Blood Biochemical Components, Antioxidant Status Biomarkers, Lipid peroxidation, Productive Performance and Carcass Characteristics of Broiler Chicks Supplemented with Alpinia galangal Rhizomes Extract during Heat Stress

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ABSTRACT

Aim: The present study aimed to explore the effects of different levels of dietary supplementation of Alpinia galangal rhizomes extracts (AGRE) on blood biochemical components, antioxidant status biomarkers, lipid peroxidation, productive performance (growth performance and mortality rate), and carcass characteristics of broilers under heat stress conditions (temperature-humidity index 87.5 – 93.5).

Materials and Methods: One day old Cobb broilers (n = 1000, body weight mean (44,58 g) were randomly allotted to four dietary groups. The control group was fed a basal diet without AGRE; the experimental groups received the basal diet with 250, 500 and 750 mg AGRE/kg (groups 250 AGRE, 500 AGRE, and 750 AGRE, respectively). The experimental period lasted for 6 weeks.

Results: In groups, 500 AGRE and 750 AGRE, plasma total protein, albumin, and globulin were significantly (P=0.0001) increased. In contrast, all supplementation levels of AGRE reduced (P=0.022, 0.0001, 0.007 and 0.0024) the plasma concentrations of total lipids, total cholesterol, triglycerides, and low-density lipoproteins, respectively. Antioxidant enzymes of broilers; superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and total antioxidant capacity in the blood were increased (P=0.0005, 0.05, 0.03, 0.02 and 0.0001) by adding dietary AGRE, respectively. However, malondialdehyde was reduced (P=0.0001) with increasing AGRE levels. Compared with other groups, chickens of group 750 AGRE had the best body weight gain and feed conversion ratio and the lowest mortality. Carcass traits cut up parts and yields significantly increased for chicks fed different levels of AGRE, Also group fed on AGRE recorded the highest abdominal fat compared with control group. Additional, liver, heart and gizzard weight was increased significantly in the group of chickens that received different levels of AGRE as compared with the control group. Ascending levels of AGRE treatments influenced the carcass meat, which was observed an increase in crude protein contents (P=0.004), increase in moisture and ether extract contents (P=0.0013 and 0.004) in breast meat compared to control group, respectively. However, Ascending levels of AGRE treatments caused downward in crude protein contents (P=0.004) of thigh meat compared to the control group.

Conclusion: AGRE can be considered as a rich source of phenolic and flavonoid compounds. The results of the study revealed that all tested levels of AGRE were useful as natural protection against heat stress to maintain performance, carcass traits and antioxidant status and could reduce the negative effects of heat stress in broilers.

Keywords: Alpinia galangal, antioxidant, blood, broiler performance, carcass, heat stress.

Introduction

In tropical and sub-tropical regions like Egypt, broiler chickens are faced with heat stress, which has negative effects on their homeostasis and affects their growth rate and production. Heat stress is also reflected in various physiological parameters and causes huge economic losses [1].

The adverse effect of heat stress affects broiler performance and causes an increase in oxidative stress [2, 3], which can impede disease resistance and impairs antioxidant status. Lately, it was shown that in broilers phenolic compounds have beneficial effects related to antioxidant, anti-inflammatory and antimicrobial activities [4]. These compounds are found in herbal plants Galanga (Alpinia Officinarum) is one of the most important herbal plants in Egypt. The plant cultivates from rhizomes in bunches of rigid stems up to 2 m in height with plentiful lengthy leaves that tolerate red fruit. It is native

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to Indonesia and South Asia, and cultivated in Malaysia, Laos, Thailand, and Egypt.

