Evaluation of dietary hemp seed meal as soybean meal substitution on productive performance, egg quality, and yolk fatty acid composition of laying hens

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Abstract

Hemp seed meals contain high protein and unsaturated fatty acids which have the potential as a substitute for soybean meal and yield eggs rich in unsaturated fatty acids. Therefore, the study aimed to evaluate the impact of dietary hemp seed meal as a substitute for soybean meal protein on productive performances, egg quality, and yolk fatty acid composition. A total of 120 Lohmann Brown laying hens aged 50 weeks were separated into 4 groups and 10 repetitions with 3 birds each. Birds received treatment without hemp seed meal (control group), or soybean meal substituted with 4%, 8%, and 12% hemp seed meal. Dietary 4% hemp seed meal significantly increased ($p < 0.05$) feed efficiency and egg production compared to the 8% and 12% hemp seed meal group. Meanwhile, feed intake, egg weight, body weight loss, egg shape index, albumen index, albumen weight, Haugh unit, yolk weight, yolk index, and eggshell thickness had no significant influence ($p > 0.05$). However, dietary 8% and 12% hemp seed meal significantly increased ($p < 0.05$) eggshell weight and yolk color score compared to the control and 4% hemp seed meal group. There was a significant increase ($p < 0.05$) in omega-3 fatty acid levels and a significant decrease in yolk omega-6 to omega-3 fatty acids ratio with an increase in dietary hemp seed meal. It concluded that dietary 4% hemp seed meal as soybean meal substitution was able to increase egg production, and feed efficiency compared to the level of 8% and 12% hemp seed meal. Increasing dietary levels of hemp seed meal increased eggshell weight, yolk color, and omega-3 fatty acids content, and decreased the omega-6 to omega-3 fatty acids ratio.

Introduction

Soybean meal remains the most important protein source in poultry nutrition. However, in recent years uncertain global economic conditions have caused problems in the product supply chain and/or insufficient products in the market, which has led to price increases. It has an impact on the increase in feed production costs which affects the profitability and sustainability of the poultry farming business. As a result, exploring alternative protein sources for soybean substitution in poultry diets is critical. Hemp seed meal has great potential because of its high-quality protein content, as well as unsaturated fatty acids and antioxidant compounds.

Hemp (*Cannabis sativa* L.) is widely planted for industrial purposes including cosmetics, paper, and textiles sectors (Crini et al. 2020). The average world hemp seed production in the last 5 years (2016–2020) reached 21,626 tons (FAOSTAT, 2022). This production is predicted to increase since many European countries have enacted laws regarding the legal use of cannabis which has attracted the attention of researchers in the utilization of hemp in various fields including its derivative products for the livestock sector. Hemp seed is an agroindustrial by-product of hemp seed oil extraction containing 30% oil, 30% crude protein, 32–40% neutral detergent fiber, 5.8% minerals, and 385.72 kcal/100g of energy (Wang and Xiong, 2019; Singh et al. 2022). The oil extraction increased the crude protein and decreased the energy content of hemp seed meal. Hemp seeds are high in arginine, glutamic acid, methionine, and cystine, as well as α-linoleic acid (17–19%), linoleic acid (60%), and 75–80% unsaturated fatty acids (Franek et al. 2022). However, protein and other nutrients might vary based on various factors such as climate, cultivar, maturity, and oil extraction process. On the other hand, antinutritional (phytic acid, cyanogenic glycosides, trypsin inhibitors, condensed tannins, saponins) and psychoactive cannabinoid substances especially Delta-9-tetrahydrocannabinol (THC) (Russo and Reggiani, 2015) which is found in cannabis seeds should be used in limited levels. A cannabinoid is not produced in cannabis seeds but it can be transferred from the flowers and leaves to the seeds and oil. Regarding THC, Jing et al. (2017) suggested that a THC level of no more than 0.3% in the cannabis plant can be utilized safely in animal diets. It has been observed that no cannabinoid residues were accumulated in the eggs, blood, body tissues, and organs (breast meat, belly fat, liver, kidney, and spleen) of laying hens-fed cannabis seeds (Kasula et al. 2021a).

