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A FEW HEMATOLOGICAL AND BIOCHEMICAL MARKERS OF BROILERS BREEDERS MALES ROSS-308 AT VARIOUS HESPERIDIN AND NARINGIN CONCENTRATIONS

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ABSTRACT

This study was designed to demonstrate the effect of using different levels of naringin and hesperidin on some blood and biochemical indicators and antioxidant status in aged broiler roosters. To achieve this goal, the birds were randomly distributed into six treatments as follows: The first treatment without any feed additions (NC). The second supplemented with vitamin E (20 IU per kg of feed) (PC). The third and fifth treatments received an addition of (250 mg/kg of feed) of both hesperidin and naringin, denoted as HS1 and NA1 respectively. The fourth and sixth were treated with an addition of (500 mg/kg of feed) of hesperidin and naringin, labeled as HS2 and NA2 respectively. The study aimed to investigate blood characteristics, certain biochemical indicators. When compared to treatments HS1, HS2, NC, and PC, the NA2 treatment clearly outperformed them in MCH and MCV. In comparison to the other treatments, the PC treatment showed a significant increase in (Hb), (PCV), and (TRBC). HS2 and NA2 treatments reduced WBC compared to NC. The hesperidin and naringin treatment resulted in lower quantities of Malondialdehyde. Additionally, HS2 and NA2 showed improvement in activity of the enzymes GOT and GPT, and glutathione concentrations. Conclusions derived from the study's findings imply that the addition of hesperidin and naringin enhanced the birds' immunological capacities. This improvement was followed by a possible decline in MDA levels, which may have resulted from an increase in liver functioning and antioxidant status.

INTRODUCTION

The growth rate of broiler chickens has increased by 400% as a result of selection and genetic improvement processes in hybrids between 1957 and 2007 (Zuidhof *et al*., 2014). Breeder flocks were among the stages of broiler chicken production that were impacted by these selection processes. As a result, the weight and size of male breeder chickens increased, which had a detrimental effect on their reproductive capabilities (Decuypere *et al*., 2010). Given that the natural sperm quality drop in male breeder chickens typically starts about 50 weeks of age, this may be a direct cause of early aging (Cobb Vantress, 2008). In natural mating systems, fertility tends to fall abruptly with age, even while gaining weight may not always affect sperm quality or reproduction rate. In addition, males' greater weight can mechanically diminish fecundity by impeding their capacity to properly finish natural mating (Hocking and Bernard, 1997). According to (Rosenstrauch *et al.*,1994) the heaviest rooster typically becomes dominant and interferes with subordinate males. Hesperidin, a plentiful and affordable secondary substance present in citrus fruits, is largely found in sweet oranges and lemons. In 1828, the researcher Lebreton isolated it for the first time from the interior of orange peels. Its molecular weight is 610.57 and its chemical formula is $C_{28}H_{34}O_{15}$. Orange peels, lemons, and citrus trees all contain large amounts of hesperidin. According to a study by Fotakis *et al.* (2017), adding hesperidin to poultry feed at doses of 0.7 and 1.5 grams per kilogram significantly reduced the amount of total fat and significantly increased the amount of amino acids in the plasma. Similar findings were made by Martínez-Noguera *et al.*, (2019), who discovered that adding different doses of hesperidin to the food of local male and female hens did not significantly alter their blood sugar or cholesterol levels (0, 150, 300, or 450 milligrams per kilogram of feed). However, due to the supplementation, changes in protein levels, testosterone levels in males, and estrogen levels in females were seen during the initial period (20–24 weeks). Hesperidin was given to adult white mice with hyperthyroidism at a dosage of 50 milligrams per kilogram of body weight per day in a study by (Ashry *et al.*, 2023), and this resulted in a considerable drop in the thyroid hormones T4, FT4, T3, and FT3. A considerable reduction in the levels of TNF-a, IL1B, IL4, IL6, and IL10, as well as a large rise in CD4+ levels, indicated changes in the immunological and inflammatory states, according to the study. A considerable rise in serum levels of SOD, GPx, CAT, and GSH, along with a discernible decline in MDA and NO levels, further demonstrated an improvement in oxidative status. An odorless powder in shades of light beige and yellow, naringin has no scent. It is a secondary plant metabolite and is a member of the bioflavonoid family. De Vry found naringin for the first time in grapefruit blooms in 1857 (Sharma *et al.*, 2019). The word "narangi," which means orange, is most likely the source of the name Naringin has the molecular weight of 580.5 grams per mole and the chemical formula $C_{27}H_{32}O_{14}$. Citrus fruits, notably grapefruits and oranges, naturally contain this flavanone glycoside (Chen *et al.*, 2018). In a study by Alam *et al.* (2013), the addition of naringin at a dose of 100 milligrams per kilogram of food per day resulted in significant decreases in plasma fat levels in mice fed a high-fat and high-sugar diet rich in fructose and glucose. It also improves glucose tolerance, increases insulin levels in the blood, and reduces inflammation, as evidenced by a significant decrease in the number of inflammatory white blood cells. In addition, there was a decrease in collagen deposition in the liver, heart, and around blood vessels, as well as a decrease in lipid droplet deposition in the liver. Moreover, levels of the liver enzyme AST and ALT decreased. When employed as a chelating agent in chicken feed, naringin has the ability to reduce the negative effects of lead, according to a study by Ebirim *et al.* (2023). Naringin, at concentrations of 80 and 160 milligrams per kilogram of food, either alone or in combination with lead, helped lessen the harmful effects of lead. This mitigation included a decline in anemia, a notable rise in the quantity of red blood cells, a rise in the volume of packed red blood cells, a rise in hemoglobin levels, and a decline in high blood pressure brought on by lead exposure. The study also demonstrated enhanced antioxidant status and a return to regular antioxidant enzyme blood levels. In light of this information, the hypothesis of your study aims to investigate the effects of hesperidin and naringin supplementation on some blood and biochemical parameters, as well as antioxidant levels, in broiler breeder males.

