

Effect of Hempseed (*Cannabis sativa* sp.) Inclusion to the Diet on Performance, Carcass and Antioxidative Activity in Japanese Quail (*Coturnix coturnix japonica*)

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Abstract

This study was conducted to determine the effects of hempseed (H) on performance, carcass traits, and antioxidant activity in Japanese quail (*Coturnix coturnix japonica*). A total of 192 quail with seven-days old were divided into four experimental groups with four replicates. The treatments were; i) Control diet (C, no hempseed); ii) 5% hempseed in diet (H5); iii) 10% hempseed in diet (H10); and iv) 20% hempseed in diet (H20). The body weight (BW) and feed intake (FI) of quail was determined at 7, 21 and 42 d of age. At 42 d of age four quail were slaughtered and the carcass and internal organ traits were determined. Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), nitric oxide (NO) and total protein were determined in the blood serum end of the experiment. The BW of the groups were not significant at 7 and 21 d, however in the 20% hempseed group BW decreased at 42 d ($p < 0.05$). The FI and feed conversion ratio were not significant among the treatment groups. The carcass, liver, intestine and heart weight and their percentage to carcass were significantly differ in treatment groups ($p < 0.05$). The serum MDA and NO decreased in hempseed addition ($p < 0.001$). The serum SOD, CAT and GSH-Px were increased by hempseed supplementation ($p < 0.001$). In conclusion, hempseed supplementation to quail diets may not improve quail performance traits but increase antioxidant activity in blood.

Key words: hempseed, quail, performance, carcass, antioxidant activity

Introduction

The *cannabis sativa* is an annual plant and well known as an important source of food, dietary oil and medicine for thousands of years in many western and eastern countries (Callaway, 2004; de Padua *et al.*, 1999). Hempseed both crushed and whole, remains an important grain in traditional Asiatic foods and medicines (Callaway, 2004). Industrial hemp with low levels (< 2 g/kg) of δ -9-tetrahydrocannabinoid (THC) has been permitted for cultivation in European Union countries with EU Council regulation 1782/2003 (2008). Also, EU commission has been working on regulations of hems' THC levels and usage in different areas (Callaway, 2008). Hempseed contains about 30% oil and 25% protein, 34% carbohydrates, dietary fibre, vitamins and minerals (Callaway, 2004;

Kriese *et al.*, 2004). Hempseed oil is high in polyunsaturated fatty acids (PUFAs, about 80%), and rich in two essential fatty acids such as linoleic acid (18:2 ω -6, 55.48%) and α -linolenic acid (18:3 ω -3 21.51%) (Bağcı *et al.*, 2003; Uluata and Ozdemir, 2012) which are important for optimal human health (Schwab *et al.*, 2006). Also, linoleic and linolenic fatty acids in quail (Yalcin *et al.*, 2012) and layer hens' (Gakhar *et al.*, 2012; Goldberg *et al.*, 2012; Silversides and Le Francois, 2005) diets may transfer to meat or egg via hempseed and hemp oil usage. In addition hempseed is rich in many essential amino acids, especially high levels of arginine and methionine and special peptides (Callaway, 2004; Tang *et al.*, 2006). It is well known that antioxidant activities of peptides are closely related to amino acid composition and sequences of these amino acids and hempseeds' special protein types show potential of natural antioxidant agents (Gebrekidan, 2012). The consumption of foodstuffs rich in antioxidants provides protection against cancer and cardiovascular diseases (Parker *et al.*, 2003). Uluata and Ozdemir (2012) and Chen *et al.* (2012) reported that hem-

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hempseed can be utilized for the production of formulations containing phytochemicals with significant antioxidant properties and health benefits and hempseed addition could increase the shelf life of the products as well. Additionally, it is reported that hempseed's cannabidiols have antimicrobial, immunomodulatory, antioxidative, antihypertensive and mineral binding properties (Korhonen and Pihlanto, 2003). The development of bioactive protein peptides of hempseed may contribute to new economic opportunities for increased value-added utilization of hemp (Lu *et al.*, 2010).

