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Combined modulatory effects of dietary arginine and olive leaf phenolic extract on growth performance and immune functions of broiler chickens, and meat antioxidant potential during frozen storage

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Abstract

Background Nowadays, broilers reared in intensive farming become more susceptible to oxidative stress, which impairs their performance and the quality of their products. Arginine (G) is a crucial amino acid for chickens and feeding on arginine beyond the recommended levels has been shown to positively impact the growth performance of broiler chickens and their immunity. Olive leaves phenolic extract (OLE) is a natural source of powerful antioxidants. The current study aimed to investigate the combined efficacy of these functional feed additives (G + OLE) in enhancing broilers' growth performance, immunity, and muscle development, as well as potentiating meat quality and antioxidant capacity during freezing.

Methods Broilers ($n = 250$) were randomly assigned into control (without supplementations) and four groups fed control diets plus 1.5 g/kg arginine alone (G) or with three different levels of OLE; 0.25%, 0.5% and 1% (G + OLEI, G + OLEII and G + OLEIII, respectively).

Results During whole rearing periods, G + OLE inclusion boosted efficacy on body weight gain, and feed conversion ratio in a dose-dependent manner. The postmortem pH values at 0.5, and 24 h, drip loss, and cooking loss % of meat were considerably minimized in G + OLE-supplied groups, especially at high levels. Even after 4 weeks of frozen storage, G + OLEIII, G + OLEII groups exhibited the most prominent increase in the breast meat scavenging ability for free radicals (2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, and ferric reducing antioxidant power) with an inverse minimization in lipid peroxidation attributes (malondialdehyde). Total flavonoid, and phenolic contents, total antioxidant capacity, and antioxidant enzymes' activities in the breast meat

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were significantly improved by increasing the concentrations of dietary G + OLE. Concordantly, upregulation of genes encoding immunity (immunoglobulins A, G and M), antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, peroxiredoxin-1, heme oxygenase-1, NAD(P)H dehydrogenase quinone 1, xanthine oxidoreductase, and heme oxygenase 1), and muscle development (myogenic determination factor, myogenin and mammalian target of rapamycin), and downregulation of myostatin, were remarkably recognized in G + OLE-supplied groups.

Conclusions The outcomes of the current study supported the usage of dietary G + OLE as an innovative feed supplement in the broilers industry to improve broilers` production, and meat quality during frozen storage.

Keywords Arginine, Phenolic extract, Immunostimulant, Meat quality, Growth performance, Broiler

Background

Currently, broilers` intensive farming greatly increases the productivity and profitability of chickens, however, it also increases the susceptibility of those broilers to oxidative stress [1], which can compromise their immunity and physiological functions, lowering the quality and shelf life of chicken meat and causing financial losses [2, 3]. Furthermore, the excessive utilization of antimicrobial agents in recent decades has endangered human and animal health due to the emergence of antimicrobial resistance, and the negative consequences of chemosynthetic medicines [4–7]. Thus, the public's and researchers' interest in medical herbs and plant extracts/metabolites has increased globally [8]. Phytogenics are the secondary metabolites secreted by plants as a result of interactions with their surroundings [9]. The potential of phytogenics to demonstrate growth-enhancing, immune-promoting, anti-inflammatory, and anti-oxidative capabilities has drawn interest in their usage as alternative growth promoters, immunostimulants, as well as antioxidants in broiler production [10, 11].

By using agro-industrial biomass, new phytogenics that can improve birds' welfare and health can be developed, as well as innovative solutions to present and future problems in the poultry industry. Among the dietary phytogenics supplementations utilized in the food, and broilers industry is olive leaf extract (OLE), which has a powerful antioxidant potential [12, 13]. Moreover, OLE may be utilized as a natural source of phytogenics such as oleuropein, hydroxytyrosol, tyrosol, caffeic acid, phenolic acids, rutin, luteolin, mannitol and apigenin [14]. The main constituents of OLE are polyphenols, carbohydrates, iridoids, and flavonoids [12, 15]. Polyphenolic compounds are regarded as the primary bioactive components in OLE [16]. Among these functional polyphenolic compounds, oleuropein accounts for a significant portion, ranging from 8 to 14% [17]. Additionally, flavonoids (such as luteolin, rutin, apigenin, etc.) and single phenols (including vanillin, tyrosol, caffeic acid, hydroxytyrosol, etc.) are also present in olive leaf [18]. Olive leaf extract (OLE) displayed a stronger antioxidation than vitamins C and E due to the synergic performance between oleuropeosides and other bioactive phenols [19].

The polyphenolic components of OLE can scavenge free radicals, disrupt the free radical chain reaction [20], as well as suppress the metal ion chelation, which may contribute to the antioxidant characteristics of OLE [21]. Of note, OLE has been shown to have antiviral [21], antibacterial [22], antioxidant [23], immunostimulant, and anti-inflammatory [24] properties, in addition to enhancing the storage life, quality, and antioxidant potential of chicken meat during frozen storage [21, 25]. Recently, Xie et al. [16] stated that feeding of broilers chickens on OLE enhanced meat quality by increasing levels of glutathione and total superoxide dismutase and decreasing levels of malondialdehyde in breast meat.

Arginine (G) is considered a nutritional supplement with enhancing impact on growth performance, immune system, antioxidant potential, and carcass yield when included in the poultry diet [25–28]. Unlike mammals, poultry lack a functional urea cycle, which prevents them from synthesizing endogenous L-arginine, thus it is regarded as an essential amino acid [29]. Since broilers obtain all their arginine requirements from their diet, it is essential that their feed contains an adequate amount to support protein synthesis and various metabolic, immunological, and pathophysiological processes [30]. Moreover, arginine offers additional benefits, such as acting as a precursor of nitric oxide, which plays a significant role in controlling lipogenesis, partitioning of energy, gastrointestinal mucosa development, pathogen elimination, and enhancing immune defense [6, 31]. Notably, a previous study stated that the combined use of phytochemicals blends with arginine enhanced the growth performance, health status, and immunity of poultry [27]. As far as we know, the combined effect of dietary arginine with various concentrations of OLE on broilers` growth performance, immunity, meat quality, and antioxidant potential of breast meat during frozen storage has not been previously investigated. Thus, the current study aimed to investigate, for the first time, the in vivo impact of dietary arginine with various concentrations of OLE on broiler chickens` growth performance, meat quality, and antioxidant potential of breast meat during frozen storage, as well as antioxidant enzymes, and immune-related genes expression.

Table 1 Impact of arginine with or without Olive leaf extract supplementations on broilers` growth performance parameters (starter, grower, finisher, and total rearing period)

Parameter	Experimental groups					*p*-value
	Control	G	G + OLEI	G + OLEII	G + OLEIII	
Starter period (1–10 days)						
Initial body weight	45.2 ± 1.3	45.2 ± 0.8	45.2 ± 1.3	45.4 ± 1.1	45.4 ± 5.5	0.994
Body weight, g/bird	352.5 ± 1.2^{d}	376.4 ± 4.7^{c}	383.5 ± 3.9^{b}	383 ± 4.c^{b}	392.3 ± 3.3^{a}	< 0.001
Body weight gain, g/bird	307.3 ± 0.7^{d}	331.3 ± 4.6^{c}	338.3 ± 2.7^{b}	337.6 ± 4.6^{b}	346.9 ± 3.3^{a}	< 0.001
Feed intake, g/bird	401.8 ± 1.8$^{b, c}$	396.1 ± 4^{c}	404.3 ± 4.2$^{a, b}$	409.4 ± 5.7^{a}	404.2 ± 3.8$^{a, b}$	0.001
Feed conversion ratio	1.3 ± 0.08^{a}	1.19 ± 0.009^{c}	1.19 ± 0.005^{c}	1.2 ± 0.007^{b}	1.16 ± 0.004^{d}	< 0.001
Grower period (11–20 days)						
Body weight, g/bird	1097.6 ± 18.2^{d}	1231.8 ± 1.5^{c}	1224.4 ± 2.1^{c}	1298.8 ± 14.1^{b}	1322 ± 9.3^{a}	< 0.001
Body weight gain, g/bird	745.2 ± 19.16^{c}	855.6 ± 3.9^{b}	841.2 ± 4.3^{b}	916.2 ± 16.5^{a}	929.4 ± 11.6^{a}	< 0.001
Feed intake, g/bird	1391.3 ± 12.2^{b}	1411 ± 2.9^{b}	1387 ± 3.5^{b}	1467.6 ± 33.4^{a}	1460.5 ± 8.2^{a}	< 0.001
Feed conversion ratio	1.87 ± 0.04^{a}	1.65 ± 0.01^{b}	1.65 ± 0.01^{b}	1.6 ± 0.01^{c}	1.57 ± 0.19^{c}	< 0.001
Finisher period (21–35 days)						
Body weight, g/bird	2386.8 ± 3.1^{c}	2634 ± 4.8^{b}	2630.4 ± 1.1^{b}	2636.4 ± 2.6^{b}	2718.4 ± 8.3^{a}	< 0.001
Body weight gain, g/bird	1289 ± 44.2^{b}	1402 ± 30.1^{a}	1406 ± 39.3^{a}	1337 ± 25.9^{a}	1397 ± 20.5^{a}	< 0.001
Feed intake, g/bird	2466.4 ± 4.8^{a}	2382.8 ± 3.9$^{a, b,c}$	2403.9 ± 12.4$^{a, b}$	2294.3 ± 3.8$^{b, c}$	2279.5 ± 14.5^{c}	0.001
Feed conversion ratio	1.91 ± 0.03^{a}	1.7 ± 0.007^{b}	1.7 ± 0.007^{b}	1.72 ± 0.02^{b}	1.63 ± 0.1^{c}	< 0.001
Total rearing period						
Body weight, g/bird	2386.8 ± 3.1^{c}	2634 ± 4.8^{b}	2630.4 ± 1.1^{b}	2636.4 ± 2.6^{b}	2718.4 ± 8.3^{a}	< 0.001
Body weight gain, g/bird	2341.6 ± 2.4^{c}	2588.8 ± 5.5^{b}	2585.2 ± 1.9^{b}	2591 ± 3.3^{b}	2673 ± 8.5^{a}	0.108
Feed intake, g/bird	4259.4 ± 9.4	4150.6 ± 5.9	4195 ± 8.4	4171.4 ± 31.3	4144 ± 14	< 0.001
Feed conversion ratio	1.82 ± 0.04^{a}	1.62 ± 0.004^{b}	1.62 ± 0.004^{b}	1.61 ± 0.016^{b}	1.55 ± 0.06^{c}	< 0.001

