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DOSE-RESPONSE ANALYSIS FOR THE EFFECTS OF *COFFEA ARABICA* L. ON GROWTH PERFORMANCE, HEALTH STATUS, AND ECONOMIC EFFICIENCY OF FATTENED RABBITS RAISED UNDER HIGH AMBIENT TEMPERATURE

Ibrahim T. El-Ratel[1], Khaled H. El-Kholy[1], Ayman M. Gomaa[1], A.M. Abdel-Khalek[2], Nesrein M. Hashem[3,4♦], Ali Ali El-Raghi[1]

[1]Department of Animal, Poultry and Fish Production, Faculty of Agriculture, Damietta University, 34517, Damietta, Egypt
[2]Animal Production Research Institute, Agricultural Research Center, Giza, Egypt
[3]Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt
[4]Department of Animal Production and Health, Faculty of Veterinary Medicine, CEU Cardenal Herrera University, C/Tirant lo Blanc, 7, 46115 Alfara del Patriarca, Valencia, Spain
♦Corresponding author: nesreen.hashem@alexu.edu.eg

Abstract

The dose-response analysis was used to investigate the effects of green coffee (GC) on growth performance, feed utilization, carcass traits, and health status of newly weaned rabbits. A total of 60 clinically healthy growing rabbits (5 weeks old) reared during summer season were divided randomly into three experimental groups, 20 rabbits each, and received 0, 2, and 4 g GC/kg diet, (GC0, GC2, and GC4), respectively. Growth performance (live body weight, LBW and average daily gain, ADG) and feed utilization (feed conversion ratio, FCR) as well as the dressing percentage and the relative weight of liver were improved significantly in the GC-treated groups compared to the control group. The dose-response regression analysis showed that the optimal doses were 2.75 and 3 g GC/kg diet for ADG and FCR, respectively. Moreover, GC treatments significantly decreased both rectum temperature and respiration rate compared to GC0, with an optimal dose being at 2.5 g GC/kg diet. Erythrocytes and leukocyte counts improved significantly in the GC-treated groups compared to the GC0 group. Blood protein and its fractions, liver and kidney functions, and lipid profile were quadratically improved by GC supplementation. The optimal dose was 3 g GC/kg diet for total protein and its fractions, ALT, and TG, while it was 2.5 and 2.75 g GC/kg diets for creatinine and TC, respectively. Total antioxidant capacity, superoxide dismutase, and glutathione activities were significantly higher, while the levels of malondialdehyde were significantly lower in the GC-treated groups than the GC0 group. A level of 2.5 g GC/kg diet was the optimal dose required for improving immunoglobulin A and G, which improved in blood serum of GC treated groups compared to the control. Economically, dietary addition of GC enhanced the economic efficiency of the supplemented diets, thereby improving the profitability of the fattening process. In conclusion, dietary supplementation of GC at level of 2.5–3 g/kg diet could be effectively used to enhance the growth indices, redox status, immune function, and economic efficiency of rabbits fattened during the summer season.

Key words: phytogenic antioxidants, heat stress, metabolism, growth, rabbit

Rabbit meat is a key source of animal protein in certain countries due to its high nutritional value, including a high protein content and a low cholesterol level (Siddiqui et al., 2023). Recently, rabbit production has faced many challenges including feed shortages, thus it is necessary to find readily available and alternative feed additives to sustain the rabbit industry (El Ratel et al., 2020; El-Sabrout et al., 2023).

Heat stress (HS) is a condition where homeothermic animals are unable to maintain a balance between heat production and emission (Liang et al., 2022). In many arid and semi-arid regions including Egypt, the elevated ambient temperature is over the rabbits thermoneutral zone, which causes a series of unfavorable impacts on rabbit productivity (El-Raghi et al., 2023). As known, HS has multiple adverse impacts on rabbits' health status and their productive performance. It has been revealed that HS is responsible for 9–12% increase in mortality rate, 20–25% reduction in average daily weight gain (DWG), and 8–15% decrease in feed conversion ratio (FCR); as well as negatively affects the carcass traits and the quality of meat (Yan and Li, 2008). Thus, HS poses a major challenge to the rabbit industry, made worse by recent global warming (Liang et al., 2022; El-Ratel et al., 2023; Hashem et al., 2023 a). Several former studies have investigated the prospective mitigation strategies, and the nutritional manipulation presents an effective dynamic mitigation approach. The dietary supplementation of fattened rabbit diets with phytogenic-based supplements has provided sufficient evidence as potential HS mitigation candidates, mainly through their antioxidant role (El-Ratel et al., 2019; Hashem et al., 2019, 2023).

Green coffee (GC) as a potential antioxidant candidate contains phyto-constituents, including flavone, polyphenols, chalcones, catechin, alkaloids, tannins, and flavonoids (Haile and Kang, 2019; Hashem et al., 2020). These compounds have the ability to efficiently scavenge the free radicals, activate antioxidant enzymes, and inhibit oxidative enzymes (Yan et al., 2023). The presence of different phytogenic compounds in GC may help

maintain redox homeostasis via different modes of action and pathways. Phytogenics can act as free radical scavengers, retarding oxidation chain initiation, and repairing the oxidized molecules (Hashem et al., 2020). Thus, the efficiency of minimizing oxidative stress due to the action of different phytogenic compounds may be better than other single antioxidant molecule, such as vitamins and minerals. Therefore, the aim of the current study is to examine the effect of dietary supplementation with GC on growth indices, carcass traits, physiological responses (rectal temperature and respiratory rate), hematological and biochemical variables, redox balance, and immunity status of growing rabbits reared under natural summer season conditions.

Material and methods

The present work was conducted at a private commercial rabbit farm, Mansoura City, Dakahlia Governorate, Egypt, in cooperation with Animal, Poultry and Fish Production Department, Faculty of Agriculture, Damietta University. Animal care and maintenance were conducted following the guidelines of the Egyptian Research Ethics Committee and the instructions contained in the Guide for the Care and Use of Laboratory Animals (2011).

Identification of phenolic compounds and antioxidant activity of green coffee

Ground green coffee beans (*Coffea arabica* L.) were procured from a local company (El-Mansoura city, Egypt). Separation and determination of green coffee phenolics were performed by reverse phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) using a column Alltima C18, 5 mm (150 mm × 4.6 mm id) with a guard column Alltima C18, 5 mm (Alltech). The solvent system used was a gradient of A (CH_3COOH 2.5%), B (CH_3COOH 8%) and C (acetonitrile). The best separation was obtained with the following gradient: at 0 min, 5% B; at 20 min, 10% B; at 50 min, 30% B; at 55 min, 50% B; at 60 min, 100% B; at 100 min, 50% B and 50% C; at 110 min, 100% C until 120 min. The solvent flow rate was 1 ml min^{-1} and separation was performed at 35°C. The volume injected was 10 µl. Phenolic compounds were assayed by external standard calibration at 280 nm and expressed in mg^{-1} dry matter of equivalent catechin for flavan-3-ols, equivalent coumarin for a polar aromatic compound and equivalent quercetin-3-rutinoside for flavanols. A variability of 8% was determined on 5 extractions of phenolics from the same Esterel barley sample. Antioxidant activity of the GC extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity at 517 nm (Hashem et al., 2023 b).