Cells generate small amounts of free radicals or reactive oxygen species (ROS) while performing their normal metabolic functions. Although low levels of ROS are essential in many biochemical processes, high levels of ROS may damage cells and tissue macromolecules, that is, lipids, proteins, carbohydrates and deoxyribonucleic acid (DNA) (Mates *et al.*, 1999; Tang *et al.*, 2006). The tocopherols, phenolic compounds and carotenoids present in vegetable oils are used against the oxidation reaction (Chen *et al.*, 2012; Ramadan *et al.*, 2003; Uluata and Ozdemir, 2012). Due to all these properties, in the past, hemp oil and seed had been used as a medical plant to treat some diseases (de Padua *et al.*, 1999).

It is reported that hempseed is an excellent source of nutrition. Not only traditional Chinese medicine but also recent modern human clinical trials agree that hempseed has health promoting properties which is supported by results from nutritional analyses of the seed, oil and seed meal (Callaway, 2004). Recently, to be protected from some illnesses, natural antioxidants have attracted great attention from consumers due to their reliable properties compared to synthetic ones. As a result of healthy food production strategy, use of natural antioxidants in diets is alleviating. The aim of the present study was to determine the effects of hempseed on performance, carcass and oxidative activity parameters in the blood of Japanese quail.

Material and Methods

Animal and diets

A total of (192 unsexed seven-day-old Japanese quail chicks (*Coturnix coturnix japonica*) were individually weighed, wing banded and distributed into 4 treatment groups with 4 replicates and 12 chicks per cage. Each cage was furnished with a heater, two wateriers and feeders. The rearing cage dimensions were 50×90×20 cm (wide, length, height). Replicates were designated as the experimental units, and randomized with respect to the

dietary treatments. The diet treatments were as follows; 1) Basal diet, hempseed not included in diet (control=C); 2) 5% hempseed inclusion (H5); 3) 10% hempseed inclusion (H10); 4) 20% hempseed inclusion (H20). The experimental diets were offered to quail for 5 wk. Maize-soybean based diets were utilized and all formulated on similar levels of nutrient composition. The hempseed was bought from a local seed shop. All diets' compositions were prepared according to NRC (1994) recommendations. The compositions of hempseed and experimental diets used in this study were given in Table 1 and Table 2, respectively.

Measurements

The individual body weights (BW) of birds and feed intake (FI) for each subgroup were measured at 7th, 21st and 42nd d of age. Feed conversion ratio (FCR; FI/body weight gain, g/g) of groups were calculated for 7 to 21, 21 to 42, and 7 to 42 d. Feed and water were offered as *ad libitum*. Animals were exposed to 24-h light during experiment. Mortality was recorded daily and was taken into consideration to calculate FI and FCR. For carcass evaluation 16 birds (8 males and 8 females, total 64 samples) in each group were randomly selected at 42 d of age and slaughtered. Their feathers were plucked, and the carcasses were eviscerated by hand. The small intestine, large intestine, and gizzard were removed, the contents were expelled. The carcass, liver, heart, proventriculus, gizzard, and empty intestine (duodenum + ileum + jejunum + cecum + colon) were recorded individually and part yields were obtained as part weight: carcass weight X 100. Cold carcass weights were recorded after the carcasses had been stored at 4°C for 18 h.

For biochemical analysis, blood samples were obtained from venipuncture of the neck of the aforesaid animals (8 males and 8 females in each subgroups, total 64 samples). The blood samples were then kept on ice and transferred to the laboratory where they were centrifuged at 740×g for 10 min to remove the serums. The serums then were stored at -70°C until the analysis. Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), nitric oxide (NO) and total protein were determined in the blood serum according to methods below.

Analytical Methods

The fatty acid (FA) composition analysis

Fatty acid compositions of the hempseed samples were

determined according to the Agilent application catalogue. For the analysis, the oil of samples were extracted with ether and stored in eppendorf tubes from which the air was removed and replaced by nitrogen. The samples were stored at -60°C until the analysis. The oil (100 mg) was saponified with 100 μL 2 N KOH, and 3 mL hexane was added to the mixture. The mixture was vigorously shaken with a vortex for 1 min, and then centrifuged at 2900 g for 5 min. FA compositions were analyzed by a GC (Agilent 6890, USA) equipped with a Flame Ionization Detector and a 100 m \times 0.25 mm ID HP-88 column. Injector temperature was 250°C . The oven temperature was kept at 103°C for 1 min, then programmed from 103 to 170°C at $6.5^{\circ}\text{C}/\text{min}$, from 170 to 215°C for 12 min at $2.75^{\circ}\text{C}/\text{min}$, finally, 230°C for 5 min. The carrier gas was helium with a flow rate of 2 mL / min; split rate was 1 / 50. FA was identified by comparison of retention times to known standards. The results were expressed as g fatty acid / 100 g total fatty acids (%).

