Using green Technology to generate Nanoparticles loaded with Papaya in order to improve the productive Performance of heat-stressed broiler Chickens

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Abstract

This experiment was conducted on the farm of Al-Anwar Poultry Company in Babylon Governorate for 35 days from 28/11/2022 until 2/1/ 2023, 360 unsexed Ross-308 broiler chickens were used. The experimental groups included (0, 8, and 16 mg/kg feed of copper NPs (Nanoparticles), 8 and 16 mg/kg feed of copper NPs loaded on papaya plants, and 10 mg/kg feed of Papaya fed for groups (G1, G2, G3, G4, G5, and G6), respectively. All groups of the chicks were subjected to a consistent temperature of 34±1 cº for the whole duration of the trial. The study yielded the following results: There was a significant improvement in the live body weight, packed cell volume (PCV), heterophile count, and heterophil-to-lymphocyte (H/L) ratio for groups G2, G3, G4, G5, and G6 compared to group G1. Group G5 showed a significant increase in weight gain and hemoglobin levels. Additionally, groups G3 and G5 exhibited a significant increase in feed intake compared to group G1. Group G6 showed a significant improvement in feed conversion ratio compared to group G1 in the fifth week. Furthermore, groups G3, G4, and G5 showed a significant increase in red blood cell count compared to the other groups. Similarly, groups G2, G4, G5, and G6 showed a significant improvement in lymphocyte ratio. Nano-copper green synthesis can be regarded as a safe and promising nano-growth promoter in broilers when introduced to the diet at a dosage level of 8 and 16 mg/kg.

Keywords: Green syntheses, nanoparticles, Copper, papaya plants.

Introduction

Recently, a method for manufacturing nano-metals in a green way has emerged, as they are used. Plant extracts synthesizing metal nanoparticles are considered an up-and-coming technology. The synthesis of nanoparticles using a plant extract has benefits over other methods of manufacturing nanomaterials, such as physical, chemical, and even biological processes using microorganisms, because the amount produced from synthesizing metal nanoparticles using a plant extract is more tremendous. Compared to other methods (1) and significantly faster (2,3) Therefore, plant extracts are a good source for the synthesis of nanoparticles and metal oxides(4,5). Nano copper has an important role in oxygen utilization, energy metabolism, growth, and defense against oxidative stress (6), in addition to being an essential element. Some research (7) has shown that copper acts as an antioxidant by removing free radicals and preventing lipid peroxidation, which is an essential part. Integrated copper (8) and nano-copper in the diet have been shown to increase antioxidant activities. It was also shown (9) that copper nanoparticles are better than copper sulphate in enhancing the growth, productive and physiological performance of birds (10). The papaya plant gave positive results when using its leaves as additives or components in the diet of broilers (11). Ground papaya leaves can be an alternative to rare and high-cost commercial synthetic vitamin sources used in broiler production, which is a good option when we want to reduce production costs. The current study aims to synthesis nano-copper by green synthesis method and add it to the diet comparative with chemical nano-copper and papaya plant to improving growth performance of broiler exposed to heat stress.

Materials and methods

For 35 days, the study carried out on the farm of the Company of Al-Anwar Poultry in the Babylon Province from 28/11/2022 until 2/1/2023,

Three hundred sixty unsexed broiler chickens (Ross-308) were used. The chickens were raised on the ground in cages (pens) of 18 cages. The floor was draped with 4-5 cm thick cm in addition to the paper brush. In the groups experiment, there was: (0, 8, and 16 mg/kg feed of copper nanoparticles, 8 and 16 mg/kg feed of copper nanoparticles loaded on papaya plants, and 10 papaya plants mg/kg feed) for groups G1, G2, G3, G4, G5, and G6, respectively.

Green synthesis method The copper nanoparticles were obtained from NanoSunny Cooperation Company

The leaves of the papaya plant were brought from a farm in Babil Governorate (Al-Siyahi area), where they were picked from the tree, after which the PapayaPapaya (Carica papaya) (C. papaya) leaves were systematically washed with deionized water and dried in the shade for 14 days, after which the dried leaves were ground on a Form a powder using a grinder according to the method mentioned by (12) and according to the following steps:

1 -Take 10 grams of ground papaya powder, then mix it with 100 ml of deionized water in a conical flask.

2 -Heat at 60°C for 10 minutes, then cool to room temperature

3 -The extract is filtered using Whatman filter paper (1 size), then the screened material is stored at 4 degrees Celsius until use.