MATERIALS AND METHODS

The Animal Production Department, College of Agriculture, Tikrit University did this investigation in the poultry section. For this investigation, 30 Ross-308 broiler breeder males at the age of 50 weeks were used. The goal of the study was to determine the effects of hesperidin (HS) and naringin (NA) supplementation on specific hematological and biochemical parameters as well as oxidative stress markers in broiler breeder males. To achieve this goal, the hens were randomly distributed into six treatments, with each treatment comprising five birds and each hen serving as a replicate. The distribution was as follows:

- The first group: (NC) a diet without any addition.
- The second group: (PC) added vitamin E at a concentration of 20 IU / kg of feed.
- The third group: adding (1HS) at a concentration of 250 mg/kg of feed.
- The fourth group: adding (HS2) at a concentration of 500 mg / kg of feed.
- The fifth group: adding (NA1) at a concentration of 250 mg / kg of feed.
- The sixth group: adding (NA2) at a concentration of 500 mg / kg of feed.

Blood Collection

Medical syringes were used to draw blood (5 mL per roosters) from the wing vein for the purpose of collecting blood samples. After a four-week break, two blood samples were taken, and two more were taken eight weeks after the start of the therapy. The technique for dividing the collected blood into two portions is as follows:

For the fraction used for hematological assays.

1 mL of fresh blood was put into tubes with EDTA, an anticoagulant, to stop blood clotting. A number of hematological tests were conducted, including the measurement of the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), packed cell volume (PCV%), hemoglobin concentration (Hb), and white blood cell count (WBC). These experiments were carried out using Campbell's (1995) approach.

