Effect of green coffee, green tea, cinnamon and rosemary extracts on productive performance, feeding behavior, immunity and oxidative stress in broilers suffering heat stress

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Keywords: Heat stress, Green coffee extract, Green tea extract, Cinnamon extract, Rosemary extract, herbal extracts, productive performance, feeding behavior, oxidative stress.

Abstract

Global warming and adverse environmental temperature changes became one of the most harmful challenges facing the poultry industry, especially broilers, due to their fast growth rate, high metabolic rate, and increased internal temperature. In this study, 1250 Cobb 500 one day old chicks were used to evaluate the effects of different herbal extracts (Green coffee (GC), Green tea (GT). Cinnamon (CV), and Rosemary extracts (SR)) on productive performance,carcass traits,cellularimmunity, lipid markers and oxidative stress under prolonged heat stress for 12 hours daily all-over experimental period. All extracts were used at the same inclusion rate of 3 kg per ton. All herbal extracts improved body weight at 21 days and 42 days age. Similarly, total weight gain, total feed conversion ratio were improved by supplementation. On contrary control and rosemary groups consumed higher feed followed by cinnamon group, while green coffee and green tea groups had significantly lowest. Mortality was significantly reduced; however, green coffee and green tea showed better results than cinnamon and rosemary extracts. Carcass traits and dressing, breast, shoulder, and left filet percentages also improved by herbal extract supplementation. The abdominal fat percentage offered a significant reduction in treated groups, especially those that received green tea extract followed by green tea cinnamon and rosemary extracts, which showed a substantial increase under heat stress conditions with significant improvement in phagocytic index and activity. Antioxidant parameters, lipid profile, kidney and liver function, acute phase proteins were improved considerably by herbal extracts supplementation, which indicates the desirable role of herbal extracts during heat stress. It was remarkable that green coffee and green tea showed the most significant improvement in most studied parameters.

# Introduction

Chickens are sensitive to high ambient temperature as they have higher internal body temperature and no sweat glands. So, with the progressive climatic changes and the global increase in the ambient temperature became a strict and severe challenge facing the poultry industry as the increased temperature results in metabolic, hormonal, and immune system changes in birds bodies when they are unable to dissipate sufficient body heat to the environment ([Saeed et al., 2019](#_ENREF_35)). Increases in environmental temperature and relative humidity are critical components in the phenomenon of heat stress as increased humidity would hinder the bird's ability to lose body heat through evaporation. The thermo-neutral zone for chickens is 16 to 25 ̊C in which birds can show the highest performance; when the surrounding temperature begins to exceed those limits, causing heat injury ([El-Kholy et al., 2018](#_ENREF_24); [Abd El-Hack et al., 2020](#_ENREF_1); [Ilmiawati et al., 2020](#_ENREF_28)). Heat stress has a very adverse effect on productive performance, including feed intake, which will reduce body weight and growth rate. In layers, it affects egg production and quality. The incidence of eggs without shells increased in summer, and hot climates and high mortalities may occur, besides the other harmful phenomenon that occurs in heat stress such as cannibalism ([Barrett et al., 2019](#_ENREF_11)). Gastrointestinal health and performance are also adversely affected by heat stress. The integrity of intestinal enterocytes and their tight junctions is affected negatively by heat stress ([Saeed et al., 2019](#_ENREF_35)). Subsequently, the intestine's immune response due to microbial invasion would cause inflammation that influences absorption and productivity ([Zhang et al., 2017](#_ENREF_45)). The chicken immunity also affected by heat stress as many studies reported a decrease in spleen and thymus weight in hens subjected to heat stress ([Ghazi et al., 2012](#_ENREF_27)), with reducing lymphoid organs weight had also reported in broilers under heat stress ([Niu et al., 2009](#_ENREF_31)). Reduction in circulating antibodies is also said in birds under chronic heat stress and lower specific IgM and IgG levels, either during primary or secondary humoral responses ([Bartlett and Smith, 2003](#_ENREF_12)). The reduction of broilers' productivity under heat stress as a sequence of hindered feed intake and growth rate and digestibility results in high losses for the broiler industry ([Awad et al., 2020](#_ENREF_9)).