Alpinia Officinarum is the galanga consumed the majority in cooking. Alpiniagalanga rhizome comprises the flavonolgalangin [5]. The rhizome comprises oil, called galangol that upon partial percolation produces cineol, which has therapeutic properties, eugenol and pinene [6]. Galanga rhizome contains approximately 50% carbohydrates, 3-6% crude fiber, and ash, 9% protein and free amino acids, and 6-8 % fatty acids and triglycerides (on dry matter basis) depending on variety, geography, and climatic conditions [7]. Galanga rhizome extract (AGRE) contains a number of volatile oil ranged about 48% methyl cinnamate, 20-30% cineole, α-pinene, β-pinene and camphor [8, 9] phenylpropanoids [10], diarylheptanoids, and Flavonoids [11, 12] have been reported so far. However, as one of the important secondary metabolites and bioactive substances in the plant, total polyphenols from galanga rhizomes was up to 13.43 mg/g under this condition as ethanol concentration 60%, extraction time 1.0 h, extraction temperature 30 C o and ratio of liquid to material 15:1[13]. These components considered are powerful free radical scavengers [14]. Galanga rhizome extract is rich in β-Sitostero, 1 Diarabinoside [15], β-sitosteroldiglucosylcaprate[16]. Galangoflavonoside, and 1-Acetoxychavicol acetate [17].The rhizome also contains flavonoids, some of which have been identified as kaemperol, kaempferide, galangin, and alpinin. So, it is considered as a better source of antioxidative constituents than another herbs plant [18]. Jammu Ginger, Curcuma, and Turmeric have been used as natural antioxidants and had positive effects on the performance of broiler chickens [19]. Therefore, Galanga rhizome extract could be attractive antioxidants and new sources of antioxidant for animal nutrition [20].

The objective of this study was to assess the effect of dietary supplementation of AGRE as a natural antioxidant on biochemical components of the blood, biomarkers of antioxidant status, lipid peroxidation, productive performance (growth performance and mortality rate), and carcass characteristics of broilers under heat stress conditions, in order to overcome the negative effects of heat stress.

Materials and Methods

Experimental design:
One thousand unsexed one-day old Cobb commercial broilers were wing-banded, weighed and randomly distributed into four experimental groups (n = 250 in each group) in a simple randomized design experiment. The control group was fed a diet without AGRE; the experimental groups were fed the same diet with 250, 500 and 750 mg AGRE/Kg (groups 250 AGRE, 500 AGRE, and 750 AGRE, respectively). The experimental period lasted for 6 weeks (from one day to 42 days of age).

Experimental animals, housing and feed:
All chicks were reared under similar environmental, hygienic and managerial conditions on four pens (1.5 m X 2 m) during starting and growing periods (0-3 and 3-6 weeks of age) under a 22: 2 h light–dark cycle. All chickens were fed crumble feed ad libitum. Feed ingredients and chemical composition of the experimental diet are shown in Table 1. The experimental diets were pelleted and formulated to meet the recommended nutrient requirements of broiler chickens according to [21]. AGRE levels (250, 500 and 750 mg) were pre-mixed with 10 kg of each diet and successively mixed into the remaining diet to obtain the homogenous inclusion level. Chickens were reared in a well-ventilated building; fresh water was automatically available all the time by stainless steel nipples fixed in each cage. In accordance with the traditional program used for broilers, the chicks were vaccinated versus prevalent broiler diseases. During the experimental period (June−July 2018), in the house, the minimum and maximum temperatures, the relative humidity, and the temperature-humidity index ranged 26.5–33.5°C, 62–75% and 87.5–93.5, respectively. That means, during the whole experimental period broilers were under severe heat stress as described by the Livestock and Poultry Heat Stress Indices [22].

Table (1): Feed ingredients and chemical composition of the experimental diet (% DM basis)

Ingredients	Diets Composition %	
	Starter Diets (0-21 d)	Grower Diets (22-42 d)
Yellow corn	62.9	67
Wheat bran	2.2	2.31
Soybean meal (48%)	15.5	12
Corn gluten (60%)	13.3	12.5
Fish meal (72%)	0.8	0.8
Meat meal (50%)	2.1	2.1
Calcium Carbonate	1.1	1.3
Dicalcium Phosphate	1	1
Premix*	0.3	0.3
Alpinia galangal	0	0
Table Salt (NaCl)	0.3	0.24
D L.Methionine	0.1	0.04
L. Lysine	0.3	0.31
Coxistate	0.1	0.1
Total	100	100
Calculated chemical composition		
Crude protein %	22.65	20.00
ME k cal / kg	3070	3150
Ether Extract %	3.42	3.61
Crude fiber %	2.53	2.69
Calcium %	1.09	1.10
P. (available) %	0.46	0.47
Lysine %	1.06	1.00
Methionine+cysteine%	0.82	0.77

*Provided the following per kg of diet:Vit. A, 1200 IU; Vit.D. 3000 IU; Vit.E, 100 IU; Vit.C, 3 mg; Vit. K, 4 mg; VitB1, 3 mg; Vit B2, 3 mg; Vit B6, 5 mg; Vit B12, 0.03 mg; Bantothinic acid, 15 mg; Folic acid, 2 mg; Biotin, 0.20 mg; Cobalt, 0.05 mg; Copper, 10 mg; Iodin, 50 mg; Manganese, 90 mg; Selenium, 0.20 mg and Zinc, 70 mg.

