 5.83 a	3406.69± 4.36 a
Significant	N.S	N.S	N.S	N.S	*	N.S

Means with different letters differ significantly with * (P<0.05), and NS: insignificant. Treatments: T1 is a control treatment (without addition); T2, T3, T4, and T5 add 1, 2, 3, and 4 mg nano methionine-zinc/kg feed.

difference between study treatments; at the same time, T2, T4, and G5 treatments improved significantly (P<0.01) compared to T1 treatment in the second week, while in the third week, significant improvement (P<0.05) for T1, T2, T3, and T4 treatments compared T5 treatment, as well significant improvement (P<0.05) for T2, and T5 treatments compared T1 treatment. In total feed conversation ratio, the T2, T3, T4, and T5 treatments had a significant improvement level (P<0.05) above the T1 treatment.

Table 5. Effect of nano methionine- zinc in feed conversion ratio of broiler exposed to heat stress

Treatments	Mean± standard error					
	First week	Second week	Third week	Fourth week	Fifth week	Total
T1	0.998± 0.008	1.158± 0.024 a	1.515± 0.060 b	1.560± 0.120	1.852± 0.279 a	1.533± 0.064 a
T2	0.943± 0.019	1.062± 0.018 b	1.501 ±0.030 b	1.519 ±0.047	1.479± 0.031 b	1.397± 0.018 b
T3	0.908 ±0.046	1.086± 0.015 ab	1.526± 0.014 b	1.464 ±0.114	1.683± 0.046 ab	1.450± 0.004 b
T4	0.934 ±0.017	0.982 ±0.040 c	1.531± 0.069 b	1.315± 0.028	1.710± 0.038 ab	1.406± 0.020 b
T5	0.969 ±0.047	1.057± 0.008 b	1.649 ±0.019 a	1.466± 0.013	1.469± 0.071 b	1.430± 0.023 b
Significant	NS	**	*	N.S	*	*

The effect of the experiment on total mortality, shown in Table (6), noted a significant (P<0.05) increase for T1 treatment compared to T3 and T4 treatments.

The significant improvement shown by the results of the study in the productive traits (body weight, weight gain rate, and feed conversion ratio) of the birds of the T2, T4, and T5 treatments compared with the control treatment may be due to the addition of the methionine-zinc nanocomposite to the diet and its ability to mitigate the effects of heat stress. Methionine has a significant role in improving the efficiency of the utilization of nutrients, in addition to its essential role in balancing amino acids in the diet, the balance of amino acids is reflected in the synthesis of proteins within the body and thus causes an increase in the building of muscle mass as well as the formation of enzymes and hormones, building connective tissues inside the body, and forming feathers and beaks (21). In addition, nano-methionine manufactured in a green way may have a higher ability to stimulate insulin-like growth factor-1 (IGF-1) and growth hormone, which work to stimulate growth, which may increase the growth performance of birds, as nano-methionine increases the gene expression of growth hormone and (IGF-1) (22).

The deterioration of the productive performance of control treatment birds may be due to their exposure to heat stress, as studies have indicated a decline in the productive and physiological performance of birds raised in conditions of high temperatures (23), as the process of thermoregulation resulting from chronic heat stress is also responsible for the changes, behavioral and physiological damage to various organs and tissues, such as the liver and heart tissue (24). The liver is essential for maintaining the body's metabolic balance and is an important site for producing antioxidants and, thus, poor resistance to heat stress (25). In addition to the increase in the

Table 6. Effect of nano methionine- zinc in mortality % of broiler exposed to heat stress

Treatments	Mean± standard error
	Total mortality
T1	8.33±1.67 a
T2	5.00±0.00 ab
T3	1.67±1.33 b
T4	5.00±0.00 ab
T5	3.33±1.67 b
Significant	*

Means with different letters differ significantly with * (P<0.05). Treatments: T1 is a control treatment (without addition); T2, T3, T4, and T5 add 1, 2, 3, and 4 mg nano methionine-zinc/kg feed.

level of the hormone corticosterone during stress, which causes feedback on the growth hormone, causing it to decrease, the hormone corticosterone also directs the energy sources in the body to resist stress, which causes decreased growth (26).

During heat stress, the changes occurring due to exposure to high temperatures contribute to an increase in mitochondrial activity, which causes an increase in the production of reactive oxygen species. After a short period of exposure to the stress, an increase in the activity of the electron transport chain and the production of superoxide can be observed, followed by a high production of superoxide, which leads to damage to the body's proteins. Mitochondrial dysfunction and tissue damage in the event of prolonged exposure to heat stress (27). In the normal state, enzymatic and non-enzymatic antioxidants work to eliminate radicals and ensure oxidative balance (28). Methionine, which is one of the non-enzymatic antioxidants, works directly to eliminate reactive oxygen species through a complex and coordinated enzymatic cycle involving methionine sulfoxide reductase, thioredoxin, and thioredoxin reductase, preventing oxidative damage to lipids, proteins, and nucleic acids (29). Methionine can also serve as a precursor to glutathione (30). Glutathione works mainly by removing H2O2, which prevents the free radical molecule from converting to the highly reactive hydroxyl radical. During this process, glutathione passes from its reduced form (GSH) to its oxidized form (glutathione disulfide) via the action of GPx and, thus, a concentration of GSH as an indicator of cellular redox status (31).