In addition to its potential as an alternative protein source for laying hens, hemp seed meal has a favorable influence on the fat content of yolks. In previous studies, the addition of 5–25% hemp seed meal in the ration raised linoleic acid, linolenic acids, and docosahexaenoic acids in the egg (Raza et al. 2016). Similar findings demonstrated that the addition of 30% enhanced yolk omega-3 fatty acid and lowered the ratio of omega-6 to omega-3 (Neijat et al. 2016) without affecting the flavor of cooked eggs (Goldberg et al. 2012). However, our study used hemp seed meal from a new cultivar developed by Ondokuz Mayıs University, Turkey which may have different effects from previous studies. Therefore, our study aimed to evaluate the impact of using hemp seed meal cultivated in Ondokuz Mayıs University as soybean meal protein substitution on laying hens' performances, external and internal quality characteristics of eggs, and yolk fatty acid composition.

Materials And Methods

Animals and design of experimental

A total of 120 Lohmann Brown laying hens aged 50 weeks with similar body weight (BW; 2001 ± 6.733 g) were separated into 4 groups and 10 replicates with 3 birds each in a completely randomized design. The field trial was conducted for six weeks at the Research Farm of Agriculture Faculty, Ondokuz Mayıs University. Birds were housed in cages consisting of 40 compartments (3 floors; 8 rows) with each section of 52x48x75 cm. A feeder and nipple drinking water system for providing *ad libitum* drinking water were equipped in each cage and the house was environmentally controlled by maintaining an ambient temperature of 23–25°C and lighting for 16 hours per day. During treatment, mash feed was provided daily at a rate of 120 g/bird.

The ration was formulated as isonitrogenous and isocaloric (CP:15.5%; ME: 2,750 kcal/kg) to fulfill the nutritional requirements of the Lohmann Brown breed according to its production phase. Laying hens received treatment without hemp seed meal (control group), or soybean meal substituted with 4%, 8%, and 12% hemp seed meal (Table 1). Hemp seed meals produced cold press method were obtained from Cannabis Research Center, Ondokuz Mayıs University. The composition of nutrients of the hemp seed meal used is presented in Table 2.

Table 1
Composition of ingredients and nutrients of diet

Ingredients (%)	Hemp seed meal level (%)			
	0	4	8	12
Yellow Corn	53.00	53.00	53.00	53.00
Sunflower meal	12.50	12.50	12.50	12.50
Soybean meal	12.00	8.00	4.00	-
Hemp seed meal	-	4.00	8.00	12.00
Wheat bran	10.00	10.00	10.00	10.00
Vegetable oil	2.17	2.17	2.17	2.17
Dicalcium phosphate	1.36	1.36	1.36	1.36
Limestone	8.40	8.40	8.40	8.40
L-Lysine	0.03	0.03	0.03	0.03
DL-Methionine	0.04	0.04	0.04	0.04
Salt	0.25	0.25	0.25	0.25
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25
Total	100	100	100	100
Nutrients composition (% as fed)[2]				
Metabolizable energy, kcal/kg[3]	2706	2714	2723	2732
Crude Protein	15.53	15.61	15.62	15.70
Crude Fiber	4.48	4.90	5.33	5.75
Extract ether	4.85	5.36	5.86	6.37
Methionine + cystine	0.60	0.61	0.61	0.62
Lysine	0.70	0.71	072	0.73

[1] Vitamin and mineral content per kg diet : 12 000 IU of retinol; 2 400 IU of cholecalciferol; 40 mg of α-tocopherol; 6 mg of riboflavin ; 4 mg of menodione; 3 mg of thiamine; 25 mg of nicotinic acid; 5 mg of pyridoxine; 10 mg of pantothenic acid; 0.03 mg of cyanocobalamin; 1 mg of folic acid; 0.05 mg of biotin; 200 mg choline chloride; 60 mg of Zn; 60 mg of Fe; 80 mg of Mn; 0.15 mg Se, 5 mg Cu; 0.2 mg Co; 1 mg of I.

[2] Based on a calculation

[3] Metabolizable energy was calculated based on tabulated values from each ingredient (TSI, 1991)

Ingredients (%)	Hemp seed meal level (%)			
	0	4	8	12
Tryptophan	0.19	0.19	0.19	0.19
Threonine	0.57	0.57	0.57	0.58
Available phosphorus	0.32	0.32	0.32	0.32
Calcium	3.60	3.60	3.60	3.60
Sodium	0.14	0.14	0.15	0.15

[1] Vitamin and mineral content per kg diet : 12 000 IU of retinol; 2 400 IU of cholecalciferol; 40 mg of α-tocopherol; 6 mg of riboflavin ; 4 mg of menodione; 3 mg of thiamine; 25 mg of nicotinic acid; 5 mg of pyridoxine; 10 mg of pantothenic acid; 0.03 mg of cyanocobalamin; 1 mg of folic acid; 0.05 mg of biotin; 200 mg choline chloride; 60 mg of Zn; 60 mg of Fe; 80 mg of Mn; 0.15 mg Se, 5 mg Cu; 0.2 mg Co; 1 mg of I.

