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Influence of dietary epigallocatechin-3 gallate and L-arginine and its combination on early laying performance and physiological status of stressed Japanese quails

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Abstract. This study was designed to investigate the effect of dietary EGCG and L-arg supplementation to alleviate the oxidative stress induced artificially by H_2O_2 in drinking water by using 400 layer quails. The research lasted for ^ weeks and birds were distributed into 5 groups, each group had 4 replications. The groups were divided into G1: negative control, G2: addition 0.2% H_2O_2 to drinking water, G3 and G4: addition 500 mg/kg of EGCG and L-arg each. G5: addition 250 mg/kg mixture of EGCG and L-arg each. H_2O_2 was added to drinking water in G3, G4 and G5. The results revealed that feed intake, egg weight, egg mass and egg production were increased in G3, G4, G5 and G1 and the same groups led to decrease FCR and mortality compared to G2. The duodenal morphology was decreased in G2 as well. In plasma, high levels of (AST, ALT, uric acid, creatinine, glucose and total cholesterol) and low levels of (LOOH, SOD, catalase, GPx, FRAP and total protein) were in favor of G2. In conclusion, supplementation of EGCG and L-arg or their mixture in diet attenuated the detrimental effect of oxidative stress through improve productive and physiological aspects of layer quails.

1. Indroduction

Nowdays, domesticated poultry have become more susceptible to stress induced by pro-oxidant factors which lead to minimum ability to resist the stressful conditions in commercial farms. Oxidative stress (OS), is an imbalance status when reactive oxygen species (ROS) generation exceed cellular antioxidant system which has become a major issue and the subject of productivity and related scope in the poultry industry. The OS negatively influences animal health, welfare, and performance, subsequently influencing economic feasibility and the final product [1, 2]. In normal metabolic activity, ROS are generated endogenously and they react with basic cellular components such as lipids, proteins and DNA and stimulate pathways that lead to oxidative damage and dangerous diseases [3]. The two groups of ROS are free radicals and nonradicals. Free radicals are molecules which have one or more unpaired electrons and thus give reactivity to the molecule [4]. The 3 major ROS that are critical from physiological viewpoint are superoxide anion ($O2^{-\cdot}$), hydroxyl radical ($HO^{\cdot}$), and hydrogen peroxide (H_2O_2) which attack biological molecules [3, 4].

Maintaining and improving oxidative conditions is necessary for normal physiological performance in birds which is done particularly through natural nutrition by plant-derived non nutritive compounds or supplementary feed additives [5, 6, 7].

Epigallocatechin-3-gallate (EGCG) is one of 4 major catechins derived from green tea including epigallocatechin (EGC), epicatechin-gallate (ECG) and epicatechin (EC) [8]. EGCG is one of the most abundant polyphenol, which is a powerful antioxidant against lipid peroxidation and has beneficial effects on health due to its robust anti-inflammatory, antimicrobial, antiproliferative, antimutagenic, antiaging, and anticarcinogenic characteristics [8, 9, 10]. Also, the signalling pathways including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor-erythroid 2-related factor 2 (Nrf2) which fuction the regulation of antioxidant and anti-inflammatory responses can be modulated through EGCG [11].

L-arginine (L-arg) is a ubiquitous essential amino acid and nutritional additive which has a considerable effect on the productive and physiological performance of birds when included in their diet [12, 13]. It well known that birds are characterized by lack a functional urea cycle or absence of most of the enzymes involved in the urea cycle thus they are unable to synthesise arginine *de novo*. Accordingly, the absolute requirement for arginine meet their needs for protein synthesis and other functions are important and completely depended on dietary arginine [14, 15]. The magnitude of arginine requirement is highly changeable under various environmental conditions therefore the arginine needs of the poultry has been significant interest. Under normal and stressful states, arginine plays a pivotal fuction as a necessary substrate for the immune system with modulation of protective immune response [16] and increases the antioxidant defence and improve egg quality as well as bone mineralisation [5, 12]. Recently, [17] stated that trophic amino acids including arginine, threonine, and glutamine may be beneficial in birds to modulate the intestinal microbiota and mucosa and may increase the epithelial turnover rates toward improve intestinal recovery following a pathogenic insult.