Preparation of Alpinia galangal rhizome extract:

Galanga plants were obtained from different regions which were grown in El-Wahat El-Bahariya, Egypt. The humidity of Galanga rhizome was reduced by sun-drying to 9-10%, and then ground by a hammer mill and kept for subsequent processing. The seeds were ground by a hammer mill to obtain a fine powder. Dry seed powder was extracted in batches of 10 g with 100 ml ethanol (70%) for 24 h at room temperature. The extract was centrifuged at 1500 g for 20 min and filtered, and then GSE was obtained as a lyophilized powder by freeze-drying and stored at 4°C until the assay according to [23]. Chemical analysis of galanga (Alpinia Officinarum) rhizome extract was detected in Table 2.

Table (2): Phenolic and flavonoids compounds of Alpinia galangal rhizomes extract

Phenolic Compounds[a]	Contents (MG/100G) %		Flavonoid Compounds[b]	Contents(MG/100G) %	
1,8-cineole	15.3	34.7	Rutin	141.1	2.3
α-fenchyl acetate	5.71	12.5	Rosmarinic acid	196.1	3.79
α-terpineol	20.02	4.24	Quercitrin	553.5	23.58
Catechin	5.9	3.81	Quercetin	679.5	32.6
p-Coumaric acid	0.21	0.29	Naringinin	921.5	34.8
Myricetin	1.5	2.16	Hespertin	131.2	0.87
Chavicolllylphenol)	25.74	30.02	Kaempferol	239.7	1.01
Methyl eugenol	8.76	1.68	Apigenin	44.9	1.05
Eugenol	4.35	3.5	Total	13.6	100
β-pinene	1	7.1			
Total phenolic	35.39	100			

Notes: [a]analyzed according to [37]; [b]analyzed according to [36].

Blood samples and determination of biochemical parameters:

For determining blood biochemical components, blood samples (5 ml from each chick) were collected during slaughter. Plasma was separated from blood by centrifugation at 1000 x g for 20 min and stored at −20°C till assayed. Plasma total protein, albumin, glucose, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL), triglycerides and total lipids were measured by a spectrophotometer (Spectronic 21 DUSA) using commercial diagnostic kits (Combination, Pasteur Lap.) according to the manufacturers' instructions. Total protein was determined according to [25]. Albumin was determined according to the method of [26]. Plasma globulin concentration was calculated by the difference between total protein and albumin, the albumin/globulin ratio was calculated. Glucose was determined according to [27]. Plasma cholesterol, LDL- and HDL-cholesterol were determined according to the method of [28]. Triglycerides were determined according to [29]. Total lipids were determined according to the method described by [30].

Biomarkers of antioxidant status and lipid peroxidation:

Blood plasma malondialdehyde (MDA) and glutathione peroxidase (GPx) activity were assayed using the method of [31]. Superoxide dismutase (SOD) activity was assayed according to [32]. Total antioxidant capacity (T-AOC) was determined according to [33], glutathione S-

transferase (GST) by [34] and catalase (CAT) activity was measured according to [35].

Slaughtering and carcass traits:

On day 42, fifty chicks were randomly selected from each pen, fasted for 12 hr before slaughtering, weighed and manually slaughtered. Carcass weight (dressing, breast, thigh, empty gizzard, liver, abdominal fat, heart) were calculated as a percentage of live body weight. Chemical analysis of meat was done according to [24] and the values were expressed on a DM basis.

Chemical analysis:

Chemical analysis of feeds was performed as recommended by [24] for determining moisture (Method 934.01), crude protein (CP, Method 942.05), ether extract (EE, Method 920.39), ash (Method 942.05), crude fibre (CF, Method 973.18), phosphorus (Method 965.17) and calcium (Method 927.02). The fractions of phenolic compounds, flavonoid compounds and scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) of the extract of galanga (Alpiniaofficinarum) rhizome extract were determined in the Micro analysis Lab., Food Technology Research Institute, Egypt by A high-performance liquid chromatographic (HPLC). Flavonoid compounds were determined by HPLC according to the method of [36], while phenolic compounds were determined by HPLC according to [37] as shown in Table 2.

