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Tryptophan and/or canthaxanthin in quail diets: effects on performance, carcass traits, hematology, blood chemistry and hepatic antioxidant capacity

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ABSTRACT

To enhance the health and performance of poultry, novel approaches have to be created. Using appropriate nutritional interventions to enhance body physiology and thus enhance productivity is one of these approaches. The purpose of the present investigation intended to examine how growing quail physiology and growth is affected by supplementing diets with tryptophan (Trp) and/or canthaxanthin (CX). The sum of 200 unsexed, 1-week-age Japanese quails (*Coturnix coturnix japonica*), with a nearly similar body weight (BW) of 33.50 ± 1.20 g, were assigned, in random, to four experimental groups. Each group consisted of five replicates, with 10 birds per replicate. Chicks in group 1 (T1) served as the control and were fed a basal diet without any supplementation from week 1 to week 5. The second (T2) and third (T3) groups received feed supplemented with 0.01 % Trp and 0.005 % CX, respectively. The fourth group (T4) was given a diet containing a combination of 0.01 % Trp and 0.005 % CX. Results indicated that supplementation with Trp, CX, or their combination significantly ($P < 0.05$) enhanced live BW and body weight gain (BWG) at 5 weeks. No noticeable variations in carcass characteristics were found across all treatments over the whole trial duration. Blood levels of high-density lipoprotein were considerably greater in the Trp and/or CX-fed group than in the control group. Adding Trp and/orCX to quail diets significantly ($P < 0.05$) decreased the activity of liver enzymes (alanine transaminase, ALT; aspartate transaminase, AST; alkaline phosphatase, ALP), along with reduced low-density lipoprotein (LDL) levels. Birds received diets with Trp and/or CX had higher values of antioxidant indices in serum and liver ($P < 0.05$), accompanied by low values of malondialdehyde compared to control group. We concluded that adding quail diet with Trp and/orCX had positive consequences on the growth performance and some physiological indices.

Introduction

Affordable protein for human consumption is the goal of the poultry industry. Currently, one source of animal protein that may help make up for the lack of red meat is poultry (Connolly et al., 2022). Owing to their early maturation, rapid development, low area requirements for farming, immunity to the majority of illnesses, and short incubation period, quails are a variety of chicken with significant economic value

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(Rahmani et al., 2014). In general, animals exposed to different kind of stress (rapid fluctuations in ambient temperature, stocking density, transport, and immunization, etc.) under intensive production systems (Zhang et al., 2009; Batool et al., 2021). According to Lan et al. (2004), each of the above variables caused immunological imbalances and had a detrimental effect on the productivity and carcass traits of poultry. In addition, under normal physiological conditions, a cell will undertake univalent reduction on three to five percent of the oxygen it takes up, which will result in the formation of free radicals (Singal et al., 1998). Numerous studies have shown that additives that enhance health status must be added to poultry feed to support it (Arif et al., 2022; Lestingi et al., 2024; Reda et al., 2024). In broilers experiencing oxidative stress, 5-hydroxytryptophan, a tryptophan (Trp) derivative, has been demonstrated to maintain membrane fluidity (Ouyang et al., 2022). Since Trp is a key chemical that regulates behavior and physiological processes, it is eventually necessary to achieve maximal weight gain and FCR in broiler chickens above the recommendations of the National Research Council (NRC) (Dong and Zou, 2017). Trp is classified as an essential amino acid because it functions similarly to other amino acids in protein production and because its metabolism produces important compounds such quinolinic acid, kynurenic acid, serotonin, and melatonin (Jianyong et al., 2021). Trp has been linked to the control of heat shock protein 70 secretion, growth factor 1, cortisol, and testosterone (Bello et al., 2018). Accordingly, dietary supplementation with Trp can effectively reduce stresses in poultry and improve antioxidant status, immune system activation, productive performance, and meat quality (Bello et al., 2018; Yue et al., 2017).

Canthaxanthin (CX), a carotenoid with potent antioxidant properties, has been extensively studied in both in vitro (Soffers et al., 1999) and in vivo (Zhang et al., 2011; Rosa et al., 2012) studies. The efficacy of CX is attributed to its ability as scavenger of reactive oxygen species and as neutralizer of other free radicals (Bohm et al., 2012; Venugopalan et al., 2013). Also, Rebelo et al. (2020) showed that in compared to other carotenes and xanthophylls, canthaxanthin, a ketocarotenoid, demonstrates stronger antioxidant and free radical scavenging capabilities. Therefore, CX has been employed as a supplementation in poultry diets to overcome the effect of oxidative stress which could be caused by a variety of variables, such as heat stress conditions (Ma et al., 2005) and pathological conditions (Georgieva et al., 2006). The small intestine absorbs dietary CX, which is then dispersed throughout the body (Bonagurio et al., 2020). In birds, it is deposited in muscles, abdominal fats, feathers, and the liver's tissues (Surai et al., 2003; Tunio et al., 2013). CX has been demonstrated to reduce lipid peroxidation by removing free radicals (reactive oxygen and nitrogen species) in chick meat (Surai, 2012).