A robust immune system with the maximum ability and healthy gut is required to maximize broilers' producing capacity as they are directly related to broilers' health and productivity. Many studies discussed available strategies to control heat stress. One of the suggested approaches to solve the drastic effects of heatstroke is changing the rearing system to control the growth rate and minimize exposure to harmful pathogens during heat stress periods ([Abo Ghanima, 2020](#_ENREF_3)). However, nutritional manipulation and dietary supplementation seem to be the most feasible approach applied mostly in the poultry production field to restore performance during heat stress. Many feed additives had been used for controlling heat stress. Minerals, vitamins, prebiotics, and probiotics were all studied and used to reduce heat stress effects; however, herbal extracts were the most recent items that looked in their desired products to lower and control heat stress drastic results ([Tang et al., 2018](#_ENREF_39)). Because of the role of heat stress in oxidative stress, the need for using potent antioxidants prevents the formation and liberation of free radicals and then reduces oxidative stress. In this term, the use of antioxidants as vitamins, especially vitamin E and C, minerals as selenium and zinc, prebiotics as mannan-oligosaccharides and fructo-oligosaccharides, and the newest approach of using dietary supplementation of herbal extracts which possess potent antioxidant, immunostimulant, growth promoter, improve gut health and improve liver and renal functions with low cost compared to other additives ([Wan et al., 2017](#_ENREF_42)). Black seeds, Thyme, Carvacrol, Artemisia annua, rosemary, moringa, Dill, Red pepper, Chicory, Fennel, Radix bupleurum and Ginger, were of the most frequently studied herbals in heat stress control and use of such herbal extracts as nutritional additives showed noticed an improvement in performance, immunity and health status of heat-stressed birds ([Abd El-Hack et al., 2020](#_ENREF_1))

The potent antioxidant properties and antimicrobial activity of green coffee extract may be the right choice in controlling the drastic influence of heat stress. Green coffee extract's supplementation gave protective effects from oxidative stress ([Parvathy et al., 2018](#_ENREF_34)). Similarly, green tea extract reported having many desirable results in the broiler production, attributed to the antioxidant effect of its polyphenolics content, including catechin, epicatechin, epigallocatechin, gallocatechin, and epigallocatechin gallate. That is having an ([Aydogan et al., 2018](#_ENREF_10)). The lipid-lowering and potent antioxidant activity of green tea extract components and their ability to improve oxidative stress and improve growth rate may positively impact relieving heat stress ([Abo Ghanima, 2020](#_ENREF_3)). The current study aimed to evaluate and compare the effects of different herbal extracts (green coffee extract, green tea extract, cinnamon extract, and rosemary extract) in controlling the impact of hearing stress in broilers through evaluating their effects on growth performance, carcass traits, cellular immunity, serum biochemical parameters, and antioxidant enzymes and some serum metabolites.

# Materials and methods

All the investigations in the current study were carried out following the Native Experimental Animal Care Committee and approved by the Ethics of the Institutional Committee of Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Damanhour University, Egypt (DMU/VetMed-2019-/0145).

* 1. *Experimental birds, design and management*

A total of 1250 one day old Cobb 500 chicks (Brought from El Wataniya Hatcheries, Km 56 Cairo – Alexandria desert road - Alexandria - Egypt) were used for the study. Birds were allotted randomly into five equal groups each of 250 bird; the first group considered as a control group, the second supplemented with green coffee extract (GC) (10 ~ 50 Chlorogenic Acid Powder- Manufactured by Qingdao BNP BioScience Co., Ltd. – Shandong –China) at a rate of 3 kg per ton ration, the third group supplemented with green tea extract (GT) (polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline),-Manufactured by Herblink Biotech Corporation – Shaanxi – China) at inclusion rate 3 kg per ton ration, the fourth group supplemented with cinnamon extract (CV) (Eugenol and Cinnamaldehyde -Manufactured by Marudhar Foods Private LTD. – Maharashtra – India) at inclusion rate 3 kg per ton ration and the fifth group supplemented with rosemary extract (SR) (10%-20% Carnosic acid oil, 10%-95%- Carnosic acid, 5%-30% Rosmarinic acid, 25%-98% Ursolic acid-Manufactured by AHA Create LLC – Hawaii – United states) at inclusion rate 3 kg per ton ration. The dose of green coffee extract, green tea extract, cinnamon extract, and rosemary extract was according to the manufacturer's instructions, as well as, according to [El-Deek et al., (2012](#_ENREF_22)); [Ozcelik et al., (2014](#_ENREF_33)) and [YANG et al., (2019](#_ENREF_44)). Each group was subdivided into five equal replicates (n=50). All birds brooded at 33 °C, then decreased by three °C weekly till they reach 24 °C then maintained at that rate. All groups were subjected to heat stress for 12 hours daily by increasing house temperature 8 ± 2 °C (10:00 am to 10:00 pm daily) using an electric heater, while relative humidity was kept at 47 % ±  3.

