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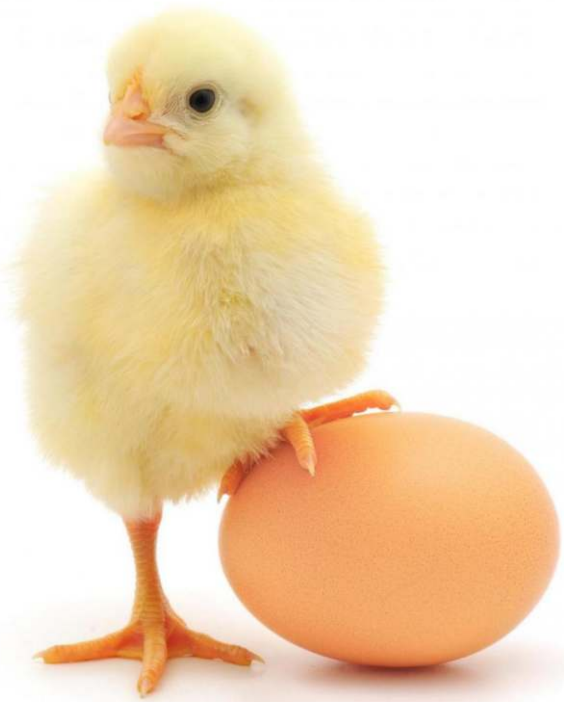

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Research Article
Egg Yolk Fatty Acids, Blood Parameters and Some Reproductive Measurements of Japanese Quail Supplemented with Chia Seeds (*Salvia hispanica* L.)

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Abstract

Background and Objective: This study was conducted to investigate the effect of supplementing Japanese quail hens with plant sources of omega-3 fatty acids on blood and reproductive traits and egg fatty acid profiles. **Materials and Methods:** A total of 240 female Japanese quail, 48 days old, were randomly distributed into 4 treatments with 3 replicates per treatment, as follows: T1 was the control and was without any addition and T2, T3 and T4 were supplemented with 4, 8 and 12 g chia seeds/kg diet, respectively. **Results:** The statistical analysis showed that T3 significantly reduced the blood uric acid, glucose, LDL, triglycerides and VLDL, while the PCV, Hb, total protein, albumin (A), globulin (G), A/G ratio, cholesterol and calcium were not affected significantly by the supplementation treatments. The egg formation hormones estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) increased significantly ($p<0.05$) in T3 compared with those in the control and other treatments, while the relative weights of ovaries and oviducts and the lengths of the oviducts did not significantly affect the egg formation hormones. The egg yolk fatty acid profiles showed significant increases in oleic, linolenic, palmitic, linoleic and stearic acids in all supplemented groups compared with those in the control. Linolenic acid increased significantly ($p<0.05$) in T3 and T4 compared with that in T1. **Conclusion:** It is concluded that the addition of chia seeds to quail diets during the laying period has a positive effect on the blood lipid profile, egg formation hormones and egg yolk fatty acid profile, especially for the more useful omega-3 fatty acids.

Key words: Blood parameters, chia seeds, egg yolk fatty acids, Japanese quail, reproductive system, sex hormones

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The applied research conducted in various areas of the poultry industry focuses on how to develop the industry in order to obtain high quality production[1] Studies have indicated that unsaturated fatty acids (PUFA) have greatly increase the economic return of the poultry industry. PUFA include monounsaturated fatty acids and polyunsaturated fatty acids and the latter include omega-3 (n-3) and omega-6 (n-6)[2] fatty acids.