The section pertaining to biochemical tests

In the section involving the biochemical assays, 3–4 mL of blood were put into tubes containing clot activator gel that were free of anticoagulants. By centrifuging the tubes for 20 minutes at a speed of 3500 revolutions per minute, serum was intended to be obtained. After obtaining serum, it was put into clean, tightly sealed tubes and kept there at a temperature of -20°C until the biochemical tests, oxidative tests, and reduction indicators were carried out. Measurements of the activity of enzymes that transport amino groups were all part of the testing. Malondialdehyde and glutathione concentrations were also tested to determine the antioxidant status.

The statistical analysis

SAS statistical analysis software, version 9, was used to collect and evaluate the study's data before the statistical analysis. The examination of the collected data was done in a completely arbitrary manner. To determine the significance of differences in the means of the attributes under research, the Duncan test (1955) was utilized with a significance level of P 0.05.

RESULTS AND DISCUSSION

According to Table (1)'s findings, the hesperidin and naringin has an impact on the aged broiler breeder hens' hemoglobin concentration, total red blood cell count, and packed cell volume. Except for the positive control therapy, which outperformed all other treatments, there were not significant differences in hemoglobin concentration levels between the supplemented treatments and the negative control treatment. Additionally, as compared to treatment T3, the negative control, and the positive control treatments, treatments NA1 and NA2, which included the addition of naringin, showed a significantly higher MCH (mean corpuscular hemoglobin) concentration. However, when contrasted with treatment HS2, no significant distinction was seen. When compared to the hesperidin supplementation treatments, the negative control treatments, and the positive control (vitamin E) treatments, treatments NA1 and NA2 involving the addition of naringin at concentrations of 250 and 500 mg/kg of feed, respectively, showed a statistically significant improvement in terms of red blood cell size. With the exception of treatment HS2, which had a significant decrease in comparison to both the negative and positive control treatments, there were no discernible differences in the total white blood count (TWBC) between the supplementation treatments and the negative and positive control (20 IU E) treatments.

Table (1): Addition of different levels of hesperidin and naringin in hematological parameters of aged broiler breeder males.

Groups	Traits					
	Hb (g/100 ml)	MCH (pg)	MCV (μm³)	TRBC (X×10⁶/mm)	TWBC (X×10³/mm)	PCV %
G1	11.36 ± 0.22 B	54.42 ± 0.74 C	179.61 ± 2.44 C	2.09 ± 0.04 B	8632.80 ± 513.95 A	37.50 ± 0.74 B
G2	12.27 ± 0.15 A	54.81 ± 1.11 C	180.90 ± 3.66 C	2.24 ± 0.03 A	8217.00 ± 390.74 AB	40.50 ± 0.50 A
G3	11.42 ± 0.18 B	54.89 ± 0.49 C	181.16 ± 1.61 C	2.08 ± 0.04 B	7258.60 ± 590.32 ABC	37.70 ± 0.59 B
G4	11.45 ± 0.12 B	56.06 ± 0.53 BC	185.02 ± 1.76 BC	2.04 ± 0.01 BC	6466.60 ± 391.02 C	37.80 ± 0.41 B
G5	11.36 ± 0.19 B	57.35 ± 1.49 AB	189.28 ± 4.94 AB	1.99 ± 0.07 BC	7642.80 ± 623.80 ABC	37.50 ± 0.63 B
G6	11.60 ± 0.11 B	59.37 ± 1.24 A	495.93 ± 4.11 A	1.96 ± 0.03 C	6831.00 ± 520.73 BC	38.30 ± 0.39 B

Different letters in the same column denote significant differences in the means of the treatments.
• T1= NC without addition, T2= PC (IU20 E), T3= HS1 (250 of hesperidin mg/kg feed), T4= HS2 (500mg of hesperidin /kg feed), T5= NA1 (250 mg of naringin / kg of feed), T6= NA2 (500 mg of naringin /kg feed).