Table 1: Basal diet formulation

|  |  |  |
| --- | --- | --- |
| Chemical analysis | Starter (1 day – 21 day) | Grower (22 – 42 day) |
| CP % | 23.12 | 20.99 |
| ME (Kcal / kg) | 3001 | 3180 |
| Calcium % | 0.99 | 0.89 |
| Potassium % | 0.54 | 0.52 |
| Available phosphorus | 0.51 | 0.46 |
| Digestible methio + Cys % | 0.93 | 0.89 |
| Digestible Lysine % | 1.43 | 1.24 |
| Digestible Methionine % | 0.59 | 0.52 |
| Digestible Arginine % | 1.25 | 1.07 |
| Digestible Tryptophan % | 0.19 | 0.17 |
| Composition: |
| Yellow Corn % | 54.1 | 58.7 |
| Soya bean meal (44% protein) % | 34.5 | 29.5 |
| Corn germ (60 % protein) % | 5.5 | 5.5 |
| Di calcium phosphate % | 2 | 1.75 |
| Lime stone % | 1.08 | 0.95 |
| Soya oil % | 1.8 | 2.3 |
| Na CL % | 0.3 | 0.3 |
| Lysine (98 %) % | 0.29 | 0.24 |
| Methionine (88%) % | 0.2 | 0.18 |
| Premix % | 0.3 | 0.3 |

Each kg of diet provides: Vit. A: 12,000 IU, Vit. D3: 5000 IU, Vit. E: 130.0 mg, Vit. K3: 3.61 mg, Vit. B1: 3.0 mg, Vit. B2: 8.0 mg, Vit. B6: 4.95 mg, Vit. B12: 0.17 mg, Niacin: 60.0 mg, Folic acid: 2.08 mg, D‐Biotin: 200.0 mg, calcium D‐Pantothenate: 18.33 mg, Copper: 80.0 mg, Iodine: 2.0 mg, Selenium: 150.0 mg, Iron: 80.0 mg, Manganese: 100.0 mg, Zinc: 80.0 mg, Cobalt: 500.0 mg. 2 Calculated according to ([Council, 1994](#_ENREF_16))

* 1. *Productive performance, feeding behavior and carcass traits*

Bodyweight of 21 and 42 days old bird and total feed intake were recorded per replicate. Total body weight gain (daily weight gain) was calculated by subtracting day old weight from the 42-day old weight. The total feed conversion ratio was calculated as total feed/total gain. Before slaughter, birds were deprived of feed for 12 h and then weighed. Five birds from each replicate slaughtered, scalded, wet plucked, eviscerated, and inner organs (Liver and gizzard, heart and spleen) weighted separately. The abdominal fats in the pelvic and abdominal cavity were collected entirely from the carcass and then weighed. The carcass was separated into cuts, including the breast (breast muscles with the sternum), thigh (average of two thighs weight), and left fillet (the de-skinned left breast muscle on the left side of the sternum), and each was weighed. Total mortality was recorded as the sum of all week's mortalities for each replicate. Feeding behavior evaluation was carried out by the total amount of feed consumed according to Clayton D. A. (1987).

* 1. *Blood biochemistry*

Two blood samples were separately collected from 5 birds from each replicate in separate labeled centrifuge tubes from the wing vein at 42 days of age—one for hematological analysis ([Dein, 1986](#_ENREF_19)), phagocytic index, and activity ([Kawahara et al., 1991](#_ENREF_29)). The other tube was left to clot and then centrifuged at 4500× g for 15 min. The serum samples were collected and preserved in a deep freezer at (−20 °C) until the analysis. total lipids (TL), triglycerides (TG), total cholesterol (TC), urea, creatinine, Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) were spectrophotometrically determined (Spectronic 1201; Milton Roy, Ivyland, PA, USA) using commercial kits (Biodiagnostic Co., Egypt). The Uric acid assay performed using the Sigma-Aldrich uric acid assay kit (Sigma-Aldrich, USA). Malondialdehyde (MDA), Glutathione peroxidase (GPx), Superoxide dismutase (SOD), Catalase activity, and Lactate dehydrogenase LDH activity assessed using the ELISA Kit (Quanti ChromTM, BioAssay Systems, USA) and (Cayman Chemical Company, USA). ELISA kits specific for chicken CRP (C-reactive protein), SAA (serum amyloid A), and TRF (Transferrin) (Wuhan Fine Biotech Co., Ltd., East Lake High-tech Development District, Wuhan, Hubei Province, China) used according to the manufacturer's instructions.

