Research Article

Leverage of Chromium Methionine Supplementation in Laying Japanese Quail's Diets on Performance, Quality, and Blood Traits Challenged by Heat Stress

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Abstract | In Egypt, the ambient temperature can stay consistently high for long periods, besides frequent hot and humid hazardous waves. A high temperature of more than 30°C causes heat stress (HS) in birds and is among the most symptoms of stress that impair poultry production. Chromium (Cr) is a trace element and has a beneficial effect Cr on human and animal health. The experiment was conducted to estimate the action of chromium methionine (Cr-Met) supplementation in laying quails (*Coturnix japonica*) under HS. A total of 180 were allotted to 5 experimental treatments (6 replicates per treatment). Treatments were: 1) Basel diet (BD), 2) 0.2 g Cr-Met/kg diet, 3) 0.4 g Cr-Met/kg diet, 4) 0.6 g Cr-Met/kg diet, and 5) 0.8 g Cr-Met/kg diet. Results showed that the performance traits, including feed conversion ratio (FCR), daily egg number, and egg mass, were affected positively by Cr-Met supplementation (P ≤ 0.05) compared to birds fed BD. All eggshell and interior egg quality traits were not affected by Cr-Met supplementation. Cr-Met administration positively affected yolk total lipid and yolk total cholesterol compared to birds fed BD as their values were decreased. Also, Cr-Met addition improves the concentricity of unsaturated fatty acids (UFA) and significantly suppresses the concentricity of saturated fatty acids (SFA) compared to birds fed BD. Likewise, dietary Cr-Met supplementation improves blood traits, including a lipid profile, kidney functions, and antioxidant profile of laying quails. In conclusion, Cr-Met supplementation to laying quails diets at 0.4 and 0.6 g Cr-Met/kg under HS enhances performance traits, yolk lipid Profile, and blood traits of quails.

Keywords | Heat stress, Quails, Blood traits, Yolk fatty acids, Chromium

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INTRODUCTION

Typical of developing countries that are very vulnerable to climate change and face multiple economic and environmental sustainability challenges, Egypt stands out as a good illustration of this. Egypt's existing arid climate will be exacerbated by climate change, with relatively high temperatures and poorer rainfall in major agricultural areas. All ecological conditions affect animal welfare, including temperature, humidity, presence of toxic contaminants, and litter quality (Utnik-Banaś et al., 2014). In the case of extreme temperatures combined with high levels of humidity, this causes severe stress in the birds, which results in a decline in production performance (Dosoky et al., 2020). HS in poultry occurs when the temperature rises beyond 30°C, and it is one of the most common indicators of stress that negatively impact chicken production indices. (Rama Rao et al., 2011). In anxious poultry, the productivity of eggs, quality of eggs, utilization of feed, and weight gains were all restricted (Mashaly et al., 2004). Antioxidant status debilitated and mineral secretion boosted in serum and liver due to HS in poultry (Khan et al., 2011). What's more, HS also accelerates lipid oxidation as more of a consequence of rising free radical production, which promotes the synthesis of reactive oxygen species and generates oxidative stress in cells (Altan et al., 2003). Acclimatization could help reduce the impacts of HS (Yalcin et al., 2001). Shelter, ventilation and cooling solutions are likely to reduce HS effectively. Because of their impracticality and high expense, several approaches have been abandoned in specific locations and farms. Instead, nutritional manipulation in poultry production could be a viable option (Shane, 1988, Khalifah et al., 2021, Hassan et al., 2022). According to the literature, antioxidant vitamins and minerals such as zinc (Chand et al., 2014), selenium, and Cr (Torki et al. 2014) may help to mitigate the negative impact of environmental stress.

Cr is a vital micronutrient for humans and animals (Nattapon et al., 2012). It aids in physiological and nutritional efficiency (Aslanian et al., 2011). Cr, which is usually found in nature as a trivalent state, is required for the activation of various enzymes and the stabilization of proteins and nucleic acids. Its principal function in metabolism is to potentiate insulin activity via an organometallic structure known as glucose tolerance factor (Mohammed et al., 2014). Cr boosts glucose glycogenesis, promotes glucose transportation, and boosts protein synthesis (Hayirli, 2005). Cr is thought to operate as an antioxidant as insulin promotes lipid peroxidation (Habibian et al., 2013). Also, Cr plays a vital role in enhancing cholesterol profile (Preuss et al., 1990). Cr shortage has been linked to slowed growth rate, glucose and protein metabolism (Pagan et al. 1995). Cr addition to poultry diets improved the growth performance variables (Habibian et al., 2013). Under environmental, nutritional, and hormonal stress, Cr's positive benefits can be shown more clearly. (Sahin et al., 2002a, Gleuktin et al., 2009).

The (NRC, 1994) made no recommendations for poultry Cr requirements, particularly quails. (Sahin et al., 2002a; Sahin et al., 2005). However, in recent years, more studies on the usage of Cr and its beneficial effects on poultry quality have been conducted. Cr supplementation can help in minimizing the adverse effects of environmental stress during the formation of the yolk (Nasiroleslami and Torki 2011). Cr in organic forms is more soluble in water and metabolized in the gastrointestinal tract to a vastly more significant amount than Cr in inorganic types (Anderson et al., 2001). Chromium linkage with L-methionine (Cr-Met) is a newly available natural Cr form whose availability and effects in growing Japanese quail have been determined (Dosoky et al., 2020).

As a result, the purpose of this study is to investigate the potential actions of Cr-Met in the laying performance and quality of quails grown in Egypt throughout the summer.

