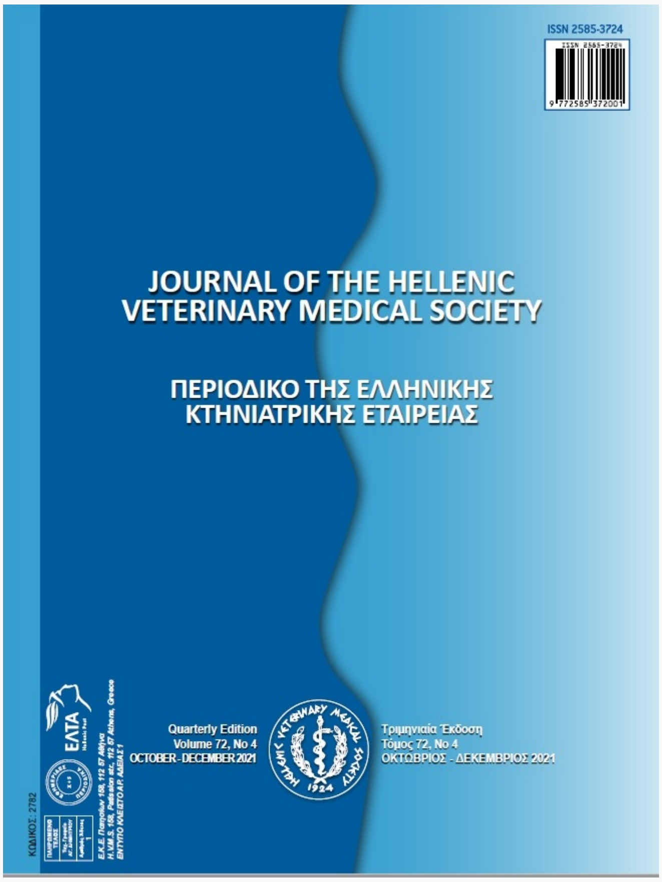
Journal of the Hellenic Veterinary Medical Society

Vol 72, No 4 (2021)



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doi: [10.12681/jhvms.29388](https://doi.org/10.12681/jhvms.29388)

J HELLENIC VET MED SOC 2021, 72(4): 3427-3434
ΠΕΚΕ 2021, 72(4): 3427-3434

Research article
Ερευνητικό άρθρο

Effect of hempseed meal on performance, egg quality and egg yolk fatty acids content in laying quails

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ABSTRACT: In this study, the laying performance, external egg quality, egg yolk colour and fatty acids profile of quails (*Coturnix coturnix japonica*) fed on diets containing hemp seed meal (HSM) were determined. During 8 weeks trial, a total of 150, 10-weeks-old laying quails were used. Five diets were formulated to contain HSM at the level of 0 (control),5, 10, 15, and 20% that represented as 0 HSM, 5 HSM, 10 HSM, 15 HSM and 20 HSM, respectively.The performance parameters were not significantly (P>0.05)influenced by the dietary HSM contents. Eggshell ratio, eggshell breaking strength, egg shape index, egg yolk index and egg yolk colour values were not significantly (P>0.05) influenced by the dietary HSM, whereas eggshell thickness was significantly (P<0.05) affected. The albumen index has been significantly (P<0.05) increased by increasing in the HSM level in the diets. The HSM supplementation to the diets was effective on fatty acid composition and total saturated fatty acids, total mono unsaturated fatty acids and total polyunsaturated fatty acids content of the egg yolk depending on the addition level. In conclusion, HSM can be used to increase egg total monounsaturated fatty acids and especially omega-3 fatty acids without unfavourable effects on the performance and egg quality parameters.

***Keywords*:** Quail; performance; egg yolk colour; eggshell quality; omega-3

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Date of initial submission: 13-10-2020
Date of acceptance: 21-12-2020