[2] Based on a calculation

[3] Metabolizable energy was calculated based on tabulated values from each ingredient (TSI, 1991)

Table 2
The nutrient content of hemp seed meal used

Nutrients content	% Dry matter
Crude protein	45.24
Extract ether	16.02
Ash	7.18
Crude fiber	18.80

Data Collecting

Productive performances

At the commencement of the experiment, the birds were weighed as the initial body weight and distributed evenly into the cage. Birds were weighed as the final weight in the sixth week. Changes in body weight were measured as the difference between the final weight and the initial weight. Feed intake was measured by subtracting the total amount of feed remaining in the feeder from the total amount of feed offered at one-week intervals. Egg production and egg weight were recorded daily. Egg production was measured by dividing the total number of eggs by the total number of laying hens on the same day. The feed conversion ratio (FCR) was calculated weekly by dividing the total feed intake by the total egg weight.

Egg Quality

Eggs were taken within the last 3 days of six weeks and kept at room temperature for 24 hours prior to measurement. Egg weight, yolk weight, and eggshell weight were measured by an electronic scale with 0.01g accuracy. Meanwhile, egg weight, eggshell weight and yolk weight were subtracted to determine the albumen weight. The eggshell thickness, width and length of the egg, albumen, and yolk were measured by a digital micrometer. Yolk and albumen thickness were measured using a digital gauge. The eggshell thickness average was calculated from the sharp, blunt, and equatorial sides. A Roche color fan of 1 to 14 scale was used to measure yolk color.

The Haugh unit (HU), egg shape index, albumen index, and yolk index were calculated using the formula :

$HU = 100 \text{ logs } (AT - 1.7\ W^{0.37} + 7.57)$ (Haugh, 1937)

Egg shape index = EL/EW x 100%

Albumen index = $AT/(AW_1 + AW_2)/2 \times 100\%$

Yolk index = YT/YW x 100%

Where AT = Albumen thickness (mm); W = Egg weight (g); EL = Egg lenght (mm); EW = Egg width (mm); AW = Albumen width (mm); YT = Yolk thickness (mm); YW = Yolk width (mm)

Yolk Fatty Acid Composition

In the last three days in weeks 4 and 6 of the field trial, a total of 90 eggs (3 eggs each replicate) were retrieved, and yolk fat was extracted with ether and measured by gas chromatography according to the method of Konca et al. (2019). Ether was used to extract the yolk fat and kept at -20°C in an Eppendorf tube for analysis. Using 3 ml of hexane and 100 µl of 2N KOH, 0.1 g of yolk fat was saponified. The mixture was vortexed for one minute and then centrifuged for five minutes at 5000 rpm. Furthermore, the fatty acid fraction was examined using gas chromatography (Shimadzu, Japan). The temperature of the injector was determined at 250°C. The temperature of the oven was maintained at 103°C for 1 minute, raised from 103°C to 170°C for 12 minutes, and then lastly at 230°C for 5 minutes. The helium mobile phase flow rate was maintained at 2 ml/minute. The fatty acid fractions were determined by comparing the retention time to the standard and was presented as percent fatty acid (%).

Statistical analysis

One-way analysis of variance (ANOVA) was employed to analyze the significance of the data using the SPSS 21.0 program. The mean difference was compared with Duncan's comparison test. The final data were provided as mean values with a standard error of the mean (SEM) and a significance level of $p < 0.05$.

Results

Laying hens productive performances

The productive performances of laying hens are provided in Table 3. In the cumulative period (1–6 weeks), FCR and egg production were significantly different ($p < 0.05$), in which the control group and the use of 4% hemp seed meal increased feed efficiency and egg production compared to 8% and 12% hemp seed meal group. In addition, there was no mortality and significant difference ($p > 0.05$) in body weight loss, egg weight, and feed intake. In the 1st week, the dietary 4% hempseed meal group yielded the highest egg production (98.57%) ($p < 0.05$), while the 12% hempseed meal group yielded the lowest egg production (88.57%). In the 2nd week, the 12% hempseed group significantly decreased ($p < 0.05$) egg production and increased FCR (74.29%; 2.69, respectively). In the 3rd, 4th, and 5th weeks, dietary 4% hempseed meal significantly increased ($p < 0.05$) egg production compared to other treatments. Likewise, the FCR value was significantly lower ($p < 0.05$) than the 8% and 12% hempseed meal groups. In the 6th week, egg production decreased ($p < 0.05$) in all hempseed meal groups.