* 1. *Tissues biochemistry*

Meat sample collection, storage, and preparation of extract were done according to [Folch et al., (1957](#_ENREF_26)). Estimation of meat triacylglycerol and cholesterol content was determined using specific kits of Biodiagnostic Co., Egypt.

* 1. *Statistical analysis*

Data were analyzed using SPSS software version 20.0, by one-way analysis of variance, general linear model, with the following model: Xij = µ + Ai + eij, where: Xij = an observational data; µ Overall mean; Ai Effect of ith treatment; eij Random error.

# Results

* 1. *Growth performance, feeding behavior, mortality, and carcass traits*

Growth performance was affected by the supplementation of herbal extracts during heat stress (Table 2). Live body weight at 21 days old was significantly increased in the green coffee group without significant difference with green tea and rosemary extracts groups, and it was considerably higher than cinnamon, which in turn showed a substantial increase over the control group (928.40 vs. 917.80 and 910.60 vs. 903.40 vs. 866.60 g, respectively). Final live body weight at 42 days old showed similar results; however, green tea showed the highest body weight without significance than green coffee and cinnamon but significantly higher than rosemary extracts, which in turn showed considerable improvement over the control group (2362.00 vs. 2337.60 and 2342.80 vs. 2327.40 vs. 2304.40 g, respectively) and total weight gain showed the same findings. Control and SR groups consumed higher feed followed by cinnamon group, while green coffee and green tea groups had significantly lowest feed intake (3734.60 and 3719.00 vs. 3683.20 vs. 3606.80 and 3619.60 g, respectively). Significant improvement by reducing total feed conversion ratio (Total FCR) was reported in green coffee and green tea groups, followed by cinnamon. Simultaneously, control and rosemary extracts showed the highest FCR (1.57 and 1.56 vs. 1.60 vs. 1.65 and 1.63, respectively).

The mortality percentage highly reduced by nutritional supplementation of herbal extracts. The most significant reduction in mortalities was represented in green coffee and green tea supplementation concerning cinnamon and rosemary extracts. They had reduced considerably mortalities than the control group (2.00 and 2.00 vs. 3.20 and 2.80 vs. 6.40 %, respectively).

Green coffee supplemented groups revealed the highest dressing percentage than green tea and cinnamon. They were significantly higher than rosemary extracts, which showed a significant increase than control group (75.27 vs. 74.10 and 73.41 vs. 71.58 vs. 68.83 %, respectively). Breast, shoulder, and left filet percentages showed similar findings. The liver, gizzard, heart, thigh, and spleen percentages showed no significant herbal medication affection with the abdominal fat percentage had significantly reduced by treatment. The green coffee group had the lowest rate, substantially different from green tea and cinnamon than rosemary extracts. They were considerably lower than control group (1.26 vs. 1.48 and 1.62 vs. 1.97 vs. 2.79 %, respectively).

