T.C.
ERCİYES UNIVERSITY
FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

EFFECT OF HEMPSEED PROCESSING TECHNIQUES ON PERFORMANCE, EGG QUALITY, FATTY ACID COMPOSITION AND BLOOD PARAMETERS IN LAYING HENS

By
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Master Thesis

September 2024
KAYSERİ

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September 2024
KAYSERİ

COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in this study was obtained in accordance with academic and ethical rules. I also state that, as required by these rules and behaviors, I have fully cited and referenced all materials and results that are not inherent in this study.

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APPROVAL OF COMPLIANCE WITH THE DIRECTIVE

The Master's thesis entitled "**Effect of hempseed processing techniques on performance, egg quality, fatty acid composition and blood parameters in laying hens**" was prepared in accordance with Erciyes University Graduate Thesis Proposal and Thesis Writing Directive.

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ACCEPTANCE AND APPROVAL

Under the supervision of Prof. Dr. Yusuf KONCA, and Dr. Rifat Ullah KHAN this thesis on the topic "**Effect of hempseed processing techniques on performance, egg quality, fatty acid composition and blood parameters in laying hens**" was prepared by Ahmed Ali SHEIKH ELMI and was accepted as master degree thesis Department of Animal Sciences and Technologies; Graduate School of Natural and Applied Sciences, Erciyes University.

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KENEVİR TOHUMU İŞLEME TEKNİKLERİNİN YUMURTA TAVUKLARINDA PERFORMANS, YUMURTA KALİTESİ, YAĞ ASİDİ BİLEŞİMİ VE KAN PARAMETRELERİ ÜZERİNE ETKİSİ

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ÖZET

Bu çalışma, kenevir tohumu işleme tekniklerinin yumurtacı tavuklarda performans, yumurta kalitesi ve yumurta sarısının yağ asidi bileşimi üzerine etkilerini belirlemek amacıyla yürütülmüştür. Çalışmada toplam 60 adet Super Nick ırkı beyaz yumurtacı tavuk kullanılmıştır. Tavuklar 3 gruba ayrılarak her tekrarlamada 2 hayvan olacak şekilde 10 tekrarlamalı kafeslere yerleştirilmiştir. Gruplar, 1: Kontrol (K, kenevir tohumu küspesi yok) , 2: %15 soğuk sıkım kenevir tohumu küspesi (C-KTK), 3: %15 sıcak sıkım kenevir tohumu küspesi (H-KTK) eklenerek oluşturulmuştur. Çalışmada tavuklar 14 günlük 8 periyotta toplam 112 gün beslenmiştir. Rasyona C-KTK ve H-KTK eklenmesi vücut ağırlığı (VA), kazanım ve VA değişimi ile yem dönüşüm oranı (YDO) üzerinde önemli bir etkiye sahip olmamıştır. C-KTK ve H-KTK eklenmesi % yumurta verimi önemli oranda artmıştır ($P<0.01$). Yumurta ağırlığı H-KTK tüketen grupta diğer gruplara yüksek bulunmuştur ($P<0.01$). C-KTK ve H-KTK grupların yumurta sarısı Roche skalası renk değerleri K grubuna göre önemli oranda yüksektir ($P<0.01$) ve yumurta sarısı parlaklık (L*), kırmızılık (a*) ve sarılık (b) değerlerini K grubuna göre önemli oranda artırmıştır ($P<0.01$). Yumurta kabuğu ağırlığı ve % oranı ve kalınlığı H-KTK grubunda C-KTK ve K grubundan daha yüksek bulunmuştur ($P<0.01$). Kontrol ve C-KTK gruplarının yumurta kabuğu yüzey alanı değerleri, H-KTK eklenen gruba göre önemli derecede yüksek bulunmuştur ($P<0.01$). Sonuç olarak; yumurta tavuğu rasyonlarında C-KTK ve H-KTK kullanımının performans ve yumurta kalite değerlerini önemli derecede iyileştirebileceği, kenevir unu işleme tekniklerinin yumurta performansı ve kalitesi üzerinde önemli derecede farklı etkilere sahip olabileceği belirlenmiştir.

Anahtar Kelimeler: Yumurta tavuğu, kenevir tohumu, performans, yumurta kalitesi

EFFECT OF HEMPSEED PROCESSING TECHNIQUES ON PERFORMANCE, EGG QUALITY, FATTY ACID COMPOSITION AND BLOOD PARAMETERS IN LAYING HENS

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ABSTRACT

This study was conducted to determine the effects of hempseed processing techniques on performance, egg quality and fatty acid composition of egg yolk in laying hens. A total of 60 Super Nick breed white laying hens were used in the study. Hens were divided into 3 groups and placed in cages with 10 replications as 2 animals in each replication. Groups were formed by adding 1: Control (C, no hempseed meal), 2: 15% cold pressed hempseed meal (HSM-C), and 3: 15% hot pressed hempseed meal (HSM-H). In the study, hens were fed in 8 periods of 14 days for a total of 112 days. The addition of HSM-C and HSM-H to the ration did not have a significant effect on body weight (BW), gain, BW change, and feed conversion ratio (FCR). The addition of HSM-C and HSM-H significantly increased % egg yield ($P<0.01$). Egg weight was found higher in the HSM-H group than in the other groups ($P<0.01$). Egg yolk Roche scale color values of HSM-C and HSM-H groups were significantly higher than in the K group ($P<0.01$) and egg yolk brightness (L*), redness (a*), and yellowness (b) values were significantly increased compared to the C group ($P<0.01$). Eggshell weight and its percentage ratio and thickness were found higher in the HSM-H group than in the HSM-C and C groups ($P<0.01$). Eggshell surface area values of the control and HSM-C groups were found significantly higher than in the HSM-H supplemented group ($P<0.01$). As a result; It was determined that the use of HSM-C and HSM-H in laying hen diets could significantly improve performance and egg quality values and that hemp flour processing techniques could have significantly different effects on egg performance and quality.

Key Words: Laying hens, hempseed, performance, egg quality

ABBREVIATIONS

a* **:** Egg yolk redness
ALT : alanine aminotransferase
AST : alanine aspartate aminotransferase
b* **:** Egg yolk yellowness
Ca : Calcium
CK : Creatine Kinase
Cl : Chlorine
CP **:** Crude protein
DM : Dry matter
EM **:** Egg Mass
EST **:** Eggshell thickness
ESW : eggshell weight
FCR **:** Feed conversion ratio
FI **:** Feed intake
HDL **:** High-density lipoprotein
Hmm **:** Albumin height
HO : hemp oil
HS : hemp seed
HSM-C **:** Hempseed meal cold processing
HSM-H **:** Hempseed meal hot processing
HU **:** Haugh unit
IBV : Infectious bronchitis virus
K : Potassium
L* **:** Egg yolk brightness value
LDL **:** low-density lipoprotein
MUFA : monounsaturated fatty acids
MUFA **:** Total monounsaturated fatty acids
Na : Sodium
ND : Newcastle disease
P **:** Level of significance
PUFA : polyunsaturated fatty acids
RRS **:** Roche color scale values
SE **:** Standard error
THC **:** Tetrahydrocannabinol

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INTRODUCTION

Up to 70% of all management expenses are related to animal nutrition, making it one of the most significant factors in determining farming efficiency (Mallick et al., 2020). Additionally, the growing interest in sustainable production systems has concentrated on alternative feeding strategies that make use of plants such as herbs, vegetables, and their by-products. These materials can be used for sustainability and profitability and produce final goods to meet consumer demand, healthy products, environmentally and economically beneficial solutions.