The mechanism of EGCG in layer quail as model for egg production is largely undiscovered and has not been previously studied yet. Although, there are many reports in literature on arginine effect in chicken broilers and layers, few studies were found in quails especially in case of layer quails. Therefore, this study was carried out to investigate the action mode by which supplemental EGCG or L-arg or its mixture in diet would improve performance, antioxidant properties and other physiological aspects of layer Japanese quails exposed artificially to OS by H_2O_2.

2.Materials and Methods

This study was performed in Poultry Farm and Laboratories of Al-Musaib Technical College, Babylon, Iraq. A total of 400 healthy females of Japanese quails (10 wk of age) with similar body weights (171±6g) were randomly divided into 5 groups containing 4 replicates per group and 20 birds in each replicate. The birds were reared for 8 weeks in 2×2 m^2 cages. They were kept to an open daily photoperiod and controlled temperature at 23°C with free choice of feed and water (Table 1) throughout the experiment. Females were divided in to the following groups: 1st group: negative control (G1), 2nd group: positive control (G2) which was exposed to oxidative stress (OS) by 0.2% H_2O_2 by drinking water whereas 3rd and 4th groups were supplemented by 500 mg/kg diet for each of EGCG and L-arg powders, respectively and 5th group was supplemented with mixture of EGCG + L-arg by 250 mg/kg diet each. The same dose of 0.2% H_2O_2 was offered to drinking water of females for 3rd, 4th and 5th groups.

EGCG and L-arg (Nanjing Health Herb Bio-Tech Co., Ltd., China) purchased from local markets. Two of these extracts were used as antioxidant substances in diet as powdery form. EGCG product contained 95% natural EGCG isolated from green tea leaves, also the puririty of L-arg was 99%. Hydrogen peroxide (H_2O_2) solution (P.P.H Stanlab Sp.J/Poland), 30% concentration diluted to 0.2% in big plastic cans every second day to keep its purity. This solution was used as prooxidant factor to induce artificially the incidence of OS. These additive materials served daily to birds in the whole experimental period. The layer productive performance was registered daily in the whole period of

experiment and these data presented as total mean (56 days, 8 weeks) for each replicate . These traits included the number of laid eggs, egg weight, feed intake and mortality. From feed intake and egg weight, the feed conversion ratio (FCR) based on (g/g) and (g/egg) was calculated. Also, egg production was calculated on hen day egg production (HD%) or on hen house egg production (HH%) as the following formulas:

$$\text{HD (\%)} = \frac{\text{total egg produced}}{\text{number of alive hens} \times 56} \times 100$$

$$\text{HH (\%)} = \frac{\text{total egg produced}}{\text{number of total hens} \times 56} \times 100$$

Egg production per hen was measured as follows:

$$\text{Egg production (egg/hen/female /56d)} = \frac{\text{HD (\%)}}{100} \times 56$$

Egg mass was measured according to this formula:

$$\text{Egg mass (egg/hen /56d)} = \frac{\text{egg number} \times \text{egg weight (g)}}{\text{number of hens}}$$

At the end of the experiment, 2 hens were randomly chosen from each replicate group (8 in each group) to register their body weight. After that, euthanasia procedure was done by decapitation. Lengths and weights of the whole gastrointestinal tract (GIT), ovary, oviduct were performed. Also, the same birds were used for histomorphological evaluation of duodenum. The intestinal duodenum was defined based on anatomic limit from the ending of gizzard (duodenum ostium) to the beginning of the of the mesentery (duodenum loop). Duodenum segments were removed and gently flushed with distilled water. Segments were placed in plastic tubes and fixed in 4% buffered formalin solution, dehydrated, and embedded in paraffin wax. The 4 μm – thick sections were stained with hematoxylin and eosin for processing. The measurements for the villus and crypt dimensions and total mucosal thickness were implemented using a DELTA$^{@}$ Optical Microscope (USA) at 40× magnification equipped with video camera (microQ Industrial Digital Camera) and computer with measurement scale and imaging software (ToupView). Ten well–oriented villi and crypts from duodenum of each slide were measured. Villus surface area was calculated as 3.1416×villus width×villus height [18].