While considerable research has shown that dietary Trp or CX can enhance broiler flocks' performance (Surai et al., 2003; Zhang et al., 2011; Rosa et al., 2012), their effects on growing quails remain poorly understood. The goal of the present study was to investigate the effects of Trp and/or CX supplementation on quail performance, carcass characteristics, and hematobiochemical measurements, aiming to establish a scientific basis for the safe use of these supplements in quail diets.

Materials and methods

Ethical approval

The study protocol was in lined with the Scientific Research Ethics Committee, Agriculture Faculty, Damietta University, Egypt. The current experiment followed the guidelines of both "Animal Research: Reporting of in Vivo Experiments" (ARRIVE) (https:// arriveguidelines.org) and the National Institutes of Health for the Care and Use of Laboratory Animals.

The present investigation was carried out over the course of 35 days at a private quail farm in Mansoura City, Egypt, as well as the Animal, Poultry, and Fish Production Department of the Faculty of Agriculture at Damietta University. All experimental procedures adhered to international ethical standards.

The experiment design and quails

The sum of 200 unsexed, 1-week-age Japanese quails (*Coturnix coturnix japonica*), purchased from local incubator with a nearly similar body weight of 33.50 ± 1.20 g, were assigned, in random, to four experimental groups. Each group consisted of five replicates, with 10 birds per replicate. Chicks in group 1 (T1) served as the control and were fed a basal diet without any supplementation from week 1 to week 5. The second (T2) and third (T3) groups received feed supplemented with 0.01 % Trp and 0.005 % CX, respectively. The fourth group (T4) was given a diet containing a combination of 0.01 % Trp and 0.005 % CX. The standard diet (basal diet) was prepared in accordance with the NRC (1994) recommendations for growing Japanese quail (Table 1). All quails were kept under similar housing settings during the experimental period. Quails in each replicate were kept in 90 × 40 × 40 cm cage which equipped with a nipple drinker and outdoor galvanized metal feeder. Fresh water and diets were provided continuously over the duration of the experiment. The ambient temperature was set at 35 °C during the first 3 days of age, then gradually lowered d by 2-3° C per week till the quails were 5 weeks old. The birds were reared in a relative humidity of 56 % to 66 % and lighting schedule of 16 h light and 8 h darkness.

Biosecurity considerations

The experiment was conducted under strict hygienic conditions. All bio-security precautions were meticulously followed.

Growth performance

The measurements of growth performance were recorded and computed biweekly/replicate at 1, 3 and 5 wks of age. The body weight gain (BWG) and feed intake were biweekly recorded and computed during each experimental period. The feed conversion ratio (FCR) was calculated as weight units of feed intake required to produce one unit of BWG (g feed intake/g BWG).

Table 1
Ingredient and nutrient composition of basal diet.

Ingredients	%
Corn	56.00
Soybean meal (44 % CP)	32.00
Plant concentrate meal (50 % CP)[1]	9.00
Limestone	1.30
Dicalcium phosphate	0.50
Vegetable oil	0.50
DL-methionine	0.10
Salt (NaCl)	0.30
Vitamins and minerals mixture[2]	0.30
Total	100
Calculated analysis[3]	
Metabolizable energy (Kcal/kg)	2919
Crude protein (CP, %)	24.00
Crude fiber (CF, %)	3.50
Calcium (Ca, %)	0.80
Available phosphorus (Ava. P, %)	0.50

[1] Plant concentrate contains (%): CP 50, CF 1.3, Ca4.72, Av P 3.1, lysine 6, methionine 2 and ME 2650 kcal/kg.

[2] Premix provided per kg of diet: vitamin A, 12.000 IU; vitamin D3, 2.400 IU; vitamin E, 30 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; vitamin B6, 5 mg; vitamin B12, 15 µg; niacin, 25 mg,Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; choline, 125,000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.

[3] According to NRC (1994).

Carcass characteristics and caecal content pH

Following a 10-hour fast, five birds per every treatment group were chosen at random on day 35 of age (the end of the experiment). Birds were weighted then slaughtered in order to evaluate the carcass characteristics and blood parameters. The birds were left to bleed after slaughtering for 120 s. Weight measurements were recorded for the carcass, liver, gizzard, heart, spleen, and intestine, and the results were converted to a percentage of the live weight. Additionally, a digital pH meter (Model 507; Crison Instruments S.A., Barcelona, Spain) was used to measure the pH of the cecal content.