Hemp (*Cannabis sativa* L.) is an annual plant belongs to the Cannabaceae family. Hemp is used in many areas including medicine, cellulose, cosmetics, oil industry and animal feed. Although it is confused with the type of plant used as illegal drug, many products can be obtained from the type known as industrial type. In fact, it is often considered as the rediscovery of the plant in recent years (Fike and Fike, 2017; Ranalli and Venturi, 2004; Yano and Fu, 2023). Due to many useful effects of hempseed, its cultivation is increasing in the world day by day. Seed of hemp contains about 94.36% dry matter, 19.1% crude protein, 6.62% crude ash, 32.99% oil and in its oil a high concentration of omega-3 fatty acid (17.69%) (Konca et al., 2019). Also it contains about 22 MJ/kg of gross energy in seeds (Callaway, 2004). In another study found that fat content of the dehulled seed is about 42 to 47% while the fat content of unhulled seed is about 25 to 34%. In hempseed cake gross energy content is 20.40 (MJ/kg) (Stastnik et al., 2020), crude protein content is 298.04 (g/kg), crude ash content is 72.6 (g/kg) and ether extract content is 96.94 (g/kg) (Šťastník et al., 2019). The psychoactive compounds present in the hempseed are Tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD). Increased concentration of THC is harmful for both humans and animals (Yang et al., 2017). A low level of THC (less than 0.3%) in hempseed is used as medicinal, seed oil crops, green fiber, and for sustainable bioenergy (Rehman et al., 2013; Ingrao et al., 2015;). Furthermore, hempseed also contains anti-nutritional factors like saponins,

trypsin inhibitors, cyanogenic glycosides, condensed tannins, and phytic acid (Russo, 2013a).

Hempseed is rich in n-3 polyunsaturated fatty acids (PUFA) which is an important part of cell membranes. It is important for normal physiological functioning and for normal health of human beings (Alagawany et al., 2019). Nutritionists recommend the intake of n-3 PUFA because of health-promoting effects like antilipidemic effects, demonstrating antithrombotic action and promoting growth (Ansari et al., 2010). Omega-3 ratio can be improved in poultry products by changing the fatty acids profile through various seeds and oils in the poultry diet (Konca et al., 2014; Alagawany et al., 2019). Therefore, including hempseed in the poultry diet may diminish egg cholesterol concentration and change egg yolks' fatty acid composition by enhancing the n-3 PUFA concentration (Silversides and Lefrançois, 2005).

Feeds used in animal husbandry are an important source of expense (65-70% of total expenses), and the main objectives are to benefit from these feeds at the highest level and to obtain the highest efficiency from the unit feed. Various processing techniques are applied to feeds to increase the utilization of feeds (Özen et al., 1999). Among these, the most commonly applied methods in practice in recent years are grinding, crushing, crushing, pelletizing, extruding and flaking.

There are various thermal treatment processes, their variation depends on the kind of heat source, and process parameter used. The types of heat treatments in animal feed processing mostly used are cooking, extrusion, pelleting, steam flaking, roasting, popping, conditioning, toasting, expending, and micronization. Thermal processing enhances digestibility, FI, carcass quality, and FCR. However, it decreases labor costs, transportation, feed waste, and storage costs. The heat works as a natural digestive enzyme to break down bonds of complex carbohydrates of grain starch and improves nutrient availability (Gurbuz, 2017).

This study investigated how hemp seed supplementation affects the performance of animals and egg quality traits with different processing techniques.

CHAPTER 1

GENERAL INFORMATION

1.1. Effects of hempseed use in poultry diets

In old World societies, hempseed has been a vital source of nourishment for thousands of years. Hempseed normally has a protein content of about 25% and an oil content of over 30%, along with significant levels of dietary fiber, vitamins, and minerals. Polyunsaturated fatty acids (PUFAs) make up more than 80% of hempseed oil, which makes it a particularly high source of the two essential fatty acids linoleic acid (omega-6) and alpha-linolenic acid (n-3). Hempseed oil typically has an omega-6 to omega-3 ratio (n6/n3) of 2:1 to 3:1, which is thought to be ideal for human health (Callaway, 2004).

Another study examined 43-week-old laying hens on a diet supplemented with 0, 50, 100, or 200 g/kg hempseed meal (HSM) for four weeks. Eggs were collected for fatty acid analysis results showed that there were no changes between treatment groups in terms of egg production, feed intake (FI), feed conversion ratio (FCR), body weight (BW) change, or egg quality (Silversides and Lefrançois, 2005).

Hempseed is enriched with abundant amounts of natural antioxidants like phytosterols, tocopherols, and phenolic compounds (Andre et al., 2016), which impart their role in decreasing the possibility of chronic diseases such as cancer, gastrointestinal disorders, dermatological diseases, immunomodulatory effects, cardiovascular disorders, lipid metabolism, and neurodegenerative diseases (Kaul et al., 2008; Prociuk et al., 2008). Many polyphenolic compounds have been found in hempseed particularly flavonoids like flavanols, flavanones, and isoflavones (Smeriglio et al., 2016; Pollastro et al., 2017). However in hempseeds main phenolic compounds are simple and complex lignanamides, which involve in many complex biological activities like anti-inflammatory activities, insecticidal effects, and feeding deterrent activities (Yan et al., 2015). Another bioactive

compound present in hempseed is tocopherol, which serves as an antioxidant by inhibiting the oxidation of unsaturated fatty acids (Kriese et al., 2004).

Hempseed is also used in organic production of broilers where there is no use of antibiotics. A poultry diet using hempseed along with the use of the specific ratio of ALA: LA (1:2 or 1:4) increases the production of antibodies against infectious bronchitis virus (IBV) and Newcastle disease virus (ND) without having any adverse effect on production performance. Hempseed contains an ample amount of iron (1680 ppm) which can be used to combat iron deficiency in children (Puthpongsiriporn and Scheideler, 2005).

Goldberg et al. (2012) investigated hens fed diets containing 4%, 8%, 12% hempseed oil, and 0, 10%, 20% hempseed for 12 weeks and found that the 12% hempseed oil inclusion had the highest total n-3 PUFA concentration in yolks compared to the control group. The supplementation of hempseed resulted in substantial decreases in lightness and increases in redness and yellowness, with the biggest alterations found in the 20% hempseed treatment. The findings suggest that including hemp in hen diets increases n-3 and PUFA ratio and egg yolk color intensity, but has no negative effects on the sensory profiles of eggs.

In a study of 19-week-old laying hens were fed a diet containing 10, 20, or 30% hempseed or 4.5 or 9.0% hemp oil. The findings showed that adding up to 30 % hempseed and 9.0% hemp oil to laying hen diets did not affect laying hen performance, egg quality, or plasma levels of metabolites (proteins, glucose, uric acid, and cholesterol) and electrolytes (Na, K, Cl, P, and Ca) (Neijat et al., 2014).

Shahid et al. (2015) showed a considerable reduction of total cholesterol of egg yolks in the 25% hempseed added group and with the 25% hempseed in feed, total monounsaturated fatty acids (MUFA) dramatically dropped while total and PUFA substantially rose. Additionally, the 25% hempseed group showed a substantial rise in total n-3 and n-6. In conclusion, the cholesterol and fatty acid composition of egg yolks has increased due to the 25% hempseed supplementation given to laying hens' diet.

In an experiment (Bazdidi et al., 2016) 5%, 10, 15, and 20 % ratio of hempseed (HS) or 2, 4, and 6% ratio of hempseed oil (HO) supplementation in hens were evaluated. The best egg production and mass, as well as the lowest FI and FCR were provided by

fed 6% HO addition. Both HS and HO supplementation reduced the egg yolk color index, although the effect of hempseed was more pronounced. Plasma's total protein level was unaffected by the diet supplements. Blood aspartate aminotransferase (AST), triglycerides, and cholesterol were considerably reduced at the highest concentration of hempseed oil6% as opposed to the highest concentration of hempseed 20 %. In contrast to hens fed 20 % hempseed, those fed a diet containing 6% hemp oil had higher blood high-density lipoprotein (HDL) levels. The study found that the inclusion of HO in the diet of laying hens improved egg quality more than the two lower concentrations of hempseed (5 and 10 percent). Additionally, the performance of laying hens after the peak was positively affected by the inclusion of HS.