4 -To synthesize copper oxide nanoparticles, 90 ml of a five mM copper sulfate ($CuSO_4$) solution is taken with 10 ml of leaf extract and allowed to reach room temperature until further color change occurs. An indicator of nanoparticle synthesis The color change was compared to the control solution (leaf extract and five mM copper sulfate).

5- The sample was converted from liquid to powder. A rotary evaporator device was used to maintain the chemical concentration of the model.

Feed group

The starter diet, which had a protein content of 23.04 percent and an energy content of 3021.45 kilocalories per kg of feed, was given to the chicks from the day they were born until the third week of their lives, at which point the growth diet, which had a protein content of 20.06 percent and an energy content of 3194.92 kilocalories per kg of feed, was introduced.

Studied traits

Productive traits

According to (13), weekly live body weight, weekly weight gain,

and feed consumption, and feed conversion ratio

Physiological traits

Cellular characteristics of Blood

At 35 days old, two birds from each group had blood drawn from the wing vein using tubes that contained an anticoagulant. In the Department of Animal Production's physiology lab (College of Agriculture, Al-Qasim Green University), tests were carried out and the following characteristics were calculated:

Numbers of red blood cells according to the method indicated by (14), numbers of white blood cells according to the procedure stated by (15) percentage packed volume of red blood cells (PCV) according to the method indicated by (16) blood hemoglobin (Hb) according to the procedure stated by (15) heterophil cells (heterophils) and lymphocytes and the ratio between them, Heterophil / Lymphocyte ratio (H/L) according to the staining method indicated by (17) and counting was performed, according to the process of (18).

Data analysis

The statistical program Statistical Analysis System (19) was used in the analysis. Data to study different groups' effects on the traits according to a completely randomized design (CRD). Significant differences between means were compared with (20) multinomial test.

Mathematical model of design:

$Yij = \mu + Ti + eij$.

Results and Discussion

1- Live body weight

The results of Table 1 indicate the effect of the groups on the average live body weight (g), as we note that there is no significant difference between the study groups in the initial weight and the first and third weeks. However, a significant superiority ($P \leq 0.05$) appeared in the second week for the G2 and G5 groups. Groups G3 and G4 were superior to groups G1 and G6. In the fourth week, there was a significant superiority ($P \leq 0.05$) for groups G2 and G5 compared to the rest. treatments G3 and G4 also outperformed groups G1 and G6. Group G6 also exceeded G1, and it appeared in the week In the fifth of the experiment, groups G2, G3, G4, G5, and G6 were significantly superior ($P \leq 0.05$) to group G1, and no significant differences occurred between groups G2, G3, G4, G5, and G6.

2- Average weekly weight gain (g/bird)

The results of Table 2 indicate the groups' effect on the weekly weight gain rate (g). The Table shows no significant difference between the experimental groups during the first, second, and third weeks. However, in the fourth week, there was a significant increase (P≤0.05) for the groups G4 and G5 compared to the rest of the groups; group G2 outperformed groups G1, G3, and G6.

Table 1. Physiological and productive response to adding copper nanoparticles and copper nanoparticles loaded on papaya plants to the diet of broiler chickens at average live body weight (gm/bird) under conditions of environmental stress (heat stress)

Group s	Average ± standard error					
	Initial weight	first week	second week	third week	fourth week	fifth week
G1	32.51 ±3.18	192.93 ±3.73	485.56 ±1.02 c	956.31 ±13.63	1.57 ±0.01 c	2033.50 ±30.23 b
G2	43.12 ±0.87	191.25 ±3.75	500.00 ±0.25 a	1001.13 ±5.62	1634.00 ±3.01 a	2137.00 ±5.00 a
G3	43.43 ±0.40	196.33 ±3.75	492.83 ±3.07 b	983.33 ±19.80	1605.67 ±2.34 b	2145.67 ±1.02 a
G4	42.91 ±0.66	187.41 ±0.65	494.91 ±1.76 b	938.67 ±27.68	1608.33 ±5.69 b	2130.00 ±1.25 a
G5	42.25 ±0.80	192.75 ±0.38	501.58 ±1.21 a	972.33 ±4.59	1645.67 ±3.39 a	2165.67 ±3.11 a
G6	42.17 ±1.44	188.41 ±1.88	486.25 ±2.17 c	930.75 ±56.99	1508.00 ±5.81 d	2146.67 ±2.03 a
Significant level	**NS**	**NS**	*****	**NS**	*****	*****

