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Effects of carob (*Ceratonia siliqua*) pod byproduct on quail performance, egg characteristics, fatty acids, and cholesterol levels

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ABSTRACT - This study was carried out to determine the effects of a carob (*Ceratonia siliqua*) byproduct (CB) supplement in diets for laying Japanese quail (*Coturnix coturnix japonica*) on performance and egg internal-external quality traits, fatty acid profile, and cholesterol content. A total of 225 female quail at 12 weeks of age were distributed into five treatment groups with three replications (15 birds in each replication). The following treatments were tested: 0% (control, no CB supplementation); 3% CB; 5% CB; 10% CB; and 15% CB in the diets. Feed and water were provided *ad libitum* to quail. The lighting program was 16 h light and 8 h dark. The experiment lasted 60 days. Treatments had no significant effects on final weight, feed intake, feed conversion ratio, egg production, and mortality. The external egg quality characteristics, shape index, shell weight, shell thickness, albumen index, yolk index, yolk weight, yolk fat, yolk color, yolk cholesterol, stearic acid, oleic acid, and heptadecanoic acid concentrations were not significantly influenced by CB supplementation to quail diets. Haugh unit, egg yolk total saturated fatty acids, total mono or polyunsaturated fatty acids, linoleic acid, gamma linolenic acid, palmitic, and palmitoleic acid contents were significantly influenced by the treatments. Carob byproduct can be used up to 15% in laying quail diets without any negative effects on performance, mortality, or internal egg quality traits.

Key Words: egg yolk, fatty acid profiles, Japanese quail, locust bean pod byproduct, production parameters

Introduction

Carob (*Ceratonia siliqua*) growing has been performed in the Mediterranean region for about 4,000 years. The world annual carob production is estimated at 310,000 t from approximately 200,000 ha (Makris and Kefalas, 2004). In Turkey, annual carob production is approximately 13,500 t, obtained from 354,000 carob trees (Biner et al., 2007).

The carob seed contains approximately 40.8 mg g^{-1} total phenol, 16.2 mg g^{-1} condensed tannins, and 2.98 mg g^{-1} hydrolyzed tannins (Avallone et al., 1997). The sugar content in the carob fruit varies between 48 and 72% and the fruit is an important source of phenols, which have significant antioxidant powers (Makris and Kefalas, 2004). Carobs have a gum content of 42 to 46%, mostly composed of natural galactomannan (Saura-Calixto, 1987).

Carob contains phenolic compounds such as gallic, syringic, and sinapic acid, being gallic acid the most abundant (1,550 mg g^{-1} dry matter) (Ayaz et al., 2007). Carob is also rich in C18:1n-9 and C18:2n-6 fatty acids and contains 0.1041 mg g^{-1} vitamin C (Gubbuk et al., 2010). However, carob byproducts have a high cellulose content and, therefore, they are restricted to up to 20% in poultry diets (Göhl, 1982). Nevertheless, supplementation of 6-9% of this ingredient in poultry diets may cause a decrease in protein and fat digestibility (13-30%) and an increase in jejunal sticky digesta (Ortiz et al., 2004). The apparent metabolic energy in geese was reported as 6.1 MJ/kg and the actual metabolizable energy as 6.6 MJ/kg. In goose diets, supplements up to 20% did not influence body weight, although 30% decreased both body weight and feed conversion ratio. Increasing carob pod ratios in diets decreased intestine (small, large, and caecum) length and gizzard weight in geese (Sahle et al., 1992).

The present study was conducted to investigate the effects of carob pod supplement in quail diets on performance and egg internal-external quality traits, fatty acid profile, and cholesterol content.

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Material and Methods

The experiment was conducted in Kahramanmaraş Province, Turkey. A total of 225 twelve-week-old female Japanese quail (*Coturnix coturnix japonica*) were distributed into five treatment groups with three replications of 15 birds each. Treatments were arranged as follows: 0% (control, no carob byproduct (CB) supplementation); 3% CB; 5% CB; 10% CB; and 15% CB in the diets. Feed and water were provided *ad libitum* to quail. The lighting program was set as 16 h light and 8 h dark. The experiment lasted 60 days.

Individual body weights of quail were determined at the beginning and end of the trial. Feed intake and feed conversion ratio of groups were determined weekly. Egg mass was calculated for the same week using egg weights and egg production rates (%). Daily egg production was recorded. Egg weight, egg internal and external quality traits (yolk weight, yolk color, Haugh unit, albumen index, yolk index, shape index, shell thickness, and shell weight), and egg yolk cholesterol were determined in the last week of the study. Mortality was recorded daily.