Another experiment showed that the addition of 15 % raw and cooked hempseed did not affect egg production of laying hens. However, the addition of raw and cooked hempseed dropped the feed consumption of hens. The cooked hempseed increased egg weight, egg mass, eggshell weight (ESW), eggshell surface area (ESA), and eggshell thickness (EST). Also, the addition of hempseed either raw and or cooked caused a decrease in palmitic, palmitoleic, and oleic acids in egg yolk, however, increased linoleic, α-linolenic, and docosahexaenoic acids compared to the control group (Konca et al., 2019).

Another research of laying hens given dietary supplements of 0, 30, 60, and 90 g/kg hempseed and the inclusion of 30 g/kg hempseed to the diet of laying hens resulted in enhanced egg production and egg mass. Only the hempseed at 60 g/kg showed a substantial rise in alpha-tocopherol levels. Hempseed at 90 g/kg reduced thickness while maintaining strength. The inclusion of hempseed reduced cholesterol levels in egg yolks in all experimental groups (Skřivan et al., 2019).

Investigation of laying hens given 5, 10, and 15% hempseed, rapeseed, and linseed cakes showed that the FI, egg mass and FCR were significantly lower at the 15% dietary hempseed cake as compared to the 5 and 10% cake groups. Egg mass and egg production were greater in hempseed groups than in linseed cakes. Increasing the dietary amount of all seed cakes resulted in a decrease in egg yolk and a higher egg albumen ratio. Boosted consumption of all three seed cakes resulted in reduced percentages of SFA as well as MUFA. The PUFA levels of linoleic and ALA were increased by increasing the amount of cake in the diet from 5% to 10% and 15%, respectively. The findings support the notion

that complex feeds containing up to 10% cakes do not adversely affect the hen's performance and provide the chance to enhance yolk fat with PUFA (Halle & Schöne, 2013).

In the broiler experiment, 15% hempseed cake inclusion into the diet resulted in lower live weight and produced a low feed conversion ratio (FCR), and the hempseed concentration (5, 10 and 15%) did not affect carcass yield. However, 15% hempseed cake had a detrimental effect on chicken growth, which in turn caused the final BW of the chicks fed hempseed cakes to be lower (Ondrej et al., 2015). Broilers fed with 5%, 10%, and 20% hempseed added diet, chicks in group fed 20% HSM had significantly higher BW gain, lower FI, and improved FCR compared to the control group (Khan et al., 2010). The researchers found that the inclusion of 20% hempseed showed significantly higher breast and leg weights compared to the other groups. Additionally, the liver, gizzard, intestines, and abdominal fat weights were significantly higher in group 20%, while heart weight showed no significant difference. Researchers claimed that *Cannabis sativa* seeds have a positive impact on muscle and visceral organ weight in broilers, making them a desirable addition to the poultry industry (Khan et al., 2009).

Hempseed inclusion on the laying hen diets revealed that hens fed the diet containing 20% hempseed exhibited significantly higher egg weights, improved FCR, and greater BWs compared to the control group. These findings suggest that the dietary inclusion of industrial hempseed at a level of 20% for laying hens is a safe practice that may positively influence their overall laying performance (Goldberg et al 2010).

Eriksson and Wall (2012) hempseed meal use in organic broiler production did not affect total production performance, mortality, or the number of *C. perfringens* in the caeca.

A total of 48 white Bovans laying hens have supplemented a diet containing 4%, 8%, 12% HO and 0 or 10%, 20% HS, which shows that hemp products in the laying hens diet enhance yolk color and n-3 FA content but not effect on the cooked eggs (Goldberg et al., 2012).

The study proved that hempseed oil and HSM can be safely used in laying hens' diets to take table eggs enriched with essential fatty acids. As increased the concentration of hempseed in the diet egg yolk became more pigmented and there was an increase in

yellowness and redness. The maximum inclusion level of hempseed in a laying hen diet was about 20% up to this level no other useful parameters were affected but there increased egg weight and n-3 fatty acids (Gakhar et al., 2012).

Halle and Schöne (2013) rapeseed, linseed, and hempseed cake (5, 10, and 15%), feed consumption, egg mass, and FCR were found to be significantly lower in 15% supplementation groups as cake ratios in the diet increased, yolk ratio decreased and albumen ratio increased. Researchers reported that the most appropriate level to be used in the diet for rapeseed, linseed, and hempseed cake was 10%. As rapeseed, linseed, and hempseed cake ratio increased in the diet, SFA decreased, but linoleic and linolenic ratios increased.

Konca et al. (2014) reported that the addition of 0%, 5%, 10%, or 20% hempseed did not affect BW, FI, FCR and egg production of quail, however, the 10% hempseed increased egg weight and egg specific gravity. The serum cholesterol, triglyceride, and HDL levels were not altered, but low-density LDL ratio was lower in the hempseed groups. Additionally, the n-3 fatty acid content in eggs increased linearly with dietary hempseed ratio.

Mahmoudi et al. (2015) used a lower dosage of hempseed (0, 25, 50, and 75 g/kg) and combination of two levels of dextran oligosaccharide (0 and 1g/kg) and found that serum triglyceride, low-density lipoprotein (LDL), total serum cholesterol, and HDL were minimal at 75 g/kg of hempseed, serum levels of HDL increased as hempseed levels increased. Diets containing 25 g/kg of hempseed also significantly reduced average daily FI and average daily gain. Dietary dextran oligosaccharide and hempseed did not significantly affect the complete blood count, antibody production, or the relative weight of the bursa and spleen in broilers. However, they did significantly increase ADG and HDL and improve the FCR when added to diets containing 1 g/kg of dextran oligosaccharide in comparison to control diets.

Ondrej et al. (2015) reported that the live weight of the broiler performance was higher in the 15% HSM supplemented group compared to the other groups in the 0, 5, and 15% HSM supplementation groups.

In a study diets containing 5%, 10%, 15%, and 20% extruded hempseed had no negative impact on BW gain, FI, or FCR. However, the inclusion of extruded hempseed significantly reduced the relative weight of breast muscle, while it did not significantly affect other carcass parts (Barani et al., 2017).

Yalcin et al. (2018) investigated the composition of egg fat after six weeks of feeding of laying quail with 5, 10, and 20% hempseed-added diets. In the 20% supplemented group, the cooking loss of breast meat was much lower. The redness of hip meat increased linearly and cubically when hempseeds were added to diets. The addition of HS to breast meat resulted in a decrease in palmitoleic and oleic FAs, but a linear increase in the amounts of linoleic and linolenic acid, however less oleic and palmitoleic fats were added to quail eggs with hempseed supplementation.

Vispute et al. (2019) examined different dosages of hempseed, either alone or in combination with dill seed, as an antibiotic growth promoter on the performance, serum biochemical, and intestinal health of broiler chickens over 42 days. Neither the carcass characteristics, such as giblet, cut-up parts, and abdominal fat yield, nor the performance traits, such as FI, BW gain, and FCR were influenced by the dietary treatments during the trial period.

Supplementation of hempseed does not affect carcass traits and growth performance however, it has a positive effect on serum lipid profile, enhances gut health, and overall increases the production performance of the broiler (Vispute et al., 2019). Konca *et al.* (2019) reported an increase in egg weight with a diet supplemented with hempseed. However, no significant difference is found in the sensory characteristics of eggs like taste, color, texture, smell, and appearance obtained from the hempseed diet supplement groups (Konca et al., 2019). The breaking strength of the tibial bone increased with the addition of hempseed ratio (30, 60, and 90 g/kg) in diets (Skřivan et al., 2019).

Rajasekhar et al. (2021) reported that adding 0, 10, 20, and 30% hempseed cake to the diets of laying hens had no effect on FI, egg production, or FCR. Also, Rajasekhar et al. (2021b) found that 10, 20 and 30% HSM supplementation to the diet increased the PUFA values including linoleic and linolenic acids in eggs and belly fat compared to the control. In addition, cannabinoids residues in eggs, blood, breast meat, body fat and belly fat were found to be below detectable levels.

