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Influence of Feeding Natural and Synthetic Lycopene on Performance, Egg Quality and Serum Parameters of Japanese Quail Layers

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ABSTRACT

Background: This study was conducted to investigate the effects of natural and synthetic sources of lycopene on performance, egg quality and some blood characteristics of Japanese quail layers.

Methods: The 252 eight-week-old quail hens were randomly divided into seven treatments with three replication. The first treatment was fed basal diet (control). Second and third treatments were given diets containing tomato fruit powder (TOM) at a level of 17 and 34 g/kg equal to 50 and 100 mg/kg lycopene, fourth and fifth treatments were given red bell pepper fruit powder (RBP) at level 16.23, 32.46 g/kg equal to 50 and 100 mg/kg lycopene, while synthetic lycopene powder at a rate of 50, 100 (mg/kg) was supplemented to sixth and seventh respectively.

Result: The final body (T4-T6), hen day egg production (except for T3 and T6), egg weight, egg mass (T4, T5), feed intake, FCR were improved as compared to control. Significant effect on the weight of eggshell, yolk, albumen and albumen (height and diameter). Yolk (height, diameter, index) improved as compared to control. Significant ($P \leq 0.05$) increase in total protein and HDL, while cholesterol (except T2 and T7), triglycerides (except T2) and LDL were decreased significantly ($P \leq 0.05$). It could be concluded that supplementary TOM, RBP and LY, have beneficial effects on laying performance, egg quality traits and some biochemical indices of quails.

Key words: Egg production, Egg quality, Laying quail, Lycopene, Red bell pepper, Tomato.

INTRODUCTION

Currently, people have attended to maintaining good health and a great body form; so, they are becoming more interested in the food they choose to eat, searching for food with high nutritional value, bioactive compounds and antioxidant ability, such as fruits and vegetables (Chavez-Mendoza, 2015).

Fruits and vegetables displayed high antioxidant activities, therefore, these foods may consider good sources of bioactive compounds, their consumption as sources of nutraceutics could be recommended particularly in groups of people where the incidence of oxidative stress-induced diseases is quite important (Bayili *et al*., 2011).

Several reports have demonstrated that some chronic conditions, such as obesity, diabetes, cancer, cardiovascular, as well as neurodegenerative, are positively affected when a diet rich in fruits and vegetables (Leite *et al*., 2011).

The interest in tomatoes (*Lycopersicon esculentum*) is raising as an agricultural product, which is an important commodity all over the world and constitutes a large part of the human diet. Tomatoes and their products are rich in antioxidant compounds such as carotenoids (especially lycopene), ascorbic acid and phenolic compounds. Regular consumption of tomatoes and their products is associated with a reduced risk of different types of cancer and cardiovascular diseases. This positive effect is due to the antioxidants present in tomatoes (Borguini and Torres, 2009).

Red bell pepper (*Capsicum annuum* L.) is an important crop usually consumed as food or spices. Pepper contains a wide range of phytochemicals, such as capsaicinoids, phenolics, ascorbic acid and carotenoids (Hamed *et al*., 2019). Recently, natural plant supplements can be used with poultry diets to strengthen the mechanisms of antioxidant protection and reduce the intensity of oxidative processes, which adversely affect the quality of poultry products (Ognik *et al*., 2016).

Lycopene is found in red fruits and vegetables. It is a bright red carotenoid pigment and tomatoes are the main source of it in the human diet. Lycopene has been shown to be the most effective antioxidant among the various common carotenoids (Sun *et al*., 2014a). Tomatoes and their products are key sources of Lycopene (Arain *et al*., 2018). Selim *et al*. (2013) reported that dietary inclusion of lycopene-enriched

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tomato by-products at a level of 1% in broilers feed under heat stress enhanced total antioxidant capacity and lowered malondialdehyde level.

The supplementation of tomato powder, red bell pepper and lycopene at 34, 32.46 (g/kg) and 50 (mg/kg) respectively, improves lipid profile, GSH-PX, SOD, reduced liver enzymes and malondialdehyde in grower quail (AL-Jrrah and Abbas, 2020). According to An *et al*. (2019), the addition of lycopene (equal to or less than 20 mg/kg diet) or tomato paste into the layers' diet is an effective nutritional strategy to enhance the oxidative stability of fresh eggs.

From this given background, the current study aimed to investigate the effect of dietary supplementation of tomato, red bell pepper powder, pure lycopene as natural and synthetic sources of lycopene on the productive performance, egg quality and serum biochemical indices of quail layers.