Experimental design

A total of 60 male APRI Egyptian line rabbits (Red Baladi × V-line) selected for litter weight at weaning (Abou Khadiga et al., 2010), weaned at 5 weeks of age with an average weight of 668.31±2.58 g were used in the experiment. Rabbits were randomly divided into three groups (20 in each group). Rabbits in the control group were fed control basal diet (CBD) without supplementation, while those in groups 2 and 3 were fed CBD containing GC at levels of 2 and 4 g/kg diet, respectively. The growing period lasted for 8 wk from 5 up to 13 wk of age. Rabbits were individually housed in galvanized wire cages (35 × 35 × 60 cm^3) supplied with feeders (*ad libitum*) and fresh water was available via an automatic nipple system, in an artificially illuminated and naturally ventilated building. All rabbits were kept under similar hygienic conditions, environmental, and managerial factors. Every morning, feces and urine on the rabbit tray floor were removed. Rabbits were exposed to normal light throughout the experimental period. The CBD was formulated to cover all essential nutrient needs of growing rabbits (De Blas and Mateos, 1998). The chemical analysis (on dry matter basis) revealed that the CBD contained organic matter (91.42%), crude protein (17.36%), crude fiber (12.37%), ether extract (2.23%), nitrogen-free extract (59.46), ash (8.58%) and digestible energy 2700 kcal/kg.

Meteorological parameters

During the experimental period, both ambient environmental temperatures (°C) and the relative humidity (%) were recorded daily using an automatic Thermo hygrometer (Dostmann GmbH and Co. KG, Wertheim, Germany). The temperature-humidity index (THI) was measured according to the method outlined by Marai et al. (2002):

$$THI = AT - \left[\left(0.31 - 0.31\left(\frac{RH}{100}\right)\right) \times (AT - 14.4)\right]$$

Where: *AT* is the ambient temperature and *RH* is the relative humidity. *THI* values were classified as follows: <27.8 (absence of HS), 27.8 to 28.9 (moderate HS), 29.0 to 30.0 (severe HS), and >30.0 (very severe HS).

Growth performance and physiological responses

During the study period, the live body weight and feed consumption were measured biweekly for each rabbit. Then the daily weight gain and feed conversion ratio were estimated by period based on aforementioned data. As part of the study, rectal temperature and respiratory rate were measured biweekly for each rabbit. Rectal temperature was measured in Celsius (°C) using a digital thermometer inserted to a depth of 4 cm into the rabbits' rectum. The respiratory rate was optically estimated using a stopwatch by counting the number of motions of the animals' flanks for one minute while in a resting position (El-Desoky et al., 2022)

Carcass measurements

At the end of experimental period, five rabbits from each group were randomly selected and prevented from

feeding for 12 hours before being slaughtered immediately after weighing. After complete bleeding, the tail, viscera, and pelt were separated as non-edible giblets. Thereafter, the carcass and edible parts including kidney, heart, and liver as well as the head, lung, and spleen were weighed as a percentage of live body weight. In addition, the length of the cecum was measured (cm). The dressing percentage = [(hot-dressed carcass weight / pre-slaughter weight) ×100].

Hemato-biochemical analysis

At the end of study period, five blood samples were drowned into sterile tubes from the slaughtered rabbits and then classified as two subsamples for different types of analyses. The first subsample was treated with an anticoagulant and used to evaluate the hematological attributes by using an automated hematology analyzer (Hospitex Hema Screen 18, Sesto Fiorentino, Italy), while the second subsample was allowed to clot and centrifuged to separate the serum samples for 15 min at 3500 rpm, then the separated serum samples were stored at –20ºC until examination.

Serum total proteins (TP), albumin (AL), glucose, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), creatinine and urea concentrations, as well as activity of aspartate (AST) and alanine (ALT) transaminases were determined using commercial kits (Bio-Merieux, Laboratory Reagents and Products, France). Antioxidants status marker, including activity of total antioxidant capacity (TAC), glutathione content (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and malondialdehyde (MDA) levels were assayed in the serum using commercial kits (Biodiagnostic, Dokki, Giza, Egypt). The serum immunoglobulins (IgG, IgM and IgA) were estimated using commercial ELISA kits, following the manufacturer's instructions.

Histology and antioxidant status of liver tissue

For histological examination, liver specimens were collected from slaughtered animals from the control and GC-treated groups and fixed in neutral formalin buffer (10%). Liver samples were dehydrated in ascending grades concentrations of ethyl alcohol, then cleared in two changes of xylol, embedded in paraffin blocks, and then cut at 4 µm sections using a microtome (Leica RM 2155). All specimens were stained with eosin and hematoxylin according to Bancroft and Gamble (2008). A digital camera microscope (Leica DM 500, Leica EC3, Leica) was used to capture the representative photomicrographs.

To assess antioxidant status of liver, samples were taken immediately after slaughtering and homogenized with potassium phosphate-buffer (pH 7.4) solution (10% w/v), and centrifuged (3500 rpm for 15–20 min). The supernatant was used to assess the levels of TAC and MDA using commercial chemical kits (Biodiagnostic, Dokki, Giza, Egypt).

Economic efficiency

Economic efficiency (EE, %) was calculated by US dollars (USD) according to the dominant prices of 1 kg feed, GC, and rabbit meat during the month of the end of experimental period: Price of 1 kg/body weight on selling was 3.3 USD, 1 kg feed cost was 0.32 USD, and 1 g GC was 0.005 USD. All aforementioned prices were collected from the local market by Egyptian pound and thereafter converted to USD according to the official price. The following economic items were calculated according to aforementioned data:

Total feed costs = [(total feed intake / rabbit × price)/ kg],

Net revenue (NE) = [price of each rabbit – total feed costs],

EE, % = [net revenue/total feed costs].

Statistical analysis

Data were edited in Microsoft Excel (Microsoft Corporation). A Shapiro–Wilk test was conducted to check for normality as described by Razali and Wah (2011). A MIXED procedure (PROC MIXED; SAS Institute, 2012) was used for assessing growth performance, feed intake, feed conversion ratio, carcass indices, hemato-biochemical parameters, redox status, immunity parameters, and inflammatory responses. The statistical model introduced an individual rabbit as a random factor, while GC levels were introduced as a fixed factor. Multiple comparisons between means were conducted according to the Duncan's multiple range tests (Steel and Torrie, 1980). A polynomial regression analysis was fitted to test the relationship between dietary levels of GC (0, 2, and 4 g/kg diet) and different parameters. Dose-response curves were fitted by the GraphPad Prism software 9.0 (GraphPad, USA). Statistical significance was accepted as P-value less than 0.05.