Table 2: Effect of herbal extracts on productive performance and carcass traits of broilers subjected to heat stress.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters  | Control | G.C | G.T | C.V | S.R | P value |
| 0 day weight (g) | 42.20±0.22 | 41.92±0.10 | 42.02±0.34 | 41.92±0.20 | 41.86±0.12 | 0.813 |
| 21 day weight (g) | 866.60±10.28c | 928.40±5.97a | 917.80±2.69ab | 903.40±1.83b | 910.60±12.32ab | ≥0.0001 |
| 42 day weight (g) | 2304.40±4.08c | 2337.60±7.53ab | 2362.00±17.96a | 2342.80±7.27ab | 2327.40±7.43b | 0.01 |
| Total feed intake (g) | 3734.60±3.61a | 3606.80±9.27c | 3619.60±12.65c | 3683.20±5.45b | 3719.00±7.75a | ≥0.0001 |
| Total weight gain (g) | 2262.20±4.18c | 2295.68±7.59ab | 2319.98±18.05a | 2300.88±7.16ab | 2285.54±7.48b | 0.010 |
| Total FCR | 1.65±0.01a | 1.57±0.01c | 1.56±0.01c | 1.60±0.00b | 1.63±0.01a | ≥0.0001 |
| Mortality % | 6.40±0.31a | 2.00±0.19c | 2.00±0.10c | 3.20±0.17b | 2.80±0.15b | ≥0.0001 |
| Dressing % | 68.83±0.23d | 75.27±0.49a | 74.10±0.25b | 73.41±0.18b | 71.58±0.50c | ≥0.0001 |
| Liver % | 4.03±0.15 | 4.19±0.14 | 4.23±0.15 | 4.18±0.18 | 3.98±0.05 | 0.651 |
| Gizzard % | 2.94±0.11 | 2.92±0.14 | 2.91±0.10 | 2.90±0.12 | 2.91±0.11 | 1.01 |
| Heart % | 0.84±0.03 | 0.88±0.03 | 0.91±0.02 | 0.89±0.03 | 0.85±0.03 | 0.347 |
| Spleen % | 0.19±0.02 | 0.20±0.02 | 0.20±0.01 | 0.19±0.02 | 0.21±0.02 | 0.927 |
| Abdominal fat % | 2.79±0.11a | 1.26±0.05d | 1.48±0.15c | 1.62±0.19bc | 1.97±0.14b | ≥0.0001 |
| Breast % | 23.30±0.71b | 29.14±0.45a | 27.80±0.90a | 28.01±0.89a | 24.51±0.63b | ≥0.0001 |
| Thigh % | 15.52±0.48 | 16.17±0.89 | 16.31±0.58 | 16.66±0.70 | 15.77±0.52 | 0.758 |
| Shoulder % | 3.75±0.29b | 4.93±0.14a | 4.32±0.36ab | 4.80±0.19a | 3.71±0.25b | 0.007 |
| Left filet % | 9.67±0.35b | 11.83±0.20a | 11.02±0.57a | 11.79±0.46a | 9.52±0.35b | 0.001 |

Means ± SEM displaying different superscript letters are significantly (p < 0.05) different from the other values within the same raw (between groups), *n*=25. Green coffee extract (GC) Green tea extract (GT) Cinnamon extract (CV) Rosemary extract (SR.)

* 1. *Meat and serum lipid profile, oxidative stress parameters, and serum metabolites*

Table 3 revealed that inclusion of herbal extracts in broiler feed had significantly reduced meat cholesterol content as green coffee and green tea supplemented birds had the lowest levels in meat extract than cinnamon and rosemary extracts. Cinnamon and rosemary extracts were lower than control but without significance (85.05 and 77.09 vs. 111.15 and 128.50 vs. 141.20 mg/dl, respectively). Similarly, meat triacylglycerol showed a significant reduction in green coffee and green tea supplemented birds had the lowest meat extract levels than cinnamon and rosemary extracts, which were lower than control but without significance (69.36 and 74.11 vs. 105.15 and 104.07 vs. 115.56 mg/dl, respectively).

The serum lipid profile had significantly affected by dietary supplementation of herbal extracts. Serum total lipids, triacylglycerol, and cholesterol showed a significant reduction in green tea and green coffee supplemented birds followed by cinnamon and rosemary extracts groups, and they showed significantly lower levels than the control group (330.68 and 351.30 vs. 490.79 and 513.93 vs. 683.89 mg/ dl, respectively)

Serum urea level showed a significant reduction in green coffee, green tea, and rosemary extracts supplemented birds than cinnamon, which was significantly lower than control group (5.07, 5.08 5.16 vs. 5.36 vs. 5.76 mmol/L, respectively). Serum creatinine in green coffee showed the lowest level than green tea, cinnamon, and rosemary extracts, and they were significantly lower than control (0.36 vs. 0.42, 0.44 and 0.43 vs. 0.62 mmol/L, respectively). ALT and AST had significantly affected by herbal medication as they were greatly reduced in treated groups than control groups. ALT findings were lowest in green coffee, and green tea than cinnamon and rosemary extracts than control (17.00 and 18.60 vs. 20.20 and 20.13 vs. 27.20 U/L, respectively). AST was lowest in green coffee and green tea than cinnamon and rosemary extracts than control (80.60 and 82.00 vs. 94.60 and 89.20 vs. 114.00 U/L, respectively)., LDH, and MDA activity showed a significant reduction in green coffee and rosemary extracts as well as a substantial decrease in green tea, and cinnamon groups as shown in table 3.