MATERIALS AND METHODS

The present study was performed at the Fish and Animal Production Department, Faculty of Agriculture (Saba Basha), Alexandria University. Samples analysis were conducted at Livestock Research Department, Arid Land Cultivation Research Institute, City of Scientific Research and Technology Applications (SRTA-City).

Housing and management

The Institutional Animal Ethics Committee of Alexandria University approved the field experiment. A total of 180 layer Japanese quails were handled. Quails were weighed separately and then randomly assigned to five experimental groups with initial average weight 152.45 g ±1.89, each with 36 birds; each group was split into six replicas, consisting of 6 birds per replicate. Throughout the testing period, which lasted from 9 to 18 weeks, quails were housed in wire-laying cages (60*43*20 cm. l*w*h) in a open house arrangement. Feed and water were supplied ad-libitum. Quails were conditioned to 16 hours of continuous light and reared under the same management. The average ambient temperature was 33 °C, with average relative humidity of 75%.

Experimental diets

Cr was added in the form of Chromium-l-methionine complex (containing 1 g of Cr:$^{kg-1}$Cr-Met) to the basal diet it was obtained from Haerbing Debang Dingli Biology Technique Co., Ltd. (Heilongjiang, China).

The basal experimental diets were formulated to cover the nutrient requirements of laying quail as NRC (1994) recommended. Composition and calculated analysis of the experimental diets are presented in Table 1.

The experimental groups received the following dietary treatments

Basel diet without supplementation (BD).

BD + 0.2 g CrMet /kg diet.

BD + 0.4 g CrMet /kg diet.

BD + 0.6 g CrMet /kg diet.
BD + 0.8 g CrMet /kg diet.

Table 1: Feed composition and calculated analysis of the experimental basal diets

Ingredients	%
Yellow corn	59.50
Soybean meal (44 %)	22.60
Concentrate (50 %) *	10.00
Di-calcium phosphate	0.40
Limestone	5.50
Sunflower oil	1.00
Vit. and min. mix. **	0.50
Salt (NaCl)	0.50
Total	100
Calculated analysis[1]	
Crude protein (%)	20.00
ME (Kcal/ Kg diet)	2903.89
Ether extract (%)	2.60
Crude fiber (%)	3.04
Methionine (%)	0.71
Methionine +Cystine (%)	0.90
Lysine (%)	1.15
Calcium (%)	2.58
Av. Phosphorus (%)	0.40

* Concentrate (contains): ME (K cal/kg) 2870, Crude protein 50%, Crude fiber 1.51%, Crude fat 1.54%, Calcium 4.29%, Phosphorus 2.39%, NaCl 0.8%, Methionine 4.6%, Methionine & Cystine 5.38%, Lysine 3.90%.

** Each kg of vitamin and minerals mixture contained: Vit. A, 4,000,000 IU; Vit. D_3, 500,000 IU; Vit, E, 16.7 g., Vit. K, 0.67 g., Vit. B1 0.67 g., Vit. B2, 2 g., Vit. B 6, .67 g., Vit. B12, 0.004 g., Nicotinic acid, 16.7 g., Pantothenic acid, 6.67 g., Biotin, 0.07 g. Folic acid, 1.67 g., Choline chloride, 400 g., Zn, 23.3 g., Mn, 10 g., Fe, 25 g., Cu,1.67 g., I, 0.25 g.,Se, 0.033 g. and, Mg, 133.4 g.

[1] Calculated according to **NRC (1994).**

Determination of performance

The number of eggs produced and egg weights for each replicate were recorded daily.The egg number, laying rate, and egg mass were calculated. Feed intake (FI) was recorded daily for each replicate, and feed conversion ratio (FCR) was calculated by dividing the amount of FI by the amount of egg mass-produced.

Determination of Eggshell quality

Eggshell quality traits were conducted weekly using all eggs of 2 days from all treatments. Indices of eggshell quality included eggshell weight percentage, egg shape index was determined according to (Romanoff and Romanoff, 1949), specific gravity (Novikoff and Gutteridge, 1949), and eggshell thickness (Voisey and Hunt, 1974).

Determination of interior Egg quality

Albumen and yolk percentages were calculated, yolk index was estimated according to (Funk, 1948). Yolk color was determined according to (Vuilleumier, 1969).

Determination of cholesterol, total lipids, and fatty acids of egg yolk

Yolk cholesterol was extracted according to (Fisher and Leveille, 1957) and analyzed according to (Allain et al., 1974). Total lipids were determined according to (Fisher and Leveille, 1957).

Fatty acids analysis, whether SFA or UFA, was carried out by gas-liquid chromatography (GLC) using Shimadzu gas chromatograph (GC-4 cm, PFE). Preparation of fatty acids methyl esters from total lipids of the sample was performed according to (Radwan, 1978).

Determination of blood traits

At the end of the experiment, six birds were randomly chosen from each treatment and slaughtered; blood samples were taken, centrifuged at 1500 rpm for 10 min, and sera were collected and stored at -20°C. Serum samples were thawed at room temperature, then total protein, albumin, creatinine, uric acid, total lipids, triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose, total antioxidant capacity (TAC), malondialdehyde (MDA) and glutathione peroxidase (GSH) were measured with a spectrophotometer (SELECTA® UV-2005) using a commercial detection kit (Bio-diagnostic, Egypt) following the manufacturer's instructions. Serum globulin levels were calculated by subtracting albumin values from total protein values (Coles, 1986).