Table 3
Body weight, feed intake, egg weight, FCR, and egg production of laying hens fed by hemp seed meal during 6 weeks.Variables

Variables	Hemp seed meal level (%)				SEM	P-value
	0	4	8	12		
1st -6th week						
Initial body weight (g/bird)	2009	2003	1999	1993	16.50	0.99
Final body weight (g/bird)	1976	1981	1977	1973	1.45	0.98
Body weight loss (%)	32.6	21.8	22.5	20.5	1.17	0.94
Feed intake (g/bird/day)	117.07	122.50	122.92	120.98	1.61	0.58
FCR	2.11^{a}	2.16^{a}	2.29^{b}	2.40^{b}	0.03	0.00
Egg weight (g/egg)	60.58	58.17	59.21	60.67	0.52	0.27
Egg production (%)	94.52^{c}	94.12^{c}	90.08^{b}	85.46^{a}	0.84	0.00
1st week						
Feed intake (g/bird/day)	117.7	119.3	126.2	121.6	2.81	0.75
FCR	2.04	2.13	2.40	2.30	0.07	0.29
Egg weight (g/egg)	59.85	58.13	59.26	61.71	0.56	0.15
Egg production (%)	97.14^{a}	98.57^{a}	94.28^{ab}	88.57^{b}	1.42	0.05
2nd week						
Feed intake (g/bird/day)	112.6	124.5	123.4	116.3	2.07	0.12
FCR	2.09^{a}	2.54^{b}	2.36^{ab}	2.69^{b}	0.07	0.01
Egg weight (g/egg)	59.82	58.75	58.94	59.51	0.58	0.91
Egg production (%)	91.43^{a}	84.29^{a}	90.00^{a}	74.29^{b}	1.84	0.00
3rd week						
Feed intake (g/bird/day)	117.09	121.34	126.12	120.38	2.35	0.61
FCR	2.37	2.26	2.42	2.41	0.05	0.64
Egg weight (g/egg)	59.59	58.62	59.32	61.41	0.59	0.39
Egg production (%)	91.56^{a}	98.10^{b}	96.84^{b}	89.73^{a}	0.92	0.01

SEM = Standard error of the mean; FCR = Feed conversion ratio; Row with a different superscript indicates a significant difference ($P < 0.05$)

Variables	Hemp seed meal level (%)				SEM	P-value
	0	4	8	12		
4th week						
Feed intake (g/bird/day)	119.94	120.40	124.25	120.13	2.45	0.92
FCR	2.70[a]	2.45[a]	3.05[b]	3.14[b]	0.07	0.00
Egg weight (g/egg)	61.57	57.10	60.39	59.90	0.73	0.16
Egg production (%)	83.68[a]	96.73[b]	87.99[a]	83.68[a]	1.38	0.00
5th week						
Feed intake (g/bird/day)	117.10	130.82	116.07	120.78	2.69	0.20
FCR	2.78	2.73	2.69	2.97	0.06	0.32
Egg weight (g/egg)	61.20	58.20	59.64	59.92	0.71	0.54
Egg production (%)	96.17[b]	97.74[b]	89.69[ab]	85.68[a]	1.57	0.02
6th week						
Feed intake (g/bird/day)	117.97	118.67	121.46	126.69	4.54	0.91
FCR	2.78	2.57	2.76	2.96	0.12	0.73
Egg weight (g/egg)	61.45	58.21	57.73	61.54	0.73	0.11
Egg production (%)	95.35[b]	89.26[ab]	81.63[a]	90.79[b]	1.52	0.09

SEM = Standard error of the mean; FCR = Feed conversion ratio; Row with a different superscript indicates a significant difference ($P < 0.05$)

Egg Quality

The effect of hemp seed meal as soybean meal substitution on egg quality is provided in Table 4. Dietary hemp seed meal up to 12% had no effect ($p > 0.05$) on egg shape index, albumen index, albumen weight, Haugh unit, yolk index and yolk weight, and eggshell thickness. However, dietary 8% and 12% hemp seed meal significantly increased ($p < 0.05$) eggshell weight and yolk color score compared to 4% hemp seed meal and the control group.