Feeding on sources rich in omega-3 type unsaturated fatty acids has an effect on various physiological activities because it improves health and reduces the risk of chronic diseases and the level of blood triglycerides and cholesterol[3,4]. These fatty acids are involved in the composition of cell membranes and the construction of cell membrane phospholipids and they work with protein and cholesterol to repair the membranes of hazardous cells and build new cells[5]. Many studies were conducted to investigate the effect of adding different sources of omega-3 or omega-6 fatty acids to animal diets on the animals' productive and physiological traits. Supplying diets with plant or animal oils containing omega-3 and omega-6 fatty acids will control the level of blood lipids and the quality of the fatty acids that make up adipose tissue in domestic animals[6]. Common carp fingerling diets supplemented with sunflower oil resulted in a significant decrease in blood triglycerides, cholesterol and low-density lipoproteins, while high-density lipoproteins were increased[7]. Similar results were found by Ezzat[8] when he fed laying hens with omega-3 fatty acids from animal or plant oil sources and reduced the levels of egg cholesterol. Egg production, egg weight, the feed conversion ratio and the qualitative characteristics were significantly improved when laying hens were fed diets supplemented with omega-3 unsaturated fatty acids[9,10] as the fatty acids reduced the concentration of malondialdehyde in the broilers when added with antioxidants, leading to a prolonged storage period[11].

Flax seeds, sun flower seeds, evening primrose, soybean seeds and chia seeds are commercially considered as good sources of oils for dietary use[12].

Chia seeds (*Salvia hispanica* L.) belong to the Lamiaceae family and are considered a good source of plant oils and could be a substitute for other oil crops[13] because of their high omega-3 fatty acid content. The percentage of oil in the seeds is 40% of the seed weight and 60% of the oil is made up of omega-3 fatty acids in the form of α-linolenic acid (ALA or LNA) while 20% of the oil is made up of omega-6 fatty acids in the form of linoleic acid. Seventy-five percent of the weight of chia seeds is in α-linolenic acid, which is the common fatty acid among the omega-3 fatty acids and can be obtained from plant oils[14].

According to Marineli *et al.*[15], α-linolenic acid is the most abundant fatty acid in chia seeds (62.80 g/100 g), followed by linoleic acid (18.23 g g^{-1}), palmitic acid (7.07 g/100 g), oleic acid (7.04 g/100 g) and stearic acid (3.36 g/100 g).

Chia seeds contain many active ingredients, including myricetin, quercetin, kaempferol and caffeic acid, which are compounds of the phenolic acids and flavonols that have anticancer, antibacterial, anti-inflammatory and antioxidant activities[16]. Additionally, it was found that the oil from chia seeds is rich in calcium, iron, phosphorus[17] and many vitamins, especially niacin, ascorbic acid, thiamine, riboflavin and vitamins A and E and it is free from mycotoxins[18] and gluten[19]. The inclusion of chia seeds in laying hen diets should increase the omega-3 and 6 fatty acids in meat and egg yolks[20] and could reduce the level of triglycerides and blood cholesterol. Therefore, chia seeds can be considered a source of natural medicines and can be used as healthy dietary supplements.

Eggs are a highly consumed animal product. Omega-3 polyunsaturated fatty acids are low in eggs. Research has tended to enrich the PUFA in food compounds to provide health benefits to humans and reach the daily recommended intake of PUFA. Since many people prefer to consume quail eggs for their high nutritional value, this study was conducted to investigate whether adding chia seeds to quail diets could enrich the quail eggs with omega-3 and 6 fatty acids, in addition to other essential fatty acids. Furthermore, the effect of chia seeds on blood parameters, including sex hormones, in addition to some measurements of the female reproductive system was investigated.

MATERIALS AND METHODS

The experiment was conducted from 31/5/2018 to 2/8/2018 at the Poultry Research Station, Abo-Gharaib, which belongs to the Ministry of Agriculture. A total of 240 quail females, 48 days old, were randomly distributed to four treatments with 3 replicates per treatment (20 quail/replicate). The birds were floor bred on shaved wood. Water and the diets were provided *ad libitum.* The house temperature was 22°C and the lighting program was set at 16 h of light followed by 8 h of darkness. The ingredients and chemical composition of the quail diet are presented in Table 1.

Chia seeds were added to the diet at the beginning of the experiment until the end. The experimental treatments were as follows: T1 was the control and was without any additions and T2, T3 and T4 were supplemented with 4, 8 and 12 g chia seeds/kg diet, respectively.