There seems to be a line in your response that compares the effects of adding naringin and hesperidin to a NC in terms of Hb, PCV, and RBC. You also said that the aforementioned measures significantly rise when naringin and hesperidin are compared to vitamin E, which serves as a positive control. The antioxidant effects of vitamin E, which may be important in protecting red blood cells, could be responsible for this improvement. Due to frequent exposure to internal and external sources of free radicals, red blood cells are particularly vulnerable to oxidative damage inside the circulatory system (Surai *et al*., 2019). Hemoglobin oxidation occurs when antioxidants fall short in their ability to protect against ROS (Reactive Oxygen Species) damage, and this is more likely to happen since ROS predominate over antioxidants (Mohanty *et al*., 2014). Since lipids make up the majority of the plasma membrane and are crucial for preserving the form and functionality of red blood cells, reducing lipid peroxidation is another way to do this (De Oliveira and Saldanha, 2010). After giving the broiler breeder male vitamin E for eight weeks, it is clear that it had a positive impact on these traits. Cook (2017), discovered that oxygen in the atmosphere is one of the most significant factors when looking at the main factors impacting the production of red blood cells in birds. The release of erythropoietin, a substance that stimulates red cell synthesis in the bone marrow, is then aided by the release of testosterone hormone from the cells in the renal interstitium. The positive effects of naringin were evident at both high and low concentrations (HS1 and HS2) on values such as MCH and MCV. A significant increase in these variables was observed, accompanied by a significant decrease in RBC compared to the NC. Opinions on the effect of naringin on RBC counts and its indices, MCV and MCH, have been conflicting. Some research has suggested a lowering effect on red blood cell counts, possibly attributed to naringin's ability to chelate certain minerals like iron and lead. This could be the reason for the decrease in red blood cell counts**.** Nevertheless, it's believed that even if naringin does reduce RBC counts, it doesn't negatively impact PCV (packed cell volume) and Hb (hemoglobin) values. These values significantly increased, supported by results obtained by Cook (2017), who found a negative correlation between RBC counts and the levels of MCV and MCH in healthy, naturally raised birds. According to Lopez-Campos *et al*. (2010), feeding fattening lambs naringin at a rate of 1.5 grams per kilogram of dry matter caused a decrease in the number of RBC, which was followed by a noticeably higher MCV and MCH in the red cells. In comparison to the NC, the treatments involving the addition of hesperidin and naringin at a rate of 500 milligrams per kilogram of feed resulted in a considerable drop in the total WBC count. Due to the anti-inflammatory qualities of hesperidin and naringin, the treated broiler breeder hens' enhanced health and immunological state can be linked to this (Kamboh *et al*., 2015). TNF-, IL-8, IL6, and pro-inflammatory cytokines can all be inhibited by naringin, and it can also stop the infiltration of white blood cells (Drummond *et al*., 2013). On the other hand, the antioxidant flavonoids hesperidin and naringin play a preventative effect that significantly boosts immunity and overall health (Choi *et al*., 2020). WBC counts did not differ significantly across the therapies involving hesperidin, naringin, and vitamin E addition. The findings of Thushina *et al*. (2015), disagree with the findings of your study about the drop in WBC counts when hesperidin was added.