INTRODUCTION

Hemp (*Cannabis sativa L.*) is an important food and fiber source, and also used in drug production due to psycohotropic compound tetrahydrocannabinol content(Cromack 1998).Hemp seed is a rich source of some nutrients such as protein (25%), fat (34%), carbohydrates (34%), as well as high levels of some vitamins and minerals content. It also contains an amount of phenolic compound and tocopherol that are beneficial for human health (Oomah et al. 2002; Kriese et al. 2004; Chen et al. 2012).Phytic acid, tannins, and trypsin inhibitors are called anti-nutritional factors and found in cannabis seed in small amounts(Russo and Reggiani 2015). Hemp seed meal is a product obtained by pressing the seeds and removing the oil from them. The product contains 30-50% protein in dry matter depending on the hemp variety used and the oil extraction methods(Malomo et al. 2014; Wang and Xiong 2019).Protein and amino acid content of HSM has high digestibility (Callaway et al. 2005; Yu et al. 2005; Hu et al. 2008; Girgih et al. 2011) The major fatty acids of hempseed oil are linoleic (60%) and alpha-linolenic (19%) acids and its total polyunsaturated fatty acids ratio is around 75 - 80% (Callaway 2004; House et al. 2010). The cultivation of hempseed has been limited by the regulations in many countries including Turkey for a long year(Anonymous 1990). Hempseed and its by-products have not subjected to the numerous researches of animal nutrition due to the restricted production. However, in some countries (Canada) with the releasing of cannabis agriculture, hempseed by-products have become able to use as animal feed(Callaway 2004; Karche 2019). The nutrient values of hemp seeds and its products are valuable enough to be a source of feed in poultry(Wang and Xiong 2019). Since poultry especially need high quality protein sources in the diet, HSM is a feed raw material that needs to be emphasized to meet their needs. In addition, it is reported that its contents of oil is quite rich in terms of unsaturated fatty acids, and it will be effective in changing the egg fatty acid content in laying hens(Gakhar et al. 2012; Konca et al. 2014).Research has shown that HSM in laying hens diet increases the amount of omega-3fatty acids found in eggs(Silversides and Lefrancois 2005; Neijat et al. 2014). Japanese quail (Coturnix coturnix japonica) is used as a model animal and is one of the smallest birds used for its egg and meat production (Shokoohmand 2008; Narinc et al. 2013). Quail provides more advantages than the chicken such as the low maintenance cost associated with its small body size (80-300g) coupled with its short generation interval, resistance to diseases and high egg production (Mills et al. 1997). Lately, quail meat has gained much popularity among consumers (Ikhlas et al. 2011). It is an ideal food for all ages due to its high meat yield, less shrinkage during cooking, being more effortless to cook, and being more easy to serve (Mountney 2012). For this purpose, it will be important to increase the studies examining the use of hemp meal in poultry. In this study, the effects of using different levels of HSM in laying quail diets on production performance, egg's internal and external quality and egg yolk fatty acids content were investigated.

MATERIAL AND METHODS

A total of 150, 10 - weeks - old laying quails (*Coturnix coturnix japonica*) were used in this study. The laying quails were divided into 5 treatment groups with 6 replicates (5 quails, each). The laying quails were reared in 33 × 40 ×28 cm size cages under the semi-controlled periphery terms (ventilation controlling system) with 16-h light - 8 h dark illumination period. Quails were provided with feed and water *ad-libitum* for 8 weeks experimental period. Quails were experimental diets were formulated to meet the nutrient requirements of National Research Council(Council 1994) (Table 1). Hemp seeds were provided from a local supplier. The seeds were pressed in a cold press machine (Karaerler Machine, NF 100 model, Ankara) at 45-50 °C and HSM was obtained by removing the oil. The HSM used in experimental diets contained 30.4% crude protein and 10.9% crude oil. Experimental diets were formulated as the control (basal diet) and the diets containing 5, 10, 15, and 20% HSM. All treatment diets were adjusted as to be isocaloric and isonitrogenic.

The measured parameters:

Quails were weighed at the first and at end the last day of the trial and the body weight changes were determined. Feed intake as determined on the 28th and 56th days of experiment. Feed conversion ratio was calculated with the ratio of feed intake (g) and egg mass (g). Egg production was recorded daily and calculated on the 28th and 56th days of experiment.

The average egg percentage (%) was detected with the formula of the (total eggs/ total quail) × 100. Egg mass was determined from the ratio of egg production (%) to egg weight (g).A total of 480 eggs (96 eggs