Blood samples was taken for analyses from the brachial vein of 12 birds from each group (3 birds per replicate) at the end of the experimental period. Blood was centrifuged at 1000 RPM for 10 min. The separated plasma was frozen at – 25°C for analysis and later transferred to the laboratory and analysed for biochemical indices. By using ready test kits (Cormay company, Poland), plasma was assayed spectrophotometrically for levels of the selected biochemical indicators, that is, total protein, albumin, glucose, creatinine, total cholesterol, triacylglycerides, uric acid, and activity both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Blood plasma of the experimental birds was also analyzed for the level of lipid peroxidation products, that is, level of lipid hydroperoxide (LOOH) and malondialdehyde (MDA) according to [19] and [20] respectively. In terms of the indicators of the antioxidative system, analysis was also carried out for the ferric-reducing ability of plasma (FRAP) according to [21]. With respect to antioxidant enzymes, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were determined on the basis of the method coined by [22] and [23]. All analyses were performed using a spectrophotometer, model Unicam 939, England. Statistical analysis was done using SAS software [24] and significance of differences between means was determined with the one-way analysis of variance test ANOVA, at a significance level of 0.05.

Table 1. Ingredients composition (%) and nutrients calculation of diet*.

Ingredients	Amount (%)
Corn	53.76
Soybean meal (44%)	29.27
Vegetable oil	4.850
Salt	0.310
Dl-Methionine	0.200
Limestone	9.500
Dicalcium phosphate	1.760
Premix[2]	0.350
Total	100.0
Nutrient composition	
Metabolizable energy, kcal/kg	2,830
Crude protein, %	17.95
Calcium, %	3.960
Phosphorus, %	0.630
Methionine, %	0.420
Lysine, %	1.050
Arginine, %	1.160
Metabolizable energy, kcal/kg	2,830
Crude protein, %	17.95

* Diet composition was calculated according to [25].
** Per 1 kilogram of premix containes: vitamin A, 8,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg; copper (copper sulfate), 10.00 mg; iodine (ethylenediamine dihydriodide), 1.00 mg; iron (ferrous sulphate monohydrate), 50.00 mg; manganese (manganese sulfate monohydrate), 60.00 mg; zinc (zinc sulfate monohydrate), 60.00 mg; and selenium (sodium selenite), 0.42 mg.

3.Results

Table 2 revealed that G2 decreased significantly the means of feed intake, egg production (HD; HH%), egg weight egg mass and egg production with increase the FCR (g/egg). In the same time it was observed that all of these traits were improved in G3, G4, G5 followed by G1.

The lowest value in GIT length was observed in G2 and G3, with decrease in small intestine length (%) in G2 and G1, whereas the G2 decreased the small intestine length (cm) and ovary weight. Also, G2 and G4 caused to decrease the total oviduct weight and length. There was no significant differences among groups regarding weights of GIT and small intestine (Table 3).

It is clear from Table 4, that all duodenal histomorphology traits which included villus height, villus width, crypt depth, villus height/ crypt depth, villus surface area and muscular layer thickness were lowered in G2. Besides, G3, G4, G5 and G1 increased these traits significantly.

Table 5 shows that G3, G4, G5 and G1 reduced levels of LOOH and elevated levels of SOD, catalase, GPx and FRAP were found in the same groups. All of these parameters were negatively registered in G2.

In Table 6, G2 increased significantly levels of total cholesterol, glucose, creatinine, uric acid, and activity both of AST and ALT with decrease in total protein level. However, G3, G4, G5 and G1 improved these parameters positively. No differences among groups were obvious with respect to

Table 2. Effect of dietary EGCG and A powders on performance (mean±standard error) of stressed layer quails.

Groups	Traits							
	Feed intake (g/hen/day)	Egg production (HD) (%)	Egg production (HH) (%)	Egg weight (g)	FCR (g/g)	FCR (g/egg)	Egg mass (g⁄hen/56 days)	Egg production (egg⁄hen/56days)
G1	26.39a±1.52	91.07ab±1.97	91.07ab±1.65	10.85b±0.03	2.44±0.05	28.97b±0.23	553.9b±11.87	51.13a±3.98
G2	20.99b±1.25	58.92c±1.96	47.63c±1.47	8.90c±0.07	2.35±0.07	35.61a±0.43	293.8c±12.8	33.12b±3.32
G3	26.85a±1.37	94.64a±1.58	94.64a±1.46	11.50a±0.54	2.33±0.08	28.36b±0.54	610.0a±35.9	53.23a±5.49
G4	27.14a±2.22	89.28±1.46	88.16b±1.49	11.20ab±0.06	2.42±0.07	30.39b±0.85	561.0b±15.87	50.24a±7.98
G5	26.60a±1.64	94.64a±1.65	94.64a±1.86	11.75a±0.03	2.26±0.01	28.10b±0.75	623.1a±14.87	53.23a±4.17

