



Evaluation of Dietary Hempseed and Hempseed Oil on Performance, Egg Quality and Some Blood Parameters in Laying Hens after Peak Period

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Abstract

The aim of this study was to recognize the effects of dietary hempseed and hempseed oil on performance, egg quality and blood parameters of laying hens. A total of 320 Hy-line 55-weeks (post-peak egg production) laying hens were randomly allotted to eight dietary treatments; each with five replicates (8 birds each). The experimental treatments were four levels of hempseed (5, 10, 15, 20%) or three levels of hempseed oil (2, 4, 6%) added to the basic diet, as well as a control group (no hempseed or hempseed oil added to the diet). The experiment lasted 12 weeks (three 4-week periods) and began at the hen's age of 55 wk. Eggs were collected daily and weighed. Birds fed 6% hempseed oil had the highest egg production and egg mass as well as the lowest feed intake and feed conversion ratio ($P < 0.05$). Increasing hempseed oil levels from 4 to 6% increased the Haugh unit in the third period ($P < 0.05$). The egg yolk color index decreased when either hempseed or hempseed oil were added to the diet though hempseed decreased yolk index to a greater degree. The treatments did not influence total protein level in plasma. The highest level of hempseed oil (6%) significantly decreased blood plasma cholesterol, triglycerides and aspartate aminotransferase compared to the highest level of hempseed (20%) ($P < 0.05$). Blood HDL-C level was higher in hens fed the 6% HO diet compared to those fed 20% HS. In conclusion, the two lower levels of hempseed (5 and 10%) had suitable effects on the performance of laying hen post-peak, and the addition of hempseed oil to diets was more effective on egg quality than hempseed addition. Therefore, we suggest that hempseed oil is a usable oilseed in laying hen diet.

Keywords

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Introduction

The oilseed of hemp (*Cannabis sativa*) is rich in nutrients and can be added to poultry feed to fulfill poultry dietary requirements, especially for energy and protein. However, hemp and its products including whole hempseed, oil, and meal, are not produced in bulk for use in commercial poultry

industry. The main bioactive component in hempseed (HS) is delta-9 tetrahydrocannabinol (THC) which it is psychoactive that affects human behavior. Hempseed products are only permitted for purposes according to the regulation (EC) No. 2860/2000 in the European United. For example,

the content of THC must be less than 0.2 and 0.3% in European United and Canada, respectively (Health Canada, 2010; Grotenhermen *et al.*, 2011).

Hempseed contains 32-34% carbohydrate, 33-35% fat, 25% crude protein and 9-11% other components including fiber, vitamins and minerals. Hempseed is secondary protein resource, slightly below soybean (25% versus 32%; Amerio, 1998; Callaway, 2004). Nonetheless, the main advantage of hempseed protein in comparison to soybean is the lack of oligosaccharide derivatives which may cause undesirable effects on stomach function (Karimi and Hayatgheybi 2006). Oilseeds such as HS are used in layer diets to provide energy and essential fatty acid requirements. The hempseed oil (HO) contains 75-80% polyunsaturated fatty acids (PUFA; Karimi and Hayatgheybi, 2006) which involve 60% linoleic acid and 17-19% α -Linolenic acid (LNA; Parker *et al.*, 2003). The HS ranks third in LNA content after flaxseed and chia (the LNA percentage in other vegetable oils is less than nine) (Gakhar *et al.*, 2012). The types of oilseed and vegetable oils in laying hen diets affect performance and egg quality parameters such as egg production, egg mass, feed intake, Haugh unit, and yolk index (Baucells *et al.*, 2000; Hosseini-Vashan *et al.*, 2009). Dietary oil quality also changes total serum lipid and lipoprotein (Mensink and Katan, 1992). Gakhar *et al.* (2012) reported that different concentrations of HS did not affect feed intake, body weight, feed conversion ratio, egg production and egg mass. In contrast, HS had a significant quadratic effect in blood activity of aspartate aminotransferase (AST) in laying hens (Neijat *et al.*, 2014). Little is known about the possibility of using HO and HS in poultry nutrition. This study was conducted to compare the effects of different levels of HS and HO on performance, egg quality and blood parameters of layers after peak of egg production.