The antioxidant activity of red AGRE was based on its scavenging activity to the stable DPPH free radical, which was determined mainly by the method described by [38]. The contents of phenolic and flavonoid compounds in the AGRE used in the present study are presented in Table 2. Furthermore, AGRE contained 35.39% total phenols, 13.6%, and total flavonoids. The scavenging activities of DPPH in AGRE amounted to 51.4%, 62.2% and 68.4% at concentrations of 25, 50 and 75 mg/kg, respectively.

Statistical analysis:

Data were statistically analyzed using the general linear model of SAS [39] as a completely randomized design. Differences among treatment means were estimated by Duncan's multiple range test [40]. Statement of statistical significance was based on ($p<0.05$).

Results

Plasma biochemical components

Data of plasma biochemical components showed that the concentration of plasma total protein, albumin, and globulin of groups 500 AGRE and 750 AGRE were (P=0.0001) higher than of the control group and group 250 AGRE (Table 3). However, there were no significant differences among the experimental groups regarding the albumin/globulin ratio and the concentration of plasma glucose. Plasma total lipids, cholesterol and triglycerides levels were (P=0.022, 0.0001 and0.007) decreased with increasing dietary levels of AGRE. Plasma HDL level was observed to decrease numerically by supplementing different levels of AGRE, but this effect was not significant. However, the concentration of LDL decreased (P=0.0024) with increasing dietary AGRE levels.

Table (3): Effect of Alpiniagalanga rhizome extract on plasma biochemical components of broiler

ITEMS	EXPERIMENTAL DIET				SEM[4]	*P*-VALUE
	Control	250 AGRE[1]	500 AGRE[2]	750 AGRE[3]		
Total protein (g/dl)	2.71[b]	2.79[b]	3.35[a]	3.47[a]	0.16	0.0001
Albumin (g/dl)	1.48[b]	1.54[b]	1.97[a]	2.0[a]	0.10	0.0001
Globulin (g/dl)	1.23[b]	1.25[b]	1.38[a]	1.47[a]	0.05	0.0001
Albumin/globulin ratio	1.2	1.23	1.42	1.36	0.09	0.147
Glucose (mg/dl)	178.23	177.12	177.26	176.2	3.56	0.439
Total lipids (mg/dl)	4.233[a]	3.865[ab]	3.714[b]	3.677[b]	0.13	0.022
Total cholesterol (mg/dl)	88.47[a]	83.01[b]	81.91[bc]	80.84[c]	1.57	0.0001
Triglycerides (mg/dl)	23.78[a]	20.19[b]	20.17[b]	20.19[b]	0.16	0.007
High-density lipoprotein (mg/dl)	91.1	89.07	90.19	87.45	3.24	0.842
Low-density lipoprotein (mg/dl)	50.09[a]	45.31[b]	45.26[b]	43.12[c]	0.001	0.0024

Notes[1]250 AGRE, 250 mg AGRE/Kg diet; [2]500 AGRE, 500 mg AGRE/Kg diet; [3]750 AGRE, 750 mg AGRE/Kg diet; [4]SEM, standard error of the mean.

[a–c] Means in a row not sharing the same superscript differ significantly ($p \leq 0.05$).

Biomarkers of antioxidant status

The effects of different levels of AGRE on blood antioxidant constituents of chickens are presented in Table 4. A significant, dose-

dependent decrease (P=0.001) of plasma MDA was observed in broilers fed diets supplemented with AGRE. An opposite effect was noticed regarding T-AOC, SOD, CAT, GPx, and GST, where the values were (P=0.0005, 0.05, 0.03, 0.02 and 0.0001) increased with increasing AGRE levels, respectively. As MDA is present in lipoproteins, this blood constituent decreased with increasing dietary AGRE level like other lipids, cholesterol, and triglycerides.

Table (4): Effect of Alpinia galanga rhizome extract on plasma biochemical components of broiler.

ITEMS	EXPERIMENTAL DIET				SEM[4]	*P*-VALUE
	Control	250 AGRE[1]	500 AGRE[2]	750 AGRE[3]		
Malondialdehyde (mmol/l)	12.44[a]	11.25[b]	8.67[c]	6.58[d]	0.27	0.0001
Total antioxidant capacity (mmol/l)	0.61[d]	0.92[c]	1.15[b]	2.12[a]	0.08	0.0005
Superoxide dismutase (U/l)	27.10[b]	32.01[ab]	36.95[a]	38.40[a]	3.43	0.05
Catalase (U/g)	490[b]	561[ab]	610[a]	630[a]	27.9	0.03
Glutathione peroxidase (U/l)	0.78[c]	1.07[bc]	1.14[b]	2.12[a]	0.06	0.02
Glutathione S-transferase (U/l)	85.1[d]	120.4[c]	179.1[b]	210.15[a]	14.37	0.0001

Notes[1]250 AGRE, 250 mg AGRE/Kg diet; [2]500 AGRE, 500 mg AGRE/Kg diet; [3]750 AGRE, 750 mg AGRE/Kg diet; [4]SEM, standard error of the mean.
[a–d] Means in a row not sharing the same superscript differ significantly ($p \leq 0.05$).