Groups: G1 (negative control) – without additives, G2 (positive control) – 0.2% H_2O_2 in drinking water, G3 – 500 mg of EGCG /kg diet and 0.2% H_2O_2 in drinking water, G4 - 500 mg of L- arg /kg diet and 0.2% H_2O_2 in drinking water, G5 - 250 mg of EGCG and 250 mg of L-arg with 0.2% H_2O_2 in drinking water
[a, b] – means within columns with different letters differ significantly at $p \leq 0.05$.

Table 3. Effect of dietary EGCG and A powders on anatomical traits (mean±standard error) of stressed layer quails.

Groups	Traits							
	Gastrointestinal tract		Small intestine			Ovary weight (g)	Total oviduct weight (g)	Total oviduct length (cm)
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (%)			
G1	98.2a±1.61	13.03±0.11	52.47a±1.76	4.23±0.22	53.43b±0.32	4.20b±0.54	6.51a±0.64	31.65a±1.00
G2	80.4b±2.65	10.26±0.23	40.35b±1.65	4.04±0.11	50.18b±0.54	3.46c±0.24	5.12b±0.35	27.48b±2.98
G3	91.1b±1.61	14.32±0.53	57.00a±1.26	4.42±0.34	62.56a±0.52	5.03a±0.64	6.54a±0.53	30.05a±1.07
G4	97.5a±2.53	12.23±0.36	55.26a±1.33	4.36±0.14	56.67a±0.34	5.15a±0.63	5.81b±0.43	29.18b±3.70
G5	99.7a±1.54	13.54±0.43	55.17a±1.34	4.12±0.12	55.33a±0.42	4.14b±0.21	7.04a±0.54	32.02a±1.01

Groups: G1 (negative control) – without additives, G2 (positive control) – 0.2% H_2O_2 in drinking water, G3 – 500 mg of EGCG /kg diet and 0.2% H_2O_2 in drinking water, G4 - 500 mg of L- arg /kg diet and 0.2% H_2O_2 in drinking water, G5 - 250 mg of EGCG and 250 mg of L- arg with 0.2% H_2O_2 in drinking water.
[a, b] – means within columns with different letters differ significantly at $p \leq 0.05$

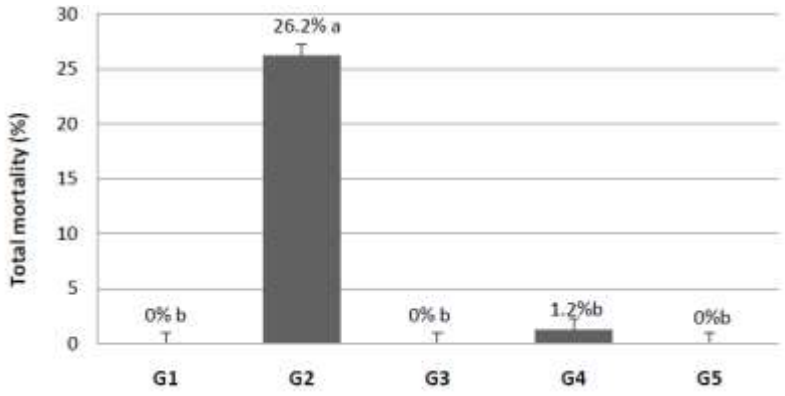

Figure 1. Effect of dietary EGCG and L-arg powders on total mortality (%) (mean) of stressed layer quails.

Groups: G1 (negative control) – without additives, G2 (positive control) – 0.2% H_2O_2 in drinking water, G3 – 500 mg of EGCG /kg diet and 0.2% H_2O_2 in drinking water, G4 - 500 mg of L- arg /kg diet and 0.2% H_2O_2 in drinking water, G5 - 250 mg of EGCG and 250 mg of L- arg with 0.2% H_2O_2 in drinking water.
[a, b] – means within columns with different letters differ significantly at $p \leq 0.05$.