The determination of malondialdehyde (MDA)

The malondialdehyde levels, as an index of lipid peroxidation was determined by thiobarbituric acid reaction according to Yoshiko *et al.* (1979). The principle of the method depends on the measurement of the pink color produced by interaction of thiobarbituric acid with malondialdehyde. The pink color absorbances are measured at 532 nm and the MDA levels are expressed as $\mu\text{mol/L}$.

The determination of Nitric oxide (NO)

Plasma nitrite and nitrate determinations are increasingly being used in clinical chemistry as a marker for the activity of nitric oxide synthase and the production of nitric oxide radicals. We performed an enzymatic methodology based on the reduction of nitrate to nitrite by nitrate reductase (E.C.1.6.6.2) from *Aspergillus* species, in the presence of β -NADPH. The concomitant oxidation of coenzyme was monitored by the decrease in absorbance at 340 nm. The method of standard addition was used to minimize the effect of interfering substances from serum. This spectrophotometric method was used to measure nitrate present in serum. Nitrite and nitrate present in serum were in proportion to total nitrogen oxides (NO_x) determined by the method. Nitrate was measured as nitrite after enzymatic conversion by nitrate reductase (E.C.1.6.6.2). Total nitrite concentration of serum was measured by using the Griess reaction (Bories *et al.*, 1995; Moshage *et al.*, 1995). Total nitrite concentration as NO levels was expressed as $\mu\text{mol/L}$.

The determination of catalase activity (CAT)

The CAT activity was measured by the method of Yasmin (1995). This method is based on the disappearance of H_2O_2 at 240 nm in a reaction medium containing H_2O_2 , potassium phosphate buffer (pH 7.0). Catalase activity was obtained from molar absorption coefficient of H_2O_2 and the absorbance change per minute. The CAT activity was expressed as ku/L .

The determination of superoxide dismutase activity (SOD)

The SOD activity was measured by the method described originally by Sun *et al.* (1988). This assay for superoxide dismutase (SOD, EC 1.15.1.1) activity involves inhibition of nitro-blue tetrazolium reduction, with xanthine-xanthine oxidase used as a superoxide generator. The enzyme activity is expressed as units/mg protein.

The determination of glutathione peroxidase (GSH-Px) activity

Glutathione peroxidase activity was measured with the method of Mishra (1990) which was modified from Paglia and Valentine (1967). The reaction contained glutathione, glutathione reductase, sodium azide, and NADPH. NADPH disappearance was monitored at 340 nm using a spectrophotometer. The enzyme activity is expressed as oxidized nmol NADPH/min/mg protein (unit/mg protein).

The determination of protein

Protein concentrations in the serum were determined using a Lowry assay (Lowry *et al.*, 1951). This method is based on the reaction of cupric ions with peptide bonds under alkaline conditions. Concentrations were calculated from a standard curve constructed using bovine serum albumin and protein levels are expressed as mg/ml.

Statistical analysis

Data were analyzed by using One-way Anova under General Linear Models of SPSS computer software (SPSS (1998)). The model included hempseed level of diets. The means were separated using Duncan's multiple range tests. The results of statistical analysis were shown as mean values and standard error of means (SEM) in the tables. Statistical significance was considered at $p < 0.05$.