Materials and Methods

Birds and housing

A total number of 320 Hy-line W-36, 55-weeks laying hens were provided from a commercial laying farm (Behparvar Com., Birjand, Iran). Hens were permitted an adaptation period of 12 d before beginning the experiment. Layers were housed in cages (dimensions 60 × 80 cm) with 12 cm² of space per bird. Temperature was maintained at 20±2°C. The lighting schedule was

16:8 hours light:dark for an experimental period of 12 weeks. Feed and water were supplied *ad libitum*. The Hy-line catalog was applied for rearing layers. All the experimental procedures were approved by Birjand University according to Animal Care guidelines. All animal research procedures were assessed and approved by the animal care committee (Wager and Kleinert, 2012).

Diets

Whole hempseed (HS) and hempseed oil (HO) were supplied from a commercial company (Khorasan oilseed Co, Mashhad, Iran). The composition of whole and oil hempseed was determined based on approximate analysis by the AOAC (1990) method. The HS and HO had 23 and 0 percent of crude protein, 32 and 100 percent of ether extract, 5.5 and 0 percent of crude ash as well as 5.550 and 11.120 Mcal/kg of gross energy, respectively. Laying hens were divided into 40 experimental units assigned to eight dietary treatments. The mean body weight of layers was 1.679±0.085 g at the beginning of the experiment. Eight diets were formulated to meet most of the nutrient requirements of Hy-line laying hens in the post-peak period (55-67 wk) as denoted in the strain's layer management guide (Hy-line W-36 management, 2016). The dietary treatments included a control group, levels of 5, 10, 15 and 20% of HS, and levels of 2, 4 and 6% of HO. Each dietary treatment was fed to five groups (replicates) of eight hens each. The diets were balanced to meet the layer requirements in Hy-line W-36 manual guide by UFFDA software. All diets were formulated to be isonitrogenous and isoenergetic (Hy-line W-36 management, 2016). The ingredient and chemical composition of the experimental diets are shown in Table 1.

Production parameters

Egg production and egg weight were recorded daily by the cage. The total feed intake was calculated weekly as the difference between feed offered and residual feed in the feeder. The egg mass and feed conversion ratios (feed consumption: egg weights) were computed weekly.

Egg quality parameters including Haugh unit score, egg shape, yolk color index (as measured by Roche yolk color fan), albumen weight percentage, yolk weight percentage, and shell

weight percentage were determined in two samples per replication that were randomly selected at the end of 28 days (10 eggs for each treatment).

Table 1. Ingredient and chemical composition of the experimental diets

Ingredient (%)	Control	Hempseed (%)				Hempseed oil (%)		
		5	10	15	20	2	4	6
Corn	58.6	57.9	53.0	48.1	43.3	41.3	41.3	41.3
Hempseed	0.00	5.00	10.00	15.0	20.0	0.00	0.00	0.00
Soybean (44% CP)	22.7	20.2	17.4	14.6	11.8	20.2	20.2	20.2
Hempseed oil	0.00	0.00	0.00	0.00	0.00	2.00	4.00	6.00
Oil	1.71	0.50	0.50	0.50	0.50	4.00	2.00	0.00
Salt	0.31	0.27	0.28	0.27	0.25	0.27	0.27	0.27
DCP	1.95	1.64	1.55	1.47	1.39	1.22	1.22	1.22
CaCO ₃	7.52	7.51	7.51	7.51	7.47	7.58	7.58	7.58
Oyster shell	3.47	3.41	3.45	3.49	3.53	3.54	3.54	3.54
Methionine	0.13	0.11	0.14	0.17	0.17	0.16	0.16	0.16
Lysine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Enzymite	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Wheat bran	2.00	1.85	4.56	7.28	9.98	18.12	18.12	18.12
Vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Threonine	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium Bicarbonate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vit A, E, D3 and K	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Chemical composition (%)</i>								
ME (Kcal/kg)	2655	2655	2655	2655	2655	2655	2655	2655
Crude Protein	15.4	15.4	15.4	15.4	15.4	15.4	15.4	15.4
Calcium	4.69	4.60	4.60	4.60	4.60	4.60	4.60	4.60
Available Phosphorus	0.500	0.450	0.450	0.450	0.450	0.450	0.450	0.450
Sodium	0.160	0.150	0.160	0.170	0.180	0.180	0.180	0.180
Methionine + Cystine	0.630	0.610	0.600	0.600	0.610	0.600	0.600	0.600
Lysine	0.780	0.740	0.690	0.650	0.610	0.760	0.760	0.760
Threonine	0.570	0.580	0.540	0.600	0.300	0.540	0.540	0.540
Linoleic acid	2.19	1.81	1.94	2.07	2.20	4.02	3.96	3.84
Fat	3.62	3.51	4.62	5.72	6.83	8.08	8.08	8.08
Fiber	2.96	2.81	2.65	2.50	2.34	2.98	2.98	2.98