Statistical analysis

The differences among treatments were statistically analyzed by one-way ANOVA using (SPSS) statistical software package for windows version 23.0. Duncan's Multiple Range-test separated the significant differences between treatment means (Duncan, 1955). The used model was:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = An observation treatment

μ = Overall mean

T_i = the treatment effect (I = 1--------- 5)

e_{ij} = The experimental random error.

RESULTS

Performance of laying quails

Data describing the influence of Cr-Met supplementation on performance of laying quails are presented in Table (2). Cr-Met supplmentation had insignificant ($P > 0.05$) effect on FI of laying Japanese quail compared to the group fed BD. The results concerning that FCR was significantly ($P \leq 0.05$) affected by incremental levels of Cr-Met. These improvements were observed in FCR of birds given 0.2,

Table 2: Effect of dietary Cr-Met on performance of laying quails.

Items	Cr-Met (g/kg diet)					SEM	P-value
	0	0.2	0.4	0.6	0.8		
Feed intake (g/hen/day)	26.80	25.66	26.94	27.04	27.03	0.226	0.256
Feed conversion ratio (g feed/g egg)	3.18[a]	2.73[b]	2.96[b]	3.00[b]	3.24[a]	0.058	0.016
Egg laying rate (%)	65.03[b]	69.59[a]	69.59[a]	71.43[a]	68.89[a]	0.991	0.007
Egg weight (g)	12.75	13.11	14.78	13.80	13.41	0.247	0.0791
Egg number (g/hen/day)	0.65[b]	0.69[a]	0.70[a]	0.71[a]	0.69[a]	0.007	0.034
Average egg weight (g)	13.01	13.47	13.01	12.62	12.13	0.206	0.334
Egg mass (g/hen/day)	8.45[bc]	9.38[a]	9.08[ab]	9.02[ab]	8.34[c]	0.131	0.021

Means followed by different lowercase letters are significantly different ($P \leq 0.05$).

Table 3: Effect of dietary Cr-Met on eggshell quality of laying quails.

Items	Cr-Met (g/kg diet)					SEM	P-value
	0	0.2	0.4	0.6	0.8		
Egg shape index	74.79	78.11	78.73	78.87	74.90	0.962	0.482
Eggshell weight(g)	1.21	1.20	1.40	1.37	1.35	0.047	0.710
Eggshell weight (%)	9.47	9.47	9.28	9.57	10.28	0.440	0.311
Specific gravity	1.07	1.66	1.08	1.11	1.10	0.055	0.067
Eggshell thickness	18.33	19.00	19.46	19.86	18.93	0.228	0.280

Means followed by different lowercase letters are significantly different ($P \leq 0.05$).

Table 4: Effect of dietary Cr-Met on interior egg quality of laying quails.

Items	Cr-Met (g/kg diet)					SEM	P-value
	0	0.2	0.4	0.6	0.8		
Albumen weight (g)	7.45	7.70	8.65	8.23	7.91	0.265	0.073
Albumen weight (%)	58.55	58.76	58.95	59.68	59.05	1.143	0.066
Yolk weight (g)	4.06	4.16	4.69	4.24	4.13	0.106	0.205
Yolk weight (%)	31.98	31.78	31.58	30.75	31.27	0.916	0.151
Yolk Index	517.66	463.2	511.46	495.23	504.86	12.138	0.683
Yolk height (mm)	12.24	12.18	13.426	12.09	12.53	0.313	0.695
Yolk Color	2.60	3.60	3.80	3.00	3.40	0.265	0.235

Means followed by different lowercase letters are significantly different ($P \leq 0.05$).

Table 5: Effect of dietary Cr-Met on yolk total lipids, yolk total Cholesterol, yolk saturated and unsaturated fatty acids of laying quails.

Items	Cr-Met (g/kg diet)					SEM	P-value
	0	0.2	0.4	0.6	0.8		
Yolk total lipids (mg/g yolk)	304.74[a]	296.87[a]	289.82[ab]	278.72[bc]	270.83[c]	2.830	0.001
Total Cholesterol (mg/g yolk)	17.28[a]	16.98[ab]	16.84[ab]	16.42[bc]	16.08[c]	0.120	0.001
Saturated fatty acids (% total fatty acids)							
Myristic acid	0.42[a]	0.346[b]	0.32[b]	0.33[b]	0.316[b]	0.009	0.001
Palmitic acid	31.56[a]	29.90[ab]	28.502[b]	26.12[c]	25.87[c]	0.468	0.001
Stearic acid	11.28[a]	11.41[a]	10.58[ab]	10.81[ab]	9.90[b]	0.164	0.020
Arachidic acid	1.77[a]	1.44[b]	1.28[c]	1.18[cd]	1.11[d]	0.038	0.001
Unsaturated fatty acids (% total fatty acids)							
Palmitoleic acid	2.20[b]	2.66[ab]	2.51[ab]	2.55[ab]	2.78[a]	0.042	0.001
Oleic acid	35.16[b]	38.16[a]	38.02[a]	38.38[a]	38.97[a]	0.395	0.001

Linoleic acid	11.28[b]	11.68[ab]	11.91[a]	12.23[a]	12.53[a]	0.148	0.050
Linolenic acid	0.81[b]	0.87[a]	0.89[a]	0.89[a]	0.911[a]	0.010	0.024

Means followed by different lowercase letters are significantly different ($P \leq 0.05$).

Table 6: Effect of dietary Cr-Met on blood traits of laying quails.