Results

The hempseed nutrient and fatty acids composition are presented in Table 1. The hempseed's dry matter, crude

Table 1. Analysed nutrient and fatty acid composition of hempseed

Item	Ratio, %
Dry matter	95.11
Crude protein	21.05
Crude oil	31.45
Crude ash	8.80
Fatty acid composition of hempseed oil*	
Palmitic acid, C16:0	6.67
Stearic acid, C18:0	2.87
Oleic acid, C18:1 (ω -9)	11.50
Linoleic, C18:2 (ω -6)	52.51
Linolenic, C18:3 (ω -3)	23.24
Arachidic acid, C20:0	0.65
Gadoleic acid, C 20:1	0.57
Behenic acid, C 22:0	0.25
ω -6/ ω -3	2.26
PUFA	87.9

*g fatty acid / 100 g total fatty acids (%).

protein, fat and ash were 95.11, 21.05, 31.45 and 8.8% respectively. The fatty acids of hempseed oil were found as linoleic acid 52.51%, linolenic acid 23.24%, ω -6/ ω -3 ratio 2.26 and PUFA 87.9%. The effects of hempseed on body weight (BW), feed intake (FI) and feed conversion ratio (FCR) values were shown in Table 3. There were no

differences statistically among the treatment groups in terms of FI, FCR and BW on d 7 and d 21 during the experimental period ($p>0.05$). However, on d 42, inclusion of 20% hempseed decreased the BW of quail ($p<0.05$).

Effect of hempseed inclusion on carcass and intestinal organ traits and their relative incidence were given in Table 4. The carcass and heart weights of the H10 group were higher than those of the other treatment groups ($p<0.05$). The liver and intestine weight in the C group was higher than those of the others ($p<0.01$) but carcass yield was lower than all hempseed supplemented groups ($p<0.05$). The gizzard weight and the gizzard and heart relative incidence were not influenced by the treatments. The intestine weight and relative incidence of H20 groups was lower than those of the other groups ($p<0.01$). The heart weight and relative incidence were not influenced by sex. The carcass, liver, gizzard, intestine weights and liver, gizzard and intestine relative incidence were lower in male quail ($p<0.05$ and $p<0.01$). Hempseed level and sex interactions were not influenced by the treatments. In general 20% hempseed inclusion decreased the carcass and internal organ weight and relative incidence.

The effects of hempseed included diets on serum mal-

Table 2. Diets feedstuff and nutrient composition

Feedstuffs	Diets, hempseed level, %			
	0 (C)	5 (H5)	10 (H10)	20 (H20)
Corn	391.40	400.00	400.00	385.11
Wheat	100.00	55.60	22.58	0.00
Soybean meal	353.94	345.41	337.33	339.20
Sunflower meal	100.00	93.82	84.84	27.23
Vegetable oil	25.76	26.00	26.00	18.37
Sodium chloride	3.46	3.53	3.59	3.64
Di-calcium phosphate	6.94	7.42	7.90	9.03
Limestone	13.48	13.37	13.26	13.20
Vitamin-mineral premix ¹	2.50	2.50	2.50	2.50
DL-Methionine	1.20	1.09	0.80	0.83
L-Lysine	1.32	1.26	1.20	0.89
Hempseed	0.00	50.00	100.00	200.00
Calculated nutrient composition²				
Dry matter, %	88.29	88.74	88.11	88.69
Crude protein, %	24.39	24.48	24.52	24.60
Calcium, %	0.80	0.80	0.80	0.80
Available phosphorus, %	0.30	0.30	0.30	0.30
Lysine, %	1.30	1.30	1.30	1.30
Methionine, %	0.50	0.50	0.50	0.50
Metabolizable energy, kcal/kg	2860.30	2865.10	2889.00	2899.60

¹Vitamin-mineral premix per kilogram of the diet, Vitamin A, 15,000 IU; Vitamin D3 2000 IU; Vitamin E, 40.0 mg; Vitamin K, 5.0 mg; Vitamin B1 (thiamine), 3.0 mg; Vitamin B2 (riboflavin), 6.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Niacin, 30.0 mg; Biotin, 0.1 mg; Calcium D-pantothenate, 12 mg; Folic acid, 1.0 mg, Choline chloride, 400 mg, Manganese, 80.0 mg; Iron, 35.0 mg; Zinc, 50.0 mg; Copper, 5.0 mg; Iodine 2.0 mg; Cobalt, 0.4 mg; Selenium, 0.15 mg assures. ²Nutrient composition of diets calculated according to NRC (1994) nutrient values of corn, wheat, soybean, sunflower meal and vegetable oil.