Means with different letters within one column differ significantly from each other. * (P<0.05), NS: not significant. And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

Table 2. Physiological and productive response to adding copper nanoparticles and copper nanoparticles loaded on papaya plants to the diet of broiler chickens in the rate of weekly weight gain (g/bird) under conditions of environmental stress (heat stress)

Group s	Average ± standard error					
	first week	second week	third week	fourth week	fifth week	total
G1	150.23 ±5.72	286.58 ±7.36	485.75 ±7.85	606.08 ±2.83 d	484.33 ±43.13 c	2012.98 ±1.19 c
G2	149.90	309.25	462.67	649.75	466.67	2038.23 ±4.78

	±3.20	±2.36	±23.74	±2.66 b	±38.30 c	c
G3	152.90 ±3.39	296.50 ±13.70	490.50 ±10.91	622.33 ±3.59 c	540.00 ±29.54 b	2102.23 ±1.74 ab
G4	144.50 ±128	307.50 ±5.14	443.75 ±23.82	669.67 ±2.16 a	521.67 ±10.89 b	2087.08 ±1.83 b
G5	150.50 ±1.08	308.83 ±4.91	470.75 ±7.42	673.33 ±3.96 a	520.00 ±35.47 b	2123.42 ±3.70 a
G6	145.91 ±0.44	297.83 ±10.83	444.50 ±53.33	577.25 ±3.25 e	638.67 ±63.74 a	2104.17 ±2.46 ab
Significant level	**NS**	**NS**	**NS**	*	*	*

Means with different letters within one column differ significantly from each other. * (P<0.05), NS: not significant. And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

Group G3 also groups G1 and G6, while group G1 outperformed G6. In the fifth week of the experiment, a significant increase (P≤0.05) appeared for group G6 compared to the rest. Groups G3, G4, and G4 also outperformed. G5 over groups G1 and G2, and in total weight gain, a significant increase (P≤0.05) was obtained for group G5 compared to groups G1, G2, and G4. Groups G3, G4, and G6 also outperformed groups G1 and G2.

3- Feed intake (g/bird)

The results of Table 3 indicate the effect of the groups on the rate of weekly feed consumption (gm), as we note that there is no significant difference between the experimental groups during the first, third, and fifth weeks. However, in the second week, there was a significant increase (P<0.05) for the groups G2, G3, G3, G4, and G5 compared to group G1. Group G5 was significantly higher in feed consumption in the fourth week compared to the rest of the groups, and no significant difference appeared between groups G1, G2, G3, G4, and G6. As for the cumulative rate of feed consumption, it increased significantly (P< 0.05) in Transactions G3 and G5 compared to the rest of the transactions. Group G2 also increased significantly compared to transactions G1, G4 and G6.

4- Feed conversion ratio (gm feed/gm weight gain)

The results of Table 4 indicate the effect of the groups on the weekly feed conversion factor (g feed/g weight gain). During the first week, a significant improvement (P≤0.01) appeared for groups G1 and G2 compared to groups G4, G5, and G6, and they also improved significantly (P≤0.01).) Group G3 compared to groups G4 and G6, and there was no significant difference between the groups studied during the second and third weeks and the cumulative average. In the fourth week, group G4 improved significantly (P<0.05) compared to the rest of the groups, and groups G2 and G5 improved compared to group G6. In the fifth week, a significant improvement (P<0.05) appeared for group G6 compared to groups G1 and G2.

Mortality %

Table 5 shows the effect of the study on mortality, noting no significant difference among groups.

Copper also works to increase the production of growth hormone (21) and thus increases the metabolism of amino acids and proteins. Copper also acts as an antioxidant as it can bind to

Table 3. Physiological and productive response to adding copper nanoparticles and copper nanoparticles loaded on papaya plants to the diet of broiler chickens in terms of feed consumption rate (g/bird) under conditions of environmental stress (heat stress)