Items	**Cr-Met (mg/kg diet)**					**SEM**	**P-value**
	0	0.2	0.4	0.6	0.8		
Total protein (mg L^{-1})	5.28[c]	5.53[c]	5.99[b]	6.02[b]	6.57[a]	0.090	0.001
Albumin (mg L^{-1})	2.40[c]	2.87[b]	3.23[b]	3.11[b]	3.70[a]	0.080	0.001
Globulin (mg L^{-1})	2.88	2.66	2.76	2.91	2.87	0.077	0.843
A/G ratio	0.88	1.13	1.24	1.09	1.49	0.072	0.091
Creatinine (mg L^{-1})	0.84	0.87	0.84	0.86	0.96	0.014	0.057
Uric acid (mg L^{-1})	5.81[a]	5.52[ab]	5.40[ab]	5.05[bc]	4.79[c]	0.092	0.002
Total lipids (mg L^{-1})	404.33[a]	392.00[ab]	393.44[ab]	377.44[bc]	372.11[c]	3.136	0.004
Triglycerides (mg L^{-1})	142.24[a]	134.11[ab]	133.56[ab]	127.89[b]	124.78[b]	2.072	0.042
Cholesterol (mg L^{-1})	181.11[a]	173.67[b]	174.78[b]	170.89[bc]	166.44[c]	1.142	0.001
LDL (mg L^{-1})	82.35[a]	79.32[ab]	76.94[bc]	73.41[cd]	70.32[d]	0.957	0.001
HDL (mg L^{-1})	36.79[c]	37.11[c]	39.95[bc]	41.31[ab]	44.33[a]	0.647	0.001
Glucose (mg L^{-1})	167.56[a]	157.44[b]	154.00[bc]	151.67[bc]	149.00[c]	1.360	0.001
Total antioxidant Capacity (ng/mL)	0.80[b]	0.87[a]	0.89[a]	0.90[a]	0.93[a]	0.011	0.002
Malondialdehyde (nmol/mL)	11.09[a]	10.52[ab]	10.29[abc]	9.98[bc]	9.51[c]	0.152	0.009
Glutathione Peroxidase (ng/mL)	30.99[c]	33.24[bc]	33.95[b]	34.53[b]	40.09[a]	0.586	0.001

Means followed by different lowercase letters are significantly different ($P \leq 0.05$).

0.4, and 0.6 g Cr-Met / kg diet. The advances in these groups reached 14.15, 6.92, and 5.66 % from the group fed BD.

The increase in laying rate reached a significant ($P \leq 0.01$) effect with different levels of Cr-Met supplmentation on diets compared to the BD group, results showed that daily egg number significantly ($P \leq 0.05$) increased by supplementations of Cr-Met with different diets levels compared to BD group. Results indicated that Cr-Met supplementation did not significantly influence average egg weight compared to BD group. Egg mass/hen/day was significant ($P \leq 0.01$) increased with different levels of Cr-Met, the highest value was recorded with birds fed 0.2 g Cr-Met/kg diet compared to the BD group.

Eggshell quality of laying quails

Data describing the effect of Cr-Met supplementation on eggshell quality traits are summarized in Table (3). The results show that egg shape index, eggshell weight (absolute and percentage), egg specific gravity, the eggshell thickness was not significantly affected by dietary supplementation of Cr-Met.

Interior egg quality of laying quails

Data describing the effect of Cr-Met supplementation on interior egg quality are summarized in Table (4). Data of albumen weight (absolute and percentage), yolk weight (absolute and percentage), yolk index, yolk height, and yolk color were not significantly affected by Cr-Met supplementation to laying quails' diet.

Yolk total lipids, total cholesterol, and fatty acids of laying quails

Results presented in Table (5) summariesed the effect of Cr-Met on total lipids and cholesterol, yolk saturated and unsaturated fatty acids in the egg yolk of laying quails. Yolk total lipids and yolk cholesterol were significantly ($P \leq 0.001$) decreased by Cr-Met supplementation. The best values in yolk total lipids and total cholesterol were recorded with Cr-Met at 0.6 and 0.8 g/kg diet. Results also showed a significant decrease in SFA content and a considerable increase in UFA by Cr-Met supplementation as compared to BD group.

Effect of Cr-Met on selected blood traits of laying quails. The results in Table (6) showed the effect of Cr-Met on selected blood parameters. Data showed that the Cr-Met induced a significant ($P \leq 0.001$) increase on blood total protein and albumin; it was observed that total protein and albumin concentrations were increased with increasing Cr-Met level in diets. The effects of Cr-Met supplementation on kidney parameters showed a significant decrease ($P \leq 0.001$) in uric acid concentration. However, creatinine concentration was not affected by Cr-Met supplementation. Cr-Met supplementations significantly decreased (P ≤ 0.05) the total lipids, total cholesterol, triglycerides, LDL, and glucose levels. However, the results indicated that HDL was significantly (P ≤ 0.01) increased compared to BD group. Also, results show a significant (P ≤ 0.01) increase in serum TAC and GSH with Cr-Met supplemented groups compared to BD group. Conversely, MDA was significantly (P ≤ 0.01) decreased in all treated groups than BD group.