Blood samples' collection

On day 35 of age, 5 quails per group were randomly chosen and sacrificed for collecting blood samples in a sterile EDTA test tube for estimating hematological measurements, *i.e.,* hemoglobin value (Hb), count of red (millions) and white (thousands) blood cells (RBCs and WBCs), lymphocytes (L), heterophils (H), and Mean Corpuscular Hemoglobin Concentration (MCHC). Another blood sample was collected in free-anticoagulant tube, allowed to clot for 20 min, centrifuged for 15 min at 3000 rpm to extract blood serum, and then kept at −20°C until biochemicals parameters analysis. The concentrations of serum total proteins (TP, g/dl), albumin (AL, g/dl), triglycerides (mg/dl), total cholesterol (mg/dl), high-density lipoprotein (HDL, mg/dl), creatinine (mg/dl), and urea (mg/dl) were assessed spectrophotometrically, along with the enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (commercial kits from Biodiagnostic Company, Giza, Egypt). Antioxidants capacity in blood serum in terms of the total antioxidants' capacity (TAC, ng/mL), reduced glutathione (GSH, mg/dL), catalase (CAT, ng/ml), superoxide dismutase (SOD, U/ml) and malondialdehyde (MDA) were performed using a spectrophotometer (Shimadzu, Kyoto, Japan) with specialized commercially available kits. The serum concentrations of Immunoglobulin G (IgG), Immunoglobulin M (IgM), and Immunoglobulin A (IgA) were analyzed using diagnostic kits obtained from Spectrum Company (Cairo, Egypt).

Antioxidants capacity in liver

The liver tissue was removed from slaughtered quails (5 per group) and was kept at −80°C for further investigation of antioxidant activity biomarkers (GSH and GSH-Px) and MDA as a lipid peroxidation marker.

Statistical analyses

Statistical analyses were conducted using the SAS software package (SAS Institute Inc., 2004). A one-way analysis of variance (ANOVA) was employed to evaluate differences among the groups, followed by Tukey's post-hoc test to identify specific pairwise differences. The threshold for statistical significance was set at $P < 0.05$, ensuring that the observed differences were not due to random variation but was statistically meaningful.

Results

Productive performance and carcass characteristics

The effects of adding Trp and/or CX in quail feeds, throughout different phases of growing period, on growth performance measurements are found in Table 2. No significant changes were recorded in LBW between control and treatment groups at the 1st week of age. In comparison to the control group, quails supplemented with Trp, CX or Trp+ CX showed improvements in LBW at 3 and 5 wks of age. Regarding to BWG, the treated groups (T2:T4) gained more ($P < 0.05$) weight through the first growth duration (1-3 wks) and overall duration (1-5 wks), whereas no changes ($P > 0.05$) were detected in the second growth duration (3-5 wks). Values of FI and FCR were not significantly ($P > 0.05$) affected by dietary addition of Trp and/or CX (Table 2). On the same context, percentages of carcass, dressing, giblets (liver, gizzard, heart, and spleen) and intestine of live body weight were not significantly affected by different experimental diets (Table 3).

Table 2
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on the growth performance of growing Japanese quail.

Variables	Control (T1)	Treatment			SEM	*P* value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
Body Weight (BW, g)						
Week 1	32.60	33.57	33.01	33.48	1.26	0.9432
Week 3	104.57 [b]	116.07[a]	122.32a	117.86a	2.43	0.0007
Week 5	180.52[b]	192.90[a]	190.89[a]	191.58[a]	2.53	0.0116
Body weight gain (BWG, g/day)						
1 - 3 weeks	5.14[b]	5.89[a]	6.38[a]	6.03[a]	0.204	0.0044
3-5 weeks	5.43	5.49	4.90	5.27	0.281	0.4664
1 −5 weeks	5.28[b]	5.69[a]	5.64[a]	5.65[a]	0.102	0.0429
Feed intake (FI, g/day)						
1 - 3 weeks	11.94	14.66	15.49	14.67	1.084	0.1472
3-5 weeks	22.99	22.69	23.55	24.09	1.536	0.9203
1 −5 weeks	16.42	17.43	17.64	17.89	0.831	0.6210
Feed conversion ratio (FCR, g feed/g gain)						
1 - 3 weeks	2.34	2.51	2.42	2.45	0.9349	0.197
3-5 weeks	4.32	4.12	4.83	4.75	0.6190	0.435
1 −5 weeks	3.11	3.07	3.13	3.18	0.9706	0.162

Means in the row with different superscript letters are significantly different at $P < 0.05$.

Blood hematology

Feeding quail on diets enriched with Trp and/or CX significantly ($P < 0.05$) increased RBCs and Hb values compared with the control group (Tables 4). Furthermore, the WBC differential count showed ($P < 0.05$) a decrease in heterophils (%) and an increase in lymphocytes (%) in response to dietary supplementation with Trp and/or CX. The values of Heterophils/ Lymphocytes (H/L) ratio were significantly ($P < 0.05$) declined by the treatments, particularly in quails fed diets containing Trp+CX (Table 4). In addition, the other hematological parameters were not significantly affected by treatments.