Control: chicks fed basal diets without any supplementations, G: chicks fed control diets fortified with 1.5 g/kg arginine (G), G + OLEI: chicks fed control diets fortified with 1.5 g/kg G plus 0.25% olive leaf extract (OLE), G + OLEII: chicks fed control diets fortified with 1.5 g/kg G plus 0.5% OLE, and G + OLEIII: chicks fed control diets fortified with 1.5 g/kg G plus 1% OLE. The data are displayed as means ± standard error of the mean (SEM). $^{a, b,c, d}$ various superscript letters in a row imply statistical significance ($p < 0.05$)

Table 2 Effect of arginine with or without Olive leaf extract supplementations on chicken breast meat quality after slaughter

Parameter	Experimental groups					*p*-value
	Control	G	G + OLEI	G + OLEII	G + OLEIII	
pH, 0.5 h	6.33 ± 0.04^{d}	6.52 ± 0.03^{c}	6.65 ± 0.03^{b}	6.7 ± 0.05^{b}	6.8 ± 0.02^{a}	< 0.001
pH, 24 h	5.4 ± 0.02^{e}	5.6 ± 0.034^{d}	5.6 ± 0.03^{c}	5.7 ± 0.3^{b}	5.9 ± 0.7^{a}	< 0.001
Drip loss, %	0.88 ± 0.03^{a}	0.76 ± 0.02^{b}	0.66 ± 0.03^{c}	0.64 ± 0.03^{c}	0.59 ± 0.04^{c}	< 0.001
Thaw loss, %	40.4 ± 0.42	40.3 ± 0.31	40.17 ± 0.08	40 ± 0.1	3.67 ± 0.32	0.062
Cooking loss, %	13.9 ± 0.26^{a}	13.2 ± 0.1^{b}	13.1 ± 0.1^{b}	13.03 ± 0.25^{b}	11.8 ± 0.26^{c}	< 0.001

Control: chicks fed basal diets without any supplementations, G: chicks fed control diets fortified with 1.5 g/kg arginine (G), G + OLEI: chicks fed control diets fortified with 1.5 g/kg G plus 0.25% olive leaf extract (OLE), G + OLEII: chicks fed control diets fortified with 1.5 g/kg G plus 0.5% OLE, and G + OLEIII: chicks fed control diets fortified with 1.5 g/kg G plus 1% OLE. The data are displayed as means ± standard error of the mean (SEM). $^{a, b,c, d,e}$ various superscript letters in a row imply statistical significance ($p < 0.05$)

Results

Growth performance attributes

The impact of dietary inclusion of G or G + OLE on the growth performance of broiler chickens is shown in Table 1. During the starter, grower, and finisher periods, BWG, and FCR were enhanced ($p < 0.01$) in all groups supplemented with G or G + OLE especially at higher levels of OLE unlike the control group. During the overall growing period, group fed G + OLEIII had considerable ($p < 0.01$) improvement efficacy on body eight (BW), body weight gain (BWG), and feed conversion ratio (FCR) regarding the control group; however, the FI did not show any considerable alterations ($p = 0.108$) among the experimental groups.

Impact of dietary arginine and arginine/olive leaf phenolic extract fortification on meat characteristics

The data concerning the meat quality are displayed in Table 2. In comparison to the control group, the postmortem pH values at 24 h were significantly ($p < 0.001$) increased in a dose-dependent manner following G + OLE fortifications. Additionally, the postmortem pH values at 0.5 h were increased, and drip loss, and cooking loss % were considerably ($p < 0.001$) minimized in G, and G + OLE -supplied groups, especially at high concentrations unlike the control group. Furthermore, the G + OLEIII group exhibited the highest levels of pH at 0.5 and 24 h (6.8 and 5.9, respectively), and the lowest cooking loss % (11.8%). Of note, G, and G + OLE dietary

supplementations didn`t affect the thaw loss % ($p = 0.062$) regarding the control group.

Antioxidant potential of chicken breast meat during frozen storage in response to dietary arginine and arginine/olive leaf phenolic extract

Table 3 illustrates the effect of dietary G and G + OLE inclusion on antioxidant and lipid oxidation indicators in chicken breast meat during frozen storage. Interestingly, dietary fortification of G and G + OLE, especially at higher concentrations displayed significant ($p < 0.001$) augmentation in the ferric reducing antioxidant power (FRAP), and enhanced chicken breast meat's capability to scavenge the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and (2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis (DPPH) free radicals at both 3 h and 4 weeks storage periods unlike the control group. At 3 h storage period, the G + OLEIII group exhibited higher ($p < 0.001$) reducing capacity of Fe^{3+} to Fe^{2+} (0.37) in contrast to the control group (0.18); meanwhile, after 4 weeks of storage period, FRAP reached its peak in G + OLEIII and G + OLEII groups (0.47, and 0.43, respectively) unlike the control group (0.26). At both 3 h, and 4 weeks storage intervals, G + OLEIII supplied group had the highest ($p < 0.001$) chicken breast meat`s capacity to scavenge DPPH (7.4, and 7.57, respectively), and ABTS (4.5, and 8.4, respectively) free radicals concerning the control group (5.89, and 6.3, and 2.4, and 5.4, respectively).

Using thiobarbituric acid reactive substances (TBARS) assay, the breast meat's malondialdehyde (MDA) content were generally increased reflecting lipid oxidation, as the duration of storage was extended. Meanwhile, dietary G + OLE fortifications markedly ($p < 0.001$) minimized MDA contents recording the lowest levels ($p < 0.001$) at both 3 h, and 4 weeks storage intervals in the G + OLEIII group (0.043, and 0.51, respectively) in comparison to the control group (0.19, and 0.8, respectively).

The total flavonoid and phenolic levels in the breast meat samples were augmented ($p < 0.001$) with increasing the concentrations of dietary G + OLE. Moreover, the G + OLEIII group displayed the most significant ($p < 0.001$) enhancement in the concentrations of total flavonoids content (TFC, 138.4 µg/g), and total phenolics (TPC, 164.6 µg/g) when compared with the control group (74.5 and 85.4 µg/g, respectively).

In the chicken breast muscle, the activities of SOD, T-AOC, GPX, and CAT enzymes were significantly ($p < 0.001$) enhanced with increasing the concentrations of dietary G + OLE when compared with the control group. Notably, the G + OLEIII group exhibited the most significant ($p < 0.001$) improvement in the levels of total antioxidant capacity (T-AOC, 12.7 U/mg protein), and SOD (124.7 U/L) enzymes unlike the control group (7.6 U/mg protein, and 52.3 U/L, respectively). Moreover, the most prominent increase in the levels of CAT (86.9, and 81.4 U/L), and GPX (343, and 364 µmol/mg) enzymes were found in G + OLEIII, and G + OLEII groups, respectively comparing with the control group (31.9 U/L, and 304 µmoL/mg, respectively).