*The vitamin premix provided per kilogram of diet: 11,000 IU of vitamin A; 3,000 IU of vitamin D3; 150 IU of vitamin E; 3 mg of vitamin K (as menadione); 0.02 mg of cyanocobalamin; 6.5 mg of riboflavin; 4 mg of folic acid; 10 mg of calcium pantothenate; 40.1 mg of niacin; 0.2 mg of biotin; 2.2 mg of thiamine; 4.5 mg of pyridoxine; 1,000 mg of choline; 125 mg of ethoxyquin (antioxidant); The mineral premix provided per kilogram of diet: 66 mg of Mn (as manganese dioxide); 70 mg of Zn (as zinc oxide); 80 mg of Fe (ferrous sulfate); 10 mg of Cu (as copper sulfate); 0.3 mg of Se (as sodium selenite); 0.4 mg of I (as calcium iodate); and 0.67 mg of iodized salt. Ground wheat was used as a carrier for the vitamin-mineral premix.

Blood biochemistry

At the end of the experiment, 5 mL of blood was removed from the bronchial wing vein of two birds per replicate then transferred to a heparinized tube. These samples were centrifuged for 15 minutes at 3000 × g to isolate plasma, which were then stored in a freezer at -20°C. Biochemical parameters in the blood were following: triglycerides, total proteins, cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and enzyme activity of aspartate

aminotransferase. The blood parameters were measured by automated analyzer spectrophotometer (Gesam Chem 200, Italy).

Statistical analysis

Data were analyzed in a completely randomized design with GLM procedure of SAS 9.12 software (2014). Tukey's procedure was used to compare the treatments means. The significance levels were based on $P < 0.05$.

Results

Performance parameters

The results for laying performance are shown in Table 2. Egg production was significantly affected by HS and HO during 63-67 weeks. Birds receiving diets containing 6% HO had the greatest egg production which was significantly different to the control group ($P < 0.05$). Egg weight and egg mass were not affected by the dietary treatments, with the exception of eggs of chickens fed 6% HO during weeks 59-63 had

statistically greater weight compared to the control ($P < 0.05$).

The inclusion of HS to base diet increased feed intake during weeks 63-67. Diet containing 20% hempseed had higher feed intake compared to control diet ($P < 0.05$). A lower feed conversion ratio was observed when hens were fed 6% HO during weeks 59-63 ($P < 0.05$). Although the lowest level of HS increased the feed conversion ratio, higher levels of HS significantly lowered FCR ($P < 0.05$).

Table 2. Laying performance of hens fed diets contained hempseed and hempseed oil in three experimental periods