Productive performance:

The effect of AGRE on growth performance of chickens reared under heat stress conditions is summarized in Table 5. Chicks receiving 750 mg AGRE/Kg diet had significantly the highest body weights at 3 and 6 weeks of age. Nevertheless, the increase of body weight in groups 250 AGRE and 500 AGRE was not significant.

In all experimental periods, chicks of group 750 AGRE had the highest body weight gain (P=0.047, P=0.039, and P=0.044) followed by group 500 AGRE.

Compared with the control group, the feed intake of groups 250 AGRE, and 500 AGRE was not significantly increased (by 0.38%, 2.91%, and 4.15%, respectively).

Table (5): Growth performance and mortality of broiler chicks fed experimental diets containing different levels of *Alpinia galanga* rhizome extract.

ITEMS	EXPERIMENTAL DIET				SEM[4]	*P*-VALUE
	Control	250 AGRE[1]	500 AGRE[2]	750 AGRE[3]		
Body weight (g)						
Initial BW	43.2	46.41	44.77	43.95	0.41	0.457
Week 3	1059.5[b]	1081.7[b]	1154.8[ab]	1200.3[a]	43.2	0.005
Week 6	2029.1[b]	2079.4[b]	2134.6[ab]	2279.7[a]	52.3	0.0001
Body weight gain (g)						
Week 1-3	1016.3[b]	1035.29[b]	1110.03[a]	1156.35[a]	41.2	0.047
Week 3-6	969.6[b]	997.7[b]	1039.8[ab]	1079.4[a]	45.7	0.039
Week 1-6	1985.9[b]	2032.99[b]	2149.83[ab]	2235.75[a]	37.8	0.044
Feed intake (g)						
Week 1-3	1075	1080	1105	1120	23.3	0.654
Week 3-6	2319	2327	2388	2415	22.1	0.713
Week 1-6	3394	3407	3493	3535	37.3	0.842
Feed conversion ratio (g/g)						
Week 1-3	1.06[a]	1.04[a]	1.00[ab]	0.97[b]	0.001	0.0024
Week 3-6	1.17[a]	1.14[a]	1.11[ab]	1.08[b]	0.013	0.0013
Week 1-6	1.71[a]	1.68[a]	1.62[ab]	1.58[b]	0.004	0.004
Mortality (%)						
Week 1-3	3.06[d]	2.51[c]	2.12[b]	1.79[a]	0.13	0.0001
Week 3-6	3.84[d]	2.37[c]	2.00[b]	1.12[a]	0.10	0.0001
Week 1-6	7.11[d]	5.12[c]	4.46[b]	3.15[a]	0.75	0.0001

Notes[1]250 AGRE, 250 mg AGRE/Kg diet; [2]500 AGRE, 500 mg AGRE/Kg diet; [3]750 AGRE, 750 mg AGRE/Kg diet; [4]SEM, standard error of the mean.
[a–b] Means in a row not sharing the same superscript differ significantly ($p \leq 0.05$).

In comparison to all other groups, the FCR of group 750 AGRE was significantly improved (P=0.0001).

Results concerning mortality revealed that increasing dietary AGRE supplementation led to a reduction in mortality during all experimental periods (P=0.0001) in a dose-dependent manner.

Carcass traits

Results of carcass traits are shown in Table 6. Generally, the group fed on different levels of (AGRE) showed (P=0.045, 0.005 and 0.0001) the highest dressing, breast, and thigh percentages, respectively, while the control group had the lowest percentages. Liver, heart, gizzard and abdominal; fat percentages were (P=0.041, 0.039, 0.41and 0.006) increased for the broiler fed on levels of (AGRE) as compared to those fed on the control diet.