Table 3 Impact of arginine with or without Olive leaf extract supplementations on the antioxidant potential of chicken breast meat during frozen storage

Parameter	Experimental groups					*p*-value
	Control	G	G + OLEI	G + OLEII	G + OLEIII	
FRAP, 3 h	0.18 ± 0.03^{d}	0.23 ± 0.03^{c}	0.25 ± 0.02^{c}	0.31 ± 0.01^{b}	0.37 ± 0.01^{a}	< 0.001
FRAP, 4 weeks	0.26 ± 0.002^{c}	0.31 ± 0.003^{c}	0.37 ± 0.002^{b}	0.43 ± 0.003^{a}	0.47 ± 0.015^{a}	< 0.001
DPPH, 3 h	5.89 ± 0.02^{e}	6.20 ± 0.02^{d}	6.30 ± 0.04^{c}	6.90 ± 0.04^{b}	7.40 ± 0.04a	< 0.001
DPPH, 4 weeks	6.30 ± 0.07^{e}	6.70 ± 0.08^{d}	6.90 ± 0.03^{c}	7.15 ± 0.05^{b}	7.57 ± 0.06a	< 0.001
ABTS, 3 h	2.40 ± 0.03^{e}	3.30 ± 0.04^{d}	3.60 ± 0.03^{c}	4.30 ± 0.07^{b}	4.50 ± 0.06^{a}	< 0.001
ABTS, 4 weeks	5.40 ± 0.05^{c}	5.70 ± 0.2^{c}	6.03 ± 0.59^{c}	7.67 ± 0.04^{b}	8.4 ± 0.16^{a}	< 0.001
TBARS, 3 h	0.19 ± 0.006^{a}	0.17 ± 0.016^{a}	0.12 ± 0.01^{b}	0.09 ± 0.01^{b}	0.043 ± 0.02^{c}	< 0.001
TBARS, 4 weeks	0.80 ± 0.03^{a}	0.71 ± 0.04^{b}	0.63 ± 0.03^{c}	0.58 ± 0.03^{c}	0.51 ± 0.02^{d}	< 0.001
Total flavonoids (µg/g)	74.50 ± 1.1^{d}	102.75 ± 3.2^{c}	105.10 ± 1.1^{c}	128.73 ± 1.40^{b}	138.40 ± 1.80^{a}	< 0.001
Total phenolic compounds (µg/g)	85.40 ± 0.68^{e}	127.30 ± 0.95^{d}	143.60 ± 4.90^{c}	150.90 ± 1.20^{b}	164.60 ± 4.50^{a}	< 0.001
SOD (U/mL)	52.25 ± 5.10^{e}	63.95 ± 3.60^{d}	78.68 ± 1.60^{c}	112.90 ± 3.10^{b}	124.66 ± 4.30^{a}	< 0.001
GPX (µmol/mg)	304.38 ± 1.70^{d}	332.45 ± 6.30^{c}	345.65 ± 3.60^{b}	364.00 ± 5.50^{a}	368.48 ± 24.30^{a}	< 0.001
CAT (U/L)	31.92 ± 1.90^{d}	47.23 ± 2.50^{c}	58.04 ± 0.79^{b}	81.42 ± 6.90^{a}	86.91 ± 5.30^{a}	< 0.001
T-AOC (U/mg protein)	7.6 ± 0.57^{d}	9.2 ± 0.08^{c}	10.2 ± 0.71^{b}	11 ± 0.06^{b}	12.7 ± 0.07^{a}	< 0.001

FRAP: ferric reducing antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TBARS: thiobarbituric acid reactive substances; SOD: superoxide dismutase; GPX: glutathione peroxidase; CAT: Catalase; T-AOC: total antioxidative capacity. Control: chicks fed basal diets without any supplementations, G: chicks fed control diets fortified with 1.5 g/kg arginine (G), G + OLEI: chicks fed control diets fortified with 1.5 g/kg G plus 0.25% olive leaf extract (OLE), G + OLEII: chicks fed control diets fortified with 1.5 g/kg G plus 0.5% OLE, and G + OLEIII: chicks fed control diets fortified with 1.5 g/kg G plus 1% OLE. The data are displayed as means ± standard error of the mean (SEM). $^{a, b, c, d, e}$ various superscript letters in a row imply statistical significance ($p < 0.05$)

Gene expression profiles of immune markers in response to dietary arginine and arginine/olive leaf phenolic extract

Figure 1 shows the relative mRNA transcription levels of immune-related genes following dietary fortification with G, and G + OLE in broilers at both 22-and-35 days of age. At 22 days of age, birds in G + OLEIII group showed the highest expression levels of immunoglobulin (*IgA)* (4.54-fold), and *IgG* (5.12-fold) genes when compared to the control group. Moreover, the expression levels of *IgM* (4.89-and 4.80-fold, respectively), *IgA* (3.31-and 3-fold, respectively), and *IgG* (4.87-and 4.70-fold, respectively) genes reached their peaks in G + OLEIII, and G + OLEII groups, respectively concerning the control group at 35 days of age.

Genes coordinated muscle mass development in response to dietary arginine and arginine/olive leaf phenolic extract

The RT-qPCR analysis for genes related to muscle development is displayed in Fig. 2. Notably, all groups receiving G and G + OLE exhibited a significant ($p < 0.05$)

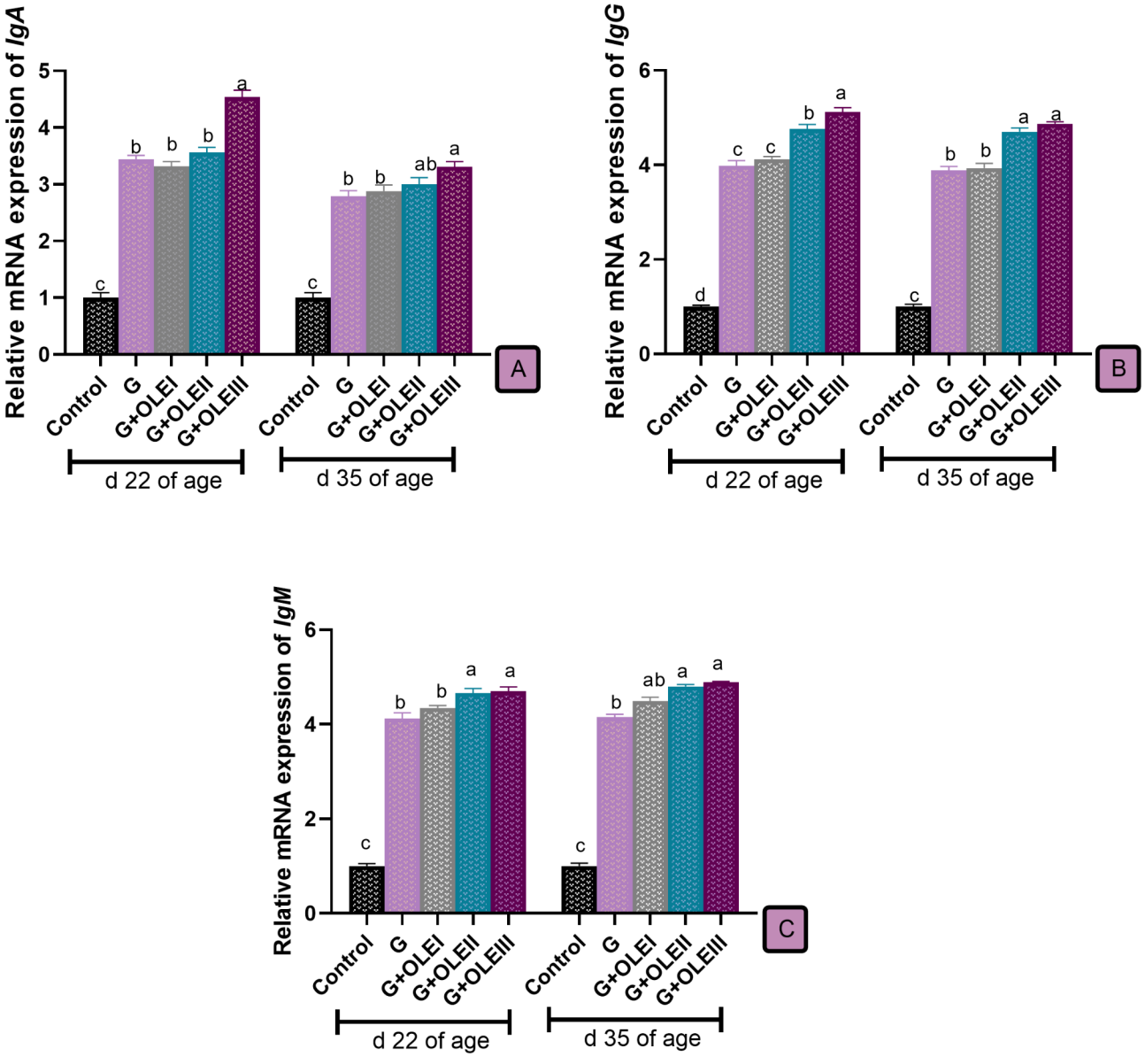

Fig. 1 Relative mRNA transcription levels of broilers' immune-related genes including IgA (immunoglobulin A; **A**), IgG (immunoglobulin G; **B**), and IgM (immunoglobulin M; **C**) in intestinal samples of broiler chickens fortified with arginine (G) and G/olive leaf extract (OLE) at 22-and-35 days of age. The data are displayed as means ± standard error of the mean (SEM). [a, b,c, d] various superscript letters imply statistical significance ($p < 0.05$). Control: chicks fed basal diets without any supplementations, G: chicks fed control diets fortified with 1.5 g/kg arginine (G), G + OLEI: chicks fed control diets fortified with 1.5 g/kg G plus 0.25% olive leaf extract (OLE), G + OLEII: chicks fed control diets fortified with 1.5 g/kg G plus 0.5% OLE, and G + OLEIII: chicks fed control diets fortified with 1.5 g/kg G plus 1% OLE