Table 4
Egg quality of laying hens fed by hemp seed meal

Variables	Hemp seed meal level				SEM	P-value
	0%	4%	8%	12%		
Egg shape index	80.42	78.08	76.84	78.81	0.52	0.10
Albumen weight (%)	64.22	64.77	64.69	64.73	0.53	0.98
Haugh unit	92.66	90.80	90.55	94.81	1.09	0.50
Albumen index	11.96	11.39	11.17	12.70	0.37	0.48
Yolk weight (%)	28.85	28.50	27.85	27.76	0.49	0.85
Yolk index	42.27	41.42	43.96	46.55	0.89	0.18
Yolk color	11.55^{a}	12.01^{ab}	12.25^{b}	12.50^{b}	0.11	0.01
Eggshell weight (%)	6.93^{ab}	6.74^{a}	7.46^{b}	7.51^{b}	0.12	0.05
Eggshell thickness (mm)	0.28	0.27	0.29	0.29	0.01	0.11
SEM = Standard error of the mean; Row with a different superscript indicates a significant difference ($P < 0.05$)						

Yolk Fatty Acid Composition

The compositions of yolk fatty acids in the 4th and 6th weeks are presented in Table 5 and Table 6. In the 4th week, the total of omega-3 fatty acids increased significantly ($p < 0.05$) with an increase in dietary hemp seed meal, in which the 8% and 12% hemp seed meal groups were higher than that of the control and 4% hempseed meal groups. In the 6th week, the total of omega-3, omega-6 fatty acids, and polyunsaturated fatty acids (PUFAs) increased significantly ($p < 0.05$) with the administration of 8% hemp seed meal. The total of saturated fatty acids (SAFAs) was not affected ($p > 0.05$) by dietary hemp seed meal. Meanwhile, the omega-6 to omega-3 fatty acids ratio decreased significantly ($p < 0.05$) with increasing hemp seed meal inclusion both in the 4th week and the 6th week. Eicosanoic acid and heptadecanoic acid as monounsaturated fatty acids (MUFAs) decreased significantly ($p < 0.05$), while linoleic acid, alpha-linolenic acid, docosahexaenoic acid, and eicosadieonic acid (PUFAs) increased significantly with increasing use of hemp seed meal either in the 4th or 6th week. The SAFAs, MUFAs, and PUFAs of yolk were dominated by hexadecanoic acid, oleic acid, and linoleic acid, respectively.

Table 5
Yolk fatty acid composition of laying hens fed by hemp seed meal at 4th weeks

Fatty acids (%)	Hemp seed meal level (%)				SEM	P-value
	0	4	8	12		
Saturated fatty acids (SFAs)						
Palmitic/Hexadecanoic acid (C16:0)	21.16	21.21	20.97	21.06	0.20	0.98
Myristic/Tetradecanoic acid (C14:0)	0.21	0.21	0.20	0.23	0.00	0.08
Margaric acid (C17:0)	0.25[b]	0.20[a]	0.20[a]	0.17[a]	0.01	0.00
Stearic/Octadecanoic acid (C18:0)	7.05	7.02	7.30	7.48	0.10	0.32
Arachidic acid (C20:0)	0.18[c]	0.12[a]	0.17[c]	0.14[b]	0.01	0.00
Lignoseric/tetracosanoic acid (C24:0)	0.18[a]	0.14[a]	0.17[a]	0.33[b]	0.02	0.02
Monounsaturated fatty acids (MUFAs)						
Heptadecenoic acid (C17:1)	0.14[b]	0.13[a]	0.13[a]	0.12[a]	0.00	0.05
Eicosanoic acid (C20:1)	0.26[b]	0.26[b]	0.25[b]	0.21[a]	0.01	0.00
Palmitoleic acid (C16:1)	2.82	2.78	2.72	2.79	0.04	0.83
Oleic acid (C18:1)	41.32	41.29	41.21	41.11	0.36	1.00
Nervonic acid (C24:1)	0.21	0.20	0.22	0.16	0.01	0.31
Polyunsaturated fatty acids (PUFAs)						
Alpha-linolenic acid (C18:3)	0.79[a]	1.03[b]	1.23[c]	1.26[c]	0.04	0.00
Docosahexanoic acid (C22:6)	0.56[a]	0.67[ab]	0.86[c]	0.76b[c]	0.03	0.00
Mead Acid (C20:3)	1.39	1.36	1.25	1.25	0.02	0.06
Linoleic acid (C18:2)	23.19	23.04	22.8	22.66	0.29	0.93
Dihomo-γ-linolenic acid (C20:3)	0.20[b]	0.13[a]	0.12[a]	0.12[a]	0.01	0.00
Eicosadieonic acid (C20:2)	0.18[a]	0.22[b]	0.17[a]	0.18[a]	0.01	0.00
SFAs	29.01	28.89	29.00	29.40	0.27	0.93
MUFAs	44.74	44.65	44.52	44.38	0.40	0.99
PUFAs	26.29	26.43	26.41	26.21	0.30	0.99