The green coffee and rosemary extracts treated group showed a markedly increase in GPx and SOD activity, followed by green tea and cinnamon group. In contrast, herbal treated groups showed a significant decline in catalase activity. The C. reactive protein showed a significant decrease in green coffee and green tea followed by cinnamon and rosemary extracts concerning control-treated one, as well as Serum amyloid was markedly decreased in all herbal treated groups relating to control one. Transferrin was significantly reduced in the green coffee group, followed by the green tea, cinnamon, and rosemary extracts group concerning the control one.

* 1. *Immunity markers and hematological picture*

Table 4 showed that there was a significant increase in phagocytic index and activity in green coffee, cinnamon, and rosemary extracts group flowed by green tea concerning control one, there was a substantial increase in total leukocytic count accompanied by marked eosinophilia in green coffee, and green tea followed by cinnamon, and rosemary extracts concerning control-treated one.

Table 3: Effect of herbal extracts on Meat and serum biochemical parameters and serum metabolites and oxidative stress enzymes of broilers subjected to heat stress.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters  | Control | G.C | G.T | C.V | S.R | P value |
| Meat cholesterol | 141.20±5.39a | 85.05±9.02c | 77.09±5.02c | 111.15±7.77b | 128.50±3.10ab | ≥0.0001 |
| Meat triacylglycerol | 115.56±6.23a | 69.36±5.06b | 74.11±6.31b | 105.15±3.73a | 104.07±5.89a | ≥0.0001 |
| Serum total lipids | 683.89±18.54a | 330.68±25.94 c | 351.30±39.03 c | 490.79±26.06 c | 513.93±27.72 b | ≥0.0001 |
| Serum triacylglycerol | 185.44±6.26a | 122.14±4.77c | 121.22±3.87c | 143.34±7.85b | 148.08±9.34b | ≥0.0001 |
| Serum cholesterol | 171.14±5.74a | 105.96±5.25c | 109.61±4.52c | 132.19±5.63b | 139.84±5.62b | ≥0.0001 |
| Urea | 5.76±0.05a | 5.07±0.06c | 5.08±0.04c | 5.36±0.05b | 5.16±0.05c | ≥0.001 |
| Creatinine | 0.62±0.01a | 0.36±0.01c | 0.42±0.01b | 0.44±0.01b | 0.43±0.02b | ≥0.001 |
| ALT | 27.20±0.49a | 17.00±1.05c | 18.60±0.75c | 20.20±0.66b | 20.13±0.71b | ≥0.001 |
| AST | 114.00±0.71a | 80.60±0.87c | 82.00±0.45c | 94.60±1.21b | 89.20±2.35b | ≥0.001 |
| LDH | 397.60±8.84a | 320.20±2.56c | 333.00±1.55b | 340.00±1.70b | 330.00±1.14bc | ≥0.001 |
| MDA | 3.65±0.10a | 1.86±0.12c | 2.47±0.22b | 2.28±0.20bc | 1.84±0.11c | ≥0.001 |
| GPX | 16.80±0.37c | 28.80±0.86a | 22.60±1.94b | 24.20±1.80b | 25.60±1.69ab | ≥0.001 |
| SOD | 58.40±0.93c | 88.20±1.46a | 78.80±2.75b | 86.00±3.08a | 84.20±2.67ab | ≥0.001 |
| Catalase | 2.52±0.03a | 1.48±0.09c | 1.78±0.10bc | 1.88±0.17b | 1.74±0.13bc | ≥0.001 |
| CRP | 2.56±0.04a | 1.50±0.04d | 1.53±0.03d | 1.77±0.05b | 1.67±0.08c | ≥0.001 |
| SAA | 295.40±3.56a | 171.20±6.00b | 173.80±3.54b | 190.00±6.97b | 187.80±8.40b | ≥0.001 |
| TRF | 1.89±0.01a | 1.42±0.03d | 1.48±0.04c | 1.56±0.04bc | 1.62±0.03b | ≥0.001 |