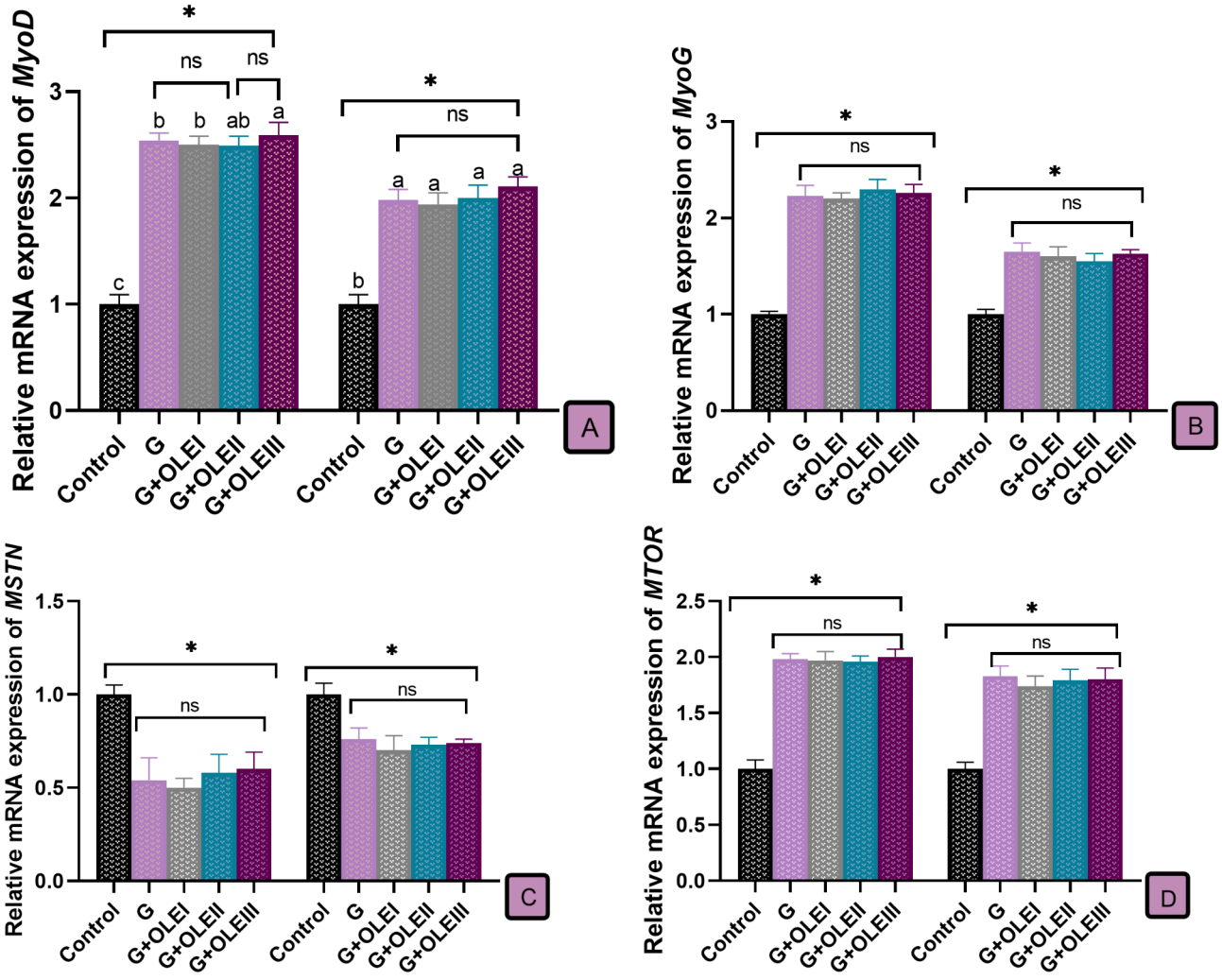

Fig. 2 Impact of dietary arginine (G) and G/olive leaf extract (OLE) on the transcription of genes related to muscle development in the breast meat samples of broilers at 22-and-35 days of age (**A-D**). **A**: *MyoD* (myogenic determination factor); **B**: *MyoG* (myogenin); **C**: *MSTN* (myostatin), and **D**: *mTOR* (the mammalian target of rapamycin). The data are displayed as means ± standard error of the mean (SEM). [a, b,c] various superscript letters imply statistical significance ($p < 0.05$). Control: chicks fed basal diets without any supplementations, G: chicks fed control diets fortified with 1.5 g/kg arginine (G), G + OLEI: chicks fed control diets fortified with 1.5 g/kg G plus 0.25% olive leaf extract (OLE), G + OLEII: chicks fed control diets fortified with 1.5 g/kg G plus 0.5% OLE, and G + OLEIII: chicks fed control diets fortified with 1.5 g/kg G plus 1% OLE

increase in the expression levels of myogenic determination factor (MyoD), mammalian target of rapamycin (mTOR), and myogenin (MyoG) genes, alongside a decrease in the expression of *MSTN* gene at both 22 and 35 days of age. At 22 days of age, the most significant increase in the expression of *MyoD* gene was observed in G + OLEIII group (2.59-fold), followed by G + OLEII group (2.49-fold) regarding the control group. Of note, there were no significant variations in the expression of myostatin (MSTN), *mTOR*, and *MyoG* genes among all G, and G + OLE -supplied groups at both 22 and 35 days of age.

Genes coordinated antioxidant mediators in response to dietary arginine and arginine/olive leaf phenolic extract

Analyzing the expression levels of antioxidant enzymes encoding genes at 35 days of age is displayed in Fig. 3. Dietary inclusion with G + OLE upregulated ($p < 0.05$) the expression levels of NAD(P)H dehydrogenase quinone 1 (NQO1) and nuclear factor erythroid 2-related factor 2 (*Nrf2*) genes in a dose-dependent manner regarding the control group. Among all experimental groups, birds fed G + OLEII and G + OLEIII achieved maximum ($p < 0.05$) expression levels of catalase (*CAT*, 2.11-and 2.24-fold, respectively) and *SOD-1* (1.56- and 1.77-fold, respectively) genes. Compared to the control group, the birds in the G + OLEIII group showed the highest significant ($p < 0.05$) mRNA expression levels of *GPX-1* (1.45-fold),