Blood chemistry

The dietary addition of Trp and/orCX exerted insignificant impacts on serum values of ALB, ALB/GLOB ratio, glucose, urea and ALT but increased the values of TP, GL, and HDL. All supplements reduced ($P < 0.05$) blood serum values of triglycerides and total cholesterol, while

Table 3
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on the carcass traits, relative organs weight and caecal content pH of Japanese quail.

Variables	Control (T1)	Treatment[1]			SEM	*P* value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
Slaughter weight (g)	172.40	183.60	181.40	186.00	2.626	0.9622
Carcass (%)	68.61	69.56	68.79	71.68	2.836	0.8622
Dressing (%)	73.20	75.24	75.32	75.66	1.523	0.9526
Liver (%)	5.51	5.44	5.81	5.45	0.87	0.9891
Gizzard (%)	2.18	2.31	2.31	2.29	0.461	0.9961
Heart (%)	1.10	0.94	1.04	0.926	0.270	0.9620
Spleen (%)	0.107	0.106	0.120	0.120	0.017	0.8710
Intestine (%)	3.90	4.50	4.58	4.66	0.432	0.4856
Caecal content pH	6.58	6.48	6.59	6.63	0.152	0.9215

Table 4
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on hematology of Japanese quails.

Variables[1]	Control (T1)	Treatment[2]			SEM	P value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
Hb (g/dl)	11.10[b]	12.55[a]	12.81[a]	13.06[a]	0.511	0.0146
RBCs (10^6/mm^3)	3.78[b]	3.90[ab]	4.01[ab]	4.09[a]	0.088	0.0205
Heterophils (%)	32.25[b]	26.69[a]	26.60[a]	25.46[a]	1.74	0.0495
Lymphocytes (%)	57.45[b]	52.97[a]	51.56[a]	51.67[a]	1.30	0.0262
Heterophils/ Lymphocytes	0.56[a]	0.50[b]	0.52[b]	0.49[b]	0.034	0.0354
MCHBC (g/dL)	33.72	34.72	34.76	34.95	1.26	0.9013

Means in the row with different superscript letters are significantly different at $P < 0.05$.
[1] HB: Hemoglobin, RBCs: red blood cells,.
[2] Trp= Tryptophan (0.01 %); CX= Canthaxanthin (0.005 %); Trp+CX= 0.01 % Tryptophan mixture with 0.005 % Canthaxanthin.

HDL level was elevated ($P < 0.05$) comparing with T1 group (Table 5). Birds fed Trp+CX diet showed the lowest triglycerides and total cholesterol in compared to the other experimental groups.

Antioxidant indices

The influence of adding Trp and/or CX in growing quail diet on the levels of TAC, GSH, SOD, CAT and MDA in serum at 5 wks of quail age is tabulated in Table 6. The Trp and/or CX-treated groups showed a significant increase ($p \le 0.05$) in TAC, GSH, SOD, and CAT levels, along with a significant decrease ($p \le 0.05$) in MDA concentration compared to the T1 group (control). The improvement values of these five blood antioxidant indicators were observed in CX and Trp+ CX groups (T3 and T4) with almost no different between them.

Table 5
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on biochemical parameters of Japanese quails.

Variables[1]	Control (T1)	Treatment[2]			SEM	P value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
Total proteins (TP, g/dl)	5.61[b]	6.24[a]	6.27[a]	6.55[a]	0.114	0.0002
Albumin (AL, g/dl)	2.21	2.52	2.57	2.53	0.119	0.1668
Globulin (GL, g/dl)	3.40[b]	3.72[ab]	3.71[ab]	4.02[a]	0.137	0.0428
AL/GL ratio	0.65	0.69	0.69	0.64	0.052	0.8368
Glucose (mg/dl)	268.26	279.50	283.47	280.70	05.63	0.2748
Triglycerides (mg/dl)	278.98[a]	119.79[b]	104.07[bc]	96.50[c]	07.31	0.0001
Total cholesterol (mg/dl)	242.79[a]	221.80[b]	191.82[bc]	172.24[c]	17.31	0.0487
HDL (mg/dl)	37.69 [c]	43.06[b]	48.76[a]	51.91[a]	01.38	0.0001
Creatinine (mg/dl)	0.86 [a]	0.61 [b]	0.51[b]	0.59[b]	0.083	0.0488
Urea (mg/dl)	1.57	1.32	1.28	1.29	0.089	0.1068
AST (IU)	180.56[a]	164.22[b]	150.89[b]	147.50[b]	10.20	0.0323
ALT(IU)	50.07	52.56	55.85	53.24	01.29	0.0793

[a] .
[b] .
[c] Means in the row with different superscript letters are significantly different at $P < 0.05$.
[1] HDL: high density lipoprotein; AST: aspartate amino transferase; ALT: alanine amino transferase.
[2] Trp= Tryptophan (0.01 %); CX= Canthaxanthin (0.005 %); Trp+CX= 0.01 % Tryptophan mixture with 0.005 % Canthaxanthin.