MATERIALS AND METHODS

Experimental design and feeding program

This experiment was carried out between May and July 2018 in the Quail Research Unit, Animal Production Department, Agriculture College, Basrah University. A total of 252 Japanese quail laying hens (*Coturnix coturnix japonica*) of eight weeks of age were weighed. The initial body weight was comparable at the beginning of the experiment. The birds were randomly assigned to seven treatments, each of three replicates with 12 birds. All diets were formulated to meet the nutrient requirements of the quails. Basic quail's diet was provided *ad libitum* containing 19.92% crude protein and 2894 ME (kcal/kg) diet. The calculated analysis and ingredients of the laying diet were demonstrated in Table 1. The following seven dietary treatments were prepared by adding different levels of tomato fruit powder (TOM) and red bell pepper fruit powder (RBP) to the basal diet (BD) as a source of natural lycopene, the content of which was previously estimated from lycopene (Table 2).

Experimental diets were designed as follows: First treatment was fed BD or control. Diet supplemented with 17 and 34 g/kg TOM equal to 50 and 100 mg/kg lycopene served as second and third treatments respectively. Fourth and fifth treatments were fed diet supplemented with RBP (16.23, 32.46 g/kg equal to 50 and 100 mg/kg lycopene. The sixth and seventh treatments were fed diet supplemented with 50 and 100 mg/kg pure lycopene (LY), respectively. Hens were housed in cages (4575×70×cm) in an experimental house on a 17-hour lighting program until 16 weeks of age.

Determination of performance parameters

The average live body weights (BW) of quails were recorded at the beginning and at the end of the experiment and body weight change (BWC) was calculated from these averages. The number of eggs laid was recorded daily and egg production (EP) was calculated as %. Feed consumption, egg mass (EM) and egg weights (EW) were recorded at two weekly intervals. Feed conversion ratio (FCR) was calculated by determining the amount of feed consumed per one kg of the egg.

Evaluation of egg quality

The egg's internal qualities were measured by gently breaking the eggs using a scalpel on a table with a glass cover in order to measure the contents, which were taken on a flat surface. The yolk was carefully separated from the albumen for weighing. The albumen weight was calculated by subtracting the weight of yolk and shell from the weight of the whole egg. The albumen and yolk height (mm) were measured using electronic caliper. Yolk index was determined according to the following formulae:

$$\text{Yolk index} = \frac{\text{Yolk height (mm)}}{\text{Yolk diameter (mm)}} \times 100$$

(Romanoff and Romanoff, 1949).

Table 1: The ingredient and chemical composition of basal diet.

Ingredient and composition	%
Maize	30.0
Wheat	35.0
Soybean meal (44%)	23.0
Protein concentration (44%)	5.0
Oil plant	1.50
Limestone	4.60
Dicalcium phosphate	0.50
Premix	0.20
Salt	0.20
Total	100
ME (kcal/kg)	2894
Crude protein %	19.92
Ether extract %	2.45
Crude fiber %	3.67
Calcium %	2.30
Phosphorus available %	0.34
Methionine %	0.37
Methionine + Cystine %	0.71

Table 2: Proximate analysis and lycopene content of tomato and red bell pepper powder (% on a dry weight basis).

Component (%)	Tomato	Red bell pepper
Dry matter	85.08	91.52
Organic matter	91.64	93.28
Total ash	8.36	6.72
Crude protein	14.53	13.13
Ether extract	3.71	11.47
Crude fibre	10.60	23.90
Nitrogen free extract	62.80	44.78
Metabolized energy (kcal/kg)*	2793.86	2729.04
Lycopene (mg/100 g)	293.58	307.99

* ME was calculated according to Lodhi *et al.* (1976).

Determination of serum biochemical parameters

Three quails per treatment were randomly selected at the end of the experiment (16th week), to determine blood parameters. Blood samples were collected in 20-ml tubes without heparin for biochemical assays and centrifuged (3000 rpm, 15 min, 25°C) to obtain plasma. Serum samples were stored at -20°C until analyzed, for total protein by a colorimetric method using a commercial kit. Serum cholesterol and triglycerides were determined according to Tietz (1999), using commercial kits (Spinreact, Spain), HDL-c, according to Warnick and Wood, (1995), LDL-c was determined by the difference between total cholesterol and HDL-c with triglyceride divided by five as the equations described by Friedewald *et al*. (1972) and Wilson (1998), Calcium and Phosphorous were measured using commercial kits (Randox). Egg yolk cholesterol were estimated using a kit manufactured by (Valtek, Chile), according to Berrio and Hebert, (1990).