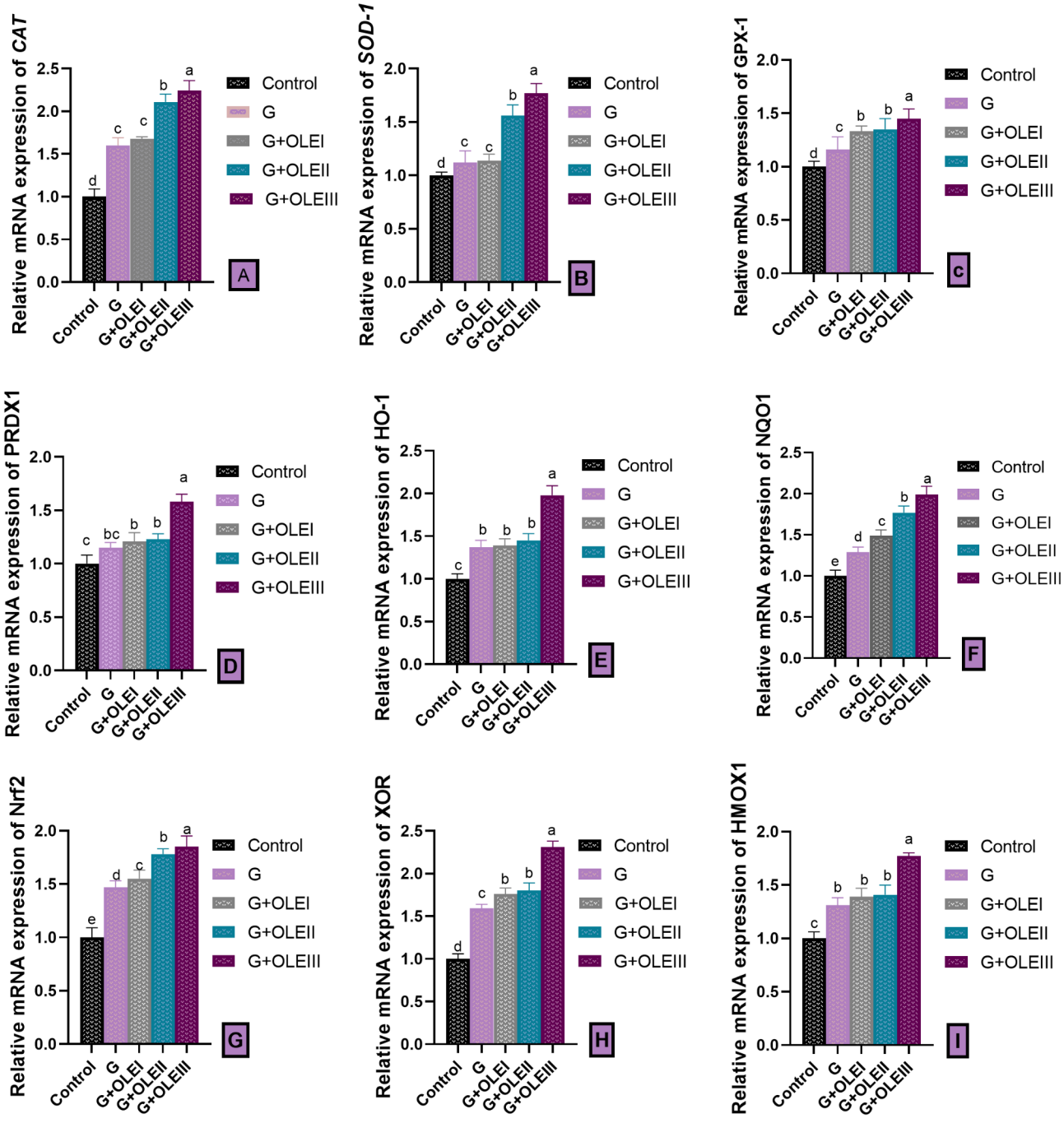

Fig. 3 Relative mRNA expression levels of genes encoding antioxidant enzymes in the breast meat samples of broilers fortified with dietary arginine (G) and G/olive leaf extract (OLE) at 35 days of age (**A-I**). **A**: *CAT* (catalase); **B**: *SOD-1* (superoxide dismutase 1); **C**: *GPX-1* (glutathione peroxidase 1); **D**: *PRDX-1* (peroxiredoxin-1); **E**: HO-1 (heme oxygenase-1); **F**: *NQO1* (NAD(P)H dehydrogenase quinone 1); **G**: *Nrf2* (nuclear factor erythroid 2-related factor 2); **H**: *XOR* (xanthine oxidoreductase), and B: *HMOX1* (heme oxygenase 1). The data are displayed as means ± standard error of the mean (SEM). [a, b,c, d,e] various superscript letters imply statistical significance ($p < 0.05$). Control: chicks fed basal diets without any supplementations, G: chicks fed control diets fortified with 1.5 g/kg arginine (G), G + OLEI: chicks fed control diets fortified with 1.5 g/kg G plus 0.25% olive leaf extract (OLE), G + OLEII: chicks fed control diets fortified with 1.5 g/kg G plus 0.5% OLE, and G + OLEIII: chicks fed control diets fortified with 1.5 g/kg G plus 1% OLE

peroxiredoxin-1 (*PRDX-1* 1.58-fold), heme oxygenase-1 (HO-1, 1.98-fold), NAD(P)H dehydrogenase quinone 1 (NQO1, 1.99-fold), xanthine oxidoreductase (XOR, 2.31-fold), and heme oxygenase 1 (HMOX1, 1.77-fold).

Discussion

Stressful environments can negatively impact broilers raised in modern intensive farming, which increases the likelihood of infectious illnesses, minimizes the quality

and shelf life of chicken meat, and causes significant economic losses [28, 32]. Notably, arginine and OLE are considered nutritional supplements with improving effects on growth performance, immune defense, and antioxidant capacity when included in the broilers` diet [13, 33]. As far as we know, the combined effect of dietary arginine with various concentrations of OLE on broilers` growth performance, immunity, meat quality, and antioxidant potential of breast meat has not been investigated yet. Therefore, our principal goal was to investigate the in vivo efficacy of dietary arginine with or without OLE inclusion on growth performance, immunity, meat quality, and antioxidant capacity of broiler chickens. Herein, the growth performance of broilers chicken throughout the different rearing stage was improved in groups supplemented with G or G + OLE, especially those contains higher concentrations of OLE, unlike the control group. In agreement with our results, a previous work stated that dietary arginine supplementation significantly enhanced FCR when compared to the control group during starter, grower, and overall growing periods of broilers, in addition to increasing BW during the finisher period of broilers [34]. These findings could be attributed to the significant role of arginine in the production of proline, glutamine, polyamines, protein, and ornithine [35]. Similarly, improved FCR of brown-egg layers was noticed following to dietary inclusion on mixture of arginine and phytogenic [27]. In accordance, the combination of arginine, vitamin E, and oils blend promoted the BWG, and FCR of broilers during starter, grower, and finisher periods [35]. Moreover, dietary fortification of OLE as feed supplement considerably boosted BWG, and FCR in broilers [36]. The growth-enhancing impact of OLE may be attributed to the presence of polyphenols, especially oleuropein, which enhance the digestibility of nutrients via stimulating the activities of gut enzymes [12] and promoting the appetite and food consumption [37], besides its antimicrobial, antioxidant, and immunostimulant properties [12, 24]. In this aspect, our results reveals a synergistic impact between arginine, and OLE in promoting overall growth performance attributes of broiler chickens. Skeletal muscles make up almost 50% of a broiler's total BW, and they have an important impact on the quantity of produced edible meat and the productivity of the broilers [38]. Thus, we examined the efficacy of dietary G, and G + OLE supplementation on broilers` meat quality attributes, as well as the transcription levels of genes encoding muscle development, and protein synthesis. Herein, the quality of broilers` breast meat was greatly improved as supported by increasing the postmortem pH values at 0.5, and 24 h, and decreasing the drip loss, and cooking loss %, especially at high concentrations of OLE, when compared to the control group. These results concur with the outcomes of Saleh et al. [13] who stated that OLE increased the pH of poultry meat, which may be attributed to the its bioactive compounds. In accordance, a recent study showed that supplementing broilers` diet with OLE at 0.3, 0.4, and 0.5% concentrations substantially minimized the cooking loss % unlike the control group [29]. Similarly, an earlier study described that dietary OLE had minimized the drip loss, and cooking loss % of broilers` meat unlike the control group [39]. These findings may be linked to the ability of OLE`s phenolic bioactive components to improve meat's capacity for holding sarcoplasmic substances, and stabilize cell integrity, thus decreasing drip loss percentage [39]. Moreover, previous studies proved that OLE improved the broilers` muscle capacity to retain water, thus increasing the water-holding capacity of chicken meat, and minimizing cooking loss, and drip loss percentages [16]. On the contrary, previous studies stated that dietary arginine supplementation did not affect the postmortem pH, drip loss, and cooking loss % in broilers [34]. Additionally, a recent work reported that supplementing broilers` diet with a combination of arginine, vitamin E, and oil blend minimized the drip loss % of meat, but it did not affect the cooking loss %, and the postmortem pH of meat [35].Notably, there is a substantial correlation between the immune system, the overall health of animals, and their antioxidant defense system. Animals` health and performance can be negatively impacted by higher reactive oxygen species levels produced in stressed animals, which can cause considerable cell damage, and lipid peroxidation [32, 40, 41]. Additionally, higher quantities of free radicals promote lipid peroxidation, which causes oxidative stress, and raises MDA concentration leading to meat deterioration after slaughter [42–44]. The primary endogenous antioxidant enzymes.