Groups	Average ± standard error					
	first week	second week	third week	fourth week	fifth week	Cumulative
G1	132.75 ±4.48	297.67 ±8.23 b	563.75 ±8.76	868.08 ±1.97 b	1205.58 ±21.20	3067.83 ±3.59 c
G2	128.08 ±2.31	325.41 ±6.32 a	568.50 ±16.23	885.08 ±2.07 b	1219.92 ±31.84	3127.00 ±3.06 b
G3	136.50 ±8.63	339.33 ±9.17 a	570.00 ±14.01	902.25 ±1.87 b	1268.75 ±14.53	3216.83 ±5.98 a
G4	140.17 ±3.16	326.08 ±5.74 a	543.83 ±12.26	860.41 ±2.32 b	1207.25 ±34.72	3077.75 ± 5.51 c
G5	143.25 ±0.76	335.50 ±4.53 a	575.25 ±5.42	1240.75 ±2.30 a	1240.75 ±24.30	3194.83 ±2.80 a
G6	141.83 ±1.30	316.91 ±7.46 ab	561.91 ±13.81	871.91 ±3.57 b	1189.17 ±8.41	3080.83 ±2.59 c
Significant level	NS	*	NS	*	NS	*

Means with different letters within one column differ significantly from each other. * (P<0.05), NS: not significant. And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

Table 4. Physiological and productive response to adding copper nanoparticles and copper nanoparticles loaded on papaya plants to the diet of broiler chickens according to the feed conversion factor (g/bird) under conditions of environmental stress (heat stress)

Group s	Average ± standard error					
	first week	**second week**	**third week**	**fourth week**	**fifth week**	**Overall rate**
G1	0.883 ±0.009 c	1.038 ±0.009	1.160 ±0.008	1.435 ±0.04 ab	2.540 ±0.29 a	1.411 ±0.06
G2	0.854 ±0.007 c	1.052 ±0.03	1.231 ±0.03	1.363 ±0.04 b	2.639 ±0.16 a	1.428 ±0.03
G3	0.890 ±0.04 bc	1.151 ±0.08	1.163 ±0.04	1.460 ±0.08 ab	2.361 ±0.11 ab	1.405 ±0.03
G4	0.970 ±0.02 a	1.060 ±0.01	1.229 ±0.04	1.291 ±0.08 c	2.314 ±0.06 ab	1.373 ±0.02
G5	0.951 ±0.01 ab	1.086 ±0.02	1.223 ±0.03	1.365 ±0.14 b	2.403 ±0.13 ab	1.406 ±0.01
G6	0.971 ±0.01 a	1.065 ±0.03	1.323 ±0.17	1.520 ±0.08 a	1.903 ±0.21 b	1.356 ±0.02
Significant level	**	NS	NS	*	*	NS

Means with different letters within one column differ significantly from each other. * (P<0.01), (P<0.05) NS: not significant. And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

Table 5. Effect of adding Copper and copper nanoparticles green synthesis to the diet in the mortality % of broiler (Ross 308)

Group s	Mean± standard error Mortality
G1	6.33 ± 1.70
G2	3.67 ± 1.00
G3	0.00 ± 0.00
G4	3.67 ± 1.00
G5	0.00 ± 0.00
G6	3.67 ± 1.00
Significant	N.S.

Means with different letters within one column differ significantly from each other. NS: not significant. And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

free radicals and get rid of them by giving them two electrons. Copper is also involved in the formation of ceruloplasmin, superoxide, and catalase, as these compounds are characterized by their antioxidant activity (22) the addition of copper nanoparticles as an alternative to antibiotics can have similar benefits on the growth performance of broilers (23). Copper nanoparticles manufactured in green are considered to enhance the performance of chickens (23). The growth of broilers increases because their doses are lower and because of their small size, which can improve absorption in the digestive system (24). Copper nanoparticles also preserve other mineral elements, such as iron and zinc, by binding to them to prevent them from disintegrating or forming. Organic compounds, such as enzymes and immune proteins, raise the resistance level and, thus, the productivity of birds (25) (26). All of these properties can reduce the burden of heat stress to which birds are exposed, and this is clear from the high amount of feed consumed. In groups G3 and G5 (2), there was also an improvement in physiological characteristics and a decrease in the H/L ratio in the groups adding copper and papaya sources compared to the control group .