Table 3. Effects of treatments on the body weight (BW), feed intake (FI) and feed conversion ratio (FCR) in Japanese quail

Day	Treatments				SEM	P
	C	H5	H10	H20		
Body weight, g						
7	21.26	22.34	21.75	22.58	0.51	NS
21	110.91	114.60	114.22	115.21	2.39	NS
42	183.62 ^a	175.34 ^{ab}	183.40 ^a	168.50 ^b	3.94	*
Feed intake, g/day						
7 to 21	8.24	7.92	8.84	7.41	0.67	NS
21 to 42	25.58	23.77	27.33	22.82	1.51	NS
7 to 42	18.64	17.43	19.93	16.63	1.26	NS
Feed conversion ratio, g FI/g BWG						
7 to 21	1.19	1.33	1.34	1.26	0.57	NS
21 to 42	5.80	8.19	6.10	9.83	0.95	NS
7 to 42	2.88	3.53	2.98	3.81	0.08	NS

^{a-c}Values with different superscript in a row differ significantly * $p < 0.05$. C, H5, H10, H20; hempseed ratio in diet as 0, 5, 10 20% respectively; SEM: standard error of means. NS: non significant.

Table 4. Effect of hempseed (H) in quail diet on carcass and intestinal organ traits

Treatment	Absolute weight, g					Relative incidence, %				
	Carcass	Liver	Gizzard	Intestine	Heart	Carcass	Liver	Gizzard	Intestine	Heart
C	125.6 ^b	4.19 ^a	3.97	9.16 ^a	1.54 ^b	67.08 ^b	3.33 ^a	3.15	7.27 ^a	1.22
H5	124.9 ^b	3.55 ^b	3.74	7.40 ^b	1.46 ^b	70.85 ^a	2.84 ^b	3.00	5.91 ^b	1.17
H10	133.9 ^a	3.86 ^{ab}	3.87	7.68 ^b	1.74 ^a	70.60 ^a	2.89 ^b	2.89	5.72 ^{bc}	1.29
H20	122.5 ^b	3.16 ^b	3.71	6.14 ^c	1.51 ^b	72.00 ^a	2.59 ^b	3.03	5.05 ^c	1.23
Sex										
Male	124.0 ^b	2.86 ^b	3.41 ^b	6.28 ^b	1.55	72.91 ^a	2.31 ^b	2.75 ^b	5.07 ^b	1.25
Female	129.5 ^a	4.52 ^a	4.23 ^a	8.91 ^a	1.57	67.36 ^b	3.51 ^a	3.27 ^a	6.90 ^a	1.21
Interactions										
C*M	124.9	3.12	3.62	7.24	1.50	71.19	2.51	2.91	5.82	1.20
C*F	126.4	5.26	4.31	11.07	1.58	62.97	4.15	3.38	8.72	1.24
H5*M	118.7	3.00	3.24	6.23	1.43	72.68	2.52	2.74	5.23	1.20
H5*F	131.0	4.10	4.23	8.56	1.50	69.01	3.15	3.25	6.60	1.14
H10*M	129.5	2.84	3.56	6.38	1.74	74.07	2.21	2.76	4.96	1.34
H10*F	138.3	4.88	4.17	8.98	1.74	67.13	3.58	3.02	6.47	1.24
H20*M	122.9	2.48	3.20	5.25	1.53	73.69	2.02	2.60	4.29	1.25
H20*F	122.2	3.85	4.23	7.03	1.48	70.32	3.16	3.45	5.82	1.22
SEM										
H	2.62	0.18	0.16	0.40	0.06	1.08	0.14	0.11	0.29	0.04
Sex	1.86	0.13	0.11	0.29	0.05	0.76	0.10	0.08	0.21	0.03
H*sex	3.71	0.25	0.22	0.57	0.09	1.53	0.20	0.15	0.42	0.05
Probability										
H	*	**	NS	**	*	*	**	NS	**	NS
Sex	*	**	**	**	NS	**	**	**	**	NS
H*sex	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a-c}Values with different superscript in a column differ significantly * $p < 0.05$, ** $p < 0.01$; C, H5, 10 20: hempseed ratio in diet as 5, 10 20%; SEM: standard error of means; NS: non significant.

ondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and nitric oxide (NO) activity showed in Table 5 and Figs. 1, 2, 3, 4 and 5. The serum protein levels were not affected by the hempseed addition into quail diets ($p > 0.05$). The serum MDA and NO levels were decreased by hempseed addi-

tion into quail diets ($p > 0.05$). In this study, all hempseed supplemented groups serum SOD, CAT and GSH-Px levels were increased compared to control group ($p < 0.001$). The serum MDA, SOD, CAT and GSH-Px values in male quail were higher than female, however in female serum NO level was higher than males ($p < 0.01$). Interactions

Table 5. Effects of hempseed inclusion to quail diets on serum malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase activity (GSH-Px) and nitric oxide (NO)

Groups	Protein (g/dl)	MDA ($\mu\text{mol/L}$)	NO ($\mu\text{mol/L}$)	SOD (U/mg protein)	CAT (kU/L)	GSH-Px (U/mg protein)
C	3.74	2.86 ^a	18.95 ^a	0.294 ^d	19.07 ^d	13.74 ^d
H5	3.75	2.62 ^b	18.34 ^b	0.309 ^c	19.12 ^c	15.46 ^c
H10	3.75	2.60 ^b	17.63 ^c	0.316 ^b	19.19 ^b	16.02 ^b
H20	3.75	2.49 ^c	17.46 ^c	0.328 ^a	19.34 ^a	17.13 ^a
Sex						
Male	3.7 ^b	2.15 ^a	17.68 ^b	0.32 ^a	19.23 ^a	15.92 ^a
Female	3.8 ^a	2.13 ^b	18.51 ^a	0.31 ^b	19.12 ^b	15.41 ^b
SEM						
Hempseed level	0.02	0.02	0.12	0.002	0.016	0.18
Sex	0.003	0.01	0.02	0.0004	0.004	0.22
Probability						
Hempseed level	NS	**	***	***	***	***
Sex	**	**	**	**	**	**
Interaction	NS	NS	**	*	*	NS

^{a-c}Values with different superscript in a columns are differ significantly; *** p <0.001; ** p <0.01, * p <0.05. C, H5, H10, H20: hempseed ratio in diet as 0, 5, 10 20% respectively; SEM: standard error of means; NS: non significant.

(hempseed and sex) affected serum NO (p <0.01), SOD and CAT values (p <0.01).

Discussion

The compositional analysis showed that hempseed contained 21.05% protein and 31.45% oil. These results were similar to the results obtained by Callaway (2004) and House *et al.* (2010). Also the fatty acids of hempseed oil were found C18:2, 52.51%; C18:3 23.24% and ω -6/ ω -3 ratio 2.26. Similar fatty acid content and ω -6/ ω -3 ratio were reported by Callaway (2004) and Uluata and Ozdemir (2012). The inclusion of hempseed to layer (Gakhar *et al.*, 2012), quail (Yalcin *et al.*, 2012) and feedlot (Gibb *et al.*, 2005) diets showed an increase in high ratio ω -6 and ω -3 fatty acids.

Hempseed supplementation to quail diet did not affect BW on d 7 and d 21 but inclusion of 20% hempseed decreased the BW of quail on d 42 (p <0.05). The FI and FCR values were not affected by the treatments. Silver-sides and Lefrancois (2005) and Gakhar *et al.* (2012) showed that including up to 20% hempseed meal in diets did not significantly influence the BW of laying hens. Also, similar results were obtained by Gibb *et al.* (2005) who reported that 0, 9 and 14% hempseed inclusion to feedlot cattle did not change BW, BWG and FI of steers. However, in contrast to our results, Khan *et al.* (2010) found that 20% hempseed inclusion to diets increased BW of broilers. However, as a processed product hempseed cake inclusion to broiler diets did not affect perfor-

mance traits (Eriksson and Wall, 2012). Analysis results showed that hempseed contained high percentages of crude ash (8.80%, Table 1) and cellulose (House *et al.*, 2010). This ratio is considerably high when compared to some traditional feeds such as corn, wheat and soybean meal. High crude ash and cellulose levels in diets may cause lower BW due to decreasing of energy and digestibility of feeds. On the other hand, it is reported that hempseed has high quality protein content and amino acid composition (Callaway, 2004). However, due to addition of hempseed some amino acid content may be excessively increased in diet, and imbalance of amino acid ratios in diet may have an antagonistic effect and lower amino acid bioavailability. Another reason of lower BW in H20 group may also be high related to THC content increase in the diet (Callaway, 2004). Unfortunately, there are only a few hempseed inclusion experiments in the literature to discuss and compare with our results.