Results

Phenolic components detected in GC

The high-performance liquid chromatography analysis (HPLC) of GC revealed that among the 9 phenolic compounds identified here, the major phenolic components in GC were chlorogenic, caffeine, catechin, ellagic, feluric, and catechol. The antioxidant activity of the GC extract as indicated by DPPH scavenging capacity was 89.88% (Table 1).

Meteorological parameters

Results of meteorological parameters during the months of experimental period (July and August) are presented in Table 2. The overall average of ambient temperature (AT), relative humidity (RH), and the temperature–humidity index (THI) were 32.12±0.78°C, 63.50±1.96%, and 30.10±0.64, respectively. The present values of THI revealed that the growing rabbits suffer from severe heat stress.

Table 1. Phenolic compounds of green coffee (GC) detected by HPLC and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

Phenolic components	Concentration (ppm)
Chlorogenic	3569.24
Caffeine	3289.06
Catechin	1544.87
Ellagic	649.91
Ferulic	634.40
Catechol	413.24
Pyrogallol	81.86
3-OH Tyrosol	36.25
Coumarin	16.01
DPPH scavenging activity (%)	89.88

Table 2. The mean values of ambient temperature (AT), relative humidity (RH), and temperature–humidity index (THI) during the experimental period

Parameters	July	August	Overall
AT (°C)	32.75±0.21	31.50±0.19	32.12±0.18
RH (%)	61.25±1.25	65.75±3.61	63.50±1.96
THI	30.54±0.22	29.68±0.15	30.10±0.14

Growth performance and feed utilization

The effect of dietary supplementation of GC on the growth indices and feed efficiency of growing rabbits during the experimental period are presented in Table 3. Generally, values of final LBW and ADG were significantly higher in the GC-treated groups than the GC0 group (P<0.05). With respect to feed intake, the dietary treatment had no significant effects on feed consumption (P=0.9578). Meanwhile the feed utilization (lower FCR) improved significantly in the GC-treated groups compared to the GC0 group (P<0.05). The polynomial regression analysis indicated the optimal doses for overall ADG and FCR were 2.75 and 3 g GC/kg diet, respectively (Figure 1 A and B).

Carcass traits

Results of carcass characteristics are illustrated in Table 4. The dietary treatments exerted significant effects on dressing percentage and the relative weights of liver (P=0.0380, and 0.0457, respectively). The dressing percentage and the relative weight of liver were significantly higher in the GC2 group compared to the GC0 (P<0.05). The results of regression analysis indicated that the dietary treatment by GC affected the dressing percentage and the relative weight of liver quadratically (P=0.0141 and 0.0072, respectively). The dietary treatment had no significant effects on the relative weights of head, heart, kidney, spleen, lung, and abdomen fat as well as cecum length (P>0.05).

Table 3. Growth performance and feed utilization of growing APRI rabbits exposed to high ambient temperature as affected by dietary treatments (GC0: 0 g/kg diet, GC2: 2 g/kg diet, and GC2: 4 g/kg diet)

Variables	Treatments			SEM	P-value[1]		
	GC0	GC2	GC4		TRTS	lin	quad
Live body weight (g) at:							
5 wks of age	671.05	666.70	667.20	3.931	0.8740	0.7636	0.7891
9 wks of age	1262.85 b	1399.60 a	1379.80 a	9.160	<.0001	<.0001	<.0001
13 wks of age	1950.25 b	2080.10 a	2068.35 a	14.910	<.0001	<.0001	0.0006
Average daily gain (g)							
5–9 wks of age	21.21 b	26.18 a	25.45 a	0.296	<.0001	<.0001	<.0001
9–13 wks of age	24.55	24.30	24.59	0.461	0.7202	0.9506	0.6386
overall (5–13 wks of age)	22.84 b	25.24 a	25.02 a	0.273	<.0001	<.0001	0.0003
Feed intake (g /day) at:							
5–9 wks of age	65.15	65.25	64.05	1.401	0.7019	0.5952	0.7167
9–13 wks of age	113.45	111.65	110.15	1.680	0.8090	0.1536	0.9398
overall (5–13 wks of age)	89.20	88.05	88.75	1.838	0.9578	0.8513	0.6566
Feed conversion ratio (g feed/g gain)							
5–9 wks of age	3.07 a	2.50 b	2.53 b	0.061	<0.0001	<.0001	0.0003
9–13 wks of age	4.63	4.59	4.50	0.097	0.5169	0.3713	0.5530
overall (5–13 wks of age)	3.91 a	3.50 b	3.56 b	0.083	0.0021	0.0061	0.0271

[1]TRTS – treatment effect, lin – linear regression, and quad – quadratic regression.
a, b – means within a row without a common letters differ at P<0.05.

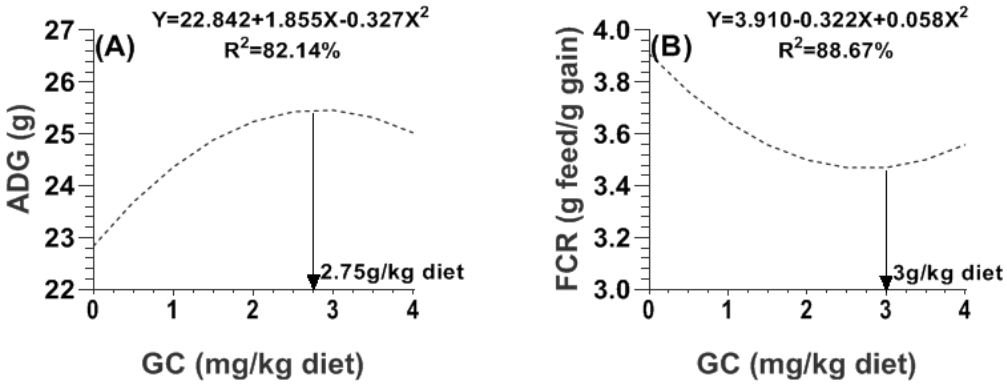


Figure 1. A polynomial regression analysis between dietary levels of GC and overall average daily gain (A – ADG), and feed conversion ratio (B – FCR)

Table 4. Carcass traits of growing APRI rabbits exposed to high ambient temperature as affected by dietary treatments (GC0: 0 g/kg diet, GC2: 2 g/kg diet, and GC2: 4 g/kg diet)

Variables	Treatments			SEM	P-value[1]		
	GC0	GC2	GC4		TRTS	lin	quad
Pre-slaughter weight (g)	2089	2084	1935.6	47.403	0.1843	0.1284	0.1163
Carcass traits (as % of pre-slaughter BW)							
dressing	57.94 b	60.72 a	59.31 b	0.733	0.0380	0.0163	0.0141
head	5.28	5.10	5.15	0.170	0.5653	0.6821	0.7492
liver	3.17 b	3.56 a	3.25 b	0.047	0.0457	0.0415	0.0072
heart	0.31	0.32	0.32	0.033	0.8450	0.4952	0.5527
kidney	0.98	0.92	0.98	0.056	0.9704	0.8234	0.8812
edible giblets	4.46	4.80	4.55	0.168	0.0844	0.2145	0.1386
spleen	0.12	0.11	0.13	0.019	0.5099	0.4712	0.3862
lung	0.73	0.70	0.71	0.045	0.8364	0.9234	0.9751
abdomen fat	0.76	0.79	0.92	0.081	0.8079	0.8436	0.7929
cecum length (cm)	11.53	12.63	11.50	0.748	0.7979	0.7362	0.6792

[1]TRTS – treatment effect, Lin – linear regression, and Quad – quadratic regression.
a, b – means within a row without a common letters differ at P<0.05.