DISCUSSION

Temperatures of 32°C or above are considered to affect the performance of laying hens. Prior studies have connected high ambient temperatures to reduced FI, egg production, egg weight, and a higher FCR (Panda et al., 2008). According to Sahin et al. (2004), the addition of Cr can mitigate some of the negative impacts of HS on production performance. Our results indicate that Cr-Met enhanced the production performance of laying quails. Similary, (Karami et al., 2018) reported that giving laying hens low Cr-Met diets (0.4 g/kg) did not influence the number of eggs laid, egg weight, or egg mass when HS was administered. Also, Mirfendereski and Jahanian (2015) observed that adding Cr-Met significantly enhanced egg production. Nevertheless (Torki et al., 2014) found no significant effect on egg production and egg mass in hens supplemented chromium picolinate (0.4 g/kg). It was discovered that Cr-Met supplementation had no effect on FI but has a beneficial impact on FCR, Sahin et al. (2002b) and Piva et al. (2003), also discovered that supplemental Cr had no effect on FI but increased FCR in laying hens. However, (Mirfendereski and Jahanian, 2015) found that Cr did not influence FCR. Various experimental circumstances and characteristics, such as supplementation ways (in diet or water), provided quantities, stress intensities, stress types, and accessibility, all have a part in different findings.

Results showed that Cr-Met supplementation showed non-significant effect on egg quality traits. However, most of the egg quality traits were enhanced. Our findings contrast those of Torki et al. (2014), who discovered that giving HS laying hens a diet containing 0.2 g/kg Cr increased eggshell weight and thickness. Sahin et al. (2002a) found that HS hens given 0.4 g/kg Cr had a higher eggshell weight. Attia et al. (2015) discovered that Cr-supplemented meals enhanced shell thickness considerably.

Because of the inclusion of Cr-Met, which may lessen the effect of HS, the egg's specific gravity was enhanced, although not significantly in this investigation. The rationale for these seemingly coincidental eggshell thickness and specific gravity discoveries may be attributed to:-(i) Cr may contribute to egg quality preservation by acting as a structural component of albumen or as a protein binder, (ii) Cr is required for the synthesis of ovomucin, which is responsible for the structure of albumen gels, and (iii) Cr stimulates the transmission of metal ions (most likely magnesium) into egg albumen during the plumping process in the uterus. It is possible to use the specific gravity as a proxy to estimate both the thickness of the shell and its strength (Roberts, 2004). As a result, it's logical to expect that a thicker shelled egg will have a higher specific gravity. Cr as a single supplement boosted egg shape index in HS birds, according to (Karami et al., 2018). Additionally, Cr-Met had no effect on yolk weight or yolk index within the control under HS; these findings were similar to (Piva et al., 2003), who discovered that Cr did not affect yolk weight or yolk index, but (Sirirat et al., 2013), found that laying hens fed diets enriched with nanoparticles chromium picolinate (0.3 and 0.5 g/kg) had lower egg yolk weight than control chickens. Our findings indicated a slight rise in yolk color, although it was not statistically significant. Adding Cr to the diet improved yolk color in HS laying hens (Torki et al., 2014). Local strain hens fed Cr showed higher albumin weight, yolk index, and yolk weight than those fed control diets (Attia et al., 2015).

According to our findings, Cr-Met reduced the concentration of total lipids and cholesterol in the yolk. In line with our results, Attia et al. (2015) discovered that chromium supplementation in laying hens during hot summer conditions reduced egg yolk total cholesterol, total lipids, and triglycerides compared to the control. In addition, (Sahin et al., 2004) discovered that supplementing laying hens' diets with Cr lowers cholesterol levels in their yolks.

Consumers need to know how many nutrients meat and eggs have and how much UFA and cholesterol they contain. Eggs' nutritional characteristics are connected to the content of fatty acids in eggs. SFA influences the pathophysiology of cancer and heart disease associated with diet (Briggs et al. 2017). In contrast, UFA, renowned for their health benefits, such as reduced thrombotic risk and decreased heart disease incidence in humans, have been shown to have a positive impact on human health (EFSA 2010). Enhanced quantities of UFA in the eggs and higher antioxidant qualities in the eggs, were all seen in the current study, demonstrating that feed supplementation with Cr-Met can be a method to improve nutritional features of quail egg yolks. The addition of Cr-Met in quail meals improved the final product by reducing undesired SFA content and enhancing good UFA.

The results indicated that Cr supplementation increased serum total protein and albumin concentrations. Similar increase in serum total protein concentration was observed by Torki et al. (2014), who added 0.2 or 0.4 g/kg Cr (as Cr picolinate) to diets of HS laying hens. However, Ma et al. (2014) observed that the serum albumin concentration of laying hens was not influenced by the addition of 0.2, 0.4, or 0.6 g/kg Cr (as Cr propionate). Results demonstrated a reduction in uric acid concentration while not affecting creatinine levels. According to Ma et al. (2014), a 0.2 g/kg Cr supplement reduced uric acid content by 31%. However, karami *et al.* (2018) discovered that Cr had no effect on serum uric acid levels, and Samanta et al. (2008) found that serum uric acid concentrations were identical in broilers fed 0.5 or 1 g/kg Cr (as Cr picolinate) and those fed a control diet. This study's reduction in uric acid might be related to this supplement's antioxidant activity (Onderci et al., 2003) as uric acid has been linked to antioxidant qualities in birds (Tsahar et al., 2006). Although we don't know why creatinine levels decreased, these data imply that Cr-Met had no negative impact on renal function.

According to our findings, Cr-Met treatment resulted in a decrease in fatty acid profile. It is consistent with our results that supplementing diets with 0.2 or 0.4 mg/kg Cr reduced triglycerides and cholesterol levels of laying chickens, as demonstrated by Torki et al. (2014). Additionally, Mirfendereski and Jahanian (2015) showed that the application of 1 g/kg Cr-Met lowered the cholesterol levels of laying hens. Conversely, Ma et al. (2014) found that adding 0.2, 0.4, or 0.6 g/kg Cr to diets did not affect triglycerides content of laying hens (as Cr propionate).