parameters	weeks	Control	Hempseed (%)				Hempseed oil (%)			SEM	P-value
			5	10	15	20	2	4	6		
Egg production (%)	55-59	73.6	76.9	71.4	75.1	74.7	74.4	78.1	80.0	1.95	0.095
	59-63	64.6	66.3	67.5	68.6	65.5	66.6	66.8	68.5	1.57	0.609
	63-67	70.7 ^b	75.0 ^{ab}	77.0 ^a	77.2 ^a	75.3 ^{ab}	77.4 ^a	77.1 ^a	79.0 ^a	1.32	0.005
Feed Intake (g)	55-59	96.3	95.4	91.1	91.0	97.7	96.0	92.8	94.2	2.27	0.339
	59-63	98.1	96.8	94.8	94.6	97.3	96.2	96.5	97.4	1.30	0.527
	63-67	95.3 ^{ab}	96.6 ^{ab}	93.5 ^{ab}	92.9 ^b	97.5 ^a	94.3 ^{ab}	92.6 ^b	95.5 ^{ab}	0.956	0.008
Egg Weight (g)	55-59	67.1	70.1	65.5	77.8	69.9	67.1	73.4	68.9	3.79	0.392
	59-63	69.3	72.4	70.2	62.6	62.9	67.4	62.0	69.2	5.17	0.759
	63-67	65.7	65.7	64.4	62.9	64.3	67.3	63.9	64.2	1.79	0.756
Egg Mass (g/bird/day)	55-59	48.0	54.0	47.2	54.4	52.2	51.2	54.4	55.0	2.55	0.239
	59-63	43.6 ^b	51.1 ^{ab}	50.1 ^{ab}	49.5 ^{ab}	46.6 ^{ab}	49.1 ^{ab}	48.9 ^{ab}	55.0 ^a	2.18	0.050
	63-67	49.2	46.7	52.4	48.8	48.3	51.1	51.3	55.3	2.44	0.553
FCR (g Feed: g egg production)	55-59	2.14	1.82	2.03	1.70	1.95	1.98	1.76	1.76	0.122	0.162
	59-63	2.38 ^a	1.95 ^{ab}	1.95 ^{ab}	1.99 ^{ab}	2.18 ^{ab}	2.01 ^{ab}	2.00 ^{ab}	1.81 ^b	0.113	0.446
	63-67	1.99 ^{ab}	2.23 ^a	1.78 ^b	1.98 ^{ab}	2.25 ^a	1.89 ^{ab}	1.94 ^{ab}	1.97 ^{ab}	0.090	0.013

^{a,b}Means within a row that do not have a common superscript are significantly different ($P < 0.05$).

Egg quality parameters

The results for egg quality parameters are shown in Table 3. Dietary treatments did not have a significant effect on shape index, yolk percentage, shell percentage, and albumen percentage. The diets containing 4 and 6% HO significantly affected Haugh unit ($P < 0.05$), with reduced and elevated values, respectively. The highest level of HO (6%) compared to the highest level of HS (20%) led to a greater increase in yolk index during weeks 55-59 ($P < 0.05$). The yolk color index decreased in HS and HO diets during the second and third periods (59-63 and 63-67 weeks), but not the first period (55-59 weeks).

Blood biochemistry

The blood biochemical parameters of hens fed diets containing HS and HO are presented in Table 4. Diets containing 6% HO resulted in the lowest blood cholesterol, plasma triglycerides, and LDL-C concentrations among all treatment groups, but the highest concentration of HDL-C ($P < 0.05$). There was not a significant difference in plasma cholesterol concentration among other dietary treatments. Plasma total protein was also not affected by dietary treatments. Activity of plasma aspartate aminotransferase was also most significantly reduced in 6% HO diets ($P < 0.05$) whereas the highest level of HS (20%) did not have a significant impact.

Table 3. The Egg quality parameters of laying hens fed diets contained hempseed and hempseed oil