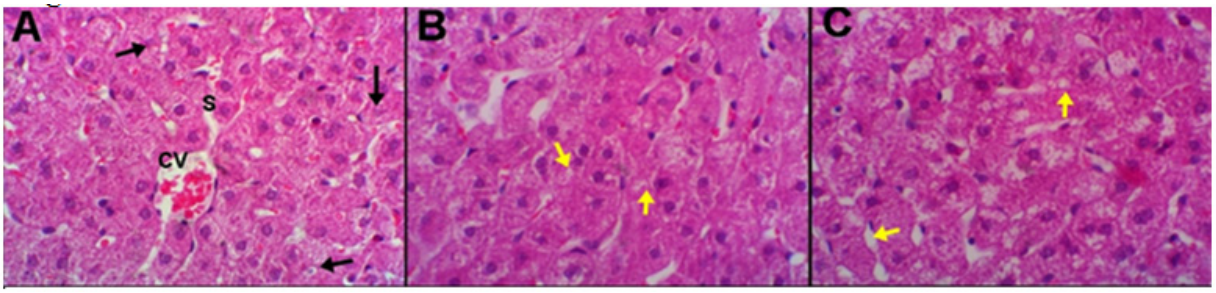


Figure 2. Representative photomicrograph of the GC0 (A), GC2 (B), and GC4 (C) treated groups

Histopathology findings

Herein, the representative photomicrographs of liver supported the obtained data of blood profile, redox status (Figure 2 A, B, and C). The control group showed a minimal periportal fibrosis characterized by diffuse, marked ballooned hepatocytes with intracellular vacuolation, necrosis nucleus (black arrows) and focal per portal cellular infiltrates with small numbers of macrophages(s). Meanwhile, the GC2 treated group revealed nearly normal histological appearance of hepatic cell and beside minimal micro vacuoles in hepatocytes (yellow arrow). However, the GC 4 treated groups showed a mild to moderate swollen hepatocytes with intracellular micro vacuolar (yellow arrows). Image magnification= 400x.

Physiological responses

Results of physiological responses including the rectal temperature (RT) and respiration rate (RR) are illustrated in Table 5. Both GC2 and GC4 groups had significantly lower values of RT and RR than the GC0 group (P<0.05). The quadratic relationship between GC levels and the values of RT and RR indicated that the optimal dose was 2.5 g GC/kg diet (Figure 3 A and B).

Hematological attributes

The effects of dietary treatments on the hematological parameters are illustrated in Table 5. The values of hemoglobin and red blood corpuscles were significantly higher in GC treated groups than the control group (P<0.05). Meanwhile, the dietary treatments significantly decreased white blood cells count compared to the control (P=0.0124). The regression analysis indicated that the GC treatment affected hemoglobin concentration linearly (P=0.0064; Figure 4 A). On the other hand, the increased levels of GC affected quadratically the red blood corpuscles (P=0.0285) and white blood cells count (P=0.0285), the optimal dose was 3 g/kg diet (Figure 4 B and C). No significant differences (P>0.05) were shown in the values of platelets count, MCV, MCH, and MCHC between all treated groups and the control (P>0.05).

Blood profile

As presented in Table 6, the majority of blood metabolites were statistically (P<0.05 or 0.001) affected by the dietary treatment. Serum concentrations of TP and albumin were increased quadratically by GC treatment, the optimal dose was established at 3 g GC/kg diet (Figure 5 A and B). However, globulin concentration was not affected significantly by the dietary treatments (P=0.1700).

Table 5. Hematological indices of growing APRI rabbits exposed to high ambient temperature as affected by dietary treatments (GC0: 0 g/kg diet, GC2: 2 g/kg diet, and GC2: 4 g/kg diet: GC4)

Variables[1]	Treatments			SEM	P-value[2]		
	GC0	GC2	GC4		TRTS	lin	quad
Physiological responses							
rectal temperature (°C)	39.73 a	38.61 b	38.79 b	0.140	<.0001	<.0001	0.0007
respiration rate (breathes/min)	130.53 a	114.8 b	117.5 b	1.317	<.0001	<.0001	<.0001
Hematological indices							
HGB (g/dL)	10.87 b	11.85 a	11.92 a	0.270	0.0106	0.0064	0.1217
RBCs (× 10^6/mm^3)	4.14 b	5.98 a	5.90 a	0.097	0.0473	0.1115	0.0285
WBCs (× 10^3/mm^3)	7.03 a	5.08 b	5.24 b	0.088	0.0002	0.0001	0.0124
PLT (× 10^3/mm^3)	274.76	296.65	295.25	26.938	0.8157	0.6135	0.6818
HCT (%)	30.81	30.55	30.55	2.106	0.9789	0.8609	0.9194
MCV (µm^3)	65.64	66.38	65.36	2.413	0.9503	0.9342	0.7628
MCH (pg)	24.18	24.37	23.74	2.616	0.9881	0.9183	0.9113
MCHC (g/dl)	35.69	34.88	35.53	4.323	0.9900	0.9787	0.8916

[1]HGB, hemoglobin; RBCs, red blood corpuscles; WBCs, white blood cells count; PLT, platelet count; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. [2]TRTS – treatment effect, lin – linear regression, and quad – quadratic regression. a, b – means within a row without a common letters differ at P<0.05.