The reason for the deterioration of most of the productive characteristics of the birds in the G1 control group may be due to exposure to heat stress, which leads to a defect in the digestive processes and an increase in the speed of the passage of nutrients into the digestive canal and a lack of benefit from the nutrients (27),it also leads to a significant decrease in the activity of the critical amylase enzyme. In the digestion of carbohydrates and the enzymes trypsin and chymotrypsin, which are essential in the digestion of proteins, this reduces the availability of amino acids that the body needs to build muscles when birds are exposed to heat stress (27), as research has indicated that heat stress hinders the absorption of nutrients from the small intestine (28).heat stress also leads to an imbalance in metabolic processes as a result of decreased secretion of thyroid hormones (Triiodothyronine (G3) and Thyroxine (G4) (29, 30), and when exposed to heat stress there is a deficiency in processing Blood goes to the digestive system, and the blood supply goes to the respiratory system, brain, and skin to get rid of heat, and thus there is a deficiency in digesting and utilizing nutrients (31, 32) with an increase in the need for cellular energy, which leads to an increase in the formation of free radicals in the mitochondria through cellular respiration and thus the occurrence of oxidative stress (33), as free radicals affect the structure of the body's protein, as free radicals work to oxidize the peptide bond. Which binds amino acids and also causes oxidation of amino acids.

Cellular characteristics of Blood

Preparation of red blood cells, white blood cells, hemoglobin percentage (pcv), and hemoglobin value at 35 days old.

The results of Table (6) indicate the effect of the groups on the number of red blood cells, white blood cells, blood hemoglobin percentage (pcv), and hemoglobin value, as we notice that in red blood cells, there was a significant superiority ($P<0.05$) of the groups G3, G4, and G5 compared to the groups G1, G2, and G6, there was no significant increase in white blood cells and blood counts. There was a significant increase ($P<0.05$) for groups G2, G3, G4, G5 and G6 compared to group G1. As for hemoglobin concentration, there was a significant increase ($P<0.05$) for the group . G5 compared to groups G1 and G6.

The percentage of heterophil cells, the rate of lymphocytes, and the H/L ratio at 35 days of age.

The results of Table (7) indicate the effect of the groups on the percentage of heterophil cells, the rate of lymphocyte cells, and the H/L ratio at the age of 35

Table 6. Physiological and production response to adding copper nanoparticles and copper nanoparticles loaded on papaya plants to the diet of broiler chickens on cellular blood characteristics at 35 days of age under conditions of environmental stress (heat stress)

Group s	Average ± standard error			
	Red blood cells x 10 6 (mm 3 blood)	White blood cells x 10 3 (mm 3 blood)	Blood heap PCV%	Hemoglobin gm/100ml
G1	2.51 ±0.45 b	30.50 ±0.50	25.50 ±0.50 b	8.50 ±0.16 b
G2	2.73 ±0.65 b	31.50 ±0.50	29.00 ±1.00 a	9.67 ±0.33 ab
G3	3.94 ±0.05 a	31.50 ±1.50	29.50 ±0.50 a	9.83 ±0.17 ab
G4	3.09 ±0.05 a	32.50 ±0.50	28.50 ±1.50 a	9.50 ±0.50 ab
G5	3.99	31.50	30.50 ±0.50	10.16

	±0.10 a	±0.50	a	±0.16 a
G6	2.82 ±0.03 b	31.00 ±1.00	24.50 ±0.04 a	8.16 ±0.02 b
Significant level	*	N.S.	*	*

Means with different letters within one column differ significantly from each other. * (P<0.05) NS: not significant. And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

Table 7. Physiological and productive response to adding copper nanoparticles and copper nanoparticles loaded on papaya plants to the diet of broilers in heterophil and lymphocytes and the H/L ratio at 35 days of age under environmental stress (heat stress)

	Average ± standard error		
Group s	**Heterophil (%)**	**Lymphocyte (%))**	**H/L ratio**
G1	26.00±1.00 a	62.50±1.50 b	0.41 ±0.01 a
G2	18.50±1.50 b	69.00±2.00 a	0.26 ±0.03 b
G3	18.00±1.00 b	61.00±1.00 b	0.29±0.02 b
G4	19.0±1.00 b	67.50±2.50 a	0.28 ±0.01 b
G5	22.00±1.00 b	69.50±1.50 a	0.31 ±0.02 b
G6	18.00±1.00 b	68.50±1.50 a	0.26 ±0.01 b
Significant level	**	*	*

Means with different letters within one column differ significantly from each other. * (P<0.01), (P<0.05). And groups 0, 8 & 16 (CNPs), 8 & 16 (CNPs green syntheses, 10 mg/kg papaya plant for groups G1, G2, G3, G4, G5, and G6 respectively.

days, where we notice a significant increase in the percentage of heterophil cells and the ratio of heterophil cells to H/L lymphocytes in favor of the G1 group compared to the rest of the groups. There was no significant difference between groups G2, G3, G4, G5, and G6. As for the percentage of lymphocytes, groups G2, G4, G5, and G6 were increased significantly (P<0.05) compared to groups G1 and G3.