Hempseed and its by-products have been used from ancient times until today as a medicinal plant (de Padua *et al.*, 1999). Today, there are still many unknowns about hempseed and its oil's nutritional value and effects when used in animal and human diets. The development and characterization of the bioactive contents in novel foods are required to provide scientific evidence to improve their quality and nutritional value (Yu *et al.*, 2005). The present study was designed to evaluate the inclusion of hempseed on performance and serum lipid antioxidant properties of Japanese quail. Hempseed contains 30% oil which is high in linoleic (LA) and alpha-linolenic acids

(ALA) (Callaway, 2004; Uluata and Ozdemir, 2012). The LA and ALA are necessary in body as essential fatty acids and protect cell uniformity in the diet for healthy individuals (Goldberg *et al.*, 2012).

In the H10 group the carcass and heart weights were higher than those of other groups. However, in the C group the liver weight was higher than 5 and 20% H groups and intestinal weight were higher than those of the other groups. When considered the relative incidence of carcass in the C group's carcass ratio was lower and liver and intestinal incidence was higher than those of the hempseed supplemented groups. The gizzard weights and incidences were not influenced by the treatments. Male quail carcass and internal organ traits without carcass incidence were lower than those of females. Khan *et al.* (2010) reported that 20% hempseed in broiler rations did not affect positively dressing percentage at 42 d. Callaway (2004) reported that hempseed has some beneficial effect on health in human and animals therefore it is hypothesized its inclusion may increase quail performance traits. However, in this study superior results were not observed in performance traits but increased carcass percentage.

The serum protein levels were not affected by the hempseed addition into quail diets. In this study, protein levels of all diets were adjusted at similar levels. On the other hand, hempseed protein level (21.2%, Table 1) was similar with the experimental quail diets (24%), therefore serum protein levels were not significantly affected by the hempseed inclusion. There are not enough studies results for comparison of our results with others. However, Karimi and Hayatghaibi (2006) reported that hempseed in rat diet after 20 d caused an increase in blood protein levels compared to blood protein level at first day of the experiment. Although, their experiment lack of control diet, there is a comparison with first and last day of the experiment to evaluate rat blood component.

The MDA, a carbonile group produced during lipid peroxidation, is used widely in determining oxidative stress and formed as an end product of lipid peroxidation (Sumida *et al.* (1989). Lipid peroxidation is a chain reaction providing a continuous supply of free radicals as it involves the oxidation of polyunsaturated fatty acids contributes to the loss of cellular functions through the inactivation of membrane enzymes and even of cytoplasmic proteins and consequences cell damage. On molecular level of lipid peroxidation, the importance of oxidative membrane damage is twofold (Stark, 2005). In the present study, the serum MDA levels were decreased by hempseed addition into quail diets. This may show that hempseed inclusion

to quail diets has a positive effect against to lipid peroxidation in the serum of Japanese quail. On the other hand hempseed contains some special protein types and amino acids (Lu *et al.*, 2010) and high ratio of ω -3 and ω -6 fatty acids which may protect the integrity of the cell membrane (Uluata and Ozdemir, 2012). Hu *et al.*, (2008) reported that serum MDA levels were decreased in test rats those fed by diets with hemp seed oil at the end of 90-day trial period compared with control groups of contained soybean oil and the normal diet. Also, it is reported that extract of cinnamon (Faix *et al.*, 2009; Ciftci *et al.*, 2010), rosemary (Yesilbag *et al.*, 2011), oregano (Botsoglou *et al.*, 2002) decreased MDA levels in broilers and quails (Bulbul *et al.*, 2012).