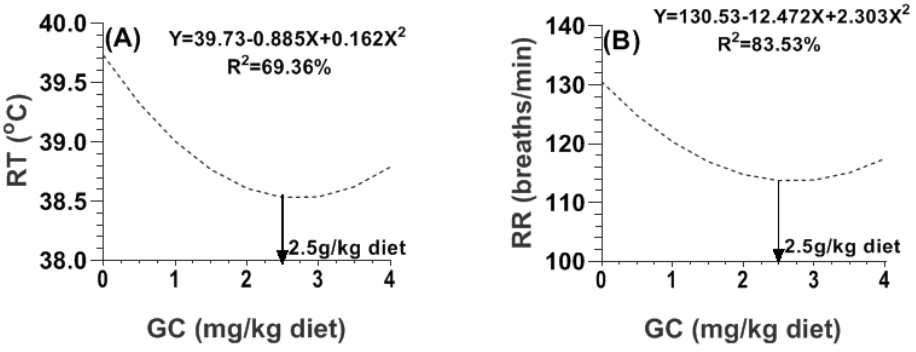


Figure 3. A polynomial regression analysis of rectal temperature (A – RT) and respiration rate (B – RR) on dietary levels of GC

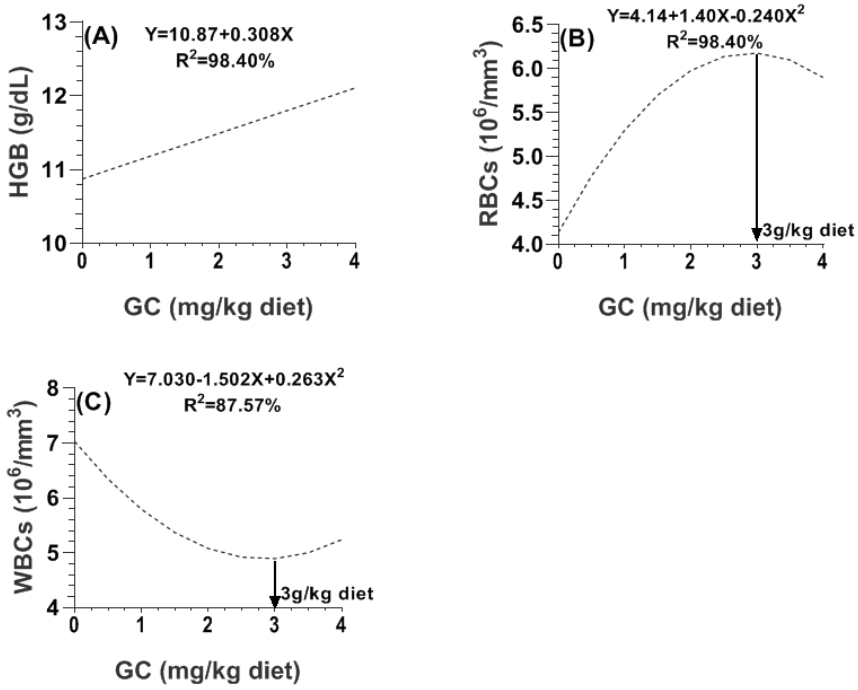


Figure 4. A polynomial regression analysis of hemoglobin (A – HGB), red blood corpuscles (B – RBCs), and white blood cells count (C – WBCs) on dietary levels of GC

Table 6. Blood profile of growing APRI rabbits exposed to high ambient temperature as affected by dietary treatments (GC0: 0 g/kg diet, GC2: 2 g/kg diet, and GC2: 4 g/kg diet)

Variables[1]	Treatments			SEM	P-value[2]		
	GC0	GC2	GC4		TRTS	lin	quad
TP (g/dL)	5.85 b	6.37 a	6.35 a	0.135	0.0054	0.0036	0.0477
AL (g/dL)	3.05 b	4.12 a	4.03 a	0.072	<.0001	0.0271	<.0001
GL (g/dL)	2.80	2.25	2.32	0.163	0.1700	0.1137	0.2927
AST (IU/L)	30.51	26.52	25.11	1.317	0.0722	0.0692	0.5087
ALT (IU/L)	56.04 a	37.52 b	38.62 b	1.584	<.0001	<.0001	0.0005
Creatinine (mg/dL)	0.92 a	0.76 b	0.80 b	0.020	0.0003	0.0002	0.0388
Urea (mg/dL)	41.77 a	33.69 c	34.49 bc	1.692	0.0022	0.1103	0.0010
Glucose (mg/dl)	87.61 a	78.25 b	75.58 b	2.772	0.0026	0.0012	0.1387
TC (mg/dL)	87.13 a	75.46 b	77.45 b	4.534	<.0001	<.0001	0.0002
TG (mg/dL)	81.70 a	57.48 b	59.78 b	2.314	<.0001	<.0001	0.0053
HDL (mg/dL)	36.62	38.55	39.60	1.812	0.4622	0.2298	0.8333
LDL (mg/dL)	26.90	24.28	24.68	1.971	0.1110	0.0986	0.0726

[1]TP, total protein; Al, albumin; GL, globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein. [2]TRTS – treatment effect, lin – linear regression, and quad – quadratic regression. a, b, c – means within a row without a common letters differ at P<0.05.

With respect to the liver functions, GC treatments significantly reduced the activity of blood serum ALT compared to the control group. The polynomial regression analysis indicated that the optimal concentration was at 3 g/kg diet (Figure 5 C). Non-significant differences were observed in the level of AST enzyme between the control group and all treated groups (P>0.05).

For kidney function, the concentrations of urea and creatinine were significantly lower in CG-treated groups than the control group (P<0.05). The quadratic regression analysis showed that the optimal doses were established at the levels of 2.5 and 3 g/kg diet, respectively (Figure 5 D and E). the GC treatments significantly decreased blood serum glucose compared to the control, being in a linear decrease with the increased levels of GC (P=0.0012; Figure 5 F).