Statistical analysis

Data were analyzed by one-way ANOVA procedure using the program of the Statistical Package for Social Sciences (SPSS) software (2012). The Least Significant Difference (L.S.D.) test was used to assess differences between treatments and probability values ≤0.05 were taken to indicate statistical significance.

RESULTS AND DISCUSSION

Production performance

The initial body weight (IBW), final body weight (FBW), BWC, EP, EW, EM, FI and FCR are presented in Table 3. In the present study, there were significant differences in FBW, BWC, EP, EW, EM and FCR due to dietary TOM, RBP and lycopene (LY) (P≤0.05). Significant improvements in FBW in (T4, T5, T6), EP (except in T3, T6), EW, EM (except in T6) and FCR, while there was a significant (P≤0.05) reduction in the feed intake in supplementary groups compared with the control.

The improvement in FBW and productive performance for supplementary treatments may be due to presences of lycopene in additive materials and its role as a powerful antioxidant that able to reduce the harmful effects of free radicals and prevent oxidation of fat, proteins and nucleic acids and that the improvement in productive traits may be related to its antioxidant properties (Wang, 2012), as well, lycopene, is known to have strong antioxidant effects (An *et al*. 2019). The reasons for the increase in egg production of quails when fed tomato paste (17g/kg) and lycopene (20 mg/kg) attributed to the antioxidant property of lycopene, which was enhanced by a decrease in MDA concentrations and an increase in the level of lycopene in the blood serum. These findings were in line with Sahin *et al*. (2006), who reported that dietary lycopene at 100 ppm increased egg production in Japanese quails. Similar findings have been also found by An *et al*. (2019), when laying hens achieved higher egg production as their diets containing tomato paste. Whereas, in a previous finding by Sun *et al*. (2014a), dietary lycopene (range of 20 to 80 mg/kg) did not affect laying performance in Chinese native breeding hens. In contrast to our findings, both egg weight and egg production were significantly lower in the lycopene (20 mg/kg) of the diet group compared to control (An *et al*. 2019).

Egg quality traits

The egg geometric traits of experimental birds are presented in Table 4. In the present experiment, all parameters of egg *i.e.*, eggshell weight , yolk weight, yolk height , yolk diameter, yolk index, albumin weight, albumin height, albumen diameter, were recorded significant differences among treatments. With regard to the eggshell weight, the results showed that T4 and T5 treatments were superior compared to the other treatments. Also, in yolk weight, T3, T4 and T5 are superior to the rest. All supplementary treatments are superior to control in yolk height (except T4 and T5), diameter and yolk index. Treatments six and seven are superior to all treatments (except T2) in albumin weight. In albumin height, T2 treatment decreased significantly compared to the control. In albumen diameter, the T2 treatment was superior

Table 3: Effect of dietary TOM, RBP and lycopene on the performance of laying quail at 8-16 wk. of age.

Parameters	Dietary groups							SEM	*P*<
	T1	T2	T3	T4	T5	T6	T7		
Initial live weight (g)	187.33	197.67	194.67	204.33	205.00	198.00	194.33	2.445	0.550
Final body weight (g)	196.63^{b}	205.76^{ab}	202.31^{ab}	209.63^{a}	210.14^{a}	208.60^{a}	205.36^{ab}	2.315	0.05
Body weight change (g)	9.30^{ab}	8.09^{ab}	7.57^{ab}	5.30^{b}	5. 95^{b}	10.60^{a}	11.02^{a}	0.628	0.05
Hen day egg production (%)	82.53^{c}	87.20^{ab}	83.8^{bc}	89.80^{a}	89.34^{a}	81.61^{c}	90.54^{a}	0.878	<0.001
Egg weight (g)	11.60^{b}	12.31^{a}	12.53^{a}	12.65^{a}	12.74^{a}	12.53^{a}	12.28^{a}	0.094	0.005
Egg mass (g/bird/d)	9.66^{d}	10.69^{bc}	10.52^{bc}	11.35^{a}	11.39^{a}	10.23^{cd}	11.13^{ab}	0.145	<0.001
Feed intake (g/bird/d)	27.94^{a}	26.94^{cd}	24.65^{f}	25.78^{e}	26.55^{d}	27.17^{bc}	27.46^{b}	0.235	<0.001
Feed efficiency (g feed/g egg)	2.89^{a}	2.52^{bc}	2.35^{de}	2.27^{e}	2.33^{de}	2.65^{de}	2.47^{cd}	0.047	<0.001

Means in the same row with no common superscript are different significantly (P≤0.05). T1 (control), T2 and T3 (Tomato powder at the levels of 17 and 34 g/kg in basal diet), T4 and T5 (Red bell pepper at the levels of 16.23 and 32.46 g/kg in basal diet) which was equivalent to 50 and 100 mg/kg lycopene respectively, T6 and T7 (lycopene powder at the levels of 50 and 100 mg/kg in basal diet) respectively.

to all the treatments, with an insignificant difference from the control. The results of the study reported here are inconsistent with the findings of Omri *et al*. (2017), who confirmed that dietary supplementation of ground linseeds (4.5%), dried tomato (1%) and sweet pepper powder (1%) mix, had no beneficial effect on shell weight, albumen weight and egg yolk weight of Novogen white laying hens.