Parameters	weeks	Control	Hempseed (%)				Hempseed oil (%)			SEM	P-value
			5	10	15	20	2	4	6		
Shape Index	55-59	69.9	72.2	71.3	72.7	74.2	71.1	72.6	72.3	1.09	0.245
	59-63	72.9	71.6	72.5	72.4	72.0	71.5	70.4	71.3	0.664	0.237
	63-67	72.7	72.0	72.7	71.1	71.5	72.6	72.6	71.5	1.05	0.899
Haugh Unit (HU)	55-59	83.5	84.1	85.2	87.1	85.0	85.6	85.8	86.5	2.25	0.961
	59-63	83.6	86.1	83.8	82.3	82.4	82.3	80.0	82.4	2.12	0.698
	63-67	83.2 ^a	86.3 ^a	80.5 ^{ab}	83.6 ^a	85.4 ^a	80.6 ^{ab}	77.2 ^b	85.9 ^a	1.29	0.0002
Yolk Index	55-59	38.9 ^{ab}	38.2 ^{ab}	39.9 ^{ab}	38.1 ^{ab}	36.0 ^b	38.9 ^{ab}	38.8 ^{ab}	41.0 ^a	1.22	0.027
	59-63	40.0	38.1	41.2	40.1	38.6	39.2	40.1	39.5	1.11	0.616
	63-67	40.6	38.7	38.6	39.9	40.7	39.3	38.0	39.1	1.04	0.535
Yolk Color index	55-59	4.00	3.40	3.80	3.80	3.60	3.80	4.00	4.00	0.173	0.194
	59-63	5.00 ^{ab}	5.20 ^a	4.60 ^{abcd}	5.00 ^{ab}	4.80 ^{abc}	4.20 ^{cd}	4.40 ^{bcd}	4.00 ^d	0.173	0.0002
	63-67	6.60 ^a	6.20 ^{ab}	5.40 ^{ab}	5.40 ^{ab}	5.60 ^{ab}	5.20 ^b	6.00 ^{ab}	6.20 ^{ab}	0.287	0.016
Albumen percentage	55-59	57.6	57.3	58.1	58.4	57.8	58.4	57.0	58.7	1.41	0.987
	59-63	57.5	59.1	56.3	57.8	56.8	57.1	58.9	57.4	0.952	0.440
	63-67	56.3	58.1	56.7	57.0	58.2	56.4	57.7	58.6	1.25	0.937
Yolk percentage	55-59	29.5	29.3	28.9	28.9	28.5	27.9	29.0	28.5	1.23	0.989
	59-63	29.4	28.3	31.1	28.6	29.8	30.2	28.7	29.7	0.794	0.143
	63-67	31.1	29.4	30.5	30.0	29.9	29.7	29.7	28.3	0.963	0.936
Shell percentage	55-59	12.8	13.3	12.5	12.5	13.6	13.5	13.9	12.1	0.563	0.528
	59-63	13.1	12.6	12.6	13.5	13.3	12.6	12.4	12.8	0.487	0.639
	63-67	12.7	12.8	12.8	12.9	12.9	12.7	12.6	13.9	0.423	0.498

^{a-d}Means within a row that do not have a common superscript are significantly different ($P < 0.05$).

Table 4. Plasma constituents of laying hens fed diets containing hempseed and hempseed oil

Parameters	Control	Hempseed (%)				Hempseed oil (%)			SEM	P-value
		5	10	15	20	2	4	6		
Cholesterol (mg/dL)	100 ^{ab}	106 ^a	99.2 ^{ab}	103 ^a	137 ^a	102 ^a	109 ^a	47.2 ^b	11.1	0.003
HDL-C (mg/dL)	48.0 ^b	45.4 ^b	57.5 ^b	50.3 ^b	50.3 ^b	48.2 ^b	48.1 ^b	76.8 ^a	5.30	0.001
TG (mg/dL)	829 ^{ab}	672 ^{bc}	963 ^a	736 ^{abc}	864 ^{ab}	900 ^{ab}	896 ^{ab}	547 ^c	47.2	0.0002
LDL-C (mg/dL)	476 ^{ab}	402 ^b	538 ^a	428 ^{ab}	520 ^{ab}	516 ^{ab}	519 ^{ab}	248 ^c	24.8	0.0001
TP (mg/dL)	4.71	5.08	5.16	5.05	4.97	5.06	5.41	3.70	0.350	0.102
AST (U/L)	229 ^a	208 ^a	195 ^{ab}	203 ^a	219 ^a	218 ^a	209 ^a	158 ^b	8.62	0.001

^{a,b}Means within a row that do not have a common superscript are significantly different ($P < 0.05$).

TG: Triglyceride; HDL-C: High-density lipoprotein Cholesterol; LDL-C: Low-density Lipoprotein Cholesterol; TP: Total protein; AST: Aspartate aminotransferase.