Table 6
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on antioxidants capacity in blood of Japanese quails at 35 days of age.

Variables[1]	Control (T1)	Treatment[2]			SEM	P value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
TAC (ng/mL)	1.41[c]	1.68[b]	1.85[ab]	1.91[a]	0.058	0.0011
GSH (mg/dl)	46.67[b]	65.69[a]	69.62[a]	69.95[a]	4.412	0.0051
SOD (U/mL)	23.44[b]	29.87[ab]	34.88[a]	34.48[a]	2.23	0.0075
CAT (ng/ml)	0.21[b]	0.29 [a]	0.32[a]	0.34[a]	0.021	0.0020
MDA (nmol/mL)	1.10[a]	0.92[b]	0.66[c]	0.70[c]	0.049	<.0001

Means in the row with different superscript letters are significantly different at $P < 0.05$.
[1] TAC= total antioxidant capacity, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde.
[2] Trp= Tryptophan (0.01 %); CX= Canthaxanthin (0.005 %); Trp+CX= 0.01 % Tryptophan mixture with 0.005 % Canthaxanthin.

Antioxidant in liver

Results in Table 7 indicated a significant enhancement ($P < 0.05$) in the antioxidant status in the liver of growing quails against various oxidative stressors by adding Trp and/or CX in quail diets. In this concern, GSH-Px activity was statistically ($P < 0.05$) stimulated, while MDA concentration was statistically ($P < 0.05$) reduced for treated groups compared to untreated group. The greatest effect was noticeably documented for quails treated with Trp and CX (T4). On the other hand, there were no differences ($P > 0.05$) in the serum levels of GSH among the trial groups.

Immunoglobulins

Results in Table 8 show that immunoglobulins, except IgM, of growing quail were significantly ($P < 0.05$) elevated by the addition of Trp and/or CX. In this concern IgG and IgA were increased ($P < 0.05$) for Trp and CX-treated groups in comparison with the control group. The greatest improvements were notably observed in quails from the Trp+CX-administrated group (T4). It is worth noting that the differences between the T2 and T3 groups in the levels of IgG and IgA were not statistically ($P > 0.05$) significant.

Discussion

In the current work, dietary experimental additions did not affect feed intake; however, both final BW and BWG showed improvements. Similar to feed intake, measurements of carcass characteristics remained unaffected. These results align with findings from earlier research on Trp and CX (Duarte et al., 2013; Mund et al., 2020). The Trp role in enhancing BWG is linked to its influence on maintaining secreting of

Table 7
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on antioxidants capacity in liver tissue of Japanese quails at 35 days of age.

Variables[1]	Control (T1)	Treatment[2]			SEM	P value
		Trp (T2)	CX (T3)	Trp + CX (T4)		
GSH (ng/ml)	0.16	0.11	0.14	0.12	0.023	0.4399
GSH-Px (ng/ml)	0.21[c]	0.24[bc]	0.27[ab]	0.29[a]	0.015	0.0079
MDA (nmol/mL)	5.90[a]	5.11[ab]	4.84 [b]	4.54[b]	0.286	0.0228

Means in the row with different superscript letters are significantly different at $P < 0.05$.
[1] GSH: reduced glutathione, GSH-Px: glutathione peroxidase, MDA: malondialdehyde.
[2] Trp= Tryptophan (0.01 %); CX= Canthaxanthin (0.005 %); Trp+CX= 0.01 % Tryptophan mixture with 0.005 % Canthaxanthin.

Table 8
Effect of dietary addition of tryptophan (Trp) and/or canthaxanthin (CX) on immunoglobulins of Japanese quails at 35 days of age exposed to heat stress conditions.

Variables[1]	Control	Treatment[2]			SEM	*P* value
		Trp	CX	Trp + CX		
IgG (ng/ml)	0.35[c]	0.49[b]	0.56[ab]	0.61[a]	0.033	0.0002
IgA (ng/ml)	0.27[a]	0.33[ab]	0.39[a]	0.42[a]	0.029	0.0130
IgM (mg/dl)	0.60	0.69	0.75	0.73	0.052	0.1921

Means in the row with different superscript letters are significantly different at $P < 0.05$.
[1] IgG: immunoglobulin G, A and M.
[2] Trp= Tryptophan (0.01 %); CX= Canthaxanthin (0.005 %); Trp+CX= 0.01 % Tryptophan mixture with 0.005 % Canthaxanthin.

insulin from pancreas (β-cells) in the organisms (Kim et al., 2020). Insulin, as a growth hormone, boosts BWG by accelerating nutrient metabolic rate. In quail, there is a high link between stress and production performance. Tryptophan is a key precursor of serotonin and has been implicated in reducing stress and altering biological processes (Bai et al., 2017). Regarding CX impacts, it is possible that carotenoids like CX play a growth-promoting role since they can activate metabolism and digestion (Amar et al., 2001) and further promote biogenesis and development in (Ohno et al., 2011).