Serum metabolites

The results for serum metabolite contents of quail hens are summarized in Table 5. The addition of TOM, RBP and LY to the diets at 16 weeks of age had a significant (P≤0.05) effect on serum total protein, cholesterol, triglycerides, HDL-c, LDL-c, calcium and egg yolk cholesterol levels. Serum total protein was significantly (P≤0.05) increased in dietary groups (except in T7) in comparison with the control. Serum lipid profile showed significant reductions (p≤0.05) in total cholesterol (TC) (at T3-T6 groups), triglycerides (TG) (at T3-T7 groups), LDL-c levels in all dietary groups in comparison with the control as a result of inclusion TOM, RBP and LY to quails diet. However, HDL-c and calcium (except T3) were increased (p≤0.05) in supplemented treatments compared to control. There was no significant difference with regard to VLDL-c and phosphorus among treatments (Table 5). Sun *et al.* (2014b) stated that dietary lycopene in the range of 20 to 80 (mg/kg) diets lowered TC, but not a triglyceride in breeding hens. While, they found an age-dependent effect of lycopene on cholesterol, when dietary lycopene lowered serum TC and HDL at 35 days, but not at 21 or 28 days in breeders (Sun *et al*., 2014a). Current results support work done by other authors that stated significantly lower total cholesterol and LDL-c concentration (Sahin *et al*., 2006; Palozza *et al.,* 2012; Mulkalwar *et al*., 2012; Reda *et al*., 2022), in the birds and rabbits that received tomato and tomato derivatives and lycopene in diets. With respect to increasing serum HDL-c, Sun *et al*. (2015) confirmed a positive effect of *in ovo* inclusion of lycopene in hatching eggs, that increased serum HDL-c and regulating lipid metabolism in birds. As reported in our previous research study (Abbas and AL-Jrrah, 2020), dietary TOM or RBP and LY (50, 100 mg/kg), in quail diets, were decreased significantly TC, TG, LDL-c and VLDL-c levels, with improvment in HDL-c in all supplementary treatments at 49 days of age. Furthermore,

Table 4: Effect of dietary TOM, RBP and LY on egg quality traits at 16 weeks of age.

Parameters	Dietary groups							SEM	*P*<
	T1	T2	T3	T4	T5	T6	T7		
Shell weight (g)	0.97^{bc}	1.01^{bc}	1.09^{ab}	1.26^{a}	1.19^{a}	0.97^{bc}	0.93^{c}	0.035	0.05
Yolk weight (g)	5.18^{d}	5.74^{bc}	6.15^{ab}	6.05^{ab}	6.16^{a}	5.65^{c}	6.09^{ab}	0.090	<0.001
Yolk height (mm)	10.67^{c}	12.07^{b}	12.14^{b}	12.25^{ab}	12.65^{a}	12.16^{ab}	11.91^{b}	0.137	<0.001
Yolk diameter (mm)	26.78^{d}	27.88^{bc}	28.02^{b}	27.66^{bc}	28.62^{a}	27.37^{c}	27.59^{bc}	0.132	<0.001
Yolk index (%)	39.84^{b}	43.29^{a}	43.32^{a}	44.28^{a}	44.12^{a}	44.43^{a}	43.17^{a}	0.399	0.001
Albumen weight (g)	5.21^{bc}	5.61^{ab}	5.05^{c}	5.16^{bc}	5.26^{bc}	5.85^{a}	5.85^{a}	0.089	0.021
Albumen height (mm)	5.75^{a}	4.40^{b}	5.65^{ab}	5.17^{ab}	4.66^{ab}	4.80^{ab}	5.55^{ab}	0.177	0.05
Albumen diameter (mm)	25.79^{ab}	26.54^{a}	24.83^{bc}	23.98^{c}	24.62^{c}	24.16^{c}	24.11^{c}	0.231	0.002

Means in the same row with no common superscript are different significantly (P≤0.05). T1 (control), T2 and T3 (Tomato powder at the levels of 17 and 34 g/kg in basal diet), T4 and T5 (Red bell pepper at the levels of 16.23 and 32.46 g/kg in basal diet) which was equivalent to 50 and 100 mg/kg lycopene respectively, T6 and T7 (lycopene powder at the levels of 50 and 100 mg/kg in basal diet) respectively.