Feed dry matter, crude protein (%) (total N in samples × 6.25), crude fat (%), crude ash (%), and crude fiber (%) analyses were carried out according to AOAC (2005). Starch content was determined by the polarimetric method, according to Karabulut and Canbolat (2005). Total sugar content was determined through the method described by Dubois et al. (1956). The metabolizable energy value of CB used in the diets (kcal/kg) was calculated as follows: ME = 239 × [(0.1551 × crude protein) + (0.1669 × starch) + (0.3431 crude fat) + (0.13 × sugar)] (Hartel, 1986) (Tables 1 and 2).

In the egg yolk, fatty acid composition was determined according to IUPAC IID19 (IUPAC, 1987). To determine yolk cholesterol levels, the egg was boiled in water and then the yolk was separated and homogenized. From these samples, 0.1 g was placed in a glass tube and mixed with 4 mL isopropyl alcohol (99.5% pure) and then read at a 520-nm wavelength in a spectrophotometer (Spectra Max plus 384); the amount of cholesterol in the egg yolks was calculated using a formula proposed by Boehringer Mannheim Gmbh Biochemica (1989).

Data were analyzed by the one-way ANOVA under the general linear model procedure of SAS computer software (Statistical Analysis System, version 9.1). The model included the CB level of diets. Means were separated using Duncan's multiple range tests. The results of statistical analysis were shown as mean values and standard error of the mean in the tables. Statistical significance was considered at P<0.05.

Table 1 - Analyzed nutrient content of carob pod byproducts

Nutrient	Amount (%)
Dry matter	86.5
Crude protein	4.0
Crude fat	0.83
Crude ash	3.0
Starch	5.7
Sugar	37.5
Crude fiber	9.66
Calcium	0.34
Total phosphorus	0.089
Metabolic energy (MJ/kg)[1]	6.735

[1] Metabolic energy was calculated according to Hartel (1986).

Table 2 - Composition and nutrient content of diets used in the experiment

Ingredient (%)	Carob pod byproduct level (%)				
	0	3	5	10	15
Maize	46.58	40.76	37.20	30.30	24.14
Full fat soybean	11.00	19.44	24.30	25.00	21.96
Soybean seed meal	25.00	18.38	15.00	16.30	20.36
Sunflower seed meal	4.10	5.00	5.00	3.40	2.00
Carob pod byproduct	0.00	3.00	5.00	10.00	15.00
Soybean oil	4.50	4.70	4.83	6.20	7.86
Dicalcium phosphate	1.18	1.22	1.16	1.28	1.38
Limestone	7.10	6.96	6.98	6.97	6.74
Methionine	0.09	0.09	0.08	0.10	0.11
Common salt	0.20	0.20	0.20	0.20	0.20
Vitamin + mineral premix[1]	0.25	0.25	0.25	0.25	0.25
Total (kg)	100	100	100	100	100
Calculated nutrient composition					
Dry matter (%)	90.97	90.80	90.69	90.62	90.60
Crude protein (%)	20.5	20.5	20.5	20.5	20.5
Crude fat (%)	8.43	9.98	10.87	12.08	12.90
Crude ash (%)	10.21	10.11	10.14	10.16	9.98
Crude fiber (%)	3.59	3.93	4.06	4.19	4.37
Calcium (%)[2]	3.02	2.99	3.00	3.04	2.99
Total phosphorus (%)[2]	0.60	0.59	0.60	0.60	0.60
Available phosphorus (%)[2]	0.34	0.34	0.33	0.32	0.32
Lysine (%)[2]	1.11	1.11	1.11	1.14	1.16
Methionine (%)[2]	0.43	0.43	0.43	0.43	0.43
Metabolizable energy (MJ/kg)[3]	11.69	11.69	11.69	11.69	11.69

[1] Each kg of diet contained: vitamin A, 12.000 IU; vitamin D3, 2000 IU; vitamin E, 35 mg; vitamin K_3, 5 IU; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.015 mg; vitamin C, 50 mg; D-biotin, 0.045 mg; niacin, 20 mg; calcium D pantothenate, 6 mg; folic acid, 0.75 mg; choline chloride, 12.5 mg; manganese, 80 mg; iron, 60 mg; zinc, 60 mg; copper, 5 mg; iodine, 1 mg; cobalt, 0.2 mg; selenium, 0.15 mg; canthaxanthin, 15 mg; and ß-apo-8'-carotenoic acid ethyl ester, 5 mg.
[2] Calculated according to NRC (1994).
[3] Calculated according to Hartel (1986).

Results and Discussion

There were no significant differences among the treatment groups in terms of final weight. There were no significant differences among the treated groups with regard to feed intake. The carob byproduct contains high levels of tannins (Alumot et al., 2006; Karabulut et al., 2006), which can reduce feed intake and body weight of goat kid (Silanikove et al., 2006). The sweet taste of the CB supplement may result in higher feed intake (Silanikove et al., 2006; Kotrotsios et al., 2010). In the current experiment, quail fed supplemented diets consumed more feed than the control group and this increased feed intake may have been a result of the sweet taste of the CB supplement (Table 3).