Discussion

Previous research and current findings show high nutritional value of hempseed and hempseed oil for laying hens, in relation to their uses as a source of dietary energy, protein, and essential fatty acids (Parker *et al.*, 2003; Callaway, 2004). The present data revealed that overall hen performance (feed intake, egg production and feed conversion ratio) was ameliorated by the inclusion of graded levels of either HS (up to 20%) or HO (up to 6.0%) to layer diets. Although dietary HS and HO

ameliorated the performance of layers, they decreased the hen performance in the two first adaptation weeks. The addition of hempseed (up to 20%) to basal diets did not significantly influence feed intake, egg production, and feed conversion ratio (Silversides and Lefrancois, 2005; Gakhar *et al.*, 2012). Silversides and Lefrancois (2005) reported that four weeks of graded levels (0, 50, 100, or 200 g/kg of diet) of hempseed meal did not significantly affect egg production or feed consumption in 42-wk-old

DeKalb laying hen, despite reductions in BW (Silversides and Lefrancois, 2005).

Similar to this finding, an increase in egg and yolk weights were found in birds fed high levels of HS or HO (Neijat *et al.*, 2014). The increase in egg weight may be related to an enhancement of yolk weight (Johnston and Gous, 2007). The growing of egg mass in hens fed higher levels of HO (6%) may be due to greater availability of fatty acids, especially linoleic acid (Grobas *et al.*, 2001). Filardi *et al.* (2005) and Neijat *et al.* (2014) reported that the fat sources had no effect on eggshell quality. In agreement with these researchers, we also observed that eggshell was not affected by dietary treatments. The current study supports laying hen diets could contain HS and HO without affecting measurements such as shape index, shell weight, and albumen weight. The inclusion of hempseed (8% and 12%) did not show a significant effect on feed intake, body weight, feed conversion rate, egg production, and egg mass (Gakhar *et al.*, 2012), which can provide additional evidence for the safety and efficacy of these potential feed ingredients for laying hens. Hempseed protein, although somewhat limiting in lysine, has been shown to be highly digestible in rodent studies (House *et al.*, 2010).

The reduction of triglycerides in the blood after the intake of n-3 PUFA (Van Elswyk *et al.*, 1991; Fritsche *et al.*, 1991) may significantly reduce egg cholesterol, as was shown after feeding a hen HS or HO diets (Scheideler *et al.*, 1998). In laying hens, published ranges of plasma protein levels include 3.5 to 5.5 mg/dL for total protein (Gyenis *et al.*, 2006). In the current study, plasma protein values for all groups of hens were within a different range of 3.70 to 5.41 mg/dL. Since hemp products provide both energy and protein to meet metabolic requirements, they do not affect blood protein (Neijat *et al.*, 2014). Karimi and Hayatghaibi (2006) reported that plasma

total protein of laying hens was not affected by diets containing HS.

The blood lipid of laying hens fed HS or HO was significantly different. A significant depression in blood LDL-C and cholesterol were observed in these birds, especially in 6% HO. Similar findings were reported in the reduction of cholesterol, TG, and LDL-C in layers with HS diets (Karimi and Hayatghaibi, 2006). Researchers try to decrease the cholesterol and LDL-C in human food to decrease the cardiovascular risk because hyperlipidemia is known as a big risk factor that stimulates coronary disease and atherosclerosis (Callow *et al.*, 2002; Kerényi *et al.*, 2006).

Due to the vital importance of liver, the current study attempts to measure aspartate aminotransferase enzyme level in plasma. Neijat *et al.* (2014) reported that inclusion of HS in diets of laying hens had a significant quadratic effect in levels of AST. In addition, the inclusion of hempseed oil did not affect plasma AST levels. The latter data may indicate that higher levels of hempseed (i.e. 30%) and hempseed oil (i.e. 10%) can be well tolerated by the hens, but an early adaptation of birds to hemp-derived products during the rearing period may be required, particularly when used at higher levels.

Conclusion

Supplementation of HS and HO to laying hen diets may ameliorate the egg quality and blood lipids without any undesirable effects on performance.

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