In a study found that 4% HSM addition increased FCR and egg production compared to the 8% and 12% HSM groups. However, there were no significant effects on FI, egg weight, BW loss, egg shape index, albumen index, albumen weight, Haugh unit, yolk weight, yolk index, or eggshell thickness. Additionally, the inclusion of HSM significantly increased n-3 fatty acid levels and decreased the yolk n-6 to n-3 fatty acids ratio (Öztürk et al., 2024).

In a study of laying quail, HSM 5%, 10%, 15%, and 20% ratio was used in diets. The performance parameters were not significantly affected by the dietary HSM contents. However, eggshell thickness was found to be significantly influenced while the albumen index increased with higher levels of HSM in the diets. Additionally, supplementation with HSM had an effect on the fatty acid composition and total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) contents of the egg yolk based on the level of addition. In conclusion, it was observed that adding HSM can increase total MUFA and n-3 fatty acids without adverse effects on performance or egg quality parameters (Cufadar et al., 2021).

Razmaitė et al. (2022) compared effects of 10% hempseed, rapeseed, and camelina seed cakes addition in quail diets. In the hempseed cake group, egg yolk L*, and a* values were lower than other groups. The highest saturated fatty acids (SFAs) and lowest MUFAs were found in the yolk hemp cake group.

Taubner et al. (2023) investigated that effects of enzyme secretion (cellulase, amylase, xylanase, pectinase, inulinase, invertase maltase, lactase, lipases, and proteinases) of hempseed and flaxseed in the digestive tract in the broiler digestive system. Addition of flaxseed and hempseed to the diet increased enzyme activities of cellulase, amylase, xylanase, pectinase, maltase, invertase, proteinase and lipase activities. As results of these increments of enzymes secretion with hempseed and flaxseed addition, growth performance increased in broilers.

Tufarelli et al. (2023) noted hempseed cake addition 0, 5 and 10 % to broiler diets did not affect final BW, gain, FI, FCR, carcass (eviscerated carcass, breast, and abdominal fat), meat chemical composition, color, and pH24. Total n-6 and n6:n3 ratios in the thigh and breast muscle of chickens fed HSM were significantly lower in the HSM-supplemented group than in the control group, and the n-3 ratio was significantly higher than in the

control group. At the same time, breast muscle TBARS and hydroxyperoxide values were significantly higher in the HSM-supplemented groups.

In laying hens, the addition of 10% hempseed did not significantly impact laying hen performance, however, higher hempseed levels (20% and 30%) negatively affected egg-laying performance. The inclusion of hempseed also improved albumen quality, with the highest Haugh Unit (HU) values observed in the 30% hempseed group. Additionally, hempseed significantly influenced yolk color, causing a decrease in yellowness; the control group had dark yellow yolks, while the 30% hempseed group had very pale yellow yolks (Taaifi et al., 2023).

Hemp is annual herbaceous plant in the Cannabaceae family that has historically been grown for the production of fiber and seeds. Hempseed has a high mineral content, high vitamin content, tocopherols, beneficial polyunsaturated fatty acid, and substantial amount of crude protein and phenolic compounds (Shariatmadari, 2023).

Kanbur et al. (2023) demonstrated that neither egg production nor egg quality was impacted by any of the included hempseed oils. However, hempseed oil affected the color characteristics of the yolk.

Šťastník et al. (2019) reported that the red color was higher in the 15% added group with 0.5 and 15% HSM expeller addition.

Razmaitė et al. (2022) with the addition of 10% rapeseed, hempseed and camelina seed cakes, egg yolk L* value in rapeseed and camelina groups was found to be significantly lower than HSM group, but a* value was found to be lower in HMS group. The highest PUFA values were found in HMS group.

Darmawan and Ozturk (2023) reported that the effect of 15% HSM supplementation in broiler diets on the weight of carcass, abdominal fat, liver, duodenum, cecum, and the relative length of the duodenum was insignificant.

Sopian et al.(2024) noted that adding hempseed products to the laying hen’s diet enhances n-3 and n-6 fatty acid content, but decreases FI, and weight gain. However, hemp products did not feed the conversion ratio in hens.

Juodka et al. (2024) reported that 10% HSM and camelina meal addition negatively affected the BW, feed consumption, and FCR coefficient values of quails, but did not significantly affect the carcass yield, edible internal organs and abdominal fat ratio. HMS addition increased total MUFA values compared to the control group, but did not cause an increase in total SFA ratio.

1.2. Feed processing techniques and effectiveness

In animal husbandry, feeds must be evaluated correctly to obtain the highest efficiency from a unit of feed. However, not all nutrients in the feed used are evaluated by the animal and a significant part of them is thrown away without any use. To prevent this, the application of some processing techniques gives useful results. One of these is thermal processes. Thermal processes include pelleting, micronizing, roasting and boiling, etc. (Özen et al., 1999).

Grinding the diet ingredients to the appropriate particle size is the first step in making pelleted chicken feed (Vukmirović et al., 2016). Grinding is the critical step in the processing of poultry feed, with substantial implications for feed quality and chicken performance. The primary goals of grinding feed ingredients for poultry are to achieve constant fitness to improve palatability, decrease surface reactivity, and facilitate mixing with other feed components (Refaay and El Sayed, 2016). The grinding method and feed form, such as mash or pellets, can significantly affect the microbial composition, and intestinal and digestive enzyme activities (Engberg et al., 2002).

Faugno et al. (2016) investigated variable screw rotation speed, seed preheating, and extraction temperatures to maximize oil yield. Using a mechanical screw press with speed and temperature sensors, the researchers found that high extraction temperatures, slow screw speeds, and seed preheating significantly increased oil yield. Under these optimum conditions, oil yield reached 79.47±0.12% (w/w), representing a 25% increase over the lowest yield among the tested conditions.

Feed processing techniques have changed dramatically over time, necessitating greater accuracy to meet the demands of modern consumers, particularly concerning livestock feed's nutritional content. Animal feed needs to be nutritionally balanced and up to the higher standards that today's consumers demand to be produced in a modern livestock

facility. The feed can take on a variety of forms depending on the processing technique used, such as pellets, flaked grains, and extruded nuggets (Gurbuz, 2017).

Martínez et al. (2017) employed Box-Behnken designs to optimize low-temperature (50 °C) screw-press extraction of sesame oil. The primary processing parameters investigated were seed moisture ratio (SMC), speed of press (PS), and die of restriction (RD). Pilot plant scale extractions achieved a peak oil recovery (OR) of 71.1% at 12.3% SMC, 4 mm RD, and 20 rpm PS. Theoretical models were used to scale up the process to an industrial scale, predicting a maximum OR of 74.4% at 8.03% SMC, 10 mm RD, and 20 rpm PS.

Deli et al. (2011) searched how the physical parameters of a screw press machine affect the oil yield from *Nigella sativa* seeds. Factors like the size of the nozzle (6, 10, and 12 mm), speed of extraction (21, 54, 65, and 98 rpm), and shaft screw diameter (8 and 11 mm) were evaluated. It was found that the combination of an 8 mm shaft screw, 21 rpm speed, and 6 mm nozzle size yielded the highest oil percentage. The results also showed that oil yield generally decreased with increased nozzle size and rotational speed when using an 8 mm shaft screw. Conversely, with an 11 mm shaft screw, the highest oil yield occurred at 65 rpm with 6 and 10 mm nozzles. The study highlighted significant differences in oil yield at different temperatures, with higher yields at 50°C and lower yields at 100°C. Overall, physical parameters like nozzle size, shaft screw diameter, rotational speed, and temperature significantly influence the oil yield of N. sativa seeds.

Pradhan et al. (2011) examined how moisture content, cooking temperature, and time affect oil yield from Jatropha seeds using a screw press expeller. Optimal conditions achieved a 73.14% oil recovery at 9.69% moisture content and 110°C for 10 minutes. Cooked seeds improved oil recovery by 7% compared to uncooked seeds. As moisture decreased, the pressing rate and sediment content varied, but the oil quality remained acceptable.