No significant differences were observed in feed conversion ratio of the treated groups. This result was similar to the report of Yıldırım and Kaya (2011), in which ground CB supplement in broiler diets did not affect feed conversion ratio of broiler at 28 to 35 days of age. The pulp of the CB also contains significant amount of tannin. The tannins in CB have a detrimental effect on the growth of goat kid; however, they have triglycerides and a cholesterol-lowering effect in blood (Silanikove et al., 2006). Yıldırım and Kaya (2011) determined that supplementation of 0, 5, 10, and 20% CB decreased body weight gain, but increased feed intake and feed conversion ratio of broilers. In another experiment (Sahle et al., 1992), despite the increase in feed intake of broiler and geese fed diets supplemented with carob, there was no decline in body weight or feed conversion ratio. There was no significant difference among treatments in terms of mortality rate (Table 3).

Table 3 - Some performance values and mortality of quail fed diets containing different levels of carob pod byproduct

Parameter	Carob pod byproduct level (%)					SEM	P-value
	0	3	5	10	15		
Initial weight (g)	263.6	263.4	265.3	265.1	263.6	16.25	0.95
Final weight (g)	270.3	268.3	265.4	266.5	265.8	36.57	0.84
Feed intake (g)	40.47	42.12	40.01	43.15	41.74	2.68	0.20
Feed conversion ratio	3.30	3.28	3.16	3.47	3.20	0.01	0.09
Mortality (%)	8.89	8.89	6.67	6.67	6.67	4.44	0.58

SEM - standard error of the mean.

The treatments did not have significant effects on egg production, cholesterol contents, external and internal egg characteristics such as egg weight, eggshell weight, thickness, albumen index, yolk index, egg shape index, egg yolk color, egg yolk weight, and yolk fat in the overall experimental period ($P>0.05$). On the other hand, Haugh unit values of control and 3% CB-supplemented groups were lower than those of the other CB-supplemented groups ($P<0.05$) (Table 4). However, Silanikove et al. (2006) noted that CB contains condensed tannins, which may bind the dietary lipids in the gastro intestine and cause a hypocholesterolemic effect on the body. Perez-Olleros et al. (1999) reported that CB is rich in cellulose, which may reduce total cholesterol and low-density lipoprotein in laboratory animals. This was not the case in the current experiment.

There were no differences among groups in terms of yolk palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), and total unsaturated fatty acid concentrations (Table 5). Although there was a tendency of decrease in palmitic acid (C16:0), stearic acid and oleic acid (C18:1) concentrations in egg yolk with increasing CB supplementation, this decrease was not significant. A similar trend was observed in total saturated fatty acid and total monounsaturated content of egg yolk ($P<0.05$). In addition, the palmitoleic acid (C16:1) concentration decreased with increasing CB supplementation ratios in diets and palmitoleic acid content of CB-supplemented groups was significantly lower than that of the control group.

The treatment had a significant ($P<0.05$) effect on the linoleic acid content of yolk. The increase in linoleic acid of yolk is possibly associated with CB and soybean oil, which are very rich in linoleic acid (Gubbuk et al., 2010).

Table 4 - Some egg quality traits and yolk cholesterol content of quail fed different diets containing carob pod byproduct

Parameter	n	Carob pod byproduct level (%)					SEM	P-value
		0	3	5	10	15		
EP (%)	3	73.67	72.80	72.13	74.67	73.27	27.29	0.98
EW (g)	3	12.27	12.84	12.59	12.46	13.04	0.12	0.14
ESW (g)	3	1.84	1.90	1.73	1.68	1.72	0.00	0.09
ST (mm)	3	0.21	0.21	0.20	0.21	0.21	0.00	0.36
AI (%)	3	3.56	3.64	3.42	3.48	3.31	0.02	0.16
YI (%)	3	48.52	49.39	49.53	48.76	49.11	1.46	0.82
ESI (%)	3	75.93	78.19	78.20	77.14	77.48	1.95	0.31
CFV[1]	3	9.40	9.07	9.35	9.23	8.75	0.16	0.34
HU	3	90.13a	90.00a	88.17b	87.93b	87.90b	0.83	0.02
EYW (g)	3	4.07	4.22	4.19	4.14	4.22	0.01	0.34
EYCF (%)	3	30.77	28.72	29.43	29.72	30.35	1.01	0.19
EYC (mg)	3	73.64	75.15	65.22	63.11	64.41	50.00	0.18

EP - egg production; EW - egg weight; ESW - egg shell weight; ST - shell thickness; AI - albumen index; YI - yolk index; ESI - egg shape index; CFV - color fan value; HU - Haugh unit; EYW - egg yolk weight; EYCF - egg yolk crude fat; EYC - egg yolk cholesterol; SEM - standard error of the mean.
[1] DSM Nutritional Products.
a,b - values with different letters in a row differ significantly ($P<0.05$).