Kawamura et al. (2020) suggested that carotenoid consumption can promote protein anabolism, contributing to increased muscles' mass. Such findings align with our study, where BWG and FBW of quail received either CX-containing diets alone or mixed with Trp, were greater compared to the control group, reflecting improved growth performance. Similar results have been reported for Trp (Duarte et al., 2013; Mund et al., 2020) and CX (Zhang et al., 2011; Bonagurio et al., 2020). Researchers propose that the ability of Trp or carotenoids to mitigate oxidative stress and reduce energy expenditure may be responsible for the observed increase in protein levels. Additionally, Fawzy et al. (2022) demonstrated that diets supplemented with CX significantly enhanced the digestive enzymes activities, including protease, amylase, and lipase, more than CX-free diet. These positive effects of dietary addition of Trp and/or may be reflect on improvement of growth performance of quail in current experimental groups in compared to control group. So, according to this current study, dietary Trp and/or CX supplementation could boost the growth performance of quails during growing period.

Hematobiochemical analyses demonstrated that dietary supplementation with Trp and/or CX yielded significant improvements in Hb value and RBCs count. There is a lack of available data regarding the impact of dietary supplementation with Trp and/or CX on the blood constituents of growing Japanese quails. It's interesting to note that blood biochemical measurements typically correlate with health status; specifically, these characteristics serve as indicators of birds' physiological and nutritional status. Accordingly, quails with ideal hematobiochemistry are more likely to be healthy and exhibit better performance. As reported by El-Kholy et al. (2022a), hematological examinations are helpful in determining the level of blood damage as well as in detecting a variety of disorders. Based on the findings of this study, dietary supplementation with Trp and/or CX significantly enhanced hematological parameters. All measured values remained within the normal range for Japanese quails, demonstrating the lack of any health issues. Nonetheless, variations in hematological parameters are frequently employed to ascertain the various states of the body and to recognize stress resulting from dietary, clinical, or environmental variables. The RBCs count is a measure of the blood's ability to carry oxygen, and it's used to determine the animal health status (Sergent et al., 2004). Our results suggest that birds receiving diets supplemented with Trp and/or CX had higher RBCs count and Hb value at the week 5 of age compared to those receiving the non-supplemented diet. This effect is evident, as RBCs and Hb are in close relationship within the bloodstream. Further, the growing quail fed Trp and/or CX had higher percentages of lymphocytes and heterophils in their blood in comparison with those consumed only the basal diet. Lower values of H/L ratio were recorded in birds supplemented with Trp and/or CX in compared to control group. The rise in the blood H/L ratio (as a stress indicator) is indicative of heightened activity of the hypothalamic-pituitary-adrenal (HPA) axis. Trp or CX may be suppress the activity of HPA and lowers H/L ratio. The findings of this study, which indicate a higher lymphocytes and a lower heterophils, align with the results of prior research conducted by Orawan and Aengwanich (2007). It has been documented that smaller avian species tend to exhibit a reduced leukocyte count. Heterophils are essential to the immune system, serving as the initial line of defense against antigens, and they are effective in managing physiological stress, as noted by Lazăr et al. (2012), which may explain the observed low heterophils % in the current study.

In the current study, the increase in blood glucose in treated groups compared to control group could be a sign of adaptation by gluconeogenesis process, enabling quails under stress conditions to maintain amino acid balance. The glucose levels seemed to rise in tandem with the previously observed patterns of N retention. Heavier FBW of quails in treated groups were possibly associated with higher blood levels of TP relative to the untreated group.

Changes in blood values of creatinine, urea, AST, and ALT are used as indicators to assess the health status of the organism. Urea and creatinine levels serve as indicators of renal function damage (El-Kholy et al., 2024), while ALT and AST levels assess liver function in the body (El-Kholy et al., 2022b). With the exception of creatinine and AST, the trends of the other liver and kidney function indices were comparable among all trial groups. Interestingly, creatinine and AST levels stayed within the acceptable normal values, showing similar patterns among the treated groups (T2, T3, and T4). These findings suggest that including Trp and/or CX in quail feed, at the tested levels, is unlikely to negatively affect the kidney and liver functions of growing quails.