Table 4. Effect of dietary EGCG and A powders on duodenal histomorphology (mean±standard error) of stressed layer quails.

Groups	Traits					
	villus height (μm)	villus width (μm)	crypt depth (μm)	villus height/crypt depth	villus surface area ($\times 10^3 \mu m^2$)	muscular layer thickness (μm)
G1	621.4a±8.98	92.4b±1.86	94.4a±0.42	6.58b±0.12	180.3b±12.87	92.8a±0.23
G2	410.7c±5.97	82.5c±1.87	82.2c±0.43	4.99c±0.51	106.4c±11.87	77.3b±0.53
G3	626.3a±6.86	106.1a±1.97	89.2b±0.45	7.02a±0.41	208.7a±11.45	91.5a±0.34
G4	610.5b±4.97	94.4b±1.45	91.1ab±0.42	6.70b±0.32	210.7a±17.87	82.2a±0.39
G5	640.4a±4.87	102.1a±1.76	90.3a±0.53	7.09a±0.24	205.4a±15.98	93.6a±0.75

Groups: G1 (negative control) – without additives, G2 (positive control) – 0.2% H_2O_2 in drinking water, G3 – 500 mg of EGCG /kg diet and 0.2% H_2O_2 in drinking water, G4 - 500 mg of L- arg /kg diet and 0.2% H_2O_2 in drinking water, G5 - 250 mg of EGCG and 250 mg of L- arg with 0.2% H_2O_2 in drinking water.
[a, b] – means within columns with different letters differ significantly at $p \leq 0.05$.

triglycerides and albumin values. No dead birds were recorded by G1, G3, G4, G5 and high mortality percentage was in favour of G2 (figure 1)

Table 5. Effect of dietary EGCG and A powders on plasma oxidation indicators (mean±standard error) of stressed layer quails.

Groups	Traits					
	LOOH (μmol/L)	MDA (μmol/L)	SOD (U/ml)	Catalase (U/ml)	GPx (U/L)	FRAP (μmol/L)
G1	40.00b±0.36	2.145±0.14	271.2b±12.8	504.2b±23.87	1.76a ±0.11	132.3a±15.96
G2	47.78a±0.74	2.344±0.13	205.1c±15.9	451.4c±21.98	1.12c±0.12	111.3c±11.45
G3	30.49c±0.53	2.110±0.10	332.2a±13.6	515.2b±31.8	1.70b±0.11	129.7b±12.4
G4	41.49b±0.45	2.250±0.12	283.8b±12.7	621.3a±28.8	1.91a±0.12	127.5b±13.64
G5	35.84c±0.47	2.162±0.114	235.7b±15.3	543.7b±26.8	1.80a±0.10	137.2a±14.9

Groups: G1 (negative control) – without additives, G2 (positive control) – 0.2% H_2O_2 in drinking water, G3 – 500 mg of EGCG /kg diet and 0.2% H_2O_2 in drinking water, G4 - 500 mg of L- arg /kg diet and 0.2% H_2O_2 in drinking water, G5 - 250 mg of EGCG and 250 mg of L- arg with 0.2% H_2O_2 in drinking water.
[a, b] – means within columns with different letters differ significantly at $p \leq 0.05$.

Table 6. Effect of dietary EGCG and A powders on plasma biocahemical indices (mean±standard error) of stressed layer quails.

Traits	Groups				
	G1	G2	G3	G4	G5
Total cholesterol (mmol/L)	235.1c ±0.05	288.2a ±0.07	213.8c±0.03	232.2c±0.06	246.8b±0.06
Triglycerides (mmol/L)	22.50±0.01	22.63±0.02	22.51±0.01	22.35±0.02	22.24±0.01
Glucose (mmol/L)	9.90b±0.30	11.01a±0.99	9.11b±0.98	9.23b±0.95	8.91b±1.00
Total protein (g/dl)	3.87a ±0.01	2.51b±0.08	3.93a ±0.06	3.53a ±0.01	3.76a ±0.05
Albumin (g/dl)	1.40 ±0.85	1.48 ±0.74	1.43±0.54	1.45 ±0.73	1.46±1.00
Creatinine(μmol/L)	4.40b ±0.85	4.91a ±0.74	4.52b±0.54	4.22b ±0.73	4.32b±1.00
Uric acid (μmol/L)	372b ±0.85	394a ±0.74	375b±0.54	372b ±0.73	374b±1.00
AST (U/L)	245.6b±21.9	348.3a±23.0	264.3b±16.8	260.5b±15.8	218.3c±1.96
ALT (U/L)	5.35b±0.43	6.12a±0.53	5.36b±0.83	5.28b±0.42	5.46b±0.63