Table 5 - Fatty acid content of egg yolk of Japanese quail fed diets containing different levels of carob pod byproduct

Parameter	n	Carob pod byproduct level (%)					SEM	P-value
		0	3	5	10	15		
Palmitic acid (C16:0)	3	24.77	25.17	24.30	23.13	22.73	1.49	0.14
Stearic acid (C18:0)	3	8.04	8.20	7.55	8.13	7.91	0.53	0.75
Oleic acid (C18:1)	3	41.32	39.61	38.91	36.49	36.30	4.97	0.08
Linoleic acid (C18:2)	3	17.40c	20.69bc	22.81ab	24.37ab	26.02a	7.18	0.05
Gamma linolenic acid (C18:3n6)	3	0.79c	1.06bc	1.29ab	1.52a	1.54a	0.05	0.01
Palmitoleic acid (C16:1)	3	3.06a	2.14b	2.16b	1.43b	1.43b	0.15	0.00
Heptadecanoic acid (C17:0)	3	0.16	0.17	0.19	0.20	0.23	0.00	0.08
Total saturated fatty acids	3	33.53ab	32.84a	30.68a	30.03b	30.27b	1.78	0.03
Monounsaturated fatty acids	3	45.20a	42.61ab	42.04ab	38.79b	38.60b	6.17	0.04
Polyunsaturated fatty acids	3	18.39c	22.05bc	24.45ab	26.19ab	27.89a	8.62	0.02
Total unsaturated fatty acids	3	63.59	64.66	66.49	64.98	66.48	3.68	0.34

a,b,c - values with different letters in a row differ significantly.
SEM - standard error of the mean.

The gamma linolenic acid (C18:3n6) and total polyunsaturated fatty acid contents of egg yolk increased with increasing CB supplementation ratios in the diets ($P<0.05$). The linoleic acid and gamma linolenic acid contents were the lowest in control group and the highest in 15% CB-supplemented group (Table 5). In the 15% CB-supplemented group, total polyunsaturated fatty acid contents were higher than in control and in 3% CB-supplemented groups.

The gamma linolenic acid is promptly elongated into dihomo-gamma-linolenic acid, necessary for production of series 1 prostaglandins (Horrobin, 1992). The linolenic acid obtained from the feed is initially converted into gamma linolenic acid and then to series 1 prostaglandins, which acts as an important anti-inflammatory agent in the body (Sprecher et al., 1995). In addition, the linolenic fatty acid is needed in the body for brain, skin, and bone health functions. Carob pod byproduct supplementation caused an increase in gamma linolenic fatty acid content of egg yolk and, therefore, it may be used to produce functional eggs.

Moreover, the oleic fatty acid ratio of quail eggs was high because of the soy oil, which is a rich source of oleic acid. In a previous study, carob oleic acid (C18:1n9) content was reported as 42.92% (Gubbuk et al., 2010). It was noted that when soy oil was supplemented in the feed, the egg yolk palmitic, stearic, and oleic fatty acid contents decreased (Sim and Bragg, 1978). The effects of treatments on egg yolk oleic acid content were not significant. Decreasing oleic acid content was observed with increasing CB and soy oil supplementation ratios.

There was a significant decrease in monounsaturated fatty acids of yolk with increasing level of CB supplementation (Table 5), despite the rather rich CB (45.68%) in monounsaturated fatty acids, whereas CB supplementation increased the polyunsaturated fatty acid concentration of yolk. In the current study, polyunsaturated fatty acid concentration of yolk ranged from 18.39 to 27.89%. The concentration of polyunsaturated fatty acid of yolk was considerably higher than that reported by Choi et al. (2001), who found that the polyunsaturated fatty acid concentration of a normal egg was 12%. The differences between the two studies is possibly associated with the differences in chemical composition of diets used in the experiments (Table 5).

The intake of polyunsaturated fatty acid has been reported to reduce the risk of atherosclerosis and coronary heart disease (Lada and Rudel, 2003; Jakobsen et al., 2009). It is well known that foods rich in omega 3 have great benefits for the human health (Mozaffarian and Wu, 2011). Therefore, the changes in yolk contents due to CB supplementation obtained in the present experiment may be important in the nutrition of people with cardiovascular disorders.

Conclusions

Supplementation of carob byproduct in diets has no detrimental effect on body weight, feed intake, egg internal and external quality traits, and some yolk fatty acid components of laying quail. Therefore, carob byproduct can be used in quail diets up to 15% as an inexpensive feed source. Supplementation of carob byproduct to quail diets may be an advantage for hyperglycemic poultry. However, the low protein and energy content of carob byproduct should be taken into consideration when included into quail diets.

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