The increase in the number of red blood cells in groups G3, G4, and G5 may be due to the addition of various copper sources in the fourth and fifth groups, as copper has an essential role in stimulating the process of forming red blood cells in the bone marrow (Erythropoiesis) (34). The increase in the number of red blood cells, in turn, increases the percentage of PCV and hemoglobin concentration, as (35) indicated an increase in the number of red blood cells when adding nano-copper to the diet of broilers exposed to heat stress, as Copper plays a role. It is vital for hemoglobin synthesis (36). The significantly higher hemoglobin in group G5, fed green nano-copper, may be attributed to the greater availability of nano-copper for hemoglobin synthesis (35). Our results are consistent with (37), who found an increase in red blood cells, hemoglobin, and erythrocytes after adding nano-copper to the diet of broilers.

The increase in the percentage of heterophil cells and the H/L ratio and the decrease in the rate of lymphocytes in the control group may be due to exposure to heat stress, as the hormone corticosterone, whose concentration increases during heat stress, stimulates the release of heterophil cells from the bone marrow and decomposes the lymphocytes, so the H/L ratio is disturbed and rises. In birds exposed to heat stress (38) Lymphocytes (39). As for the improvement in the proportions of lymphocytes and heterophils and the H/L ratio in the groups of adding Copper from its various sources, this may be due to its antioxidant role and the activation of oxidative enzymes such as the SOD enzyme (40). This is also evident in the improvement in the condition of Copper. Antioxidants (glutathione enzyme and SOD as well as heat shock protein) in groups G4 and G5 in the results of our experiment (Table).

Conclusion

It is concluded from the results of the current study that nano-copper manufactured in a green synthesis, nano-copper, and papaya plants improved the productive and physiological characteristics of broilers exposed to heat stress.

Conflict of interest

The authors declare no conflict of interest.

References

1. **Saif, S.; Tahir, A.; Chen, Y. 2016.** Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications. Nanomaterials, 6, 209. doi:10. 3390/nano6110209.

2. **Makarov, V.v.; Love, A.J.; Sinitsyna, O.v.; Makarova, S.S.; Yaminsky, I. 2014**. Taliansky, M.E.; Kalinina, N.O. 'Green' Nanotechnologies: Synthesis of Metal Nanoparticles Using Plants. Acta Naturae., 6, 35–44.

3. **Ahmed, S.; Ahmad, M.; Swami, B.L.; Ikram, S. A. 2016.** Review on Plants Extract Mediated Synthesis of Silver Nanoparticles for Antimicrobial Applications: A Green Expertise. J. Adv. Res, 7, 17–28. doi:10.1016/j jare.2015.02.007.

4. **Ppn, V.K.; Shameem, U.; Kollu, P.; Pammi, S.V.N. 2015.** Green Synthesis of Copper Oxide

Nanoparticles Using Aloe vera Leaf Extract and Its Antibacterial Activity Against Fish Bacterial Pathogens Thermoelectric materials and perovskite solar cells View project Investigating magnetic properties of doped cerium oxide nanoparticles View project, (n.d.). doi:10.1007/ s12668-015-0171-z.

5. **Bordbar, M.; Sharifi-Zarchi, Z.; Khodadadi, B. 2017.** Green Synthesis of Copper Oxide Nanoparticles/Clinoptilolite Using Rheum Palmatum L. Root Extract: High Catalytic Activity for Reduction of 4-Nitro Phenol, Rhodamine B, and Methylene Blue. J. Sol-Gel Sci. Technol., 81, 724–733. doi:10.1007/s10971-016-4239-1.

6. **Linder, M.C. 1991**. Copper and metabolic regulation. In 'Biochemistry of copper'. pp. 241–300. (Springer US: Boston, MA).