Muangrat et al. (2018) explored how drying temperatures (60, 75, 90°C) and press head temperatures (60, 75, 90°C) affect oil yield from Sacha inchi (Plukenetia volubilis L.) seeds using a single screw press machine. Non-dried samples consistently produced higher oil yields compared to dried ones across all press head temperatures. Sacha inchi oils were rich in PUFA, especially n-3and n-6. Phenolic compound content varied from 30.46 to 120.32 mg GAE/100g oil. The oils had DPPH IC50 values from 317.33 to 663.54

mg/mL and ABTS IC50 values from 215.48 to 301.75 mg/mL. Additionally, the oils showed peroxide values of 1.94–2.27 meq O2/kg, iodine values of 102.66–104.05 g I2/100g, saponification values of 182.18–195.11 mg KOH/g, acid values of 1.94–5.90 mg KOH/g, and free fatty acid percentages ranging from 0.97% to 2.97%.

Kopacz et al. (2021) investigated the impact of raw rapeseed cake (RRC), hydrobarothermally-treated rapeseed cake (HRC), and fermented rapeseed cake (FRC) on the performance of laying hens, and the quality, fatty acid profile, and oxidative stability of their eggs over 12 weeks. The control group received soybean meal as the main protein source, while the experimental groups were fed diets containing 20% RRC, HRC, or FRC. Hydrobarothermal treatment and fermentation reduced glucosinolate and phytate phosphorus in the rapeseed cake. Hens in the HRC and FRC groups showed higher laying performance similar to the control group, although RC negatively affected eggshell quality across all experimental groups. Albumen quality was highest in the FRC group. Dietary inclusion of RRC, HRC, and FRC resulted in a decrease in saturated fatty acids and an increase in n-3 and n-6 polyunsaturated fatty acids, improving the n-6/n-3 ratio in egg yolks. These treatments also reduced superoxide dismutase activity in egg yolks, with FRC eggs showing the highest catalase activity and lowest lipid peroxides. The sensory quality of eggs was not compromised, with FRC eggs receiving the highest overall score. In conclusion, FRC is recommended for inclusion in layer diets due to its positive impact on laying performance, albumen quality, and sensory quality of eggs.

By-product feedstuffs can be categorized into stalks and residues, like by-products from residues of fruits and vegetables, grains- and legumes-milling industry, and the oil industry. Feedstuff manufacturing from waste addresses waste and environmental pollution issues. Utilizing new agro-industrial by-products, such as vegetable and fruit residues may support sustainable feedstuff production. These by-products offer significant value, mitigating food crises to some extent. This review discusses their nutrition and methods to improve the quality of agro-industry by-products as unconventional feeds (Yang et al., 2021)

Abdilova et al. (2023) explored the optimization of sunflower seed oil pressing in Kazakhstan, focusing on grinding degrees and screw rotation speeds. Using an oil pressing machine, we examined the effects of plate hole diameters (5 mm, 4 mm, 3 mm,

and 2 mm) and screw speeds (30 rpm to 250 rpm) on oil yield. Results indicated that increasing both grinding degree and screw speed improved oil yield. Optimal conditions were identified as a 4 mm grinding degree and 60 rpm screw speed, achieving the highest yield of 15.6 g/s. Additionally, higher pressure during pressing significantly reduced residual oil content in the seed cake. Increased screw speeds and finer grinding elevated power consumption and cake density, impacting energy demand and cake compactness. These findings offer valuable insights for enhancing sunflower oil pressing efficiency by selecting appropriate grinding degrees and screw rotation speeds.

Kiss et al. (2020) explored how different rotational speeds and nozzle diameters of a screw press impact walnut kernel processing. Variables measured included oil pressing temperature, yield, total phenolic compounds (TPC), FAs and antioxidant capacity. The results indicated that pressing conditions may affect the antioxidant capacity and TPC in both bagasse pellets and pressed oils. A lower nozzle diameter increased oil yield but also raised oil pressing temperature, affecting fatty acids. Saturated fatty acids positively correlated with higher pressing temperatures, while polyunsaturated fatty acids were positively correlated but not significantly. Monounsaturated fatty acids were more negatively impacted by higher temperatures, showing greater susceptibility compared to polyunsaturated and saturated fatty acids.

Mridula et al. (2020) optimized oil expelling parameters for dehulled and whole sunflower seeds under variable moisture and press head temperatures using Box-Behnken design. Key variables included oil recovery, press rate, residual oil in meal, sediment content, oil and meal temperature, and free fatty acid (FFA). The findings suggest that using 68.64% dehulled and 31.36% whole sunflower seeds with 6% moisture at a press head temperature of 71.5 °C is optimal for oil expelling.

Jirarattanarangsri and Muangrat (2022) compared the quality of Assam tea seed oil obtained via supercritical CO_2 extraction and screw press methods. The supercritical CO_2 method yielded higher oil (12.94–16.53%) compared to the screw press (9.96-12.18%). Higher temperatures in both methods decreased saponin content but increased flavonoid content in CO_2 extraction. Supercritical CO_2 extraction reduced acid value and free fatty acids with temperature rise, unlike screw press extraction. Both methods' oils were rich in oleic, linoleic, and palmitic acids. Increased temperatures also enhanced phenolic

compound extraction, boosting antioxidant capacity. These findings suggest that supercritical CO_2 extraction is a competitive, eco-friendly alternative for high-quality oil production.

Mirzaxmedov et al. (2023) investigated the optimization of screw press parameters for extracting flaxseed oil to maximize oil recovery while maintaining quality. Flaxseed oil is prized for its high unsaturated fatty acid content and low n-6/n-3 ratio, offering health benefits but being prone to oxidation. Using Response Surface Methodology, the size of the cake outlet (1 mm) and screw speed (155.89 rpm) were identified as optimal, achieving an oil recovery of 41.4%. Although higher screw speeds increased oil recovery, the cake outlet size had a more significant impact. The highest acid and peroxide values were 0.71 mg KOH/g and 7.71 meq/kg, respectively. Sediment content ranged from 9.12% to 14%. Despite increased extraction temperatures, the n-6/n-3 ratio remained beneficially low.

1.2.1. Thermal treatments

In addition to feed processing techniques like grindings, which reduces the particle size of the feed, and then continues with crushing, with the development of technology, different techniques have been developed using heat treatment, steam, and pressure applications. It is reported that processing techniques also affect the digestion rate, location, and distribution of protein, starch, and cellulose in feed (Mathison, 1996). Inadequate feed processing practices at low temperatures and durations increase the feed cost and cause the expected benefit from processed feeds not to be seen, and excessive processing causes a decrease in animal performance due to a decrease in dry matter consumption (Hutjens et al., 2000).

Due to its multi-step, versatile, thermal/mechanical nature, extrusion cooking has opened up a wide range of food applications. There is uncertainty about how extrusion cooking affects nutritional quality. Destroying anti-nutritional factors, gelatinizing starch, increasing soluble dietary fiber, and lowering lipid oxidation are some of the positive benefits (Singh et al., 2007).

The method of extrusion involves pushing the material (mixture or feed-stuff) into the barrel using variously shaped screws and pressing it through the die at the end of the

barrel. Short time and high temperature are the fundamentals of the extrusion process; the high temperature can be directly caused by preconditioning and steam injection (wet extrusion) friction (dry extrusion), or their combinations. In wet extrusion, the treated material's humidity can reach 80% whereas in dry roughly 30% (Levic and Sredanovic, 2010).

Martínez et al. (2013) determined how seed moisture content and pressing temperature impact the oil recovery and quality of almond oil (AO), along with the effectiveness of natural and synthetic antioxidants on its oxidative stability. Different seed moisture contents (4-12%) and pressing temperatures (20-60°C) were tested. Higher moisture content generally increased oil recovery, with the highest recovery (79.3%) at 8% seed moisture and 40°C pressing temperature. All tested extraction conditions maintained acceptable oil quality.