NO is produced at the cellular level from L-arginine catalyzed by nitric oxide synthase, a very important signaling molecule (Nathan, 1992). NO is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including vasodilatation, neuronal messenger, immunological response, antimicrobial and antitumor activities (Beckman and Koppenol, 1996; Balakrishnan *et al.*, 2009). However, NO participates in several cell processes as a highly reactive free radical (Moncada *et al.*, 1991). NO in the body is oxidized within seconds; in the presence of oxygen and the oxidation end-products of NO can oxidize cell lipids (Hassanpour *et al.*, 2009) consequently, cell damage occurs. In the current study, hempseed inclusion to diets caused a linear decrease in the serum NO levels. The control group serum NO level was higher than all other groups ($p < 0.001$). These results revealed that the inclusion of hempseed to quail diets exhibited well in NO scavenging activity. The present study shows that hempseed inclusion to diet may potent nitric oxide scavenging activity. Similar results with green tea were obtained by Hassanpour *et al.* (2010) which showed green tea supplementation to broiler diets reduced NO metabolites as free radical group.

The SOD, GSH-Px, and CAT are antioxidative enzymes act as the first step of antioxidant defense and protection cells against damage of free radical groups (Tang *et al.*, 2003). In the body, increase of these antioxidant enzymes enhances in scavenging activity for oxygen free radicals. In this study, all hempseed supplemented groups' the serum SOD, CAT and GSH-Px levels were increased compared to control group. Furthermore, when hempseed inclusion rate were increased, in a linear increase in the level of serum SOD, CAT and GSH-Px was observed ($p < 0.001$). These findings showed that hempseed inclusion increased total blood antioxidant enzyme capacity. Hu *et al.* (2008)

found that serum SOD and GSH-Px levels increased significantly at the end of 90-day feeding with hempseed oil. Hempseed and its oil contain some phenolic compounds, n3 and n6 fatty acids (Callaway, 2004), special peptides and protein types (Lu *et al.*, 2010) and they have protection ability against harmful effects of ROS in the body (Gebrekidan, 2012; Uluata and Ozdemir, 2012). In different experiment results showed that vegetable oils such as rosemary and garlic (Ancsin *et al.*, 2009) and cinnamon oil (Cifteci *et al.*, 2010) in broilers and essential oil's mixture of different plants (Bozkurt *et al.*, 2012) increased GSH-Px levels in layers.

Taken together, these results showed that the hempseed inclusion at 5, 10 and 15% levels to Japanese quail diet improved antioxidant status of animals. Because, hempseed has special fatty acids composition (Uluata and Ozdemir, 2012), phenolic compounds (Chen *et al.*, 2012; Uluata and Ozdemir, 2012), special proteins and amino acids (Gebrekidan, 2012), antimicrobial, immunomodulatory, antioxidative, antihypertensive and mineral binding properties (Korhonen and Pihlanto, 2003). In addition to Yu *et al.* (2005) reported that hempseed oil has better oxygen radical absorbing capacity than many plant oils. Therefore it has unique material to protect against ROS and protect cell membrane integrity. The researchers claimed that findings are significant for the discovery and development of natural antioxidants from hemp by-products (Callaway, 2004; Chen *et al.*, 2012; Korhonen and Pihlanto, 2003; Uluata and Ozdemir, 2012; Yu *et al.*, 2005). Results of the our study are confirming the findings of Callaway (2004) who reported that hempseed oil is a functional food for animal and humans and for animal feeding long-standing utility as an important food resource.

Conclusion

In conclusion, according to our results, up to 10% hempseed may use in quail diet without negative effect on performance and carcass traits and hempseed inclusion may increase carcass yield. In this experiment hempseed inclusion did not cause any significant change in serum protein levels, but caused decrease in serum MDA and NO levels and increase in serum SOD, CAT and GSH-Px as antioxidant parameters. These parameters revealed that hempseed have good potential as a natural antioxidant material for health improvement and disease prevention. In addition, hempseed ingredients as natural antioxidant source may contribute to longer duration of shelf life of animal products. There is no enough experiment on effect

of hempseed inclusion in animal diets and its nutritive and antioxidant efficacy in animals (Botsoglou *et al.*, 2002). Further experiments are needed to determine the effects of hempseed in poultry feeding.

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