Groups: G1 (negative control)– without additives, G2 (positive control) – 0.2% H_2O_2 in drinking water, G3 – 500 mg of EGCG /kg diet and 0.2% H_2O_2 in drinking water, G4 - 500 mg of L-arg /kg diet and 0.2% H_2O_2 in drinking water, G5 - 250 mg of EGCG and 250 mg of L-arg with 0.2% H_2O_2 in drinking water.
[a, b] – means within rows with different letters differ significantly at $p \leq 0.05$

4. Disussion

Different productive and physiological impairments caused by a chronic state of OS exhibited in birds reared under stress (G2) in current study. For instance, decrease in feed intake, FCR, egg production indexes with high mortality, which were accompanied with lowering histomorphology traits of duodenum, anatomical investigations for GIT and reproductive tract. The OS causes an escalating generation of harmful ROS as a result of physiological imbalance between the production of antioxidants and oxidants substances in the blood which has a negative role by attack biological molecules, such as lipids, proteins, and DNA [1, 3] which causes in turn the damage the membrane function and composition, transcription, translation, RNA processing [1]. H_2O_2 is one of the most important ROS. It leads to oxidation and induces the cytotoxicity when accumulated in high levels in living tissues. It can be controlled not only by catabolism but also by excretion because it is very ubiquitous and diffusible within and between cells [26]. Many studies have confirmed the role of H_2O_2

in the stimulation of the oxidative and toxic stress when supplemented to drinking water of quails [27] or chickens [7, 28, 29] with adverse effects on productive and physiological case. H_2O_2 consumed orally leads to starting a chain reaction leading to the OS output through increasing the amount of oxygen production in the stomach, which enters the blood stream and causes increase lipid peroxidation in all tissues and organs [7, 28, 29]. Lowering in egg production and depression in FCR in oxidation -exposed quails might be due to reduction in feed intake and failure in utilisation of nutrients with deterioration in health status [5]. As a result, increased total mortality in G2 might refer to that OS contributes to many pathological conditions and diseases, such as, asthma, cancer, neurological disorders, atherosclerosis, hypertension, diabetes, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and other cellular effects [4].

Moreover, G2 causes lowering of increased indicators of lipid peroxidation in plasma (MDA and LOOH), and decreased indicators of the antioxidant defense system in plasma (SOD, catalase, and GPx activities, and FRAP). Most of biochemical parameters including total cholesterol, glucose, total protein, creatinine, uric acid and activity both of AST and ALT measured in plasma for G2 were also adversely affected under stress. Major defense mechanism of antioxidant enzymes is to convert active oxygen molecules into nontoxic compounds. For example, CAT is found in peroxisomes and acts in concert with SOD to convert and detoxify H_2O_2 into H_2O and O_2 and help to dispose of H_2O_2 generated in organelles. Also, GPx removes H_2O_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG) [30].