that control ROS and shield cells from oxidative stress are GPX, SOD, and CAT [45]. Furthermore, oxidative stress may affect the redox-sensitive signaling pathway, and transcription factors, which may compromise the normal metabolic processes of the cell. The transcription factors *NRF2*, *HO-1*, *NQO1*, *PRDX-1*, *XOR*, and *HMOX1* are thought to play important regulatory functions in the cells` oxidative stress response by upregulating the transcription of phase-2 detoxification enzymes, and antioxidant proteins [46, 47]. The addition of phytogenics rich in polyphenols has the ability to scavenge free radicals and helps to prevent ROS harmful impact in order to preserve normal metabolic processes and maintain the animals` antioxidant capacity, which in turn enhances the quality of meat and extends its shelf life after slaughter [48]. In the present work, the activities of T-AOC, GPX, SOD, and CAT enzymes were enhanced in the broilers` breast muscle following dietary G, and G + OLE inclusion when compared with the control group. Additionally,

in parallel with increasing the activities of antioxidant enzymes, dietary fortification with G + OLE upregulated the expression levels of *CAT*, *SOD-1*, *GPX-1*, *HO-1*, *NQO1*, *PRDX-1*, *XOR*, *Nrf2*, and *HMOX1* genes, in a dose-dependent manner in the muscle samples unlike the control group, which suggested the G + OLE antioxidant activity. Accordingly, *Nrf2* signaling pathway controls the transcription of antioxidant genes, which is considered to be the most critical pathway in the cellular antioxidant mechanism as its activation via feeding on antioxidants, can ameliorate oxidative stress in chickens [49]. Additionally, birds in the G + OLEIII group showed the lowest TBRAS development after 3 h and 4 weeks of storage in contrast to the excessive TBARS content found in the control group. This demonstrated the ability of OLE to protect broilers` meat against lipid peroxidation during storage via scavenging free radicals [50]. Our results are in agreement with the findings of previous studies, which indicated that supplementing broilers' diets with OLE significantly enhanced the levels of T-AOC, GPX, CAT [16], and SOD enzymes, and minimized the MDA level [33] in the breast muscle, unlike the control group. Likewise, previous literature indicated that OLE significantly minimized the TBARS in broiler meat during frozen storage unlike the control group [13].These findings could be linked to the free-radical-scavenging, and antioxidant activities of OLE, which might be attributed to its higher levels of polyphenolic compounds such as oleuropein, hydroxytyrosol, tyrosol, caffeic acid, phenolic acids, rutin, luteolin, and apigenin [14, 51]. The protective role of polyphenols in biological processes is linked to their capability for reduction of α- tocopherol radicals, metal chelation, antioxidant enzymes` activation, and electrons` transfer [52]. In consistent with our outcomes, earlier literatures showed reduction in TBARS level in broilers meat during frozen storage following dietary supplementation with combination of arginine, vitamin E, and oils blend [31], and blend of arginine and phytogenics [27]. Similarly, enhanced oxidative stability was observed following the addition of higher concentrations of olive by-products to the diets of broiler chickens [53, 54]. Likewise, fortifying broilers` diet with phytogenic feed additives significantly upregulated the expression level of HO-1, CAT [32, 55], NQO1, SOD-1, GPX-1 [47, 56], HMOX1 [56], and PRDX1 [47, 64] genes, in addition to increasing the T-AOC [47]. Of note, the DPPH has been widely used to investigate compounds' capacity to scavenge free radicals [57, 58]. According to Tayade et al. [59]. and Ibrahim et al. [75], there was a strong correlation between the TPC abundance and DPPH radical scavenging activities. Furthermore, the ABTS radical test is a useful tool for determining a compound's antioxidant activity [60]. The current work described that even after 4 weeks of storage, the scavenging capability of ABTS, and DPPH free radicals, as well as FRAP assay were increased following dietary G + OLE inclusion, particularly at higher concentrations, showing their significant antioxidant properties. Additionally, our results showed that TFC and TPC levels in the breast meat samples were augmented with increasing concentrations of dietary G + OLE fortification. In consistent with our findings, previous reports stated that dietary OLE inclusion significantly enhanced the activities of T-AOC [61], GPX enzyme, TPC, TFC, DPPH [61], and ABTS scavenging, and minimized the TBARS in broilers owing to the free-radical-scavenging, and chelating abilities of polyphenols found in OLE [62]. Similarly, dietary arginine supplementation enhanced the activities of T-AOC, CAT, and FRAP, and minimized MDA levels in quails [63, 64]. Likewise, when compared to the control group, supplementing broilers` diet with olive by-products significantly increased the levels of TFC, TPC, T-AOC, GPX, and SOD enzymes, and DPPH scavenging, in addition to minimizing the TBARS level due to the presence of polyphenolic compounds that scavenge free radicals [65].

Poultry's immune system and antioxidant system activity are positively correlated, providing defense against invasive pathogenic microorganisms. Broilers raised in intensive farming face stressful conditions, thus enhancing their immune defense through food rich in natural antioxidants can help cope with this problem [28]. Additionally, the immune system is crucial for preserving the health of broilers. Phytogenics have an enhancing impact on the poultry immune system via improving the production of immunoglobulins (Igs), interferon-γ release, and lymphocyte activity [55, 66, 67]. Immunoglobulins have a significant role in immunological activities such as opsonization, phagocytosis, and neutralization of harmful microorganisms, making them important parts of the humoral immune defense [58]. Furthermore, IgM, IgG, and IgA are the three main Igs isotypes that react to both systemic and local infections [68]. In this context, dietary G, and G + OLE fortification, especially at higher concentrations, enhanced broilers` humoral immunity as proved by increasing the expression levels of IgM, IgG, and IgA genes when compared to the control group at both 22-and-35 days of age. At 22 days of age, birds in G + OLEIII group showed the highest expression levels of IgA, and IgG genes when compared to the control group. Moreover, the expression levels of IgM, IgG, and IgA genes reached their peak in G + OLEIII, and G + OLEII groups concerning the control group at 35 days of age. In agreement with our findings, earlier literature showed that dietary arginine inclusion significantly increased the level of IgM, and IgA in broilers unlike the control group, but there were no variations in the level of IgG among experimental groups [26]. Similarly, supplementing broilers diet with a combination of arginine, vitamin E, and oil

blend significantly enhanced the levels of IgG, and IgM when compared to the control group [35]. These findings may be linked to the ability of arginine to enhance pro-B lymphocyte development and B lymphocyte re-release from bone marrow, which in turn encouraged Igs secretion from B lymphocytes [29, 69]. In accordance, dietary phytogenics supplementations promoted the levels of IgM [70], IgG, and IgA in chickens [70, 71]. Our findings are also in accordance with the outcomes of previous studies, which displayed that dietary OLE fortification significantly enhanced the levels of IgM, and total Igs [12] in fish. This could be explained by the antioxidant, anti-inflammatory, and antimicrobial properties of OLE, which enhanced the immune defense via improving the activities of Igs, and pro-inflammatory cytokines, consequently reducing inflammation and the growth of pathogens [12]. Notably, sustaining appropriate muscle development depends on the balance between two significant pathways; insulin-like growth factor-1 (IGF-1)/mTOR (positive regulator), and the myostatin-signaling pathway (negative regulator) [72, 73]. To the best of our knowledge, none of the previous studies that supplemented G + OLE in broilers` diets examined their effect on muscle development. In this context, at both 22-and-35 days of age, dietary G, and G + OLE triggered the synthesis of muscle protein via upregulating the MyoD, mTOR, and MyoG genes, and reduced protein degradation via downregulating the MSTN gene in the examined broilers` muscle concerning the control group, which came in parallel with the previous improved meat quality findings. Such muscle development primarily depends on the protein build-up in myofibers, which is brought about by the mTOR signaling pathway activation [38]. In agreement with our findings, recent literature displayed that dietary arginine supplementation increased the transcription level of myogenic genes in chickens [74] that augmented its application for enhancing muscle development.

Conclusions

Our interesting results reveals that the optimal combination of functional feed additives, including arginine and olive leaf extract, enhances the muscle-building capacity of broiler chickens by upregulating the expression of genes related to breast muscle development, thereby maximizing their growth performance. Additionally, the immune regulatory defense in broiler chickens was enhanced with the dietary inclusion of G + OLE especially at higher level of OLE. Besides, supplemental arginine and olive leaf extract at the level of 1.5 g/kg of G plus 1% of OLE enriches broiler's meat with powerful antioxidants that can scavenge free radicals under frozen condition, thereby guarantee satisfactory oxidative stability in poultry meat offered for human consumers. Therefore, the promising properties of arginine and olive leaf extract blend created many avenues for their utilization as innovative futhe broilerilerl feed supplements in broiler chickens` industry.

Methods

Ethical approval

All research procedures were performed under the rules and authorized regulations of the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Zagazig University, Egypt with approval number (ZU-IACUC/2/F/258/2023).

Arginine and Olive leaf extract

Arginine was obtained from Sigma-Aldrich (MO, USA). Additionally, the production of the (OLE) was carried out as previously described [75].