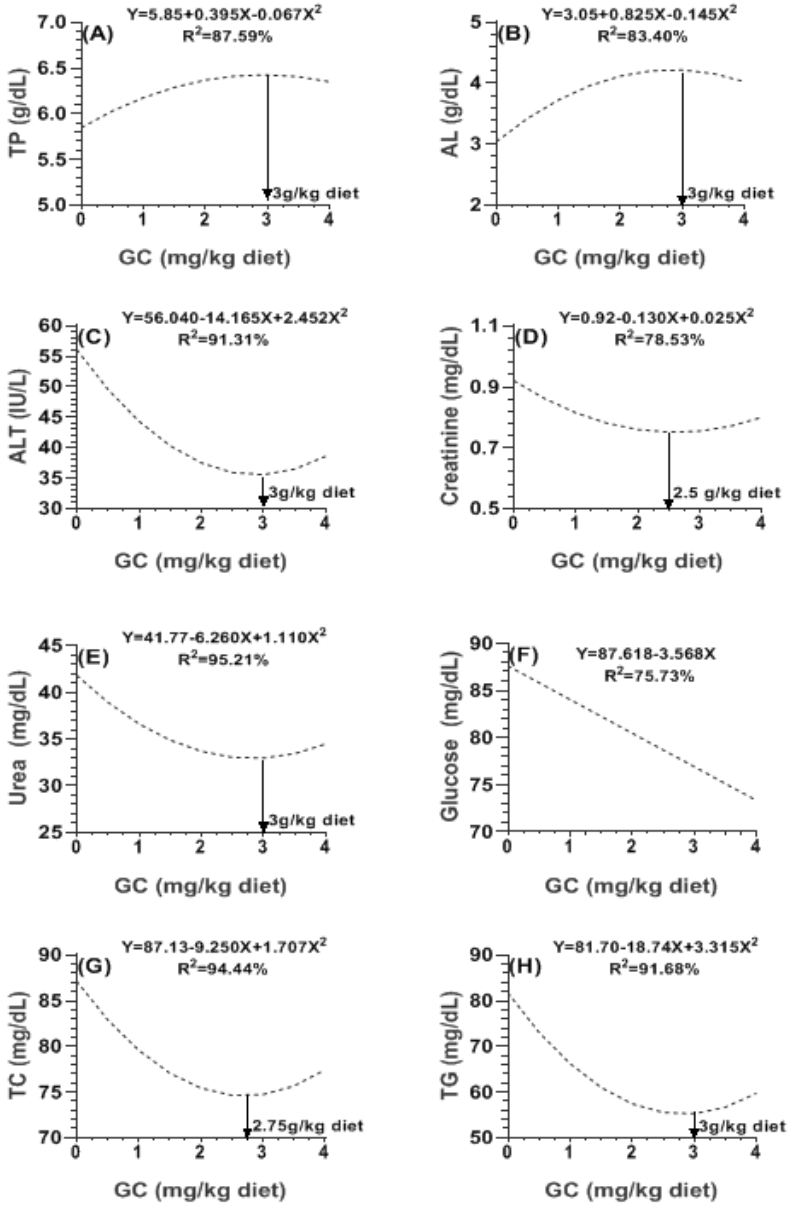


Figure 5. A polynomial regression analysis of total protein (A – TP), albumin (B – AL), alanine aminotransferase (C – ALT), creatinine (D), urea (E), glucose (F), total cholesterol (G – TC), and triglyceride (H – TG) on dietary levels of GC

Regarding blood lipid profile, the values of TC and TG decreased significantly (P <0.0001) due to the GC treatment. The regression analysis indicated that the optimal doses were observed at levels of 2.75 and 3 g GC/kg diet for TC and TG, respectively (Figure 5 G and H). Meanwhile, the high- and low-density lipoprotein did not differ significantly between the control and the treated groups (P>0.05).

Redox and immunity status

Results in Table 7 clearly indicated that there were significant positive effects of GC treatments on redox status concerning the levels of serum TAC (P=0.0157), SOD (P=0.0016), and GSH-Px (P<0.0001), and the levels of MDA in blood serum and liver tissue (P=0.0002 and <0.0001, respectively). The levels of serum TAC were significantly higher in the GC2 treated groups than the control group, however non-significant difference was observed between the GC4 treated group and the control (P>0.05) and the polynomial regression analysis indicated that the optimal dose was 2.5 g GC/kg diet (Figure 6 A). The activities of SOD and GSH-Px were significantly higher in the GC treated groups than the control group (P<0.05). The results of quadratic regression showed that the optimal doses were 2.5 and 3 g GC/kg diet for SOD and GSH-Px, respectively (Figure 6 B and D). Regarding the oxidative stress, the levels of MDA in serum and liver tissue were significantly reduced in GC-treated groups compared to the control group (P<0.05) and they optimally improved at 3 and 2.75 g GC/kg diet, respectively (Figure 6 C and E).

Table 7. Redox status and immunity response of growing APRI rabbits exposed to high ambient temperature as affected by dietary treatments (GC0: 0 g/kg diet, GC2: 2 g/kg diet, and GC2: 4 g/kg diet

Variables[1]	Treatments			SEM	P-value[2]		
	GC0	GC2	GC4		TRTS	lin	quad
Redox status							
TAC (ng/mL)	0.26 b	0.44 a	0.38 ab	0.032	0.0157	0.0595	0.0171
GSH (mg/dl)	13.90	14.85	14.97	1.252	0.4384	0.3247	0.1754
SOD (U/mL)	0.20 b	0.41 a	0.36 a	0.019	0.0016	0.0027	0.0115
MDA (nmol/mL)	10.84 a	8.08 b	8.30 b	0.440	0.0002	0.0002	0.0082
GSH-Px (mg/dL)	3.50 b	5.29 a	5.21 a	0.042	<.0001	<.0001	<.0001
TAC liver (nmol/ml)	0.90	1.08	1.04	0.053	0.0514	0.0556	0.0994
MDA liver (nmol/ml)	5.72 a	3.41 b	3.66 b	0.067	<.0001	<.0001	0.0002
Immunity							
IgG (mg/dl)	489.93 b	535.32 a	526.66 a	3.761	<.0001	<.0001	0.0009
IgA (mg/dl)	150.99 b	165.20 a	166.12 a	3.286	0.0085	0.0053	0.1085
IgM (mg/dl)	126.53 b	138.76 a	135.41 ab	2.669	0.0025	0.0020	0.0441

TAC, total antioxidant capacity; GSH glutathione; SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M. [2]TRTS – treatment effect, lin – linear regression, and quad – quadratic regression. a, b – means within a row without a common letters differ at P<0.05.

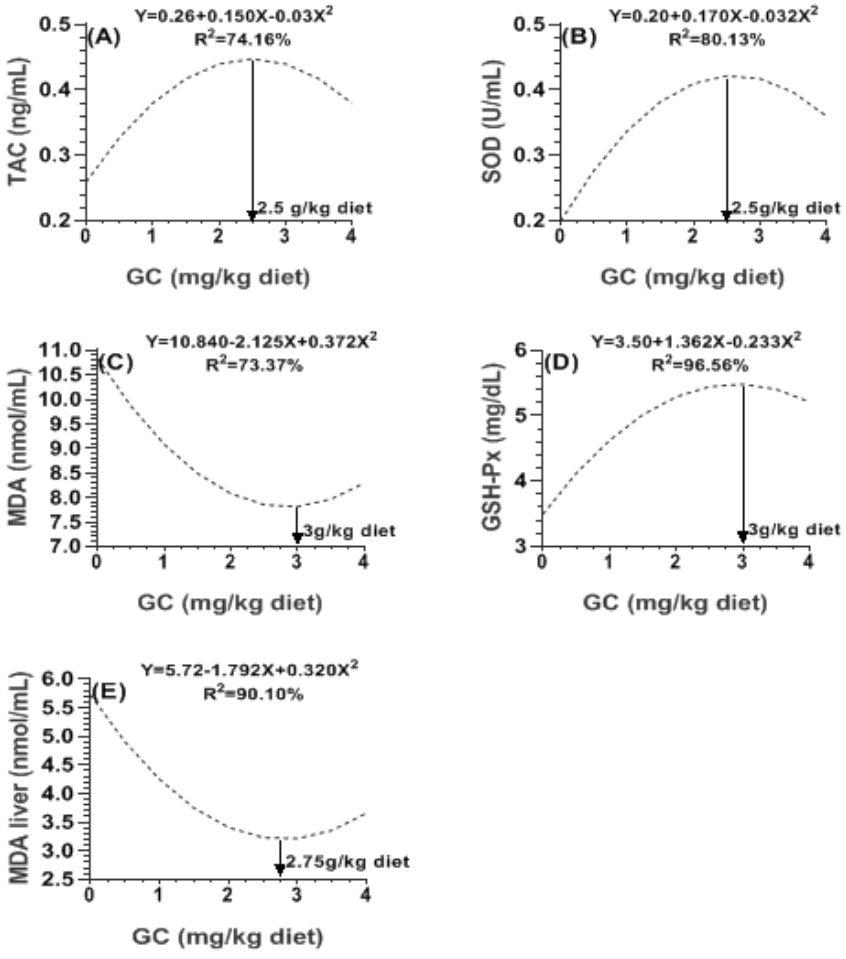


Figure 6. A polynomial regression analysis of total antioxidant capacity (A – TAC), superoxide dismutase (B – SOD), malondialdehyde (C – MDA), glutathione peroxidase (D – GSH-Px), malondialdehyde in liver (MDA liver) on dietary levels of GC