Pelleting is the most widely used thermal processing technique in the poultry industry. The pelleting technique is designed to agglomerate ingredients particles using, moisture, temperature, mechanical action, and pressure. Pelleted diets may increase weight gain, FCR, and FI in broilers. Moreover, this improved performance is only possible provided the pellet remains intact until ingested many factors influence the pellet's physical quality, including feed ingredient particle, feed moisture, feed nutritional composition, conditioning temperature, and time. Despite this importance, these parameters are not always managed correctly, therefore, pelleted may not contain a large of undamaged pellets (Corzo et al., 2011; Keysuke et al., 2015).

There are various thermal treatment processes, their variation depends on the kind of heat source, and process parameter used. The types of heat treatments in animal feed processing mostly used are cooking, extrusion, pelleting, steam flaking, roasting, popping, conditioning, toasting, expending, and micronization. Thermal processing enhances digestibility, FI, carcass quality, and FCR. However, it decreases labor costs, transportation, feed waste, and storage costs. The heat works as a natural digestive enzyme to break down bonds of complex carbohydrates of grain starch and improves nutrient availability (Gurbuz, 2017).

Massuquetto et al. (2019) found that pelleted diets improved the performance (weight gain, FCR and FI) of broilers caused by the conditioning and pelleting process, which increased energy and nutrient digestibility.

Ouchbani et al. (2021) explored how the temperature of the heating ring on a screw press extraction cylinder affects argan oil yield and quality from unroasted kernels. Using a mechanical screw press, temperatures of 60, 80, 100, 120, 140, and 160°C were tested at a screw speed of 5 rpm. Results showed that the highest oil yield was obtained at 80°C (53-55% for crude oil, 47% for clarified oil). At lower temperatures (60°C), the process was hindered by residue particles causing cylinder jams. The actual temperature of the extracted oil ranged from 45-68°C. While temperature significantly affected quality parameters like free acidity, peroxide value, and UV absorbance, all extracted oils met extra virgin grade standards. The optimal heating ring temperature of 80°C thus maximizes yield without compromising oil quality.

Zlatković et al. (2024) examined how screw frequency, nozzle diameter, and press head temperature affect fodder radish seed oil (FRSO) yield, throughput, and fatty acid composition. Using a quadratic equation and response surface methodology, the study identified the highest FRSO yield (17.61%) and a 51.3% extraction efficiency with a 50 Hz screw frequency, 80°C temperature, and an 8 mm nozzle. All pressing factors significantly influenced FRSO throughput. The oil's fatty acid composition included C18:1 (30.9%), C22:1 (20.8%), C18:2 (14.9%), and C18:3 (11.9%), consistent with other fodder radish oil studies.

Amalia Kartika et al. (2006) showed that adjusting the position and spacing between reversed screw elements improved oil yield. Higher yields were achieved by moving these elements apart and using smaller pitch elements. Optimal conditions for the highest yield (85%) and best meal quality (residual oil content <13%) were 120°C, 75 rpm, and 19 kg/h. Lowering barrel temperature and feed rate, while increasing screw speed, boosted energy input, particularly specific mechanical energy. All tested oil samples maintained high quality, with acid values below 2 mg of KOH/g and phosphorus content under 100 mg/kg.

Rotimi and Rotimi Moses (2014) examined the performance of a mechanical screw press for soybean oil extraction. The test assessed expelling efficiency, oil yield, and extraction

losses at various kneading temperatures. Soybean oil content ranges between 18-20%, with the test soybeans having a 10.7% moisture content. Results showed oil yield between 6.61-14.22%, expelling efficiency from 32.26% at 50°C to 68.13% at 90°C, and extraction losses from 5.39-9.90%. The highest expelling efficiency was 69.13% at 90°C. Statistically significant differences in mass flow rates were noted across temperatures. The machine performed well compared to others, simplifying operations and reducing labor intensity.

Rabadán et al. (2017) focused on optimizing pistachio oil extraction using hydraulic and screw presses, evaluating yield, physicochemical properties, sensory attributes, and the quality of defatted pistachio flour. Hydraulic press extraction showed no significant differences in yield or physicochemical characteristics, but sensory analysis favored oil from roasted pistachios (100 °C/30 min). Screw press extraction revealed differences in yield and sensory qualities, with a lower rotational speed (17 rpm) providing higher yields and more consumer-preferred oil. Differences in moisture, fiber, nitrogen, protein, and ash content were noted in pistachio flour from both presses. The findings suggest prioritizing yield and sensory evaluation over physicochemical characteristics. Pistachio flour, a by-product, was high in protein.

Karaj and Müller (2021) looked into the quality of Jatropha curcas oil extracted using a Komet D85-1G mechanical screw press and compared it with the chemical extraction (soxhlet) method and DIN 51605 standards. The quality of J.curcas oil was generally within acceptable norms, showing minimal variation due to different screw press parameters. Most chemical properties were within limits, except for AV, FFA, TC, P, Ca, K, and WC, while CR and Na met the standards. The highest temperatures were noted at point T3, correlating well with oil quality. Maintaining extraction temperatures below 90°C at the compression zone and 70°C at the oil outlet ensures oil quality suitable for direct use as fuel or biodiesel production.

Roncero et al. (2021) explored almond oil extraction using screw press (SP) and hydraulic press (HP) methods. The SP achieved the highest oil yield at 100–150°C. While HP yielded better quality oils due to less aggressive treatment, these oils had lower sterol content. The fatty acid composition, predominantly unsaturated fatty acids (>90%), remained consistent across both methods. Operational conditions did not significantly

affect oil performance or composition, but sensory tests revealed distinct differences between HP and SP oils. Additionally, the defatted almond flours from both methods were high in protein and fiber, with higher fat content in flour from HP pressing cake.

Wang et al. (2022) examined screw press, hydraulic press, and Soxhlet extraction methods, the study evaluated rapeseed and flaxseed oil yield, sensory qualities, physicochemical properties, and the composition of major and minor components. Soxhlet extraction significantly increased oil yields by 30.10%-73.90% for rapeseed oil and 6.30%-54.40% for flaxseed oil compared to other methods. Roasted pretreatment also boosted oil yields by 4.10%-25.00% for rapeseed and 6.70%-23.15% for flaxseed compared to untreated samples. The screw press method resulted in higher linolenic acid and vitamin E contents, with cold-pressed rapeseed oil showing a 1.50%-23.80% increase in linolenic acid. Soxhlet extraction improved total phenol content but resulted in higher acid and peroxide values, indicating lower oil quality. The hydraulic press method yielded oils with excellent sensory scores. Major volatile compounds in the oils included aldehydes, acids, alcohols, heterocycles, and ketones. Extraction methods and pretreatment did not significantly affect triglyceride compositions and contents. The study suggests that selecting the appropriate oil extraction technique and pretreatment can optimize oil yield and quality for large-scale production.

Sakdasri et al. (2023) explored how moisture ratio, feed rate, temperature, and impact the yield and thymoquinone ratio of cold-pressed black cumin seed oil using a screw press. The parameters were optimized with a Box–Behnken design, identifying optimal conditions by superimposing response surfaces for oil yield and thymoquinone content. Thymoquinone content remained stable at 60°C, while moisture significantly influenced both oil yield and thymoquinone content. The maximum oil yield (31.67 wt%) was achieved at a 45 g/min feed rate, 70°C, and 6% moisture, though with low thymoquinone content (5.14 mg/mL). High thymoquinone content (~12.00 mg/mL) resulted in lower oil yields (10-13 wt%). Optimal conditions were a feed rate of 50–54 g/min, 15–18% moisture, and 55–60°C, yielding 22 wt% oil and 10 mg/mL thymoquinone. The study underscores the balance required between optimizing oil yield and maintaining high thymoquinone content.

This study investigated how hempseed supplementation affects the performance of animals and egg quality, fatty acid composition and blood traits with different processing techniques.