As a result of our observations, the glucose concentration gradually decreased as the amount of Cr-Met increased. Mirfendereski and Jahanian (2015) and Torki et al. (2014) observed that supplementing laying hens with 0.5 and 1 g/kg Cr reduced glucose concentrations. Inversely, Ma et al. (2014) found that feeding laying hens 0.2, 0.4, or 0.6 g/kg Cr (as Cr propionate) did not affect their blood glucose levels. Cr has been proposed to be a physiologically active mineral because it is a component of a biomolecule known as chromodulin. Chrmodulin is a component of the insulin signaling pathway and seems to influence glucose and lipid metabolism through the action of insulin (Vincent 2000).

Tissue damage, bird health problems, and financial losses are all linked to oxidative stress, which occurs when free radical generation outpaces antioxidant defenses (Panda and Cherian 2014). Minerals including zinc, copper, manganese, and selenium have been advocated as external antioxidants (Willcox et al., 2004). The current study found that Cr-Met acts as an antioxidant by increasing GSH activity in the blood and lowering MDA levels. Our findings agree with those of Attia et al. (2015), who discovered that adding chromium resulted in less ($p \leq 0.05$) MDA concentration than control in laying hens under Egyptian summer conditions. In rats, administration of Cr picolinate reduced MDA synthesis, a marker of lipid oxidation, and Cr functioned as an antioxidant, according to Preuss et al. (1997). Also, MDA in serum was significantly reduced in rats fed a Cr-supplemented diet, according to Anderson et al. (2001). Augmenting with trace organic minerals reduced the peroxidation of lipids (Bun et al., 2011) and enhanced the activity of GSH (Ma et al., 2011). For their potential to reduce oxidative stress, Mathivanan and Selvaraj (2003) discovered that Cr supplements in layer hens' diets act as antioxidants. There are several components in both egg yolks (phospholipids) enzymes and whites (ovalbumin, ovotransferrin, phosvitin) are linked to antioxidant properties (Carocho et al., 2013). In conclusion Cr-Met could be supplemented to diets of laying quails at 0.4 and 0.6 g Cr-Met/kg for its benefits on performance, egg quality traits, increasing the fatty acid profile of eggs and enhanced the blood traits including (lipid profile, kidney function and antioxidant activity) under HS.

CONFLICT OF INTEREST

No conflict of interest.

NOVELTY STATEMENT

The authors have developed the composition of laying quails diet by natural feed additives (chromium methionine). The results of the leverage of chromium methionine on laying quails diets on performance, quality and blood traits challenged by heat stress are published for the first time.

AUTHORS CONTRIBUTION

AMK & WMD: Experiment idea and design. AMK, WAK & SAA: Executing the experiment and lab analysis. TAE: statistical analysis. AMK & WMD: write the manuscript. SMZ & HSZ: revised the manuscript.

REFERENCES

Allain CC, Poon LS, Chan CS, Richmond WC, Fu PC (1974). Enzymatic determination of total serum cholesterol. Clinical chemistry, 20(4), 470-475. https://doi.org/10.1093/clinchem/20.4.470

Altan O, Pabuccuoglu A, Altan A, Konyalioglu S, Bayraktar H (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. British Poultry Science, 44: 545-550. https://doi.org/10.1080/00071660310001618334

Anderson RA, Roussel AM, Zouari N, Mahjoub SJ, Matheau JM, Abdelhamid, AK (2001). Potential antioxidant effects of zinc and chromium supplementation in people with type

2 diabetes mellitus. J. Am. Coll. Nutr. 20, 212–218. https://doi.org/10.1080/07315724.2001.10719034

Aslanian A, Noori K, Abolfazl AD, Habib AS, Shahnaz R, Naser MS (2011). Evaluate the effect of Cr methionine on performance and serum metabolite in growing-finishing male broiler. J Basic Appl Sci Res 1(11): 2442-2448.

Attia, KM, Tawfeek FA, Mady MS, Assar MH (2015). Effect of dietary chromium, selenium and vitamin c on productive performance and some blood parameters of local strain dokki-4 under Egyptian summer conditions. Egypt. Poult. Sci. J. 35(1). 14:21

Briggs MA, Petersen KS, Kris-Etherton PM (2017). Saturated fatty acids and cardiovascular disease: Replacements for saturated fat to reduce cardiovascular risk. Healthcare. 5: e29. https://doi.org/10.3390/healthcare5020029.

Bun S, Guo Y, Guo F, Ji F, Cao, H (2011). Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. Poult. Sci. 90:1220–1226. https://doi.org/10.3382/ps.2010-01308

Carocho M, Ferreira IC (2013). A review on antioxidants, pro-oxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxi., 51: 5–25. https://doi.org/10.1016/j.fct.2012.09.021

Chand N, Naz S, Khan A, Khan S, Khan RU (2014). Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. Int. J. Biometeorol. 58:2153–2157. https://doi.org/10.1007/s00484-014-0815-7

Coles E (1986). Veterinary clinical pathology, 4th Ed. W.B. Saunders in Philadelphia. Pp.