Briefly, the olive leaves were ground using a blender after being dried for eight minutes at 120° C in a vented oven. The extraction method involved the use of ultrasonic assistance and a 1/20 (w/v) ratio of Milli-Q water (Sigma-Aldrich MO, USA) as the extraction solvent. The extract underwent filtration using Whatman filter paper (Sigma-Aldrich, St. Louis, MO, USA), freeze-dried using LyovaporTM L-200 (Fisher Scientific Ltd, MA, USA), and then kept at -20° C. The phenolic profile was ascertained as earlier pronounced [76], and high-performance liquid chromatography with diode-array detection (HPLC-DAD) and an external calibration curve using a corresponding standard was used to quantify the phenolic compounds in OLE, mg/g (oleuropein, 36.74 ± 0.05; vanillic acid, 0.062 ± 0.03; hydroxytyrosol, 0.54 ± 0.04; caffeic acid, 1. 79 ± 0.04; verbascoside, 1.22 ± 0.05; luteolin, 0.56 ± 0.04; apigenin-7-glucoside, 0.61 ± 0.06; rutin, 0. 431 ± 0.06; luteolin − 7 glucoside, 0.97 ± 0.05; vanillin, 0.659 ± 0.03; tyrosol, 0.33 ± 0.01 and diosmetin, 1.14 ± 0.09).

Experimental design and feeding protocol of broiler chickens

Two hundred and fifty, 1-day-old, male Ross broiler chicks from a commercial local poultry hatchery were used in the current experiment. The chicks were weighed upon arrival and sorted into five experimental groups in floor pens at random. Each group comprised fifty chicks raised for 35 days after being evenly divided into five replicates. Throughout the 35-day trial, all chicks had unlimited access to food and water and every bird was grown in perfectly hygienic conditions as per the Ross Broilers Management Guide [77]. All birds were offered coccidiostat-free, and antibiotic-free food in the mash form for the starter (1–10 d), grower (11–20 d), and finisher (21–35 d) stages, as listed in Table 4, following the criteria of Ross broiler nutrition specifications [77]. As stated by the

Table 4 Ingredient and nutrient composition of the basal diets

Ingredient (%)	Starter (1–10 d)	Grower (11–20 d)	Finisher (21–35 d)
Soybean meal	34.7	30.85	25.65
Corn	58.8	61.40	62.52
Soybean oil	1.8	3.1	4.1
Common salt	0.30	0.30	0.30
Dicalcium phosphate	1.50	1.20	1.50
Calcium carbonate	1.50	1.50	1.50
L-Lysine HCL (Lysin, 78%)	0.35	0.31	0.28
L-Arginine (98.5%)	0.15	0.12	0.10
DL-Methionine (Methionine, 99%)	0.15	0.15	0.15
Anti-mycotoxin	0.10	0.10	0.10
Choline chloride	0.20	0.20	0.20
Premix*	0.90	0.90	0.90
Nutrient composition			
CF (%)	2.60	2.61	6.2
EE (%)	4.25	5.59	2.6
CP (%)	23.05	20.46	19.48
Lysine (%)	1.43	1.30	1.18
Methionine (%)	0.48	0.46	0.43
Arginine (%)	1.50	1.36	1.23
Arginine/ lysine	1.05	1.05	1.04
Available phosphorus (%)	0.67	0.4	0.4
Ca (%)	1.30	0.93	0.87
Metabolizable energy (kcal/kg)	3005	3101	3202

* Vitamin premix provided for each kilogram of diet: Zn (sulfate and oxide), 120 mg; Cu (sulfate), 14 mg; Fe (sulfate), 100 mg; Mn (sulfate and oxide), 30 mg; Se (selenate), 0.3 mg; I (iodide), 1.2 mg; cyanocobalamin, 15 µg; biotin, 300 µg; pyridoxine, 6 mg; pantothenate, 12 mg; thiamine, 4 mg; niacin, 50 mg; riboflavin, 7 mg; tocopherol acetate, 70 mg; folate, 3 mg; menadione, 2.5 mg; retinol, 6000 IU; cholecalciferol; 10.000 IU

Association of Official Analytical Chemists [78], chemical analyses were performed on all feed ingredients and diets for crude fiber, ether extract, crude protein, and moisture. The five experimental groups were as follows; control (chicks fed basal diets without any supplementations), G (chicks fed basal diets supplemented with 1.5 g/kg (G), G + OLEI (chicks fed basal diets supplemented with 1.5 g/kg G plus 0.25% OLE), G + OLEII (chicks fed basal diets supplemented with 1.5 g/kg G plus 0.5% OLE), and G + OLEIII (chicks fed basal diets supplemented with 1.5 g/kg G plus 1% OLE).

Measurement of growth performance

Average BW and FI were determined during the starter, grower, and finisher phases and the total rearing period (35 days) to estimate BWG and FCR as previously pronounced [7, 79–81].

Sampling

At 22-and-35 days of age, five chicks/ replicate were chosen randomly and euthanized by cervical dislocation for blood and tissue sampling according to according to the American Veterinary Medical Association guidelines for the euthanasia of animals [82] and before euthanasia birds were anesthetized via intraperitoneal injection of sodium pentobarbital (50 mg/kg) infused intravenously into the wing vein. After that breast meat samples aseptically were handled and stored at -20° C. The obtained breast meat and intestinal samples were utilized for analyzing the meat quality, and antioxidant potential, and for subsequent gene expression analysis by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) technique.

Analysis of meat quality via estimating meat pH, thaw, cooking, and drip loss

The breast meat samples were utilized to estimate the postmortem pH (at 0.5, and 24 h) via by pH meter. Additionally, drip, and thaw, cooking loss in meat samples were determined as earlier pronounced [83, 84]. In brief, drip loss percent is the percentage of weight lost when a sample is left hanging in a closed plastic bag at 4 °C for 72 h. Then, the same sample was utilized for determining both cooking and thaw loss following storage at -20 °C. The thaw loss percentage represents the proportionate weight loss of a meat sample before frozen storage at -20 °C and following an overnight defrosting at 4 °C. The cooking loss percentage represents the proportionate weight loss of a sample following cooking in an open plastic bag in a water bath at 70 °C for 40 min, followed by cooling.

Antioxidant potential of meat samples

The breast meat was cut into approximately 3 cm^2 cubes 6 h after the bird was sacrificed and handled, and then we removed any visible fat and connective parts. These muscle cubes were blended with distilled water, homogenized, centrifuged, and utilized to determine total antioxidant markers as free radical scavenging assay utilizing DPPH, FRAP, and ABTS, TBARS assay, antioxidant enzymes, total flavonoid, and phenolic contents.

Ferric reducing antioxidant power assay

On meat homogenates, FRAP assay was performed as described by Lavanya et al. [85]. Following homogenization in potassium phosphate buffer (Thermo Fisher Scientific, USA), the meat samples were centrifuged to separate the supernatant. After that, 1 mL of the supernatant was taken and put into 3 mL of FRAP buffer, which contained 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine) (Sigma-Aldrich MO, USA) in 40 mM HCl (Sigma-Aldrich MO, USA), and 20 mM Fe_2Cl_3 (Sigma-Aldrich MO, USA) was transferred to 300 mM acetate buffer. The absorbance at 593 nm was determined right after mixing, and $FeCl_2$ (Sigma-Aldrich MO, USA) was used to prepare a standard curve. µM of Fe^{2+}/gram of moist muscle tissue was the antioxidant power of the samples.

Free radical scavenging activity via DPPH

The muscle samples' scavenging capacity was assessed via DPPH as formerly pronounced [86]. In brief, the meat samples were centrifuged after being homogenized in distilled water to collect the supernatant. After combining the supernatant with DPPH radical solution, and ethanol it was incubated for ten minutes in a dark room. At 517 nm, the absorbance reading was then recorded. The DPPH radical scavenging capacity was defined as µM/gram of moist muscle tissue.

Antioxidant activity via ABTS

The modified Trolox-equivalent antioxidant capacity method was used to determine the overall antioxidant activity of breast meat [86]. In summary, the production of $ABTS^+$ radical cations was stimulated by combining 14 mM ABTS with an equal volume of 4.9 mM potassium persulfate (Sigma-Aldrich MO, USA). The reaction was then incubated for 12–16 h at room temperature in the dark. Subsequently, one Ml of $ABTS^+$ solution (Sigma-Aldrich MO, USA) was combined with 10 µL of meat homogenate, and properly mixed, and the absorbance was measured at 734 nm after 60 s.

Thiobarbituric acid-reactive substance assay (TBARS)

The thiobarbituric acid-reactive substance assay was used to assess lipid oxidation both on the first day and four weeks following storage as formerly reported A Zeb and F Ullah [87]. Five grams of meat sample were treated with 27 mL of 3.83% perchloric acid (Sigma-Aldrich MO, USA), homogenized for 1 min, and filtered through filter paper. The supernatants were then treated with 2 mL of thiobarbituric acid (Sigma-Aldrich MO, USA) and incubated for 20 min at 100° C in a water bath. Following a quick cooling to room temperature and a 15-minute centrifugation, the spectrophotometer was used to measure the absorbance at 532 nm. The readings were then represented as milligrams of MDA per kilogram of meat and the findings were computed using the standard curve.