Antioxidant indicators

The statistical analysis of Table (2) demonstrates the impact of varying the amounts of hesperidin and naringin added for various times on the levels of the GOT and GPT enzymes in mature broiler breeder males. The levels of the enzymes GOT and GPT in the treated birds were found to be significantly lower in all treatments with hesperidin and naringin at both low and high dosages compared to the NC treatment. Additionally, compared to the positive control treatment and the other hesperidin and naringin addition treatments, NA2 had a much lower level of enzymes. Treatments G3 and G4 also showed a significant improvement in the levels of the enzymes GOT and GPT in the treated birds when compared to the positive control treatment, while treatment T5 did not show significant differences when compared to the positive control treatment in terms of enzyme levels. The concentrations of MDA in all addition treatments were much lower than in the NC, which supports the findings from Table 3 that the lipid oxidation process has improved. Comparing the sixth treatment to the other addition treatments and the PC treatment, NA2 (G6) in particular had a noticeable impact in lowering the amount of MDA. The amount of MDA in the treated birds' serum did not differ significantly between the T4 and T5 and the positive control treatment. In contrast to the other addition treatments and the PC treatment, HS1 revealed a much higher amount of MDA. The findings show that all addition treatments significantly increased the serum level of glutathione in comparison to the NC treatment. The amount of glutathione was also significantly higher in the T6 compared to the other, with the exception of the PC treatment. In contrast to the other treatments, the G3 exhibited the lowest significant level of glutathione. The amount of glutathione in the treated birds did not significantly differ between the G4 and G5 treatments. The results from Table 2 indicate a significant decrease in lipid peroxidation and its biological index in the body, as evidenced by the decrease in the level of (MDA) in all HS and NA. Additionally, the addition of vitamin E, which is known for its strong antioxidant properties, that's led to a significant reduction in MDA levels compared to the negative control treatment. It's worth noting that the significant decrease in MDA levels observed in treatments G4 and G5, which included hesperidin and naringin, respectively, did not show significant differences compared to the vitamin E treatment. Furthermore, NA2 (G6), exhibited a greater significant reduction in MDA levels compared to the PC treatment. These results were reflected in the increase in the level of glutathione (GSH) in the serum of the treated roosters. The findings do, in fact, highlight the importance of antioxidant flavonoid substances like hesperidin and naringin in fending off reactive oxygen species (ROS) when their levels rise in the body. According to some research, these substances have an advantage over antioxidants since they prevent oxidative stress by maintaining physiological levels (Buchner *et al*., 2020; Abdul-Majeed and, Rahawi,2022). The double bonds in unsaturated fats found in cell membranes as well as a number of cell organelles and bodily organs are all targets of ROS generation. This can result in oxidative stress, raise (MDA) levels, damage proteins and genetic material, as well as produce carcinogenic substances such 8-hydroxy-2-deoxyguanosine (8-OHdG), which can cause mutations. These elements each have the ability to activate different transcription factors and cause cell death (Jiang *et al*., 2019). Naringin functions as an antioxidant by regulating and lowering the depletion of antioxidants like SOD, CAT, GSH, and GPX in the liver (Thangavel *et al*., 2012 ; Amen *et al*.,2023), which is in light of the significance of antioxidant enzymes and their preservation. Nitric oxide (NO-), one of the main oxidizing agents, is also decreased by naringin (Wang *et al*., 2017). Furthermore, when administered to rats at a rate of 200 mg/kg body weight, hesperidin serves as a strong free radical scavenger, avoiding oxidative stress and stopping the loss of antioxidants like SOD, CAT, and GSH in the liver and immunological organs (Estruel-Amades *et al*., 2019).When hesperidin and naringin were added to the diet of white leghorn chickens at rates of 0.5 and 4 g/kg feed, respectively, Lien *et al*. (2008)observed a significant rise in SOD concentration in serum and a significant drop in MDA levels in serum.

Table (2): Addition of different levels of hesperidin and naringin in some antioxidant parameters and Testosterone hormone of aged broiler breeder males.

Groups	Traits			
	GOT (IU/L)	GPT (IU/L)	MDA (μmol/mol)	GSH (μmol/mol)
G1	165.40 ± 3.05 ^{a}	42.80 ± 0.44 ^{a}	29.39 ± 1.48 ^{a}	5.43 ± 0.30 ^{e}
G2	134.20 ± 3.38 ^{d}	34.80 ± 1.31 ^{d}	11.76 ± 2.09 ^{c}	10.85 ± 0.81 ^{ab}
G3	146.60 ± 2.31 ^{b}	40.70 ± 1.47 ^{b}	18.45 ± 2.56 ^{b}	8.78 ± 0.37 ^{d}
G4	138.60 ± 1.31 ^{c}	37.10 ± 0.84 ^{c}	11.28 ± 0.77 ^{c}	10.19 ± 0.41 ^{bc}
G5	133.40 ± 2.88 ^{d}	34.40 ± 1.46 ^{d}	12.70 ± 1.56 ^{c}	9.76 ± 0.92 ^{c}
G6	129.00 ± 2.21 ^{e}	31.10 ± 0.72 ^{e}	8.58 ± 0.76 ^{d}	11.16 ± 0.90 ^{a}