SEM = Standard error of the mean; Row with a different superscript indicates a significant difference ($P < 0.05$)

Fatty acids (%)	Hemp seed meal level (%)				SEM	P-value
	0	4	8	12		
Omega-6 (n-6)	23.56	23.38	23.08	22.95	0.29	0.90
Omega-3 (n-3)	1.35[a]	1.70[b]	2.09[c]	2.02[c]	0.067	0.00
n-6/ n-3	17.51[c]	13.82[b]	11.17[a]	11.42[a]	0.565	0.00

SEM = Standard error of the mean; Row with a different superscript indicates a significant difference ($P < 0.05$)

Table 6
Yolk fatty acid composition of laying hens fed by hemp seed meal at 6th weeks

Fatty acids (%)	Hemp seed meal level (%)				SEM	P-value
	0	4	8	12		
Saturated fatty acids (SFAs)						
Palmitic/Hexadecanoic acid (C16:0)	21.01	21.1	20.55	21.97	0.21	0.10
Myristic/Tetradecanoic acid C14:0	0.20[a]	0.23[b]	0.21[a]	0.24[c]	0.00	0.00
Margaric acid (C17:0)	0.21[c]	0.19[b]	0.21[c]	0.16[a]	0.01	0.00
Stearic/Octadecanoic acid (C18:0)	7.23[a]	7.05[a]	7.78[b]	7.34[a]	0.08	0.01
Arachidic acid (C20:0)	0.09[a]	0.11[ab]	0.16[c]	0.12[b]	0.01	0.00
Lignoseric/tetracosanoic acid (C24:0)	0.12[a]	0.13[a]	0.18[c]	0.16[b]	0.01	0.00
Monounsaturated fatty acids (MUFAs)						
Heptadecenoic acid (C17:1)	0.13	0.13	0.12	0.12	0.00	0.19
Eicosanoic acid (C20:1)	0.37[b]	0.27[a]	0.26[a]	0.29[a]	0.01	0.00
Palmitoleic acid (C16:1)	2.80[b]	3.05[c]	2.65[a]	3.07[c]	0.04	0.00
Oleic acid (C18:1)	43.56	41.76	40.96	42.39	0.41	0.14
Nervonic acid (C24:1)	0.16[a]	0.18[b]	0.25[c]	0.19[b]	0.01	0.00
Polyunsaturated fatty acids (PUFAs)						
Alpha-linolenic acid (C18:3)	0.80[a]	1.10[b]	1.26[c]	1.07[b]	0.04	0.00
Docosahexanoic acid (C22:6)	0.43[a]	0.70[b]	0.85[d]	0.74[c]	0.03	0.00
Mead Acid (C20:3)	1.05[a]	1.38c	1.23[b]	1.23[b]	0.03	0.00
Linoleic acid (C18:2)	21.51[a]	22.28[bc]	23.02[c]	20.59[a]	0.27	0.00
Dihomo-γ-linolenic acid (C20:3)	0.11[a]	0.12[b]	0.11[a]	0.10[a]	0.00	0.01
Eicosadieonic acid (C20:2)	0.22[a]	0.23[b]	0.24[b]	0.23[b]	0.00	0.04
SFAs	28.85	28.80	29.08	29.98	0.27	0.39
MUFAs	47.01	45.38	44.23	46.06	0.44	0.15

SEM = Standard error of the mean; Row with a different superscript indicates a significant difference ($P < 0.05$)

Fatty acids (%)	Hemp seed meal level (%)				SEM	P-value
	0	4	8	12		
PUFAs	24.10[a]	25.79[b]	26.69[b]	23.94[a]	0.32	0.00
Omega-6 (n-6)	21.83[ab]	22.63[bc]	23.36[c]	20.91[a]	0.27	0.00
Omega-3 (n-3)	1.22[a]	1.79[b]	2.11[c]	1.81[b]	0.07	0.00
n-6/ n-3	17.89[d]	12.64[c]	11.10[a]	11.55[b]	0.57	0.00

SEM = Standard error of the mean; Row with a different superscript indicates a significant difference ($P < 0.05$)

Discussion

Hemp is categorized as a *Cannabis sativa* plant with a tetrahydrocannabinol (THC) content of less than 0.3% (Congressional Research Service, 2019). Hamp seeds contain more than 30% oil, of which 80% are polyunsaturated fatty acids high in omega-3 which have been discovered to be able to enhance performance and omega-3 enrichment in eggs. Hamp seed meal extracted from oil contains 25–30% protein depending on the variety (Konca et al. 2019). Therefore, hemp seed meal has the potential to be utilized as a protein source. The European Monitoring Center of Drug Addiction has determined that ham seed meal can be safely added to approximately 30% of chicken diets (EFSA, 2011).