Estimation of total flavonoid contents

Total flavonoid content was determined as earlier stated [88]. Briefly, one milliliter of double-distilled water (Sigma-Aldrich MO, USA) was combined with 0.25 mL of the specimen. After that, 0.5 ml of 1 M NaOH, 0.075 ml of 10% $AlCl_3$ (Oxoid, UK), and 0.075 ml of $NaNO_2$ (Oxoid, UK)were put in that order, then double-distilled water was added (up to 2.5 mL). With the UV–visible spectrophotometer, the solution's absorbance was measured at 410 nm. Quercetin (Sigma-Aldrich MO, USA) was utilized as a reference for measuring the total flavonoid content. The findings were presented as µg of quercetin equivalents (QE)/ milligram.

Determination of total phenolic contents

Samples of breast meat were tested for TPC using the method outlined previously [89]. Two hundred and fifty µL of 50% Folin-Ciocalteu reagent (Sigma-Aldrich MO, USA), 2.5 mL of distilled water (Sigma-Aldrich MO, USA), and 500 µL of 95% ethanol (Sigma-Aldrich MO, USA) were mixed with a 100 µL homogenized meat specimen. After 5 min, 250 µL of 5% Na_2CO_3 (Sigma-Aldrich MO, USA) were mixed with the resulting mix, vortexed, and allowed to sit in a dark room for one hour. The samples' absorbance was then measured at 725 nm using a spectrophotometer. The breast meat TPC was determined as gallic acid equivalent (mg gallic acid/100 g meat).

Estimation of antioxidant enzymes

Using commercial assay kits (Sigma-Aldrich, MO, USA), T-AOC and the activity of antioxidant enzymes including SOD, GPX, and CAT were measured in meat filtrates as per the manufacturer's instructions.

Gene expression analysis via reverse transcription-quantitative polymerase chain reaction assay

Following the dietary inclusion of G, and G + OLE, the mRNA transcription intensities of genes encoding intestinal immunity [*IgA*, *IgG*, and *IgM*] and muscle development [*mTOR*, *MyoD*, *MyoG*, and *MSTN*] and antioxidant enzymes [*HO-1*, *NQO1*, *Nrf2*, *GPX-1*, *SOD-1*, catalase (*CAT*), *PRDX-1*, *XOR*, *and HMOX1*],, in the chickens` breast meat samples were performed by RT-qPCR technique. Following the manufacturer's instructions, total RNAs were extracted from meat samples preserved with RNAlater (Qiagen, Germany) using the QIAamp RNeasy Mini kit (Qiagen, Germany). The measurement and evaluation of RNA purity were then done spectrophotometrically using a NanoDrop® ND-1000 (Thermo Scientific, USA). Using the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Germany) and gene target primers, RT-qPCR assays were performed in triplicate on the MX3005P real-time PCR machine (Stratagene Co., USA) following the manufacturer`s protocol. The investigated gene quantities were measured using the comparative cycle threshold, CT ($^{2-\Delta\Delta}$Ct approach) [90], representing the results as fold changes, and normalized to the endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene as well as compared to the calibrator. Melting curve exploration was performed to remove the possibility of nonspecific amplifications. The sequences of primers encoding housekeeping and investigated genes utilized in RT-qPCR assay are listed in Table 5.

Statistical analysis

The outcomes were statistically examined using the SPSS Inc. software version 20 (IBM Corp., NY, USA).

Table 5 Primers' sequences used for quantitative reverse transcription polymerase chain reaction analysis

Specificity/ Target gene	Primer sequence (5'-3')	Accession No.
House keeping		
GAPDH	F: GGTGGTGCTAGCGTGTTA	NM205518
	R: CCCTCCACAATGCCAA	
Antioxidant enzymes		
NQO1	F: TCGCCGAGCAGAAGAAGATTGAAG	NM_001277620.1
	R: CGGTGGTGAGTGACAGCATGG	
HO-1	F: AAGAGCCAGGAGAACGGTCA	NM_205344
	R: AAGAGCCAGGAGAACGGTCA	
Nrf2	F: GAGCCCATGGCCTTTCCTAT	NM_001007858.1
	R: CACAGAGGCCCTGACTCAAA	
GPX-1	F: AACCAATTCGGGCACCAG	HM590226
	R: CCGTTCACCTCGCACTTCTC	
SOD-1	F: GGCAATGTGACTGCAAAGGG	NM_205064.1
	R: CCCCTCTACCCAGGTCATCA	
CAT	F: GGGGAGCTGTTTACTGCAAG	NM_001031215.2
	R: GGGGAGCTGTTTACTGCAAG	
PRDX-1	F: CTGCTGGAGTGCGATTGT	NM_001271932.1
	R: GCTGTGGCAGTAAAATCAGGG	
XOR	F: GTGTCGGTGTACAGGATACAGAC	NM_205127.1
	R: CCTTACTATGACAGCATCCAGTG	
HMOX1	F: ACACCCGCTATTTGGGAGAC	NM_205344.1
	R: GAACTTGGTGGCGTTGGAGA	
Muscle development		
mTOR	F: CATGTCAGGCACTGTGTCTATTCTC	XM_417614.5
	R: CTTTCGCCCTTGTTTCTTCACT	
MyoG	F: GGAGAAGCGGAGGCTGAAG	NM_204184.1
	R: GCAGAGTGCTGCGTTTCAGA	
MyoD	F: CAGCAGCTACTACACGGAATCA	NM_204214.2
	R: GGAAATCCTCTCCACAATGCTT	
MSTN	F: ATGCAGATCGCGGTTGATC	NM_001001461.1
	R: GCGTTCTCTGTGGGCTGACT	
Immune related genes		
IgA	F: ACCACGGCTCTGACTGTACC	S40610.1
	R: CGATGGTCTCCTTCACATCA	
IgM	F: AGGAGACAGGACTGGAATGCACAA	XM_025906584.1
	R: GGAGGCAGTATAGGTATCATCCTC	
IgG	F: GAGGGAAGGGAAGAGTTACAGC	X07174
	R: GTGTTCCTGTAGACGCTCTTGC	

GAPDH: glyceraldehyde 3-phosphate dehydrogenase; *PRDX-1*; peroxiredoxin-1; *XOR*; xanthine oxidoreductase; *HMOX1*; heme oxygenase 1; *HO-1*: heme oxygenase-1; *NQO1*: NAD(P)H dehydrogenase quinone 1; *Nrf2*: nuclear factor erythroid 2-related factor 2; *GPX-1*: glutathione peroxidase 1; *SOD-1*: superoxide dismutase 1; *CAT*: catalase; mTOR: the mammalian target of rapamycin; *MyoD*: myogenic determination factor; MyoG: myogenin; MSTN: myostatin; *IgA*: immunoglobulin A; *IgG*: immunoglobulin G; *IgM*: immunoglobulin M

Shapiro-Wilk's and Levene's testing were used to determine the experimental groups` normality and homogeneity, respectively. Additionally, to determine the effectiveness of dietary G, and G + OLE supplementation, a one-way ANOVA test and Tukey's test were utilized. The standard error of the mean (SEM) was used to express variations in the experimental trial findings at a significant threshold of $p < 0.05$. GraphPad Prism Version 8 (CA, USA) was used to create the graphs.

Abbreviations

G	Arginine
OLE	Olive leaf extract
BW	Body weight
BWG	Body weight gain
FI	Feed intake
FCR	Feed conversion ratio
SEM	Standard error of the mean
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing/antioxidant
TBARS	Thiobarbituric acid reactive substances
ABTS	2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
TFC	Total flavonoid content
TPC	Total phenolic contents
FRAP	Ferric-reducing antioxidant power
TEAC	Trolox-equivalent antioxidant capacity
T-AOC	Total antioxidant capacity
SOD	Superoxide dismutase
GPX	Glutathione peroxidase
CAT	Catalase
HO-1	Heme oxygenase-1
NQO1	NAD(P)H dehydrogenase quinone 1
Nrf2	Nuclear factor erythroid 2-related factor 2
GPX-1	Glutathione peroxidase 1
SOD-1	Superoxide dismutase 1
CAT	Catalase
PRDX-1	Peroxiredoxin-1
XOR	Xanthine oxidoreductase
HMOX1	Heme oxygenase 1
mTOR	Mammalian target of rapamycin
MyoD	Myogenic determination factor
MyoG	Myogenin
MSTN	Myostatin
IgA	Immunoglobulin A
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase

Supplementary Information

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Supplementary Material 1

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Author contributions

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Data availability
Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate
The study was performed in compliance with the ARRIVE guidelines. All experiments were performed in accordance with relevant guidelines and regulations that were permitted by the Institutional Animal Care and Use Committee (ZU-IACUC/2/f/258), Faculty of Veterinary Medicine, Zagazig University. In the cuurent study, the animals were purchased so the written informed consent from owners was not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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