7. **Shao, X-p, Liu, W-b, Xu, W-n, Xia, W, Jiang Y. 2010**. Effects of dietary copper sources and levels on performance, copper status, plasma antioxidant activities and relative copper bioavailability in Carassius auratus gibelio. Aquaculture 308, 60–65. doi:10.1016/j.aquaculture. 2010.07.021**.**

8. **Johnson MA, Fischer JG, Kays S.E. 1992.** Is copper an antioxidant nutrient? Critical Reviews in Food Science and Nutrition 32, 1–31. doi:10.1080/ 10408399209527578**.**

9. **Al-Bairuty GA, Boyle D, Henry TB, Handy RD. 2016**. Sublethal effects of copper sulphate compared to copper nanoparticles in rainbow trout (Oncorhynchus mykiss) at low pH: physiology and metal accumulation. Aquat. Toxicol., 174, 188–198.

10. **Mroczek-Sosnowska N, Łukasiewicz M, Adamek D, Kamaszewski M, Niemiec J, Wnuk-Gnich A, Scott A, Chwalibog A, Sawosz E. 2017.** Effect of copper nanoparticles administered in ovo on the activity of proliferating cells and on the resistance of femoral bones in broiler chickens. Arch Anim Nutr. 71(4):327–332.

11. **Oloruntola O. D. 2018.** serum biochemistry and histological studies in growing rabbits fed diets supplemented with Mucuna pruriens leaf meal. Arch Current Res Int.;15(1):1-10.

12. **Sankar. R., Manikandan P., Malarvizhi V., Fathima T., Shivashangari K. S., Ravikumar V. 2014.** Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy Volume 121 Pages 746-750, ISSN 1386-1425.

13. **Al-Fayad, Hamdi Abdel Aziz, Saad Abdel Hussein Naji, and Nadia Nayef Abdel 2011**. Poultry products technology. Second Edition . The second part . Ministry of Higher Education - University of Baghdad - College of Agriculture.

14. **Natt, M. P. and Herrick, C. A. 1952.** A new blood diluent for counting theerythrocytes and leucocytes of the chicken. Poultry Science, 31(4): 735- 738.

15. **Al-Daraji, Hazem Jabbar, Walid Khaled Al-Hayani, Ali Sabah Al-Hassani. 2008.** So the blood of birds is shed. Ministry of Higher Education and Scientific Research - University of Baghdad - College of Agriculture.

16. **Archer, R.K. 1965.** Hematological techniques for use on animals oxford : Blakwell scientific publication .

17. **Shen , P.F. and L.T. Patterson . 1983.** Asimplified wrights stain technique for routine avian blood smear staining . poulty Sci., 62: 923-924.

18. **Campbell, T. W. 1988.** Avian Hematology and cytology. 1st edition , Ames , I.A. lowa state university press . USA.

19. **SAS. 2018.** Statistical Analysis System, User's Guide. Statistical. Version 9.1 th ed. SAS. Inst. Inc. Cary. N.C. USA**.**

20. **Duncan, D.B. 1955**. Multiple Rang and Multiple F-test. Biometrics. 11: 4-42.

21. **Ibrahim, I. A. G., EL-Gendi, M., Nihad, A. A., Okasha, H. M. and El-Attrouny, M. M. 2022 a.** Effect of Different Dietary Copper Forms and Levels on Carcass Characteristics and Meat Quality Traits of Broilers Chickens. *Ann. Agric. Sci., Moshtohor*. 60: 1091–1102.

22. **El Basuini MF, El-Hais AM, Dawood MAO, Abou-Zeid AE-S, EL-Damrawy SZ, Khalafalla MME-S, Koshio S, Ishikawa M, Dossou S. 2016.** Effect of different levels of dietary copper nanoparticles and copper sulfate on growth performance, blood biochemical profiles, antioxidant status and immune response of red sea bream (Pagrus major). Aquaculture, 455, 32–40.

23. **Ibrahim, IA, GM EL-Gendi, AA Nihad, HM Okasha, and MM El-Attrouny. 2022.** "Potential Effects of Different Dietary Copper Sources to Improve Productive Performance, Plasma Biochemical Parameters and Oxidative Response Activities of Broiler Chickens." Journal of Animal and Poultry Production 13 (8):111-118.

24. **Mohamed.D.A , Abd El-sadek. M.S and Abdel-Wareth. A.A.A. 2022.** Green synthesis of copper oxide nanoparticles in broiler nutrition: Present perspectives and strategic future in climate change conditions. SVU-International Journal of Agricultural Sciences Volume 4 Issue (3) pp.: 203-222, 2022 Print ISSN 2636-3801.Online ISSN 2636-381X Doi: 10.21608/svuijas.2022.166077.1238.