CHAPTER 2

MATERIAL AND METHODS

2.1. Animals and experimental design

The study was approved Erciyes University's local ethics committee released the decision of the approval on 12.04.2023 and 20/120. This study was conducted at Erciyes University Agricultural Research and Application Center (ERÜTAM). A total of 60 commercial Super Nick, 37-week-old laying hens were used as animal material in the study. The chickens used in the study were vaccinated against Newcastle, infectious bronchitis, and Bursal disease during the chick and pullet period according to the vaccination program in commercial facilities. The animals were fed commercial feed for pullets and laying hens in rearing periods. Before starting the experiment, the BWs of the laying hens were weighed and distributed to the groups to minimize the live weight difference among the treatments. At the same time, 14 d adaptation periods were applied with control feed, egg records of the hens were followed and care was taken to ensure that there was no difference between BW, egg production, and egg deformity. In case the hens reduced the egg productivity they were replaced with unobserved laying hens from the farm.

In the study, chickens were housed in wire cages (42x40x46 cm) and 2 chickens in each cage. When hens were placed in cage compartments, treatment subgroups were distributed in different areas of the cages. Partitions are placed between feeders to prevent animals from consuming feed from other side compartments. In addition, the electric lighting method applied 16 hours of illumination (between 05.00 - 21.00) and 8 hours of darkness (between 21.00 - 05.00). Laying hens were placed in cages and fed study diets for 16 weeks (112 days) after 14 days of the adaptation period. It was taken as a period every two weeks and was divided into 1st Term (0-14 days), 2nd Term (14-28 days), 3rd

Term (28-42 days), 4th Term (42-56 days), 5th Term (56-70 days), 6th period (70-84 days), 7th period (84-98 days), and last 8th period (98-112 days).

The hens were equally divided into 3 treatment groups and placed in cages with 10 replications and 2 animals in each replication. The groups were formed as follows; 1: Control (C, no hempseed meal addition), 2 and 3 group 15% cold (60 °C, HSM-C) and hot (120 °C, HHS-M) pressed hempseed meal addition to the laying hen diets. The study lasted for 112 days. Water and feed were obtained as *ad libitum.* Nipple drinkers were used to meet the water needs of the chickens.

2.2. Experimental feeds

The feed ingredients (without hempseed meals) used in the experiment were purchased from a commercial feed company. The seeds were processed in a screw press speed stage 2.5, 6 mm nozzle, pressure head temperature 60 and 120 °C. The cold-processed hempseed meal (HSM-C) was produced by a screw machine and oil separated at 60 °C screw temperature. The hot-processed hempseed meal (HSM-H) was produced by a screw machine and oil was separated at 120 °C screw temperature.

The feed ingredients were formulated to meet the needs of the chickens (according to NRC, 1994). While no hempseed meal was added to the control ration, 15% cold-pressed hempseed meal was added to the second group and 15% hot-pressed hempseed meal was added to the third group. Feed premix additives were mixed homogenously with a mixer machine. Feeds were filled with plastic caps and stored with a lid closed in a shaded area. Feeds were prepared every month. Feed was offered as ad libitum. Experimental feed compositions are presented in Table 2.1.

The data collection process was carried out every 14 days throughout the research. By sampling the internal and external quality characteristics of the eggs, one egg was obtained from each chicken. Individual feed consumption was calculated for every 14 days.

2.3. Determination of body weight

The average live weight of animals at the beginning and end of the study was weighted individually with the help of a 1g sensitive scale. Body weight (BW) changes were calculated using live weight values measured at the beginning and end of the study.

Table 2.1. Experimental feed composition

Feed materials	Control	HSM-C, 15%	HSM-H, 15%
Corn	52.9	48,3	48,3
Sunflower meal (36% CP)	1.58	0	0
Soybean meal (46% CP)	24,51	15,8	15,8
Wheat	8	8	8
Limestone	9,34	9,2	9,2
Plant oil	1.58	1.7	1.7
Salt	0,36	0,35	0,35
Vitamin -mineral premix[¥]	0,2	0,2	0,2
Di-calcium phosphate	1.40	1.38	1.38
D-L-Methionine	0,13	0,10	0,10
Hempseed cake	0	15	15
Total, kg	100	100	100
Chemical composition*			
Dry matter, %	94,18	94,18	94,18
Crude protein, %	17,51	17,51	17,51
Crude fat, %	3,91	3,91	3,91
Crude fiber	2.63	2.63	2.63
Crude ash	18,76	18,96	18,96
Methionine**	0,41	0,41	0,41
Lysine**	0,87	0,87	0,87
Calcium**	3,87	3,87	3,87
Available Phosphorus**	0,39	0,39	0,39
Metabolic Energy Kcal ME/kg***	2750	2750	2750

[¥]Vitamin-mineral premix per kilogram of the diet, retinol acetate, 10.000,0000 IU; cholecalciferol, 2.500.000 IU; dl-α-tocopheryl acetate, 25.000 mg; menadione, 5.0 mg; Thiamine Hydrochloride, 2.000 mg; riboflavin, 5.000 mg; Pyridoxine Hydrochloride, 5.000 mg; Cyanocobalamin 20 mg; nicotinic acid, 40.000 mg; Calcium d-Pantothenate 12.000 mg, biotin, 50 mg; folic acid, 500 mg, Cantaxanthin, 4,000 mg; β-Apo-8-Carotenic Acid 1.000 mg; β-Apo-8-Carotenic acid 1.000 mg; Manganese (MnO), 120.000 mg; Chlorite, 30.000 mg, Carbonate 5.000 mg; Selenium 250 mg; Antioxidant, 10.000 mg. **Nutrient compositions were calculated based on NRC (1994) data of feedstuffs nutrient tables.

2.4. Determination of feed consumption and feed conversion ratio

Feed consumption (FC) was calculated by subtracting the weight of feeds left from feeds given based on subgroups for 2 weeks considering the number of dead animals in the calculation of the average FC. The average daily FC per animal was obtained by dividing the amount of feed consumed between two weighing by the number of days of feeding and the number of animals belonging to that group. Also, the feed conversion ratio (FCR) was determined by proportioning periodic feed consumption and egg mass. The FCR was calculated every 2 weeks.



Figure 2.1. Laying hens used in the experiment

2.5. Determination of egg yield

Egg production was recorded daily and each subgroup's total number of eggs in 14 days was calculated. Egg yields were calculated as both number and percentage (%), and the formula was number of eggs produced /total number of eggs that should be produced in 14 days in each subgroup x 100 was used in the calculation of % egg yield.

2.6. Determination of egg weight and internal quality characteristics

During the 14th, 28th, 42nd, 56th, 70th, 84th, 98th, and 112th days of the study, 30 eggs from each subgroup (total of 90 eggs) were taken to the laboratory for further analyses. Weight and internal characteristics such as albumin height, Haugh unit (HU), and Roche yolk color scale were determined with Egg Analyzer (Orka Food, USA). Also, CIE lab values of egg yolk color such as L* (brightness), a* (redness), and b* (yellowness) color measurements were determined with a Minolta color meter (CR-400 Minolta Co, Japan).

2.7. Determination of eggshell properties

After the eggs were broken for analyses with an egg analyzer machine, remaining eggshells were washed and dried in oven 75 °C for 24 h, then the eggshell weights (g) were determined by weighing on a precision scale sensitive to 0.1g. The eggshell percentage was calculated by dividing the shell weights: egg weight *100 formula.

Shell weight per unit area (mg/cm^2) was calculated by dividing the absolute shell weight by the egg surface area.

Egg surface area (cm^2), reported by Carter (1975).

Surface Area = was calculated with the formula 3.9782 x egg weight $^{0.7056}$.

Shell thickness (mm) was measured with the help of a digital micrometer sensitive to 0.01 mm in the sharp, blunt, and middle parts of the broken eggshells, and then the shell thickness value was determined by taking the arithmetic mean of these three measurements.