Table 1. The nutrients composition of experimental diets

Ingredients	Dietary hempseed meal (HSM) levels, %				
	0	5	10	15	20
Corn	50.10	47.70	44.85	42.35	39.90
Hempseed meal (30.4 %CP)	0	5.00	10.00	15.00	20.00
Soybean meal (45% CP)	36.00	33.10	30.30	27.40	24.50
Vegetable oil	6.20	6.50	7.00	7.40	7.75
Limestone	5.10	5.10	5.15	5.15	5.15
Dicalcium phosphate	1.80	1.80	1.85	1.85	1.85
Salt	0.25	0.25	0.25	0.25	0.25
Premix[1]	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.10	0.10	0.15	0.15	0.15
DL-Methionine	0.20	0.20	0.20	0.20	0.20
Calculated nutrients%					
Crude protein	20.01	20.02	20.04	20.04	20.05
Energy, kcal ME/kg	2904	2900	2902	2903	2902
Calcium	2.50	2.50	2.52	2.52	2.52
Available phosphorus	0.35	0.35	0.36	0.36	0.36
Lysine	1.05	1.04	1.05	1.04	1.03
Methionine	0.48	0.48	0.49	0.50	0.50
Methionine + Cystine	0.84	0.85	0.85	0.86	0.86

[1]: Premix provided the following per kg of diet: retinyl acetate, 4.0 mg; cholecalciferol, 0.055 mg; DL- α-tocopheryl acetate, 11 mg; nicotinic acid, 44 mg; calcium-D-pantothenate, 8.8 mg; riboflavin sodium phosphate, 5.8 mg; thiamine hydrochloride, 2.8 mg; cyanocobalamin, 0.66 mg; folic acid, 1 mg; biotin, 0.11 mg; choline, 220 mg; Mn, 60 mg; Fe, 30 mg; Cu, 5 mg; I, 1.1 mg; Se, 0.1 mg

for each group) were collected in the last two days of each 14- days period. Egg samples selected randomly from each sub-group and weighed and then determinated to have eggshell breaking strength, eggshell thickness, eggshell weight, egg shape index, egg yolk index and albumen index. Egg yolk height and albumen height were determined by digital height caliper. Egg yolk diameter, egg and egg albumen length and width were determined by digital caliper (Mitutoyo Inc., Japan). Egg shape(Anderson et al. 2004), egg yolk index and albumen index (Romanoff and Romanoff 1949) were computed by following formulas respectively; [Egg width/ Egg length) x 100], [(yolk height/yolk diameter) x 100], [(albumen height/(average albumen length and width)) x 100]. Eggshell breaking strength was measured using the compression test module and resistance of eggshell broad pole to pressure was determined (Orka Food Technology Ltd., Ramat Hasharon, Israel). The cracked eggshells were washed and dried, then weighed using a 0.01 g precision scale. Eggshell weight ratio was calculated using the formula: Eggshell weight (%) = [eggshell weight (g)/egg weight (g)]/100. The thickness of the egg shell (with membrane) was determined as the average of the measurements made from the blunt end with two points on the equatorial axis of the egg using a micrometer(Mitutoyo Inc., Japan).

The yolk colour was determined using Minolta CR-410 colorimeter (Konica Minolta, Osaka, Japan). The L*, a* and b* parameters correspond to the lightness (-100/+100, dark/white), redness (-100/+100, green/red) and yellowness (-100/+100, blue/yellow), respectively.

Fatty acid profile of a total 150 egg yolk (30 eggs for each group) was detected and oils were extracted by the solvent method (Ethanol/chloroform solvent) (Kovalcuks and Duma 2014). Fatty acid methyl esters of the egg yolk oils were obtained according to the method of the recommendation of the European Union (EU) regulation 2568/91(Regulation 1991). Egg yolk oils were weighed (0.10 g) into the screw-cap glass tubes and dissolved within 10.0 mL hexane. Following, 100 µL 2N potassium hydroxide solution in the methanol was added to the tubes and shaken vigorously for 30 s. The tubes centrifuged at 2500 × g for 5 min and the upper layer was taken to a small vial and stored at 0°C till analysing date(Ayyildiz et al. 2015).The fatty acid composition was detected by gas chromatography (GC) device (Shimadzu GC-2010 Plus, Japan) which had the FID detector and HP-88 column (100m ×250 µm×0.20 µm id). The temperature at the injection block was 250 °C and the column oven heat program was adjusted as 2 min at 50 °C, 4 min between 50 °C -250 °C, and 10 min at

250 °C. The carrier gas was Helium with 1.3 mL/min flow rate. Fatty acids were detected by using retention time (min) and area (%) data of identified peaks and classed with standards of fatty acids and were presented as a percentage.

The trial was designed as a complete randomized model and data were analysed by using the ANOVA procedure with Minitab (Minitab 2000). Duncan's multiple range test was used to determine the differences among treatments which found significantly different (P<0.05).