Table 1: Ingredients and chemical calculations of the basal diet

Ingredient	Percentage
Yellow corn	53.00
Wheat	9.00
Soybean meal (48%)	26.00
Protein concentrate	5.00
Vegetable oil	1.20
Limestone	5.50
Dicalcium phosphate	0.30
Total	100.00
Calculated chemical analysis*	
Crude protein	20.20
ME (kcal^{-1})	2905.00
Fat	3.90
Fiber	2.60
Calcium	2.50
Available phosphorus	0.72
Methionine+Cysteine	0.76
Lysine	1.13

*According to the NRC[21]

Blood was collected at 6 and 8 weeks of age to measure packed cell volume (PCV), hemoglobin (Hb), total protein, albumin, globulin, the albumin to globulin ratio, uric acid, cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoprotein (VLDL), calcium, glucose, estrogen, luteinizing hormone (LH) and follicle stimulating hormone (FSH).

At the end of the experiment, the birds were slaughtered and the ovaries and oviducts were sampled in order to measure their relative weight and the length of the oviduct.

The egg yolk oil was extracted according to Folch *et al.*[22] and the percentages of certain yolk fatty acids (oleic, linolenic, linoleic, palmitic and stearic) were estimated using a gas chromatography system (GC) according to Dieffenbacher and Pocklington[23]. The method involved taking 0.5 mL extracted oil and adding 0.5 mL methyl potassium hydroxide and 5 mL hexane solvent and shaking well for one minute to create 2 layers. One microliter was taken from the upper layer and then injected into the GC system. The concentrations of the oil fatty acids were calculated according to the following equation:

$$\text{Fatty acid (\%)} = \frac{\text{Area of fatty acid} \times \text{standard concentration}}{\text{Standard area}} \times \text{Dilution factor}$$

Statistical analysis: A completely randomized design (CRD) was used to study the effect of the treatments on the different p parameters. The less significant differences between the treatments were estimated using Duncan multiple range test[24]. The statistical analysis was performed using SAS/STAT Institute user's guide for personal computer[25].

RESULTS AND DISCUSSION

Blood measurements: The statistical analysis of the blood measurements at 6 weeks of age is presented in Table 2. From the values, it appears that adding chia seeds to the quail diet had no significant effect on the levels of total protein, albumin, globulin and the albumin/globulin ratio, while uric acid decreased in the supplemented groups and this decrease was significant ($p<0.05$) in T2 and T3 compared to the control.

As for blood lipids, it was noted that the LDL and total cholesterol levels were not significantly affected but mathematically, it was observed that all supplemented groups had low levels compared to that of the control. This reduction in cholesterol could be due to the presence of the omega-3 unsaturated fatty acids in the chia seeds, which could inhibit the activity of the enzymes responsible for cholesterol syntheses in the liver. These enzymes include 7 alpha-hydroxylase and beta-hydroxy methylglutaryl-CoA. The first enzyme works to convert cholesterol to bile acids, while the second one is limited to manufacturing cholesterol by the process of turning mevalonate to squalene[26]. The other reason for the blood cholesterol reduction may be the efficacy of the unsaturated fatty acids in inhibiting the manufacture of cholesterol by reducing its re-absorption from the small intestine[27,28]. Polyphenol compounds depress the reverse-cholesterol transport, resulting in a lower cholesterol absorption in the intestine and an increased secretion of bile acids[29]. This result is consistent with Al-Daraji *et al.*[30] and Al- Fadhlee[31] who noticed a decrease in the blood cholesterol levels of laying hens or quail fed diets containing different sources rich in unsaturated fatty acids (omega-3).

Concerning triglycerides and VLDL, it was found that T3 resulted in a significant ($p<0.05$) increase compared to T4 and the control. However, T3 led to a significant ($p<0.05$) decrease in LDL compared to the control. These results may be because T3 reduced the cholesterol in the quail blood (Table 2) and since the LDL molecule is the main carrier of cholesterol from its manufacturing position in the liver to the body tissues, the decrease in LDL led to a decrease in cholesterol. VLDL (which was high in the T3 treatment) has the apo protein apo-11 on its surface, making it weakly attracted to lipoprotein lipase (LPL); therefore, VLDL will turn from the liver towards the ovary where it will be combined with the protein receptors located on the germ cell membranes of egg oocytes[32].