Table 5: Serum biochemical indices and cholesterol egg yolk at 16 wk. of age of quail fed TOM, RBP and LY.

Parameters (mg/dl)	Dietary groups							SEM	*P*<
	T1	T2	T3	T4	T5	T6	T7		
Total protein (g/dl)	3.26^{c}	4.97^{a}	4.72^{ab}	5.12^{a}	3.88^{ab}	4.12^{ab}	3.49^{bc}	0.213	0.05
Cholesterol	155.03^{a}	145.74^{ab}	127.10^{bc}	127.36^{bc}	123.18^{c}	134.61^{b}	147.22^{a}	2.750	<0.001
Triglycerides	163.82^{a}	152.97^{ab}	116.44^{c}	126.17^{bc}	128.44^{bc}	130.57^{bc}	125.16^{bc}	4.875	0.05
HDL-c	48.06^{b}	64.80^{a}	70.68^{a}	75.44^{a}	65.19^{a}	68.32^{a}	66.09^{a}	2.193	0.011
LDL-c	82.89^{a}	59.22^{b}	31.13^{d}	26.33^{d}	29.62^{d}	39.46^{cd}	56.99^{b}	4.729	<0.001
VLDL-c	23.92	23.28	25.26	25.68	27.89	25.45	23.75	0.638	0.577
Calcium	2.98^{d}	6.51^{a}	4.14^{c}	6.17^{ab}	5.87^{ab}	5.20^{abc}	4.82^{bc}	0.306	0.004
Phosphorus	2.15	2.27	2.08	2.84	3.49	2.69	3.28	0.228	0.591
Cholesterol egg yolk (mg/g)	11.76^{a}	11.04^{ab}	10.53^{ab}	10.66^{ab}	10.17^{b}	10.85^{ab}	10.16^{b}	0.193	0.05

Means in the same row with no common superscript are different significantly (P≤0.05). T1 (control), T2 and T3 (Tomato powder at the levels of 17 and 34 g/kg in basal diet), T4 and T5 (Red bell pepper at the levels of 16.23 and 32.46 g/kg in basal diet) which was equivalent to 50 and 100 mg/kg lycopene respectively, T6 and T7 (lycopene powder at the levels of 50 and 100 mg/kg in basal diet) respectively. HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol.

Reda *et al*. (2022) also obtained similar results when tomato pomace fed to Japanese quail breeders. The lowering of TC levels when the quails are supplemented with TOM, RBP powder and pure LY may be related to a decrease in cholesterol synthesis through the inhibition of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase activity and expression, modulation of LDL receptor and inhibition of the activity of acyl-coenzyme A: Cholesterol acyltransferase (ACAT) (Palozza *et al.,* 2012). Moreover, various studies pointed that red pepper could decrease TC, triglyceride, LDL and increase HDL levels. The hypolipidemic effect of red pepper may be related to several factors including activation of peroxisome proliferators-activated receptor α (PPAR α) (Mueller *et al*., 2011). Concerning egg yolk cholesterol, our results showed that T5 (RBP 32.46 g/kg) and T7 (LY 100 mg/kg) achieved the lowest level of cholesterol compared to the control at 16 weeks age. Similarly, Sahin *et al*. (2006) found that supplementation of the diet with lycopene (100 mg/kg) in Japanese quail significantly decreased serum and yolk cholesterol concentrations and improved antioxidant status. Also, a significant ($P \leq 0.001$) decrease in serum and egg yolk cholesterol concentrations was observed in laying hens (Lohman LSL, hybrid), fed purified lycopene (20 mg/kg), or an equal amount of lycopene-containing tomato powder for 12 weeks (Orhan *et al*., 2021). Conversely, ground linseeds (4.5%), dried tomato (1%) and sweet pepper powder (1%) mix dietary supplementation, had no effect on cholesterol and triglycerides in fresh egg yolk of Novogen white laying hens (Omri *et al*., 2017).

CONCLUSION

It could be concluded that the inclusion of fruit powders of tomato (34 g/kg) and red bell pepper (16.23 g/kg) and pure lycopene (50 to 100 mg/kg), led to an improvement in FCR, egg production (HD%) and egg weight. Also, this addition improves serum total protein, HDL-c and reduces cholesterol concentration, triglycerides and LDL-c of Japanese quail Layers.

Conflict of interest: None.

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