In contrast to current result, Ghazaghi et al. (2024) showed increased in the hepatic enzyme AST in quail chicks received Trp, but this could be attributed to the high consumption of Trp in such conditions. In the present study, the dietary inclusion of Trp, either alone or in combination with CX, resulted in a significant ($P < 0.05$) reduction in AST levels, while having an insignificant effect on ALT. This change was associated with an increase in immune response, *e.g.*, IgG production. The variation in the response of these transaminases may be related to their inherent properties. The simultaneous rise of TP and GLB suggests that Trp and CX may exert protecting impacts, in addition to their role in protein synthesis. Blood TP is composed of ALB and GLB, including immunoglobulins. In this work, the observed rise in TP appears to be primarily due to an increase in the GLB fraction rather than the ALB. While ALB reflects protein synthesis, GLB is associated with the body's protective mechanisms. Meanwhile, the decrease in blood levels of UA in present study by the dietary addition of Trp and/or CX indicates the body's improved use of its nitrogen supplies and lower waste of nitrogen. It's interesting to note that reduced TG in the blood was linked to decreasing UA, suggesting that the blood's energy characteristics synced the use of recycling N (Abou-Elkhair et al., 2020). In another mechanism, the dietary inclusion of Trp promotes the releasing of insulin-like growth factor 1 (IGF-I), which enhances the use of amino acids and glucose. This process promotes protein synthesis and reduces protein catabolism by downregulating the mRNA expression of 20S protease and cathepsin B (Fouad et al., 2021).

Total antioxidant state reflecting the activity of enzymatic reactions and non-enzymatic process. TAC, GSH, SOD, CAT, and MDA are commonly used to evaluate oxidative stress and antioxidant defense mechanisms in biological systems (Shuai et al., 2023). The current work demonstrated that that Trp and CX possess the capacity to increase the quail birds' antioxidant levels and decrease oxidative stress. These improvements in antioxidant defense likely contributed to optimal BWG in growing quail. Alike findings have been reported in previous research of Mund et al. (2020) who observed significant increases in serum antioxidant enzyme activities, such as GSH-Px and CAT, in broiler chickens fed diets supplemented with Trp. In addition, Patil et al. (2013) highlighted that Trp, as a precursor of melatonin and serotonin plays a pivotal role in reducing the oxidation-related harms in broiler chickens. Their study reported enhanced activity of key antioxidant enzymes, including CAT and SOD, in Trp-administrated birds. Similarly, Wang et al. (2014) found that increasing Trp levels to 1.5 times the dietary recommendations effectively alleviated oxidative stress and improved feed utilization in broiler chicks subjected to stressful conditions. These findings suggest that dietary supplementation of Trp over the NRC (1994) recommendations can significantly boost blood antioxidant state, aiding in the removal of ROS and protecting cellular structures from oxidative damage. In current study, the improvement of redox status in hepatic and serum was especially observed in Trp mixture with CX groups.

Canthaxanthin has been identified as a powerful antioxidant with immune-modulating effects (Venugopalan et al., 2013). TAC is a crucial indicator for assessing both enzymatic and non-enzymatic antioxidant systems, with higher TAC levels indicating enhanced antioxidant potential. In this work, CX supplementation led to a significant increase in TAC, consistent with the results of Di Mascio et al. (1990), who demonstrated similar effects in mice. CAT, a vital enzyme in the antioxidant defense mechanism, plays a pivotal role in neutralizing hydrogen peroxide (H2O2) by converting it into H_2O and an oxygen molecule, thereby preventing oxidative injury to cells (Ighodaro and Akinloye, 2018). In this research, dietary inclusion of Trp and/or CX boosted the activity of CAT enzyme. Additionally, GSH-Px, which also removes H_2O_2, showed reduced activity in CX-treated group compared to the control. This reduction suggests that CX provided enhanced cellular safeguard (Fawzy et al., 2022). Liu et al. (2015) observed similar trends in White Pekin ducks, where dietary supplementation of Trp over the recommended amounts significantly increased both GSH-Px and CAT activities both blood plasma and liver. Likewise, Patil et al. (2013) found that Trp, a precursor of serotonin and melatonin, plays a pivotal role in reducing the oxidation-related harms in broiler chickens by enhancing the activity of CAT and SOD. In this work, dietary administration of CX and its combination with Trp caused a reduction ($P < 0.05$) in MDA levels, comparing with the control. MDA serves as an indicator of lipid peroxidation and oxidative injury (Ayala et al., 2014). The observed reduction in MDA levels suggests that CX effectively inhibits lipid peroxidation, protecting cells from free radical damage, as reported by Sahin et al. (2014). The potent antioxidant properties of CX, including scavenge free radicals (Chang et al., 2013), modification of the prooxidative/antioxidative balance, and absorb extra energy from ROS (Araujo et al., 2020; Bohm et al., 2012). In the current study, dietary CX supplementation significantly ($P < 0.05$) boosted the serum activity of SOD and GSH-Px while reduced ($P < 0.05$) MDA levels in growing quails. These findings indicate that CX can lessen oxidative stress and enhance antioxidant capacity. Combining CX with Trp further yielded improvements in TAC, decreased MDA levels, and boosted overall antioxidant activity in the growing quails. Notably, quails receiving CX and Trp supplementations exhibited higher serum antioxidant capabilities than un-supplemented quails. As a potent lipid-soluble antioxidant, CX enhances the activities of enzymatic antioxidant, as demonstrated in studies on broilers and laying chickens (Al-Gamal et al., 2021). Additionally, in vitro research has shown that CX inhibited t-butyl hydroperoxide (t-BOOH), induced production of MDA and lipid peroxidation, and scavenged free radicals (Chew and Park, 2004).