At 8 weeks of age (Table 3), the results of the blood parameter testing showed that the PCV, Hb, total protein, albumin, globulin, the albumin/globulin ratio, uric acid and calcium were not significantly affected by the experimental treatments, while the blood sugar was decreased significantly $p<0.05$) compared with that in the control. The results of the

Table 2: Effect of supplementing chia seeds on some blood parameters in quail at 6 weeks of age

	Treatments				
Items	T1	T2	T3	T4	p-value
Total protein (g dL^{-1})	6.910±0.49	7.22±0.30	7.24±0.28	6.99±0.66	NS
Albumin (g dL^{-1})	3.340±0.30	3.36±0.30	3.32±0.28	3.23±0.14	NS
Globulin (g dL^{-1})	3.580±0.60	3.86±0.10	3.92±3.17	3.77±0.67	NS
Albumin/globulin	1.020±5.33	0.87±11.15	0.87±3.17	0.91±13.31	NS
Uric acid (mg dL^{-1})	5.830±0.51^{a}	4.53±0.33^{b}	4.57±0.20^{b}	5.60±0.26^{ab}	0.05
Cholesterol (mg dL^{-1})	167.330±2.88	161.00±6.24	137.67±0.69	160.00±5.77	NS
Triglycerides (mg dL^{-1})	147.000±1.75^{b}	155.00±2.46^{ab}	163.67±3.17^{a}	142.00±2.97^{b}	0.05
HDL (mg dL^{-1})	37.750±4.20	35.73±9.93	30.53±0.63	35.47±9.73	NS
LDL (mg dL^{-1})	100.770±0.5^{a}	94.27±1.24^{ab}	74.45±3.12^{b}	96.13±1.15^{ab}	0.05
VLDL (mg dL^{-1})	29.400±0.27^{b}	31.00±0.08^{b}	32.70±0.13^{a}	28.40±0.16^{b}	0.05

^{a,b}Means in the same row with different letters are significantly different at p<0.05. NS: Non-significant, T1: Control group, T2, T3 and T4: 4, 8 and 12 g chia seeds/kg diet respectively

Table 3: Effect of supplementing chia seeds on some blood parameters in quail at 8 weeks of age

	Treatments				
Items	T1	T2	T3	T4	p-value
PCV (%)	35.33±1.33	37.33±4.66	35.33±3.17	34.50±0.28	NS
Hb (g/100 mL)	11.10±0.49	11.07±0.95	11.10±1.05	10.80±0.11	NS
Total protein (g dL^{-1})	8.20±0.34	7.10±0.14	7.40±0.51	8.21±2.60	NS
Albumin (g dL^{-1})	3.81±0.80	3.01±0.11	3.39±0.08	3.40±0.02	NS
Globulin (g dL^{-1})	4.39±0.54	4.05±0.16	3.98±0.44	3.50±0.52	NS
Albumin/globulin	0.95±0.32	0.75±0.04	0.87±0.06	1.01±0.10	NS
Uric acid (mg dL^{-1})	5.30±0.83	4.73±0.78	5.50±0.96	4.93±1.05	NS
Glucose (mg dL^{-1})	312.67±67^{a}	294.00±20.0^{a}	69.67±4.63^{b}	91.67±1.27^{b}	0.05
Calcium (mg dL^{-1})	11.46±0.49	11.23±0.55	10.20±0.37	11.17±0.35	NS
Cholesterol (mg dL^{-1})	141.27±2.33	168.00±12.8	166.33±11.86	147.50±1.99	NS
Triglycerides (mg dL^{-1})	146.87±0.54^{a}	137.07±4.23^{ab}	128.20±5.04^{b}	138.90±4.21^{ab}	0.05
HDL (mg dL^{-1})	31.39±0.51	33.02±5.16	36.96±2.63	32.39±2.88	NS
LDL (mg dL^{-1})	80.49±1.70	107.56±11.70	103.73±8.72	86.94±9.26	NS
VLDL (mg dL^{-1})	29.37±0.10^{a}	27.41±0.84^{ab}	25.64±1.00^{b}	27.78±0.84^{ab}	0.05