In the present study, the inclusion of 4% hemp seed meal produced the highest egg production and feed efficiency among all treatments in the cumulative period (1–6 weeks). On the other hand, the highest decrease in the laying hens' productive performance occurred in the 12% hemp seed meal treatment. The reduction was noticeable in the 2nd and 4th weeks of the experiment. The absence of a feed adaption phase in our study might explain the lowering of egg production in the 2nd week. Our findings contrast prior studies, which found that the inclusion of up to 15% (Konca et al. 2019) and 30% (Neijat et al. 2014) hemp seed meal did not have a deleterious impact on the laying hens' performances. Gakhar et al. (2012) stated that dietary 20% hemp seeds meal had no detrimental impact on laying hens' performances. Furthermore, the inclusion of up to 30% hemp seed meal did not affect growth, egg production, and feed intake (Neijat et al. 2014). However, the current study is similar to the results of Kasula et al. (2021b), that there was a decrease in egg production, and feed efficiency with dietary 10% hempseed meal. We assumed that the hemp variety utilized in this study had a higher antinutrient content which exceeded the hens' tolerable limits. As reported by Russo and Reggiani, (2015) that the antinutrient content is influenced by hemp varieties, where monoecious varieties are higher than dioecious varieties. When at a level of 8% and 12% hemp seed meal, accumulation of antinutrients might decrease protein digestibility and other nutrient absorption required for follicle development and ovulation consequently decreasing egg production which was shown clearly from the 4th week. However, dietary of 4% hemp seed meal might be still tolerated and had no harmful effects on laying hens, in addition, the high content of methionine, cysteine, and arginine amino acids as well as omega 3 and 6 fatty acids of hemp seed meal are favorable for egg formation (Darmawan et al. 2013; Konca et al. 2019). This reasoning is supported by the statement of Berenjian et al. (2021) that omega-3 fatty acids inclusion in laying hens' diet has a favorable impact on nutrient digestibility and intestinal morphology and their inclusion increases villi height, and surface area nutrient absorption. They further explained that improving gut health by omega-3 inclusion in the diet was associated with healthy epithelial cell regeneration and mitosis of active cellular.

According to Pojic et al. (2014), the highest antinutrient in hemp seed meal is phytic acid (22.5mg/g) followed by condensed tannin (0.23mg/g), cyanogenic glycosides (3.80μmol/g), and trypsin inhibitors (2.88TIU/mg protein). Furthermore, Russo and Reggiani, (2015) discovered that the tannin content of monoecious and dioecious varieties (4.46g/kg; 2.28g/kg, respectively) was higher than that of soybean (0.1g/kg), but not in trypsin inhibitors and saponins. Antinutrients cause physiological and functional effects in animals when consumed above tolerance levels. For instance, phytic acid can decrease protein digestibility and improve amino acids, endogenous nitrogen, and bivalent cation minerals excretion (Bernardes et al. 2022). Cyanogenic glycoside levels exceeding 10 mg/kg are hazardous to chicken health (EFSA, 2019). Also, tannins and saponins can suppress growth, feed consumption, and egg production due to their ability to reduce protein digestibility as well as nitrogen and minerals absorption (Hassan et al. 2020). Saponins and tannins can generate complex bonds with feed proteins and endogenous enzymes. Reducing egg production is also connected with saponins' astringent characteristics, which can irritate and impair intestinal motility (Russo and Reggiani, 2015). Previous studies found that a high diet tannin concentration of 0.56% (Woyengo et al. 2012) and 0.28% tannin (Torres et al. 2013) decreased aminopeptidase, growth, and feed efficiency in 42-day-old broiler chickens. In addition, it was reported that the effect of anti-nutrients was able to decrease intestinal villi height and increase crypt depth (Erdaw and Beyene, 2018). Rocha et al. (2014) and Norozi et al. (2022) stated that broiler chicken fed a diet high in trypsin inhibitors impaired pancreatic function and intestinal integrity including shortening and destruction of intestinal microvilli. Thus, a lower villus decreases the capacity for nutrient absorption, which eventually has an impact on animal performance.