Means ± SEM displaying different superscript letters are significantly (p < 0.05) different from the other values within the same raw (between groups), *n*=25. Green coffee extract (GC) Green tea extract (GT) Cinnamon extract (CV) Rosemary extract (SR)

Table 4: Effect of herbal extracts on total and differential leukocytes count and phagocytic index and broilers' activity subjected to heat stress.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Control | G.C | G.T | C.V | S.R | P value |
| WBCS | 23.60±0.07d | 24.90±0.20a | 24.55±0.15ab | 24.08±0.04c | 24.22±0.08bc | ≥0.001 |
| Phagocytic index | 1.18±0.06c | 1.72±0.04a | 1.68±0.02a | 1.48±0.02b | 1.62±0.07a | ≥0.001 |
| Phagocytic activity | 14.28±0.10d | 17.10±0.19a | 15.84±0.32b | 15.14±0.16c | 16.50±0.22a | ≥0.001 |
| Eosinophil % | 8.10±0.04c | 8.60±0.04a | 8.50±0.07a | 8.30±0.07b | 8.48±0.07a | ≥0.001 |
| Lymphocytes % | 34.96±0.14b | 36.26±0.30ab | 35.72±0.47ab | 35.92±0.62a | 36.32±0.12ab | 0.123 |
| Heterophils % | 23.34±0.12 | 23.42±0.12 | 23.36±0.12 | 23.68±0.15 | 23.20±0.09 | 0.116 |
| Basophils % | 1.07±0.01 | 1.10±0.02 | 1.07±0.02 | 1.10±0.01 | 1.08±0.00 | 0.199 |
| Monocytes % | 5.36±0.11 | 5.52±0.12 | 5.40±0.13 | 5.38±0.04 | 5.58±0.09 | 0.483 |

Means ± SEM displaying different superscript letters are significantly (p<0.05) different from the other values within the same raw (between groups), *n*=25. Green coffee extract (GC) Green tea extract (GT) Cinnamon extract (CV) Rosemary extract (SR).

# Discussion

The reported results in the current investigation had revealed that supplementation of herbal extracts had a significant influence on controlling and reducing the drastic effects of heat stress. Using herbal extracts ad feed additives in broiler ration had resulted in restoring birds' productive ability as body weight at 21 and 42-day ages, and total weight gain had significantly improved by Herbal extract treatment. In contrast, total feed intake and FCR had significantly reduced than control groups. Groups supplemented with green coffee and green tea extracts showed better results than cinnamon and rosemary extracts groups. The reduction in the control group's productivity may be attributed to the adverse effects of heat stress, causing a decrease in enterocytes' integrity. As a sequence, microbial invasion results in enteritis, impairs digestibility, and absorption and interferes with nutrient availability for broilers([Zhang et al., 2017](#_ENREF_45)). The changes that occur in blood circulation patterns during exposure to heat stress, especially for prolonged periods and changes that occur in blood pH, might also be a primary cause for growth depression during heat stress ([Cronje, 2005](#_ENREF_18)).

The antioxidant capacity of herbal extracts and their ability to restore gut function by increasing enterocyte cell wall integrity and reduce invasion of microbes and the formation of free radicals, besides their antimicrobial role, may explain their desirable impact on the performance of birds during heat stress([Saeed et al., 2019](#_ENREF_35)). A healthy intestine promotes the growth of beneficial bacteria, and in turn, increased enterocytes, absorption, and detestability improved, improving nutrient availability resulting in higher growth rate and better feed conversion ([Castillo et al., 2006](#_ENREF_13); [Wati et al., 2015](#_ENREF_43); [Shewita and Taha, 2018](#_ENREF_37); [Taha et al., 2019](#_ENREF_38)). Carcass characteristics were also enhanced by dietary supplementation of herbal extracts as dressing, breast, shoulder, and left filet percentages showed significant improvement in treatment in harmony with ([Ashour et al., 2020a](#_ENREF_7)) that proved the enhancing effect of the herbal extract on the carcass. At the same time, abdominal fat was significantly reduced, especially in the green tea and green coffee supplemented groups. The decline that occurred in muscle mass in the control group may be attributed to the effect of heat stress as it results in an increase in protein susceptibility to proteolysis over 32 °C ([Saeed et al., 2019](#_ENREF_35)), and that would result in a reduction of protein deposition in muscles, causing a decrease in muscle mass. The role of the examined herbal extracts in restoring and improving dressing and other carcass traits may be due to its antioxidant, immune-supporting and growth and health-promoting effects as these effects would result in an increase in digestive enzymes, improvement of gut health, and increased immunity and improve digestibility ([Mountzouris et al., 2011](#_ENREF_30); [Al-Sultan et al., 2019](#_ENREF_5); [Ashour et al., 2020b](#_ENREF_8)) The reduction in abdominal fat in the herbal supplemented groups may be attributed to the lipid-lowering effect of the ingredients of those herbal extracts as a sequence of their ability to increase lipolysis rate and inhibit lipogenesis process([Orlowski et al., 2018](#_ENREF_32))