Dosoky WM, Zeweil HS, Zahran SM, Ebied TA, khalifah AM, Kashyout WA (2020). Effect of Organic Chromium on Performance, Physiological and Anti-oxidative Stress Indicators of Growing Japanese Quail under High Ambient Temperature. J. Sus. Agric. Sci. 46:113-124

Duncan DB (1955). Multiple range and multiple F test. Biometrics. 11: 1-42 https://doi.org/10.2307/3001478

EFSA (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J. 8: 1–107. https://doi.org/10.2903/j.efsa.2010.1461

Fisher H, Leveille GA (1957). Observations on the cholesterol, linoleic and linolenic acid content of eggs as influenced by dietary fats. J. Nutrit. 63(1), 119-129. https://doi.org/10.1093/jn/63.1.119

Funk EM (1948). The relation of the yolk index determined in natural position to the yolk index as determined after separating the yolk from the albumen. Poult. Sci. 27(3): 367. https://doi.org/10.3382/ps.0270367

Gleuktin M, Uyanik F, Guclu BK, Eren M (2009). Effects of Cr and manganese on performance, egg quality and serum lipid levels of Japanese quail exposed to heat stress. Book Proceedings of 2nd Mediterraean Summit of WPSA, Turkey 9; 117-121.

Habibian M, Ghazi S, Moeini MM (2013). Lack of effect of dietary chromium supplementation on growth performance and serum insulin, glucose, and lipoprotein levels in broilers reared under heat stress condition. Biol. Trace Elem. Res. 153:205–211. https://doi.org/10.1007/s12011-013-9663-2

Hassan S, Hassan M, Soliman F, Safwat A (2022). Influence of hot red pepper oil in broiler diets on blood, antioxidant, immunological parameters and intestinal bacteria counts, Anim. Biotech., https://doi.org/10.1080/10495398.2021.2020132

Hayirli A, (2005). Chromium nutrition of livestock species. Nutr. Abs. Rev. Ser. B.

Karami M, Torki M, Mohammadi H (2018). Effects of dietary supplemental chromium methionine, zinc oxide, and ascorbic acid on performance, egg quality traits, and blood parameters of laying hens subjected to heat stress. J. Appl. Anim. Res. 46(1): 1174-1184. https://doi.org/10.1080/09712119.2018.1481411

Khalifah AM, El-Saadany AS, Hassan MI, Kashyout WA, Dosoky WM (2021). Impact of stevioside supplementation as feed additive in finisher broiler diets on growth performance, carcass traits, meat quality, selected biochemical parameters, and caecum microflora. Adv. Anim.Vet. Sci.9(12):2168-2175. http://dx.doi.org/10.17582/journal.aavs/2021/9.12.2168.2175

Khan RU, Naz S, Nikousefat Z, Tufarelli V, Javadani M, Rana N, Laudadio V (2011). Effect of vitamin E in heat-stressed poultry. World's Poult. Sci. J. 67 (3):469–478. https://doi.org/10.1017/S0043933911000511

Ma W, Niu H, Wang Y, Feng J (2011). Effects of zinc glycine chelate on oxidative stress, contents of trace elements, and intestinal morphology in broilers. Biol. Trace Elem. Res. 142:546–556

Ma W, Gu Y, Lu J, Yuan L, Zhao R (2014). Effects of chromium propionate on egg production, egg quality, plasma biochemical parameters, and egg chromium deposition in late-phase laying hens. Biol. Trace Elem. Res. 157:113–119. https://doi.org/10.1007/s12011-013-9875-5

Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO, Patterson PH (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. 83:889–894. https://doi.org/10.1093/ps/83.6.889

Mathivanan R, Selvaraj P (2003). Influence of dietary chromium on egg production and quality parameters in layers. Ind. J. Poult. Sci., 38: 51 – 53.

Mirfendereski E, Jahanian R (2015). Effects of dietary organic chromium and vitamin C supplementation on performance, immune responses, blood metabolites, and stress status of laying hens subjected to high stocking density. Poult. Sci. 94:281–288. p

Mohammed HH, El-Sayed BM, Abd El-Razik WM, Ali MA, Abd El-Aziz, RM (2014). The influence of chromium sources on growth performance, economic efficiency, some maintenance behavior, blood metabolites and carcass traits in broiler chickens. Global Vet., 12: 599-605.

Nasiroleslami M, Torki M (2011). Egg quality characteristics and productive performance of laying hens fed diets supplemented by Echinacea purpurea extract, immunizing and vitamin E. Glob. Veterin. 7 (3): 270- 275.

Nattapon S, Jin-Jenn L, Alex T, Tu-Fa Lien T (2012). Effect of different level of nanoparticles of Cr picolinate supplementation on performance, egg quality, retention and tissue mineral accumulation in layers. J. Agr. Sci. 5(2): 150-159. https://doi.org/10.5539/jas.v5n2p150

Novikoff M, Gutteridge HS (1949). A comparison of certain methods of estimating shell strength. Poult. Sci. 28(3): 339-343. https://doi.org/10.3382/ps.0280339

NRC (1994). Nutrient Requirements of poultry. 9th end. Natio. Acad. Press. Washington. DC USA.

Onderci M, Sahin N, Sahin K, Kilic N (2003). Antioxidant properties of chromium and zinc: in vivo effects on digestibility, lipid peroxidation, antioxidant vitamins, and some minerals under a Low ambient temperature. Biol. Trace Elem. Res. 92:139–150. https://doi.org/10.1385/BTER:92:2:139

Pagan JD, Jackson SG, Duren SE (1995). The effect of chromium supplementation on metabolic response to exercise in thoroughbred horses, in Biotechnology in the Feed Industry: Proceedings of Alltech's Eleventh Annual Symposium, T. P. Lyons and K. A. Jacques, eds. Nottingham University Press, Nottingham, UK, 249–256 (1995).