25 **Zaboli K, Aliarabi H, Bahari AA, Abbasalipourkabir R. 2013** .Role of dietary nano-zinc oxide on growth performance and blood levels of mineral: a study on in Iranian Angora (Markhoz) goat kids. J. Pharm. Health Sci., 2, 19–26.

26. **Scott A, Vadalasetty KP, Chwalibog A, Sawosz E. 2018.** Copper nanoparticles as an alternative feed additive in poultry diet: a review. Nanotechnol Rev.;7:69-93. https://doi.org/10.1515/ ntrev-2017-0159.

27. **Har, I., Rong, D.and Zhang, Z.Y. 2000**. The effect of thermal environment on the digestion of broiler. J. Anim. Physiol. A. Anim. Nutr., 83 : 59- 64.

28. **Garriga, C.; Hunter, R.R.; Amat, C.; Planas, J.M.; Mitchell, M.A.; Moretó, M. 2006.** Heat stress increases apical glucose transport in the chicken jejunum. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R195–R201.

29.Melesse,A.,Maak.S.,Schmidt.Rand vonLengerken.G.(2011). Effect of long-term heat stress on key enzyme activities and T3 levels in commercial layer hens. Int. J. Livest. Prod., 2(7): 107-116.

30.Bueno,J.p.,Gotardo.L.R.,Santos. A.M.,Litz.F.H.,Oliveri.O.C.,Alves.R.L.,Moraes.C.Aand Nascimento M. R. 2020. Effect of cyclic heat stress on thyroidal hormones, thyroid histology, and performance of two broiler strains. Int. J. Biometeorol ., 64(7):1125-1132.

31. **Wolfenson, D., Frei, Y.F., Snapir, N.and Berman, A. 1981.** Heat stress effects on capillary blood flow and its redistribution in the laying hen. Pflugers Arch., 390(1) :86-93.

32. **Cronje, P.B. 2007.** Gut health, osmoregulation and resilience to heat stress inpoultry,Aust.Poult.Sci.Symp.URL http://sydney.edu.au/vetscience/apss/documents/ APSS2007-cronje-pp9-13.

33. **Mujahid, A., Pumford, N. R., Bottje, W., Nakagawa, K., Miyazawa, T., Akiba, Y., & Toyomizu, M. 2007.** Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. *The Journal of Poultry Science*, *44*(4), 439-445.

34. **Samanta, B, A Biswas, and PR Ghosh. 2011.** "Effects of dietary copper supplementation on production performance and plasma biochemical parameters in broiler chickens." British Poultry Science 52 (5):573- 577.

35. **El-Kassas, S, Abdo, S.E., El-Naggar,k, Walied Abdo, Kirrella,A.K, NasharT.O.2018** Ameliorative effect of dietary supplementation of copper oxide nanoparticles on inflammatory and immune reponses in commercial broiler under normal and heat-stress housing conditions. Journal of Thermal Biology. https://doi.org/10.1016/j.jtherbio.2018.10.009.

36. **CLOSE, W.H. 1998.** The role of trace mineral proteinates in pig nutrition, in: LYONS, T.P. & JACQUES, K.A. (Eds) Biotechnology in the Feed Industry, Proceedings of Alltech's 14th Annual Symposium, pp. 469—474 (Nottingham, Nottingham University Press).

37. **Ognik K, Sembratowicz I, Cholewińska E, Jankowski J, Kozłowski K, Juśkiewicz J, et al. 2018.**The effect of administration of copper nanoparticles to chickens in their drinking water on the immune and antioxidant status of the blood. Anim Sci J; 89(3):579–88; https://doi.org/10.1111/asj.12956.

38. **Feizi,A.,Dadlan,F.and Asadzaehmajdi,S.(2012).**the effect of heat stress on some blood parameters ,biochemical values and humoral immunity in broiler chickens. Vete. Clin. Path.,6(3): 1621 - 1627**.**

39.**Duangjinda,M.,Tunim.S.,Duangdaen.C.andBoonkum.W.(2017).**Hsp70Genotypes and Heat Tolerance of Commercial and Native Chickens Reared in Hot and Humid Conditions. Brazili. J. Poult. Sci.,19 (1):7-18.

40. **Jaiswal, S.K., Raza, M. and Chaturvedani, A. K. 2017.** Effect of Thermal Stress on Serum Biochemical and Haematological Parameters in Broiler Chicken. IJVSBT.,12(3):19-22.