The serum lipid profile had significantly reduced by dietary supplementation of herbal extracts: especially green coffee and green tea. Our result was inconsistent with ([El-Hack et al., 2020](#_ENREF_23); [Ilmiawati et al., 2020](#_ENREF_28)). They found that green coffee has a significant declined effect on cholesterol and triglyceride, and ([Cho et al., 2010](#_ENREF_15)) suggested that green coffee reduces triglyceride and cholesterol concentrations. Increases attributed this to the lipogenesis effect of green coffee ([Ding et al., 2020](#_ENREF_20)). Herbal treated poultry showed significant improvement in uric acid, AST, ALT, LDH, and MDA activity in the same line. These data were in harmony with [Abd El-Hack et al., (2020](#_ENREF_1)) and [Abo Ghanima et al., (2020](#_ENREF_2)) in which they reported that cinnamon supplementation decreased the uric acid concentration, LDH, and MDA in heat-stressed birds, in which herbal supplementation improve the resistance of the body to heat stress with consequent blood purifier properties at high temperature. Our result revealed a significant improvement in the antioxidant activity, which in harmony with [Arif et al. (2019](#_ENREF_6)) and [Abd El-Hack et al. (2020](#_ENREF_1)). They reported the antioxidant activity of herbal and their impact on the progress of production performances. Besides, phytogenic nutrition supplementation plants may use the development of an antioxidant mechanism to carry out their successful health service activities; by directly breaking down ROS development linked to stress by inhibiting it by chelating with enzymes or trace metals ([Thring et al., 2011](#_ENREF_40)). Immunity parameter markers, phagocytic activity, and index supported the beneficial role of the tested herbal supplemented. This finding was in the same with [Farahat et al. (2016](#_ENREF_25)) and [Seidavi et al. (2017](#_ENREF_36)). They reported that green tea had been shown to have antioxidant and immunostimulant properties. Concerning the WBCS, the herbal supplementation accompanied by leukocytosis and eosinophilia, which was in harmony with [El-Deek et al. (2012](#_ENREF_22)), they reported that green teat supplementation significantly increased the WBCs due to its immunomodulatory properties. Inflammation of poultry is followed by the systemic reaction known as the acute phase process (APR) ([Cray et al., 2009](#_ENREF_17)). The APR is a specific marker of the innate immune to inflammation ([Cray et al., 2009](#_ENREF_17)). These proteins can destroy infectious bacteria and restore health ([Eckersall and Bell, 2010](#_ENREF_21)). APPs, which include C-reactive protein (CRP), serum amyloid A (SAA), and Transferrin (TRF) ([Chamanza et al., 1999](#_ENREF_14)). The amount of the APPs increase within the body due to developing inflammation ([Urieli-Shoval et al., 2000](#_ENREF_41); [Adler et al., 2001](#_ENREF_4)). This finding was in harmony with our study in which C. reactive protein, serum amyloid, and transferrin showed a significant decrease in green coffee and green tea followed by cinnamon and rosemary extracts concerning control-treated one

# Conclusion

 Herbal feed additives improve poultry production and performance under normal and under heat stress. Our finding revealed that daily intake of herbal feed additives reduces the adverse impacts of heat stress by increasing the productive performance and FCR, improving the liver and kidney function immunity (Phagocytic index), and antioxidants activity (GPx, Catalase, and SOD). The C. reactive protein, serum amyloid, and transferrin showed a significant decrease in green coffee and green tea followed by cinnamon and rosemary extracts concerning control-treated one and decreasing the abdominal fat with the improvement of the general health status of the bird under heat stress conditions.

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**Graphical abstract**