There were significant differences in the contents of moisture, protein and ether extract among the different groups. Results indicated that addition 500 and 750 mg AGRE/Kg diet significantly (P=0.0013, 0.004 and 0.004) increased moisture, protein and ether extract contents in

breast meat compared to those fed on control and 250 mg AGRE/Kg diet, respectively. Moisture and ether extract contents of thigh meat were (P=0.004 and 0.003) increased in broiler fed on diets containing 500 and 750 mg AGRE/Kg diet as when compared with control and 250 mg AGRE/Kg diet, respectively. An opposite effect was noticed regarding protein content of thigh meat, where the values were ($p \leq 0.05$) decreased with 500 and 750 mg AGRE/Kg diet.

Table (6): Carcass traits of broiler chicks fed experimental diets containing different levels of *Alpinia galangal* rhizome extract

ITEMS	EXPERIMENTAL DIET				SEM[4]	*P*-VALUE
	Control	250 AGRE[1]	500 AGRE[2]	750 AGRE[3]		
Carcass characteristics and body organs (%)						
Dressing	63.40[b]	64.42[a]	65.47[a]	66.95[a]	0.51	0.045
Breast	30.97[b]	34.25[a]	35.97[a]	36.91[a]	0.15	0.005
Thigh	25.33[b]	26.15[a]	26.97[a]	27.98[a]	0.53	0.0001
Liver	2.55[b]	2.97[b]	3.23[a]	3.27[a]	0.08	0.041
Heart	0.62[b]	0.64[ab]	0.66[a]	0.67[a]	0.05	0.039
Gizzard	1.98[b]	2.29[a]	2.14[a]	2.25[a]	0.02	0.041
Abdominal fat	1.99[b]	2.23[a]	2.37[a]	2.39[a]	0.07	0.006
Chemical composition of meat on DM basis (%)						
Breast						
Moisture	67.17[b]	67.49[b]	68.15[a]	69.01[a]	0.24	0.0013
Protein	22.71[b]	22.97[b]	23.26[a]	23.81[a]	0.21	0.004
Ether extract	1.71[b]	1.98[b]	2.09[a]	2.11[a]	0.10	0.004
Thigh						
Moisture	73.10[b]	73.12[b]	74.21[a]	74.87[a]	0.58	0.004
Protein	20.18[a]	20.07[a]	19.71[b]	19.0[b]	0.25	0.004
Ether extract	2.11[b]	2.31[b]	2.54[a]	2.7[a]	0.13	0.003

Notes[1]250 AGRE, 250 mg AGRE/Kg diet; [2]500 AGRE, 500 mg AGRE/Kg diet; [3]750 AGRE, 750 mg AGRE/Kg diet; [4]SEM, standard error of the mean.
[a–b]Means in a row not sharing the same superscript differ significantly ($p \leq 0.05$).

Discussion

Plasma biochemical components

Plasma proteins are part of the immune response where antibodies are made of albumin which is the major protein constituent of serum. The values found herein for total protein, albumin and globulin are similar to those cited by [41]. Plasma total protein and globulin of broilers fed 1.0% polyherbal feed premix diet was significantly ($p \leq 0.05$) higher than those fed synthetic vitamin C 0.1 % and control diets as shown by [42]. Blood plasma glucose was observed to move down constantly with increasing dietary levels of AGRE in the present work. A blood glucose level on control (without Alpiniagalanga L.) is almost the same with 250 AGRE, 500 AGRE, and 750 AGRE. According to [43], blood glucose will circulate in the blood and will stable in the bird, regardless of different dietary level. Much of this regulation is due to the interplay of many varieties of hormones, including glucagon, pancreatic polypeptide, insulin, and thyroxine. These hormones can regulate glucose metabolism. Usually, more than a third of glucose absorbed during a meal and is converted to lactate in the intestinal wall, buffering the peak influx.

Blood triglyceride levels on control (without AGRE) are higher than 250 AGRE, 500 AGRE, and 750 AGRE. According to [43] blood triglyceride levels is 35.20 + 16.45 mg/dL, while according to [44], the content of triglyceride is 27 mg/dL. Blood triglyceride levels in broiler decreased by adding AGRE, because of the phytochemical in AGRE, inhibit the formation of triglycerides compounds of the early work of glycerol-3-phosphate derived from glycerol, dihydroxy acetone phosphate (GPDH), and the NADH help to synthesize glycerol-3- phosphate for triglycerides and lowering the activity of glycerol-3-phosphate (GPDH) enzyme in the biosynthesis of triglycerides [45]. The active component of antioxidant flavonoids is 0.21%; it will inhibit the early stages of the reaction by release 1 hydrogen atom forming and reducing associated with one free radical. This bond will stabilize the radical peroxy that makes energy activity reduced, and finally, the content of triglycerides will decline [46]. Decreasing blood triglycerides also affected by saponins content for delaying the absorption of fat in the small intestine by inhibiting lipase activity, through the mechanism of binding triglyceride-and saponins in the intestinal lumen, and affects the metabolism of fat in the body [47].