RESULTS AND DISCUSSION

The effects of hempseed meal on performance parameters are shown in Table 2. The results showed that the effect of dietary different levels of HSM on the initial and final body weight and body weight changes of quails were not statistically significant (P> 0.05).

Similar body weight gain among quails groups indicated that HSM had no negative effect and it meet the nutrient requirements needs of birds. Konca et al. (2014) reported that different dietary levels of hempseed for quails had no effect on final body weight and body weight changes. Neijat et al. (2014) found that there was no significant difference among treatments in terms of body weight changes of laying hens, after using of dietary hempseed at 10, 20 and 30% levels. Similarly, Gakhar et al. (2012) and Silversides and Lefrancois (2005)demonstrated that laying diets containing up to 20% HSM did not significantly affect on the body weight at the end of the experiment. Contrary results were obtained by Khan et al. (2010) who stated that addition of 20% HSM to the diet increased the body weight of broilers, as well as Gakhar et al. (2012) who recorded that feed intake and feed efficiency were not affected by the addition of hempseed up to 20% to the diet in broilers.

This study revealed that adding different levels of HSM to the laying quail's diets induced no significant (P> 0.05) effect on egg production, feed intake, feed conversion rate, egg weight and egg mass. Supplementation of diets of laying hens with 10, 20 and 30% levels of HSM had no significant effection egg production and egg weight (Neijat et al. 2014).Moreover, laying diets supplemented with 20% HSM did not significantly affect the egg production, feed intake and feed conversion ratio (Silversides and Lefrancois 2005).Konca et al. (2014)detected that different levels of hempseed (5, 10 and 20%)didn't significantly affect the egg production, egg weight and egg mass. Whereas 15% raw hempseed(RHS)and 15% heat treated hempseed(HHS) added to the laying hen diets did not affect the feed conversion rate and egg yield, the feed consumption of the RHS group was less compared to the HHS and control groups. Egg weight and egg mass were not significantly different in RHS and HHS groups compared to the control group (Konca et al. 2019).

The present work demonstrated that the used levels of HSM induced no significant (P> 0.05) effects regarding the egg shell ratio and egg shell breaking strength. The egg shell thickness was significantly (P <0.05)affected by addition of HSM to the diets as level up to 5-20% decreased the shell thickness compared with control group (Table 3). Previously, Neijat et al. (2014) recorded that the egg shell weight and thickness of laying hens were not affected by diets containing HSM at levels of 10, 20 and 30%. Another study on laying hens showed that egg shell thickness was similar in groups fed with 0, 10 and 20% hempseed(Gakhar et al. 2012). In addition, Konca et al. (2019) noticed that the egg shell weight, egg shell ratio and eggshell thickness were not significantly affected with raw and heat-treated meals with hemp seeds.

Here, giving laying quails diets containing different levels of HSM didn't significantly (P> 0.05) affect on the egg shape and yolk index, while their effect on the albumin index was significant (P <0.05). The albumen index was statistically higher in groups containing 15 and 20% HSM than control group, but was statistically similar to those treated with 5 and 10% (Table 3). It has been documented that there was no significant difference in yolk and albumen height among groups fed with diets containing 4, 8 and 12% HSM (Gakhar et al. 2012).Albumin height was not affected by raw and heat-treated hemp seeds(Konca et al. 2019).

As seen in Table (3), diets containing different levels of HSM induced no statistical significant (P> 0.05) effect on egg yolk colour of quails.

Xanthophyll pigments and oxy-carotenoids are the main factors that determine the egg yolk colour, which affects consumer preferences(Vuilleumier 1969). Skřivan et al. (2019) noticed that addition of 3% hempseed to the diet of laying hens increased the egg yolk redness, while level of 9% produced adverse

Table 2. Effect of different levels of dietary HSM on performance in Japanese quail

Performance Parameters	0 HSM	5 HSM	10 HSM	15 HSM	20 HSM	*SEM*
Initial body weight, g	219.73	217.00	219.30	208.70	218.57	*3.507*
Final body weight, g,	266.12	268.36	262.30	260.61	259.40	*5.632*
Body weight change, g	46.38	51.36	43.00	51.91	40.83	*5.855*
Egg production, %	90.81	90.90	91.79	91.87	90.18	*1.516*
Feed intake, g	27.42	27.45	27.12	28.49	27.85	*0.478*
Feed conversion ratio, (Feed/Egg mass)	2.45	2.51	2.40	2.43	2.44	*0.052*
Egg weight, g	12.35	12.14	12.37	12.79	12.65	*0.158*
Egg mass, g	11.21	10.96	11.36	11.76	11.40	*0.225*