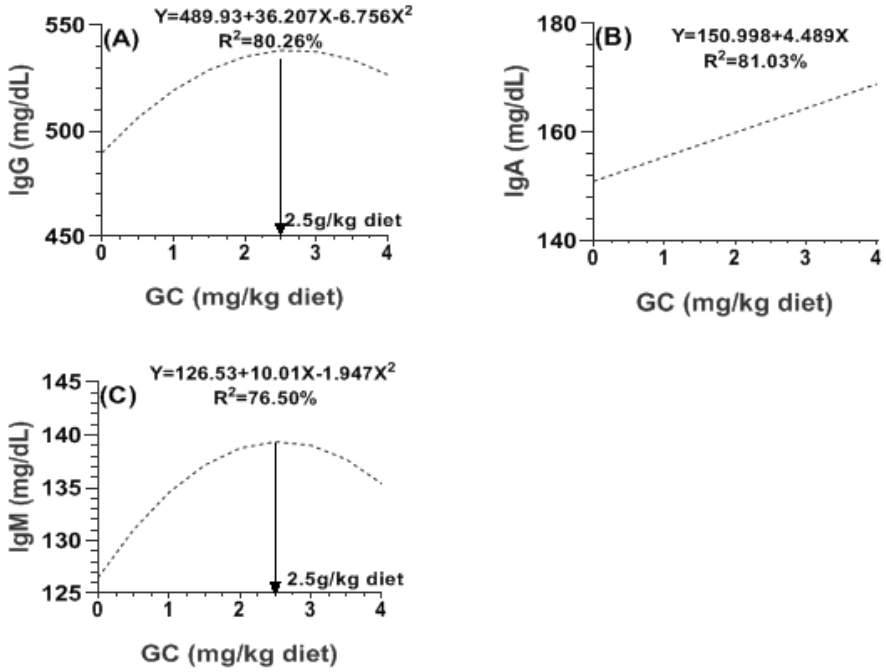


Figure 7. A polynomial regression analysis between dietary levels of GC immunoglobulin G (A – IgG), immunoglobulin A (B – IgA), and immunoglobulin M (C – IgM)

Table 8. Economic efficiency of growing APRI rabbits exposed to high ambient temperature as affected by dietary treatments (GC0: 0 g/kg diet, GC2: 2 g/kg diet, and GC2: 4 g/kg diet

Items	GC0	GC2	GC4	SEM	P-value
Live body weight (kg)	1.950 b	2.080 a	2.086 a	0.014	<0.0001
BWG/rabbit (kg)	1.279 b	1.413 a	1.401 a	0.013	<0.0001
Total revenue/rabbit (USD)	4.221 b	4.664 a	4.623 a	0.046	<0.0001
Feed intake/rabbit (kg)	4.995	4.930	4.970	0.094	0.8872
Feed cost/rabbit (USD)	1.598	1.577	1.590	0.031	0.8995
GC cost/rabbit (USD)	0.00 c	0.049 b	0.099 a	0.001	<0.0001
Total costs/rabbit (USD)	1.598	1.627	1.689	0.031	0.1059
NE/rabbit (USD)	2.623 b	3.037 a	2.934 a	0.060	<0.0001
Economic efficiency (USD)	1.655 b	1.885 a	1.746 ab	0.064	0.0589
REE	100	113.94	106.63	–	–

BWG, body weight gain; NE, net revenue; EE, economic efficiency; REE, relative economic efficiency. a, b – means within a row without a common letters differ at P<0.05.

With respect to the immunity status, results in Table 7 indicated that the dietary GC resulted in significant improvements in levels of IgG, IgA, and IgM (P<0.0001, 0.0085, and 0.0025, respectively). The increased levels of GC affected IgA linearly (P=0.0053; Figure 7 B) and both IgG and IgM quadratically (P=0.0009 and 0.0441, respectively) and the optimal doses were 2.5 g GC/kg diet (Figure 7 A and C).

Economic efficiency

Results in Table 8 illustrated the effect of dietary fortification of fattened rabbit diets by GC on economic efficiency. The GC2 treated group showed the best NE, EE and REE followed by the GC4 compared to the control group (P<0.05).

Discussion

One of the major environmental stressors facing the profitable production of rabbit meat is heat stress (HS), especially in tropical and subtropical regions, which are usually characterized by high ambient temperature combined with high humidity. The growth of rabbit requires maintaining animals under ideal microenvironment conditions to allow the expression of good quality traits

embedded in their genetic components (Oladimeji et al., 2022). Rabbits have no or few sweat glands, thus making them more susceptible to HS, which has adverse impacts on adaptation, welfare, feed intake and utilization, growth performance, redox balance, and health status (El-Raghi et al., 2023). The employment of eco-friendly feed additives to relief of the negative effect of HS remains an indispensable issue. In the present study, the effects of GC as a potential source of phytogenics with biological antioxidant activity on maintaining growth performance and carcass traits, metabolism, and redox and immune statuses of heat stressed newly weaned rabbits were explored. Among phytogenic-derived antioxidants, GC is considered as one of the excellent sources of phenolic compounds (Hashem et al., 2020). This is confirmed in our study as the HPLC analysis identified 9 phenolic compounds (chlorogenic, caffeine, catechin, ellagic, ferulic, catechol, pyrogallol, 3-OH tyrosol, and coumarin) that are known by their strong antioxidant activities in biological systems (Erskine et al., 2022).

Temperature-humidity index (THI) could be used to determine the vigor of HS induced by the elevated ambient temperature (Kang et al., 2020). In this study, the values of THI signalized that the fattened rabbits are raised under severe HS (28.9 to <30.0; Marai et al., 2002). Nevertheless, the dietary supplementation of GC boosted the heat tolerance capacity, resulting in significant improvements in the growth performance, feed utilization, antioxidant capacity, and immune function.