Alpinia galangal rhizomes extract significantly ($p \leq 0.01$) decreased the elevated levels of LDL ($p \leq 0.01$) and reduced ($p \leq 0.05$) levels of total lipids, where HDL levels were not significantly decreased. Comparable results were shown by [42]. Corresponded with the [48] reported that the levels of ginger diet reduce cholesterol, triglyceride (TG) and glucose. The phenolic compounds in AGRE were shown to be very effective in inhibiting the oxidation of LDL [49]. Moreover, [50] suggested that the high concentration of antioxidants might decrease lipid peroxidation and therefore reduce the

serum concentration of triglycerides. In this respect, [41] studied the effect of different levels of turmeric rhizome extract (0.05, 0.1, 0.15 and 0.2%) on some blood parameters of laying hens a diet and this doses caused a significant decrease in blood LDL and cholesterol while the opposite trend was observed in HDL levels. Also, [51] in a study showed that using the 0.5% extracted ginger rhizome significantly decreased total cholesterol in broilers that are not consistent with these results.

Biomarkers of antioxidant status

This result agrees with the conclusion of [52] who showed that serum MDA levels were lower when rats administration aqueous extracts of some traditional medicinal plants (including Camellia sinensis leaves, Carumcarvi seeds, Alpinia galangal rhizomes, Boswelliaserrata resins, and Cinchona officinalis bark). In the present study, the opposite effect was noticed regarding T-AOC, where the values were significantly increased with increasing AGRE levels. These results confirm that the antioxidant activity of flavonoid compounds in Alpinia galangal rhizomes is mainly due to their reduction-oxidation (redox) reactions and chemical structure [53].

Our results regarding antioxidant enzymes (SOD, CAT, GPx, and GST) are in good agreement with those of [42] who stated that in broilers a supplementation of polyherbal feed premix at 1.0g/ kg diet increased the activity of antioxidant enzymes significantly (such as SOD, GPx and CAT). Moreover, [54] noticed that adding ginger essential oil in diets of broilers at 150 mg/kg the total superoxide dismutase (TSOD) activity in liver increased vise verse Malondialdehyde (MDA) concentrations decreased compared to that in the control group, followed by those results which showed that there was no significant difference between experimental groups regarding glutathione peroxidize (Gpx), TSOD and catalase (CAT) enzymes in red blood cells under heat stress. Furthermore, the latter authors added that Alpinia galangal rhizomes polyphenols are active compounds which are involved in the control of redox homeostasis in chickens during aging. The importance of these antioxidant enzymes is due to their involvement in the clearance of superoxide and H2O2 to maintain the structure and function of biological membranes [55]. SOD plays a role in an antioxidant defense system [56]. Costa et al., [57] reported that Alpinia galangal was effective in converting the oxidized glutathione into reduced glutathione and in removing H2O2 created by oxidative stress. Flavonoids make a great contribution to the antioxidant activity of AGRE due to their effect in free radicals' elimination [58]. It seems that flavonoids from AGRE possess a significant antioxidative activity. The antioxidant constituents of AGRE comprise mainly compounds with phenolic hydroxyl groups and double bonds including tannins, flavonoids, and unsaturated fatty acids and phenolic contents, including that phenolics are the dominant antioxidant constituents of AGRE [59]. They added that polyphenolic compounds can clean off the free radicals and reduce the membrane lipid peroxidation, so they can reduce the occurrence of free radical-related diseases.