^{a,b}Means in the same row with different letters are significantly different at p<0.05. NS: Non-significant, T1: Control group, T2, T3 and T4: 4, 8 and 12 g chia seeds/kg diet, respectively

Table 4: Effect of supplementing chia seeds on some reproductive parameters

	Treatments				
Items	T1	T2	T3	T4	p-value
Ovary (%)	3.68±0.26	3.45±0.12	3.38±0.67	3.19±0.19	NS
Oviduct (%)	3.58±0.38	3.98±0.14	3.44±0.16	3.54±0.17	NS
Oviduct length (cm)	22.94±0.97	22.94±2.52	22.04±1.01	22.35±1.95	NS
Estrogen (pg mL^{-1})	99.55±0.33^{b}	94.35±0.64^{c}	131.10±0.80^{a}	82.88±0.54^{d}	0.05
FSH (m IU mL^{-1})	0.10±0.00^{d}	0.13±0.00^{b}	0.19±0.00^{a}	0.11±0.00^{c}	0.05
LH (m IU mL^{-1})	0.10±0.00^{c}	0.10±0.00^{c}	0.34±0.00^{a}	0.22±0.00^{b}	0.05

^{a,b}Means in the same row with different letters are significantly different at P<0.05. NS: Non-significant, T1: Control group, T2, T3 and T4: 4, 8 and 12 g chia seeds/kg diet, respectively

blood lipid profile showed that the total cholesterol, HDL and LDL were not affected by the addition of chia seeds to the diet, while the triglycerides and VLDL were reduced significantly (p<0.05) in the treatment that involved adding 8 g chia seeds/kg diet (T3) compared to those in the control.

The reduction in the level of triglycerides in the T3 quail at 8 weeks of age could be due to omega-3 fatty acids that may activate receptor molecules called peroxisome proliferator–active receptors (PPARs), which act as bonding agents regulating the lipid equilibrium. Activating PPARs will increase the oxidation of the fatty acids in the liver and thereby reduce the blood triglyceride level[33]. This result agrees with the findings of Al-Daraji *et al.*[30] who indicated that the use of omega-3 rich sources (flaxseed oil and fish oil) reduced the levels of blood triglycerides in quail.

Reproductive measurements: The results of the reproductive characteristic examinations in this study are shown in Table 4.

Table 5: Effect of supplementing chia seeds on the egg yolk fatty acids

	Treatments				
Fatty acid (%)	T1	T2	T3	T4	p-value
Oleic	13.000±0.28[c]	16.50±0.28[a]	17.20±0.28[a]	15.23±0.44[b]	0.05
Linolenic	8.067±0.29[c]	15.50±0.28[b]	23.80±0.28[a]	24.00±0.28[a]	0.05
Linoleic	2.000±0.28[b]	3.90±0.28[a]	3.30±0.28[a]	3.40±0.28[a]	0.05
Palmitic	16.400±0.28[d]	26.40±0.28[a]	22.30±0.28[b]	20.50±0.28[c]	0.05
Stearic	6.300±0.28[c]	13.60±0.28[a]	11.70±0.28[b]	11.20±0.28[b]	0.05

[a,b]Means in the same row with different letters are significantly different at p<0.05, T1: Control group, T2, T3 and T4: 4, 8 and 12 g chia seeds/kg diet, respectively

There were no significant differences between the treated and control groups in the relative weight of the ovaries and oviducts or in the length of oviducts. The levels of blood estrogen, FSH and LH increased significantly (p<0.05) in the T3 group compared to the control and other supplemented groups. This result indicates that adding 8 g chia seeds per 1 kg diet was optimal in this regard. Unsaturated fatty acids affect reproductive activities through different mechanisms; they are used as a source of energy during the development and maturity of ovarian follicles and even during the advanced stages of embryonic development. The addition of linoleic acid to the diet has an important effect on the growth of the ova and the differentiation of its cells, has an organized role in the meiosis of the germ follicles and protects them from collapse[34] as it increases the total number of follicles with increased volumes, especially in the mature follicles, before ovulation. The effect of unsaturated fatty acids in follicle growth and development may be due to their direct effects on the steroid hormone synthesis process in the ovary by increasing the activity of the enzymes included in the pathways of sexual steroid synthesis by stimulating the female sex hormones including estrogen[35]. In this regard, it was found that adding unsaturated fatty acids to the diet led to an increase in the ova number[21] and supplementing omega-3 fatty acids led to a significant increase in egg production[36].