It was found that adding Methionine to broiler diets reduces the mortality rate, and this could be due to the antioxidant action of Methionine, which participates in the synthesis of cysteine, S-adenosylmethionine and glutathione (32) and its direct protective effect against oxidative stress (33). It was found that methionine levels increased the gene expression of the enzymes cystathionine β-synthase, glutathione synthetase, and glutathione peroxidase in broilers exposed to heat stress (2). All of these enzymes participate in the synthesis of cysteine (which is involved in the formation of the enzyme glutathione) from Methionine and in the synthesis and metabolism of glutathione (34), which shows the apparent role of Methionine in the work of the antioxidant system and thus reducing the rate of catabolism (8). The hormone corticosterone also rapidly deteriorates protein levels in the body (35), which causes direct damage to the growth and replacement of intestinal cells, thus increasing intestinal disease and decreased absorption of nutrients. At the same time, Methionine works to adjust acid-balanced amino acids and stimulate gene expression for protein synthesis, in addition to its antioxidant role, which reduces the burden of heat stress (36).

The improvement in production performance may also be due to the role of zinc, as zinc works to increase the synthesis of metallothionein, a protein rich in cysteine, which works to suppress free radicals, zinc works as an aid to antioxidants, especially the superoxide dismutase enzyme, as well as its association with enzymes that maintain the integrity of cells that participate in the immune response (37), and zinc, can replace some mineral elements such as iron (Fe) and copper (Cu) in their places of association. It combines with cellular membranes and reduces the production of free radicals. Thus, it acts as a direct antioxidant in heat stress, and zinc works to reduce the effects of stress by preventing the process of lipid peroxidation by inhibiting glutathione depletion, zinc is also considered essential for the activity of many enzymes, participates in many enzymatic activities involved in metabolic processes in the body, and participates in the metabolism of carbohydrates, fats, and proteins (11), on the basis that the process of protein digestion Fats and starch decrease when the birds are exposed to high temperatures, in addition to the decrease in the activity of the trypsin, chymotrypsin, and amylase enzymes when the temperature rises to above 32°C. Zinc also has a role in maintaining the synthesis of metalloproteins such as growth hormone, insulin-like factor (IGF-1), and insulin. This role contributes to maintaining the average growth and development of the body, as these hormones regulate glucose absorption and cellular processes (9).

Table (7) shows the effect of combining nano methionine- zinc in some physiological traits, with no significant difference in RBC and WBC cells, as well as significant ($P<0.01$) increase for T2, T3, T4, and

T5 treatments compared T1 treatment, the T1 treatments came a higher significantly (P<0.05) in heterophil cell percentage compared T2, T4, and T5 treatments, while in lymphocyte cell significant (P<0.01) increase for T3 treatment compared T1 treatment, at the same time significant (P<0.01) improvement for T2, T3, T4, and T5 treatments compared T1 treatment.

The higher percentage of PCV in the nano-methionine-zinc treatments compared to the control treatments may be because the nano-zinc Methionine reduced the effect of heat stress on the treated birds, as exposure to high temperatures leads to a state of blood thinning due to increased water intake (1), which causes a decrease in the number of red blood cells and thus a decrease in PCV (38). Exposure to heat stress also increases the level of free radicals in the body, which affect the red blood cells, causing them to be damaged, which in turn is reflected in the PCV rate (39). Zinc also enhances the function of the thyroid in secreting the hormones T3 and T4, which leads to the formation of erythropoietin and then increases the production of red blood cells, PCV, and Hb (40). Heat stress also increases the ratio of lymphocytes to heterophils (3), as the hormone corticosterone rises during stress, which has a receptor on the surface of lymphocytes, causing their degeneration, which raises the H/L ratio, which is considered physiological evidence of the

Table 7. Effect of nano methionine- zinc in some physiological traits of broiler exposed to heat stress

Treatments	Mean± standard error					
	RBC x 10^6	WBC x 10^3	PCV %	Hetrophil	Lymphocyte	H/L ratio %
T1	3.64± 0.25	32.66± 0.33	23.33± 0.88 b	29.00± 0.57 a	61.33± 2.02 b	0.470± 0.01 a
T2	3.60± 0.29	32.00± 1.00	26.33± 0.88 a	24.67± 1.45 b	67.00± 2.08 ab	0.363± 0.012 b
T3	4.16± 0.22	32.66± 0.33	29.00± 0.57 a	25.67± 0.33 ab	69.00± 1.52 a	0.366± 0.01 b
T4	3.95± 0.07	31.66± 1.67	26.67± 1.20 a	23.00± 1.52 b	66.00± 1.73 ab	0.346± 0.02 b
T5	3.79± 0.06	30.66± 0.88	27.66± 0.67 a	24.00± 0.57 b	65.00± 0.57 ab	0.360± 0.01b
Significant	NS	N.S	**	*	*	**

Means with different letters differ significantly with ** (P<0.01) * (P<0.05), NS: not significant. Treatments: T1 is a control treatment (without addition); T2, T3, T4, and T5 add 1, 2, 3, and 4 mg nano methionine-zinc/kg feed.

occurrence of stress, methionine-zinc nanoparticles may reduce the severity of heat stress due to the antioxidant role of Methionine, as Methionine can serve as a precursor to glutathione (37), which has a significant role in scavenging radicals. In addition to its participation in the activation of thioredoxin proteins, which have a significant role in the work of the antioxidant system (36), which may reduce the severity of heat stress, zinc is also necessary for the development of lymphocytes. Zinc deficiency depletes the thymus gland and reduces the number of T-cells, nano-zinc may support the optimal development of lymphocytes, thus alleviating the effect of heat stress on birds.

Conclusion

The current study found that adding nano methionine-zinc to the diet improved the productive traits, and this was accompanied by an improvement in the condition of heat stress resistance through the indicators studied.

Conflict of interest
The authors declare no conflict of interest.

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