With regard to the thermoregulatory parameters, the supplementation of rabbit diets with GC decreased the rectum temperature, which may be attributed to their high contents from different phenolic compounds including flavones and flavonoids (Erskine et al., 2022). In same context, under high ambient temperature, rabbits begin to increase their respiration rate to achieve thermoregulation which can explain the increase in panting rate in the control group compared to the GC-treated groups. Aforementioned findings can present a sufficient explanation for the improvements in growth performance of GC-supplemented rabbit, indicating the bioactive roles of GC in thermoregulation as the most of the available energy is consumed for the important biological functions in the body such as growth rather than being expenditure in heat regulation process and respiratory evaporation (El-Desoky et al., 2022).

Rabbits that received GC supplementation showed high growth performance (higher body weights and FCR) compared to control rabbits. These results are in accordance with those found by Abdelnour et al. (2022) and Chen et al. (2022). These positive effects on growth performance can be ascribed to the action of some phenolic compounds of GC. For example, dietary supplementation with 800 mg/kg chlorogenic acid (the abundant phenolic compound found in GC in the present study) could improve the growth performance of weaned rabbits by enhancing intestinal structural integrity, improving the intestinal epithelium functions, and modulating the composition and diversity of gut microbiota (Chen et al., 2022) . In fact, this phenolic compound has a wide range of potential health benefits, including its anti-diabetic, anti-carcinogenic, and anti-inflammatory effects (Tajik et al., 2017). Moreover, chlorogenic acid, caffeine, and ferulic acid present in coffee showed inhibitory effects against wide ranges of pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Salmonella choleraesuis* (Martínez-Tomé et al., 2011; Murai and Matsuda, 2023). This effect supports the function and health of the gastrointestinal tract and thus nutrient absorption and utilization.

In the existing study, the fortification of fattened rabbit diets by 2 g GC/kg diet, resulted in significant increase in the dressing percentage compared to the untreated group. Also, there was no indication of damage to major organs under hot conditions as a result of the oxidative stress, which may due to the antioxidant properties of GC (Dogan and Celik, 2012).

A complete blood count is considered as a good signal of general health status of rabbits and their immunity responses; numerous diseases and/or HS can alter hematological parameters, especially both of erythrocyte and leucocyte counts. Herein, the addition of GC resulted in a significant increase in erythrocyte and hemoglobin concentration and decreased the WBCs in the blood, being in the accepted range, which reflects the better health status of heat stressed growing rabbits. The increased number of erythrocytes and hemoglobin concentrations play an important role in oxygen circulation and its delivery for different organs/tissues. This is a very important physiological adaptive mechanism to heat stress. Interestingly, D'Alessandro et al. (2020) found a beneficial correlation between the caffeine levels and improved antioxidant capacity of stored erythrocytes. Thus, caffeine is one of the GC phenolic compounds that can maintain erythrocytes functioning and integrity under heat stress conditions.

The liver has a definitive role in the synthesis of blood protein and other enzymes involved in heat regulation. In this regard, the higher relative weight of liver in GC treated rabbits than their counterparts in the control could be considered as a mirror to improved health status of growing rabbits at marketing age. Also, the decreased levels of liver enzymes (ALT) in this study and the increased levels of serum total protein, albumin and globulin, being in normal physiological ranges showed that the administration of GC was safe and helpful in expressing liver functions. Moreover, the significant decrease in the levels of urea and creatinine concentrations in serum of GC treated rabbits compared to control indicated a potential and positive impact of dietary GC supplementation on normal kidney function and feed utilization. Many phenolic compounds of GC have hepatoprotective effect mainly through their antioxidant, anti-inflammatory and detoxification activities (Rathee et al., 2023).

Heat stress is expected to disrupt metabolism of growing rabbits, by decreasing concentrations of total

protein, blood glucose and triglyceride, while increasing cholesterol concentrations (Liang et al., 2022). An opposite trend was observed in our study, as the metabolism was changed by a manner supporting growth process. Energy yielding metabolites such as glucose and cholesterol were lower, while total protein and albumin were higher in GC-treated rabbits than the control rabbits. These changes happened with positive effects on growth performance and without negative impacts on liver and kidney functions. In same context, some phenolic compounds existing in GC can affect glucose metabolism. For example, coffee chlorogenic acid has antidiabetic effect and can improve insulin sensitivity and thus glucose utilization by peripheral tissues (Yanagimoto et al., 2022).

It is well known that under HS conditions, there is an excessive production from reactive oxygen species (ROS), which trigger oxidative stress and deteriorate the function and/or structure of the important molecules such as nucleic acids and proteins (El-Raghi et al., 2023). The antioxidant defense system including enzymes such as SOD, GSH-PX, and GSH are responsible for maintaining ROS levels. In the present study, the dietary supplementation of GC resulted in a significant decrease in lipid peroxidation in serum and liver, but significant increases in TAC, SOD, GSH, and GSH-PX activities compared to the control. These results are in accordance with those found in studies focused on the use of the natural antioxidants (e.g., flavonoids and flavones) to maintain redox status under HS conditions (Sadeek, 2018; El-Ratel et al., 2019).

Regarding general health status, HS is involved in diminishing the immune capacity of fattened rabbits as it reduces the synthesis of cellular immunity such as IgG and IgM, which make rabbits more susceptible to pathogens and disturb their growth performance and feed efficiency (Abdelnour et al., 2020). In this study, the GC can boost the synthesis of immunological variables including IgG, IgM and IgA during summer season, offering an affirmative mean to conquer the harmful effects of HS on growing rabbits. The positive effects of GC could be attributed to its properties as anti-inflammatory, antibacterial, and antioxidant agent (Hashem et al., 2020). These properties enable GC to maintain gut eubiosis of growing rabbits. Moreover, it can balance the ecosystems of bacteria in the gut, ultimately leading to better metabolism and feed utilization (Mousa et al., 2022). These results are in accordance with those obtained in other studies using polyphenol-based supplementation (El-Desoky et al., 2021; Hashem et al., 2023 b).

Economically, several studies have proven that the HS is involved in negative effects on rabbits growth performance, leading to multiple economic losses as a result of reduced feed efficiency, higher health management costs, and increased mortality rates (Oladimeji et al., 2022; El-Raghi et al., 2023). Herein, the dietary addition of GC has been shown to enhance the economic efficiency of the supplemented diets, thereby improving the profitability of the fattening process.

Conclusion

Based on the aforementioned results, the dietary supplementation of heat-stressed growing rabbit diets with phytogenic antioxidants derived from GC can counter the adverse effects of natural heat stress on growth performance, feed utilization, antioxidants criteria, and immunity status, enhancing the general health status and economic efficiency of fattened process. These effects seem to be related to the antioxidant activity of the unique mixture of phenolic components and its multi-functional biological roles. The dose-response analysis revealed a dose of 2.5–3 g GC/kg diet can be recommended as an optimal dose range for growing rabbits raised under heat stress conditions.

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Conflicts of interest

The authors declare no conflicts of interest.

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