Different letters in the same column denote significant differences in the means of the treatments.

• T1= NC without addition, T2= PC (IU20 vat. E/kg feed), T3= HS1 (250 mg of hesperidin /kg feed), T4= HS2 (500mg of hesperidin /kg feed), T5= NA1 (250 mg of naringin / kg of feed), T6= NA2 (500 mg of naringin /kg feed).

CONCLUSIONS

Conclusions derived from the study's findings imply that the addition of hesperidin and naringin enhanced the birds' immunological capacities. This improvement was followed by a possible decline in glucose and cholesterol levels, which may have resulted from an increase in liver functioning and antioxidant status. These benefits were on par with vitamin E influence, and in some situations, they may have even been greater.

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CONFLICT OF INTEREST

The researchers provide evidence that this work does not interfere with other people's interests.

بعض المعايير الدموية والكيموحيوية لديكة امهات فروج اللحم ROSS-308 تحت مستويات مختلفة من الهسبردين والنارنجين

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الخلاصة

صممت هذه الدراسة لبيان تأثير استخدام مستويات مختلفة من النارنجين والهسبردين في بعض المؤشرات الدمية والكيموحيوية وحالة مضادات الاكسدة في ديكة امهات فروج اللحم، ولتحقيق هذا الهدف وزعت الطيور عشوائيا الى ست معاملات وبخمس مكررات كما يأتي: مثلت المعاملة الاولى معاملة السيطرة بدون أي اضافة للعلف (NC) , في حين زودت المعاملة الثانية بفيتامين E 20IU لكل كغم علف (PC), واضافة (250ملغم/كغم علف) من الهسبردين والنارنجين للمعاملتين الثالثة والخامسة على التوالي (HS1) و (NA1) ، وعوملت المعاملتين الرابعة والسادسة بإضافة (500ملغم/كغم علف) من الهسسبردين والنارنجين HS2 وNA2على التوالي. ليتم دراسة صفات الدم وبعض الصفات الكيموحيوية المعبره عن تقييم مناعة الطيور ونشاط الكبد ومقدار حالات التضرر التاكسدي. اظهرت المعاملة بالنارنجين NA2 علف تفوق معنوي في MCH وMCV مقارنة بمعاملات HS1&HS2 و NC و PC. سجلت PC ارتفاع معنوي في تركيز الهيموغلوبين Hb و PCV وTRBC مقارنة بالمعاملات الاخرى، انخفضت خلايا الدم البيض في معاملات HS2و NA2 مقارنة مع معاملة NC. ادت المعاملة بالهسبردين والنارنجين اثر خافض لمستويات المالونداي الديهايد فضلا عن ذلك سجلت HS2 و NA2 تحسن في معايير الاكسدة والاختزل كان بشكل انخفاض معنوي لنشاط الانزيمات الناقلة لمجموعة الامين مع ارتفاع مستويات الكلوتاثايون. يمكن الاستنتاج من بيانات هذه الدراسة ان اضافة الهسبرين والنارنجين حسنت من حالة الطيور المناعية قد يكون ناجم عن تحسن في حالة مضادات الاكسدة ووظائف الكبد التي كانت مماثلة لتاثير فيتامين E ان لم تتوفوق عليه.

الكلمات المفتاحية: الهسبردين، النارنجين، الدم، مضادات الاكسدة، الهرمونات.

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