Productive performance

High ambient temperature decreases growth performance, possibly because of excessive reactive oxygen species that oxidize and destroy cellular biological molecules, inhibit some ATPase activities and finally cause a variety of impairments to intestinal tissues [60]. Additionally, Stress in broilers results in a decline in body weight, feed intake, and overall feed efficiency. However, supplementation of antioxidants along with the basal diet has been scientifically well proven to improve growth and performance in birds [61]. In the present study, a progressive increase in body weight was observed with increased levels of AGRE. The improvements in body weight amounted at 6 weeks of age to 2.48%, 5.2% and 12.35% for groups 250 AGRE, 500 AGRE, and 750 AGRE, respectively. The present results agree with experiments in rabbits reported by [62], where AGRE was incorporated up to 1.0 g/kg diet without impairing performance. Furthermore, [63], confirmed that polyphenols present in

Alpiniagalanga (L.) was absorbed in sufficient levels to contribute and modulate the antioxidant activity in broiler chickens. [6], proved that antioxidant activity, total phenolic, and total flavonoid, 2-Propenoic acid, phthalic acid, palmitic acid, sandaracopimaradiene, oleic acid, octadecanoic acid, 2-[2-(4-nonylphenoxy) ethoxy] ethanol and glycidyl stearate were the major constituents of methanol extract of Kaempferia galanga rhizome. This result is

consistent with findings of [64] in broilers, where no growth depressions were observed after supplementing diets with grape polyphenols. These components present in the AGRE stimulate digestive enzymes and improves overall digestion and thus leads to increased body weight. It has been established that ginger in the diet stimulates lactic acid bacteria and reduces pathogenic bacteria such as coliforms, mesophilic aerobics, and Escherichia coli, thereby improving the absorption of nutrients leading to better bird weight [65]. Or maybe due to a lower ability galangal's to enhance insulin sensitivity [66]. In the study of [67], indicated that the morphometry of the jejunum as length and width of villi was improved by an additional 1 percent ginger in the broiler diet, which increases the absorbent surface area of the intestine and thus increases the absorption capacity, resulting in a higher gain in body weight.

The results are consistent with those of [67] who stated that body weight gain (g/bird) was found to be higher in garlic and ginger supplemented group than control and garlic and ginger mixture supplemented group. The same results were found by [68] when testing addition turmeric (Curcuma longa) to broiler chickens diets.

In the study of [61], found an increased gain in body weight in polyherbal feed premix (O. Sanctum (leaves), Terminalia chebula (fruit), P. emblica (fruit and leaves), W. Somenifera (root), and Shilajit) supplemented group compared to a control group broilers below heat stress. Also, [69, 70] observed that compared to a control group, birds supplemented by Stresroak (polyherbal formulation) showed increased body weight gain.

It has shown that the smell and/or taste of AGRE do not adversely affect the palatability of feed in broiler diets. In the other study, [71] reported that adding a mixture of medicinal plant extracts on broiler feed intake was reduced significantly compared to the control group.

In this connect [72] said that ginger extract 1% of the total treatment period showed the best feed conversion ratio so was the lowest. In the study [73], with turmeric and among treatments reported significant conversion factor. Kamel [74] reported that the feed conversion ratio improved by adding plant extracts. In the study [75], reported that adding turmeric and Aloro saw a significant effect on FCR.

Carcass traits

Supplementation of AGRE as a source of natural antioxidants under conditions of oxidative stress (where the production of free radical increases dramatically) prevents damage to major organs and systems and is economically justified [65], so in our experiment dressing weights in groups 250 AGRE, 500 AGRE, and 750 AGRE were significantly increased by 1.61%, 3.26%, and 5.6%, respectively. These results are in agreement with results of [43], where among all measured carcass traits of rabbits a dietary AGRE supplementation of 0.5% and 1.0% AGRE increased ($p \leq 0.05$) only carcass weight, dressing percentages, and carcass cuts. Furthermore, the presented results are in agreement with investigations on broilers.

For instance, [20] found that AGRE supplementation affects the carcass traits and the relative lengths of large intestine and caeca of broiler chickens under heat stress. However, these results were contrary to [72] reported that addition 1.5% of extracted ginger in broiler diets effect on the percentage of abdominal fat and thigh compared to control. In the study, [76], reported that adding 0.5 percentage of turmeric root extract to the diet caused a significant decrease in the relative weight of abdominal fat. We disagree with the trial and the trial [75].

Conclusion

The findings of this study demonstrated that dietary supplementation of AGRE at 250, 500 and 750 mg/kg improved growth performance, antioxidant status and reduced the mortality of broilers. Moreover, AGRE has a high antioxidant capacity or is an efficient free radical scavenger which is related to the presence of a mixture of polyphenolic compounds with good antioxidant properties especially for heat-stressed broilers. In the present study on broilers, the best results were obtained supplementing 750 mg AGRE/kg diet.

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical guidelines for the animal.

Conflict of interest: None declared.

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