In current data, there was a direct reflex for performance on physiological changes in response to dietary supplemental EGCG or L-arg or their mixture (G3, G4 and G5). The laying performance and physiological traits of quails under the OS condition were improved in these groups. Many polyphenols found in plant extracts for example, EGCG possesses an antibacterial activity [8] and can stimulate the microbial eubiosis in the gut, increase feed intake and endogenous secretions and combat stress, which could make nutrients available for the host and allowing more nutrients to be utilized for metabolism [31, 32]. One of the preventive benefits related with EGCG consumption to increase egg production is probably attributed to decrease number of spontaneous fibroid tumors (leiomyomas) and tumorigenesis in stressed quails oviduct with lowering the serum tumor necrosis factor-α (TNF-α) [33]). It was proven that phytochemicals derived from green tea, such as EGCG had anti-stress influences through the stimulation of Nrf2/ haeme oxygenase-1 (HO-1) pathways and alleviates the inflammation incidence in quail [6,11]. High laying performance in G3 might be also partly accounted for the inhibitory impacts of EGCG regarding to suppression of inflammation markers, such as activator protein-1 (AP-1) components (phospho-c-Jun and c-Fos), cyclooxygenase-2 (COX-2) and heat shock proteins in hepatic tissue [34]. Increase in egg production as a result of offering amounts of L-arg as antioxidant factor (G4) can attributed to specific stimulatory effect on luteinizing hormone secretion, activation the ovaries, ovarian follicles and ovulation process [35, 36]. In addition, L-arg, as a part of the arginine vasotocin hormone, (neurohypophysial hormone) plays an essential function in the primary contraction of hen's uterus through an increased binding to its receptor. which might affects the oviposition process [37]. High feed intake or low FCR in G4, as recently mentioned by [13] that L-arg has a secretagogue effects because it stimulates the release of pituitary and GIT hormones, including growth hormone, insulin, glucagon and ghrelin. These hormones could increase feed intake and protein synthesis. Furthermore, L-arg is a substrate for synthesis of many molecules, such as protein, nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, gmatine, dimethylarginine [12, 38]. L-arg carries out the metabolic regulation of protein, glucose, and fatty acids [39] with participation in the muscle energy buffering system [12]. Most putative explanations to increase feed intake in G3, G4 and G5 might be related to high demands for antioxidants in diet to counteract the oxidation and was reflected to enhance the absorption and digestibility in intestine by improving the histomorphology or morphology of gut (Tables 3 and 4).

These findings were in accordance with the results found by [33], who concluded that diet of layer quail supplemented with 200 or 400 mg of EGCG/kg increased feed intake with lowering MDA level in serum and liver. Similarly, it was observed that feeding quails exposed to heat stress for 12 weeks

with 200 or 400 mg of EGCG/kg diet increased feed intake, egg production and antioxidant biomarkers (catalase, SOD, and GPx) in liver [6]. Also, the same dose of EGCG was established by [10] who found that EGCG powder ameliorated the OS state in heat-stressed quails through linearly increase of feed intake, weight gain, carcass yield with improving feed efficiency and decrease cholesterol, triglycerides and glucose levels. Contradictory outcomes were obtained by [5] who conveyed that late laying quail exposed to heat stress and fed with 500 or 1000 g of arginine silicate inositol complex containing arginine 49.5% per kg diet had no effect on feed intake or egg production under stress conditions. Also, different findings were obtained by [35] who declared that diet with extra L-arg (1.54 and 2.05 %) alone or interaction with different levels of lysine for local Saudi layer hens did not affect performance and ovarian activity although feed efficiency improved with the higher levels of L-arg. Moreover, it was observed that there was no effects on laying performance or blood parameters of brown Leghorn laying hens containing 17 mg L-arg /kg diet [16].

Similar results to our data but for unstressed birds were mentioned by [39] who stated that Japanese quails fed 5 mg L-arg /kg increased egg weight, serum total antioxidant capacity, with reduction in MDA and triglycerides levels. Also, [40] stated that long-term L-arg graded 70, 100 and 200% supplied in diet of four purebred layer lines has increased daily feed intake during the rearing, induced higher laying intensity and daily egg mass production in the laying period. Current data was agreeable based on [36], that broiler breeder fed during the late laying period with 1.36% digestible L-arg (1,972 mg/day) has increased laying rate and serum total antioxidant capacity, however, with low MDA and had no effect on serum GPx level.

5. Concluding Remarks

The exposure to OS lowered performance variables, such as egg production, feed intake, FCR and mortality, enhanced lipid peroxidation and suppressed antioxidant mechanism defense and plasma biochemical parameters. The response in variables of supplemental EGCG or L-arg was opposite to exposure to OS. Positive alterations in these traits occurred at a greater rate in quails subjected to OS with offering dietary EGCG, L-arg or their mixture than in those reared under the OS condition without these feed supplements. Our data suggest that EGCG or L-arg elicits antioxidant influences through modulating oxidative biomarkers and plasma biochemistry, which was reflected on laying performance, duodenal histomorphology and morphometry of GIT and reproductive tract.

6. References

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