Egg yolk fatty acid profile: The statistical analysis of the egg yolk fatty acid percentages studied are presented in Table 5. In general, a significant (p<0.05) increase was found in the percentages of oleic, linolenic, palmitic, linoleic and stearic acids in quail supplemented with chia seeds compared with those of the control. T3 and T4 increased significantly (p<0.05) linolenic acid, which is an omega-3 fatty acid. A significant increase was found in oleic acid in T2 and T3 compared to that in the control. On the other hand, T2, T3 and T4 increased significantly (p<0.05) linoleic acid compared to the control. Palmitic and stearic acid increased significantly (p<0.05) in T2 compared to those in the control and other supplemented groups.

Linolenic acid is the major omega-3 fatty acid and is considered an essential source of omega-3 fatty acids, which include eicosapentaenoic (EPA) and docosahexaenoic (DHA). The human body can manufacture EPA and DHA when linolenic acid is available in food in a high enough quantity[37]. The ALA desaturation and elongation in hen livers could be the reason for the increase in the DHA in egg yolks. The body of laying hens has the ability to deposit DHA in egg yolks by converting ALA to DHA[38]. The increased percentage of fatty acids in the supplemented groups compared with that in the control could be a result of the oil quality in the chia seeds. This result is in agreement with Al-Fadhlee[31] and Ezzat[8] who found an increase in the omega-3 fatty acid percentage when laying hens were fed a diet rich in omega-3.

A significant increase was found in oleic acid in T2 and T3 compared to that in the control. On the other hand, T2, T3 and T4 had increased significantly (p<0.05) linoleic acid compared to that of the control. Palmitic and stearic acid increased significantly (p<0.05) in T2 compared to that in the control and other supplemented groups.

The double-bond unsaturated fatty acids linoleic acid (LA) and arachidonic acid (ARA) are the most prevalent in nature (called omega-6 fatty acids) and they interfere with the synthesis of the phospholipids contained in cell membranes due to the antioxidant properties of herbs, which means that they have a role in controlling the metabolism processes[39]. This role could be due to the antioxidant properties of herbs, which can stimulate metabolism. LA is the primary source of omega-6 fatty acids in humans because it can be transformed into arachidonic and linolenic acid[40]. These results indicate the importance of using chia seeds as dietary supplements to quail.

CONCLUSION

It can be concluded that adding 8 g chia seeds per 1 kg female quail diet during the laying period lowered blood uric acid, glucose, LDL, triglycerides and VLDL and increased the sex hormones responsible for egg formation: estrogen, FSH and LH. The egg yolk fatty acids (oleic, linolenic, palmitic, linoleic and stearic acids) were increased significantly in all supplemented groups. However, adding 8 or 12 g chia seeds/kg diet was characterized by an increase in the percentage of linolenic acid (omega-3 type), which led to the recommendation of this egg as a healthy food. Supplementing the quail diet with chia seeds did not affect the measurements of the female reproductive system (weight and length), blood protein, calcium or some erythrocyte indicators.

SIGNIFICANCE STATEMENTS

The results of this study proved that quail eggs can be enriched with important omega-3 and 6 fatty acids by adding chia seeds to the quail diets to provide the human body with its fatty acid requirements when the eggs are consumed. In addition, we can provide the advantage of balanced blood lipids and further increase the sex hormone-related egg formation, which means we can improve laying rates. These results encourage researchers to conduct more studies in order to investigate the exact mechanisms of action of the active ingredients in chia seeds.

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