Panda AK, Cherian G (2014). Role of vitamin E in counteracting oxidative stress in poultry. J. Poult. Sci. 51:109-117. https://doi.org/10.2141/jpsa.0130134

Panda AK, Ramarao SV, Raju MVLN, Chatterjee RN (2008). Effect of dietary supplementation with vitamins E and C on production performance, immune responses and antioxidant status of white leghorn layers under tropical summer conditions. Br. Poult. Sci. 49:592–599. https://doi.org/10.1080/00071660802337233

Piva A, Meola E, Gatta P, Biagi G, Castellani G, Mordenti A, Mordenti A (2003). The effect of dietary supplementation with trivalent chromium on production performance of laying hens and the chromium content in the yolk. Anim. Feed Sci. Tech. 106(1-4): 149-163. https://doi.org/10.1016/S0377-8401(03)00006-3

Preuss HG, Grojec PL, Lieberman S, Anderson RA (1997). Effects of different chromium compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. Clin. Nephrol. 47:325–330.

Preuss RI, Geller J, Evans GW (1990). The effect of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects, West. J. Med. 152: 41–45 (1990).

Radwan SS (1978). Coupling of two-dimensional thin layer chromatography with gas chromatography on the quantitative analysis of lipids classed and their constituent fatty acids. J. Chromatogr. Sci., 16, 538–542. https://doi.org/10.1093/chromsci/16.11.538

Rama Rao SV, Nagalakshmi D, Reddy VR (2011). Feeding to minimize heat stress. Available at: http://www.poulvet.com/poultry/articles/feeding _in summer.php.

Roberts JR (2004). Factors affecting egg internal quality and egg shell quality in laying hens. J. Poult. Sci. 41(3):161-177. https://doi.org/10.2141/jpsa.41.161

Romanoff A L, Romanoff A J (1949). The Avian Egg. New York: Wiley.

Sahin K, Onderci M, Sahin N, Gursu MF, Vijaya J, Kucuk O (2004). Effects of dietary combination of chromium and biotin on egg production, serum metabolites, and egg yolk mineral and cholesterol concentrations in heat-distressed laying quails. Biol. Trace. Elem. Res. 101:181–192. https://doi.org/10.1385/BTER:101:2:181

Sahin K, Ozbey O, Onderci M, Cikim G, Aysondu MH (2002a). Chromium supplementation can alleviate negative effects of heat stress on egg production, egg quality and some serum metabolites of laying Japanese quail. J. Nutr. 132:1265–1268. https://doi.org/10.1093/jn/132.6.1265

Sahin K, Sahin N, Kucuk O (2002b). Effects of dietary chromium and ascorbic acid supplementation on digestion of nutrients, serum antioxidant status, and mineral concentrations in laying hens reared at a low ambient temperature. Biol. Trace Elem. Res. 87:113–124. https://doi.org/10.1385/BTER:87:1-3:113

Sahin, N, Sahin K, Onderci M, Gursu MF, Cikim G, Vijaya J, Kucuk O (2005). Chromium picolinate, rather than biotin, alleviates performance and metabolic parameters in heat-stressed quail. Br. Poult. Sci., 46, 457-463. https://doi.org/10.1080/00071660500190918

Samanta S, Haldar S, Bahadur V, Ghosh TK, (2008). Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. J. Sci. Food Agr. 88:787–796. https://doi.org/10.1002/jsfa.3146

Shane SM (1988). Factors influencing health and performance of poultry in hot climates. Crit. Rev. Poult. Biol., 1:247–267.

Sirirat N, Lu JJ, Hung ATY, Lien TF (2013). Effect of different levels of nanoparticles chromium picolinate supplementation on performance, egg quality, mineral retention, and tissues minerals accumulation in layer chickens. J. Agr. Sci. 5:150–159. https://doi.org/10.5539/jas.v5n2p150

Torki M, Zangeneh S, Habibian M (2014). Performance, egg quality traits, and serum metabolite concentrations of laying hens affected by dietary supplemental chromium picolinate and vitamin C under a heat-stress condition. Biol. Trace Elem Res. 157:120–129. https://doi.org/10.1007/s12011-013-9872-8

Tsahar E, Arad Z, Izhaki I, Guglielmo C (2006). The relationship between uric acid and its oxidative product allantoin: a potential indicator for the evaluation of oxidative stress in birds. J. Comp. Physiol. 176:653–661. siol. 176:653-661. https://doi.org/10.1007/s00360-006-0088-5

Utnik-Banaś K, Żmija J, Sowula-Skrzyńska E (2014). Economic aspects of reducing stocking density in broiler chicken production using the example of farms in southern Poland. Ann. An. Sci., 14: 663-671. https://doi.org/10.2478/aoas-2014-0029

Vincent JB (2000). The biochemistry of chromium. J. Nutri. 130(4): 715-718. https://doi.org/10.1021/bi000503h

Voisey PW, Hunt JR (1974). Measurement of eggshell strength. J. Texture Stud. 5(2): 135-182. https://doi.org/10.1111/j.1745-4603.1974.tb01099.x

Vuilleumier JP (1969). The 'Roche Yolk Colour Fan '—An Instrument for Measuring Yolk Colour. Poult. Sci. 48(3): 767-779. https://doi.org/10.3382/ps.0480767

Willcox J, Ash S, Catignani G (2004). Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr. 44:275–295. https://doi.org/10.1080/10408690490468489

Yalcin S, Ozkan S, Turkmut L, Siegel, PB, (2001). Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. Br. Poult. Sci. 42:149–152. https://doi.org/10.1080/00071660120048375