Bonagurio et al. (2020) reported that the addition of CX was found to reduce MDA levels in the liver and serum of mature European quails. This reduction likely enhances defense against oxidative stress by boosting the antioxidant capabilities, enabling more effective scavenging of free radicals generated during liver and blood metabolism. Lower oxidative stress, facilitated by antioxidants, supports improvements in mitochondrial biogenesis and function (Chen et al., 2019). CX could work synergistically with certain amino acids, like Tryp, to neutralize free radicals. In this respect, Urso et al. (2015) found that CX interacts synergistically with vitamin E in broiler breeder diets. As highlighted by Johnson-Dahl et al. (2016), these antioxidant molecules may improve immune functions. This theory confirmed by the improvement of antioxidant status in hepatic and blood of birds belongs to T3 and T4 groups.

In this work we revealed that Trp supplementation enhanced the antibody production in growing quail Ghazaghi et al. (2024) showed that dietary Trp increased concentration of serum IgG in quail chicks. According to Sanchez et al. (2008), adding Trp boosts melatonin production, which, in turn, increases the nonspecific immunity through the activation of peritoneal macrophages. In addition, Chen et al. (2016) and Guo et al. (2015) showed that melatonin receptors found on immune tissues may be the mechanism by which Trp affects immunological function. Mund et al. (2020) demonstrated comparable outcomes regarding the immunological response in broiler chickens supplemented with Trp. Trp supplementation was found to be effective in regulating IgM, according to this study findings. Trp has been shown to regulate the innate immunological response and the synthesis of melatonin and serotonin (Esteban et al., 2004). In the current study, the positive effect of CX addition on humoral immunity of growing quails was in agreement with findings of Ghodratizadeh et al. (2014) on rabbits. There is lack information of the effect of CX on humoral immunity of growing quails until write this manuscript.

Dietary addition of Trp and/orCX beneficially increased IgG and IgA, and beneficially reduced MDA concentration in both of meat and blood. Additionally, it lowered blood levels of triglycerides, TC, creatinine, and AST. The antioxidant properties of these compounds may be the fundamental mechanism driving the positive effects of dietary Trp or CX (Mehri et al., 2021). Khanipour et al. (2019) proposed that Trp and its metabolites (like melatonin and kynurenine) may have a combined effect on the body's immune and antioxidant systems via controlling cytochrome P450 in the hepatocytes. Try is a precursor to several bioactive compounds that have been linked to antioxidant qualities, such as kynurenine and melatonin. Try and its metabolites have the ability to scavenge radicals, which implies that they may have a function in reducing oxidative stress in the cellular environment (Nayak and Buttar, 2016). One popular metabolite of Try that is well-known for its strong antioxidant properties is melatonin. It aids in preserving the redox equilibrium of cells by scavenging free radicals. Furthermore, another Try molecule called kynurenine has been connected to antioxidant responses by controlling oxidative stress (Johns and Platts, 2014). The relationship between Trp, its metabolites, and its antioxidant properties thought to integrate closely with the immune functions (Ghazaghi et al., 2024). Regarding CX, in vivo studies demonstrated its ability to reduce lipid peroxidation, protect against hepatocytes DNA destruction, and boost hepatic antioxidant defenses (Gradelet et al., 1998; Elia et al., 2019). CX has been shown to stimulate alkaline phosphatase activity (ALP), aiding in detoxifying lipopolysaccharides (Rebelo et al., 2020).

Conclusion

In summary, incorporating Trp and/or CX into the basal feed of quails through growth period enhanced productive performance, antioxidant capacity, and humoral immunity. However, it had no effect on carcass characteristics. Finally, the present work provides valuable new insights of the effects of dietary Trp and CX on production and physiology of quails. Moreover, it establishes a foundation for future research to explore the underlying mechanisms and long-term implications of flexible dietary strategies in poultry nutrition, beyond conventional practices.

Declaration of competing interest

The authors declare no conflict of interest.

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