Dietary hemp seed up to 12% did not affect egg shape index, albumen index, albumen weight, Haugh unit, yolk weight, yolk index, and eggshell thickness. However, dietary 8% and 12% hemp seed meal increased eggshell weight and yolk color score (Table 2). It is similar to a study by Gakhar et al. (2012) which demonstrated that dietary 20% hemp seed did not influence the egg's physical quality. Regarding yolk color, Konca et al. (2019), Bazdidi et al. (2016), and Kasula et al. (2021c) stated that the yolk color score improved as indicated by a yellowish to reddish color with increasing hemp seed meal inclusion levels. The color change is caused by the content of carotenoids, xanthophylls, and chlorophylls in hemp seeds meal that are transferred into yolks. According to Titcomb et al. (2019), the main sources of yolk color are carotenoids and xanthophylls in feed. In this study, eggshell weight (%) increased with the inclusion of 8% and 12% hemp seed compared to 4% hempseed and the control group. A previous study by Konca et al. (2019) also reported an increase in eggshell weight with dietary 15% hempseed meal. This increase indicates a high absorption and storage of minerals in the eggshell, especially calcium, phosphorus, and magnesium as the main components of the eggshell. These minerals may be contributed by hempseed meal which is a good source of micro and macro minerals. According to Farinon et al. (2020), phosphorus minerals are the most abundant in hempseed meal (890 to1170 mg/100g), followed by magnesium (240 to 694 mg/100g), and calcium (90 to145 mg/100g).

The omega-3 content in the yolk has increased significantly with the increasing dietary hemp seed meal. It revealed that the unsaturated fatty acid in hemp seed meal, particularly omega-3 and 6, could be absorbed and accumulated in yolks. According to Klir et al. (2019), hemp seed meal contained high PUFAs with 52–60% omega-6 and 15–22% omega-3. Similarly, it was reported that administration of 20% and 30% hemp seed meal enhanced unsaturated fatty acid in yolk without cannabidiol residues in blood, meat, eggs, and liver (Kasula et al.2021a). Furthermore, Mierliță, (2019) reported that dietary 20% hemp seed meal significantly improved omega-3 fatty acid and decreased the omega-6 to omega-3 ratio. However, it was interesting in our study that dietary of 12% hemp seed meal reduced total omega-6 in the 6th week (Table 6). One of the reasons for the decrease in total omega-6 was a significant decrease in linoleic acid, which was the largest omega-6 fraction. This might be due to competition between omega-3 and omega-6 for enzymes of desaturase (delta-4 and delta-6 desaturase enzymes). These enzymes determine the rate of PUFAs conversion into their respective metabolites in which they prefer to convert omega-3 rather than omega-6, consequently increasing omega-3 can inhibit the conversion of omega-6 (Simopoulus, 2006). As a result, the omega-6 to omega-3 ratio in the 12% hemp seed meal group decreased significantly from 17.51 (control) to 13.82 (4% hemp seed meal), 11.17 (8% hemp seed meal) and 11.42 (12% hemp seed meal) (Tabel 6). According to Simopoulos, (2002), the omega-6 to omega-3 ratio is important in reducing the percentage of mortality from cardiovascular disease in which omega-6 to omega-3 ratio of 50 resulted in 45% cardiovascular mortality in Europe and the United States compared to the ratio of 1 to 10 in Japan.

Conclusion

Dietary 4% hemp seed meal as soybean meal substitution can increase egg production, and feed efficiency compared to the level of 8% and 12% hemp seed meal. Increasing dietary levels of hemp seed meal increase eggshell weight, yolk color, and omega-3 fatty acids, and decreased the omega-6 to omega-3 ratio in the yolk. Further study is needed regarding the elimination of its antinutrient content which is expected to improve the level of its inclusion in the diet.

Declarations

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Author contribution All authors designed the research work and prepared the manuscript draft; Samet Hasan ABACI and Arif DARMAWAN analyzed experimental parameters; Arif DARMAWAN, Şevket ÖZLÜ, and Ergin ÖZTÜRK designed the experiments; and Arif DARMAWAN wrote the manuscript. The final manuscript was reviewed and approved by all authors.

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Data availability The datasets created or analyzed in our study are available from the corresponding author upon reasonable request.

Ethical approval All methods in this study were approved by the Animal Ethics Committee of Ondokuz Mayıs University with number 2022/53.

Conflicts of interest The authors declare no conflict of interest. Funders had no role in the design of the study, data collection, analysis or interpretation, manuscript writing, or the decision to publish the results.

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