SEM: Pooled standard error of means. Diets contain hemp seed meal at the level of 0 (control), 5, 10, 15, and 20% for experimental groups named as 0HSM, 5HSM, 10HSM, 15HSM and 20HSM respectively

Table 3. Effect of different levels of dietary HSM on eggshell quality in Japanese quail

Egg Quality Parameters	0 HSM	5 HSM	10 HSM	15 HSM	20 HSM	*SEM*
Eggshell weight ratio, %	7.97	8.45	8.09	8.56	8.26	*0.173*
Eggshell thickness, mm	0.292^{b}	0.316^{a}	0.318^{a}	0.324^{a}	0.319^{a}	*0.005*
Eggshell breaking strength, kg	1.54	1.57	1.60	1.55	1.51	*0.045*
Egg shape index	76.99	78.35	77.65	76.60	78.01	*0.551*
Egg yolk index	43.70	42.60	43.48	43.09	43.53	*0.337*
Albumen index	8.64^{b}	9.39^{ab}	9.19^{ab}	9.71^{a}	9.67^{a}	*0.221*
Egg Yolk Colour Value						
L*	53.95	52.39	51.84	52.49	52.14	*0.690*
a*	13.49	14.60	14.50	13.78	14.04	*0.566*
b*	41.99	41.36	39.79	42.31	41.76	*0.697*

[a, b]: Differences between the averages are significant in the same column with different letter (P<0.05). SEM: Pooled standard error of means. Diets contain hemp seed meal at the level of 0 (control), 5, 10, 15, and 20% for experimental groups named as 0HSM, 5HSM, 10HSM, 15HSM and 20HSM respectively

Table 4. Effect of different levels of dietary HSM on egg yolk fatty acids content in Japanese quail

Egg yolk fatty acids content (%)	0 HSM	5 HSM	10 HSM	15 HSM	20 HSM	*SEM*
Myristic acid (14:0)	0.355^{a}	0.345^{ab}	0.288^{abc}	0.275^{bc}	0.260^{c}	*0.0163*
Palmitic acid (16:0)	22.97^{a}	23.07^{a}	20.20^{b}	19.39^{b}	19.83^{b}	*0.2426*
Palmitoleic acid (16:1)	3.49^{a}	2.66^{b}	2.64^{b}	2.42^{b}	2.40^{b}	*0.1537*
Stearic acid (18:0)	9.26	10.23	10.55	10.49	10.33	*0.4483*
Oleic acid (18:1)	39.85^{ab}	37.41^{b}	41.18^{a}	40.09^{ab}	39.67^{ab}	*0.6350*
Linoleic acid (18:2)	18.93^{b}	21.57^{a}	16.60^{b}	18.09^{b}	18.28^{b}	*0.6010*
α-Linolenic acid (18:3, n3)	1.68^{b}	1.29^{b}	5.62^{a}	6.17^{a}	6.17^{a}	*0.1726*
Arachidic acid (20:0)	0.473^{a}	0.460^{a}	0.310^{b}	0.413^{ab}	0.415^{ab}	*0.0330*
Eicosapentaenoic acid (EPA; 20:5)	0.044^{b}	0.039^{b}	0.247^{a}	0.246^{a}	0.254^{a}	*0.0177*
Erucic acid (22:1)	1.84^{a}	1.89^{a}	1.18^{b}	1.21^{b}	1.24^{b}	*0.0713*
Docosahexaenoic acid (DHA; 22:6)	0.200^{b}	0.228^{ab}	0.250^{a}	0.265^{a}	0.258^{a}	*0.0114*
ΣSFA	33.29^{ab}	34.33^{a}	31.59^{bc}	30.81^{c}	31.08^{c}	*0.5001*
ΣMUFA	45.18^{a}	41.96^{b}	45.00^{a}	43.75^{ab}	43.27^{ab}	*0.6813*
ΣPUFA	20.86^{b}	23.10^{ab}	22.71^{ab}	24.75^{a}	24.95^{a}	*0.6529*
Σ omega−3	1.93^{b}	1.54^{b}	6.12^{a}	6.67^{a}	6.67^{a}	*0.1950*

[a, b, c]: Differences between the averages are significant in the same line with different letter (P<0.05). SEM: Pooled standard error of means. Diets contain hemp seed meal at the level of 0 (control), 5, 10, 15, and 20% for experimental groups named as 0HSM, 5HSM, 10HSM, 15HSM and 20HSM respectively.

effect on the color. The egg yolk colour did not increase linearly with increasing HSM levels. Chickens egg yolk colour was higher in group fed with raw hempseed than those of control and heat-treated hempseed groups(Konca et al. 2019).In the study of Goldberg et al. (2012), addition of hempseed oil to the laying hens diets induced significant decrease in egg yolk lightness(L*) but significant increase in redness(a*) and yellowness(b*) and the largest changes were observed in the group with 20% hempseed. The effect of diets containing different levels of HSM in laying quail on egg yolk fatty acid profile is presented in Table (4).

The highest level of saturated fatty acid in egg yolk oil was palmitic acid, while the unsaturated fatty acid was oleic acid. The addition of HSM caused significant ($P<0.05$) decrease in the content of palmitoleic acid. In a comparison with control group, addition of HSM at levels 10% and above induced lower palmitic acid and levels of 15% and above produced significant ($P<0.05$)reduction in myristic acid content. Oleic and linoleic acid contents were significantly ($P<0.05$) influenced by the addition of HSM to the diet. The highest oleic acid content was found in group treated with 10% hempseed whereas the highest linoleic acid in 5% hempseed treated birds. The contents of α-linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)increased significantly($P<0.05$) when the level of HSM in the diet was 10% and above. This result showed that HSM significantly ($P<0.05$) enriched the yolk fatty acid composition in favour of omega-3 fatty acids. In addition, when the level of HSM in the diet was 10% and above, the level of erucic acid decreased significantly. The effect of adding HSM to the diet on total unsaturated fatty acids, total monounsaturated fatty acids and total polyunsaturated fatty acids contents was significant. When the level of HSM in the diet was 15% and above, the content of ΣSFA decreased while ΣPUFA content increased compared to the control group.

Inclusion of HSM in to laying quails diet significantly changed fatty acid content of egg yolk ($P<0.05$). Result of the current study showed that dietary HSM may altered the fatty acid profile of egg yolk. Similar results were obtained in quails (Konca et al. 2014; Konca et al. 2019) and in chickens(Shahid et al. 2015). Raza et al. (2016) reported on a significant reduction in palmitic acid content of the egg yolk of laying hens as a result of addition of 25% hempseed to the ration. The results of the studies showed that with the addition of hempseed to the diet, the overall decrease in saturated fatty acids(SFA) in egg yolk is due to the high content of unsaturated fatty acids of hemp seeds(Konca et al. 2014; Shahid et al. 2015; Raza et al. 2016; Konca et al. 2019). According to the results of this study, the major change in fatty acid composition in egg yolk was the increase in PUFA (α-Linolenic acid, EPA and DHA). Reducing the SFA content and increasing the omega-3 amount provides an advantage for the health of consumers(Ayerza and Coates 2000). Goldberg et al. (2012) described significant increase in the amount of EPA in the egg yolk of hempseed fed hens compared to the control group. Although hempseeds do not contain docosahexaenoic-rich content, the amount of this fatty acid has been increased in the eggs of quail fed with HSM. The fatty acid docosahexaenoic is gained in eggs by two ways. It may be taken directly by diet or synthetized from α-linolenic acid(Yalçın and Ünal 2010). Similar study by Konca et al. (2019) revealed an increase in the synthesis of DHA as a result of high accumulation of α-linolenic acid. This work denotes an increase in omega-3 fatty acids in groups containing 10% and above HSM. Silversides and Lefrancois (2005) and Gakhar et al. (2012) stated that the eggs content of omega-3 fatty acids was increased with increasing the dietary hempseed. Furtheremore, Goldberg et al. (2012) found that the total omega-3 content in egg yolk was higher than the control group after addition of 12% hempseed oil to the laying hens diets.

CONCLUSION

According to the results of this study, addition of HSM to laying quail diets did not induce any negative effects on body weight change, egg production, feed intake, egg weight and egg mass. In addition, supplementation of HSM did not cause significant changes in the egg shell quality characteristics and egg yolk colour. However, 10% or more levels of HSM decreased the egg yolk SFA content but increased the MUFA and omega-3 fatty acid content. These results showed that HSM can be used to increase egg total MUFA especially omega-3 fatty acid. Comprehensive studies on the utilization of hempseed and its by-product in poultry nutrition are needed.

ACKNOWLEDGEMENT

This study was funded by Coordination of Scientific Research Projects of Selçuk University, Project Number 1840105

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