2.8. Feed material analysis

The experimental feed chemical analyses were done according to AOAC (2013). In the chemical analyses, feed's dry matter (DM), crude protein (CP), crude ash, and crude oil analyses were made by using the following methods:

Dry matter: Feed's dry matter was determined after 6 h at 105 °C in the drying oven.

Crude protein: Crude protein were determined with Dumas technology in Velp protein analyzer.

Crude ash: Dried feeds were put at 550 °C for 5 hours in an ash oven.

Crude fat: Feed's crude oil was determined with Velp fat extractor with petroleum ether.

ME calculations: It is calculated according to the TSE (1991) formula.

2.9. Blood analysis

At the end of the experiment, 10 ml of blood was taken under the wing from a randomly selected laying hen from each subgroup was centrifuged and its serum was separated and stored in eppendorf tubes at -80 ° C until it was analyzed. Serums were taken out from the freezer on the day of analysis were thawed and serum glucose, total protein, triglyceride cholesterol, high-density lipoprotein (HDL), alanine aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations were determined in the autoanalyzer with commercial kits for each parameter.

2.10. Statistical analyses

Since the study was set up and carried out in a completely randomized design, variance analysis was performed using the One-Way ANOVA procedure in the SPSS statistics program Version 22. Duncan tests were used to determine differences between averages, $P < 0.05$ level was taken into consideration for the level of significance. All data were expressed as Means± standard errors.

CHAPTER 3

RESULTS

3.1. Effect of treatments on live body weight, feed intake and feed conversion ratio

Effects of hempseed cold and hot processing techniques on BW, body weight gain, and change (%) of laying hens are shown in Table 3.1. There were no significant effects of feed processing techniques on BW, gain, and change of the laying hens (P>0.05).

Tablo 3.1. Effects of addition of cold and hot processed hempseed meal to diets on body weight (g) of laying hens

Groups	Initial body weight, g	Final body weight, g	Change, %
Control	1580±0.02	1650±0.02	0.07±0.01
HSM-C	1600±0.03	1680±0.03	0.08±0.02
HSM-H	1570±0.03	1620±0.03	0.05±0.02
P	0.744	0.355	0.436

HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability.

The effects of HSM-C and HSM-H on the daily feed intake (FI) of laying hens are given in Table 3.2. There were no significant differences between groups in terms of FI in all periods except period 3. In the 3rd period, the HSM-H group's FI was lower than the control and the HSM-C groups (P<0.01). However, there was no significant difference in feed consumption value between the control and HSM- C groups.

Effects of the addition of HSM-C and HSM-H on the FCR of laying hens are given in Table 3.3. There was no significant difference in FCR between all the groups except period 3 and in the overall period. In the period 3, the HSM-H group's FCR values was lower than those of the other groups (P<0.01); however, there was no significant

difference between the control and HSM-C group's FCR values in this period. In the overall period, the C group's FCR values were higher than the HSM-C group (P<0.01); and, there was no significant difference between the HSM-C and HSM-H added groups.

Tablo 3.2. Effects of addition of cold and hot processed hempseed meal to diets on daily feed intake (FI, g) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	112.05±2.54	117.01±5.86	113.41±6.92	0.789
2	106.63±3.04	98.57±2.65	98.82±2.08	0.065
3	111.78±3.68[a]	115.64±2.36[a]	101.36±2.81[b]	0.008**
4	114.25±3.02	116.37±3.00	115.39±2.58	0.871
5	100.74±2.88	100.15±3.10	100.60±3.51	0.990
6	115.38±1.90a	106.29±2.63b	107.33±3.75ab	0.055
7	103.78±2.69	106.32±3.91	111.78±2.11	0.196
8	113.28±2.51	109.12±3.84	109.29±2.75	0.566
Overall	109.74±1.03	108.68±0.91	107.25±0.78	0.187

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

Table 3.2. Effects of addition of cold and hot processed hempseed meal to diets on feed conversion ratio (FCR, g feed/g egg mass) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	1.88±0.07	1.94±0.10	1.95±0.10	0.852
2	1.82±0.08	1.63±0.08	1.56±0.09	0.089
3	1.87±0.07[a]	1.92±0.05[a]	1.65±0.06[b]	0.009**
4	1.94±0.06	1.85±0.09	1.86±0.07	0.597
5	1.66±0.04	1.55±0.05	1.64±0.08	0.374
6	1.80±0.06	1.62±0.07	1.76±0.12	0.298
7	1.76±0.06	1.72±0.06	1.87±0.04	0.173
8	1.90±0.06	1.80±0.08	1.85±0.06	0.575
Overall	1.83±0.02[a]	1.76±0.03[b]	1.77±0.02[ab]	0.045*

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, *:P<0.05, **: P<0.01.

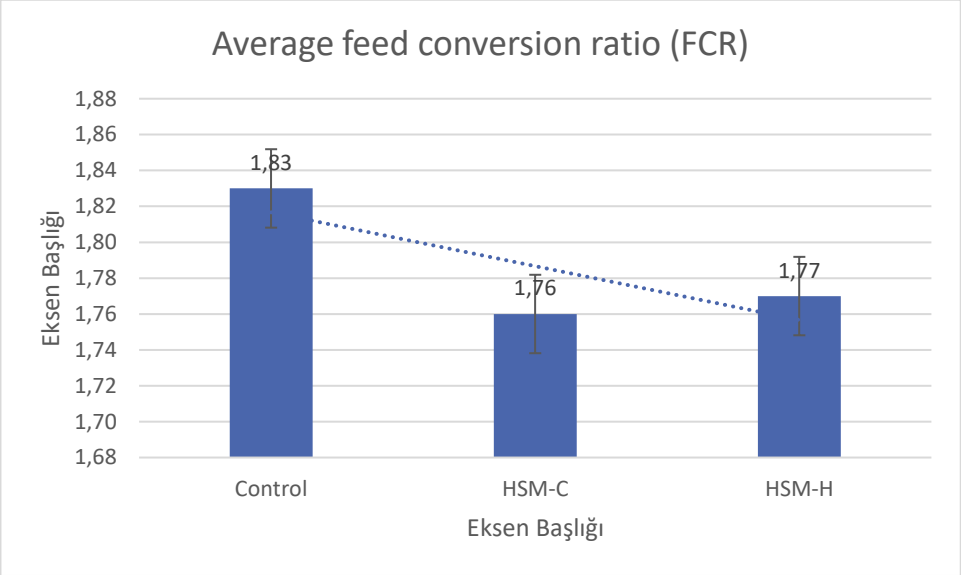


Figure 3.1. Effects of addition of HSM-C and HSM-H on average feed conversion ratio (FCR) of laying hens

3.2. Effect of treatments on egg production and egg quality traits

The effect of the addition of HSM-C and HSM-H into laying hens ration on the egg yield ratio (%) of laying hens is given in Table 3.4. Addition of HSM-C and HSM-H caused an increase in egg yield of laying hens compared to C group at period 1 and overall duration ($P<0.05$). However, there was no significant difference in egg production percentage in other periods of the experiment.

Table 3.3. Effects of addition of cold and hot processed hempseed meal to diets on egg yield (%) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	95.00±1.09^{b}	97.86±0.79^{a}	97.50±0.55^{a}	0.047*
2	97.50±0.55	95.36±1.07	97.50±1.07	0.189
3	93.93±2.38	97.50±1.20	97.14±0.89	0.248
4	94.29±2.67	97.86±0.79	97.50±1.20	0.299
5	95.36±1.60	99.64±0.36	96.43±1.41	0.057
6	95.72±1.39	98.57±0.58	97.86±0.79	0.120
7	92.86±1.77	97.14±0.71	95.36±1.31	0.093
8	93.57±1.17	95.36±1.07	95.00±1.43	0.562
Overall	94.78±0.95^{b}	97.41±0.50^{a}	96.79±0.46^{a}	0.026*

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, *: $P<0.05$.

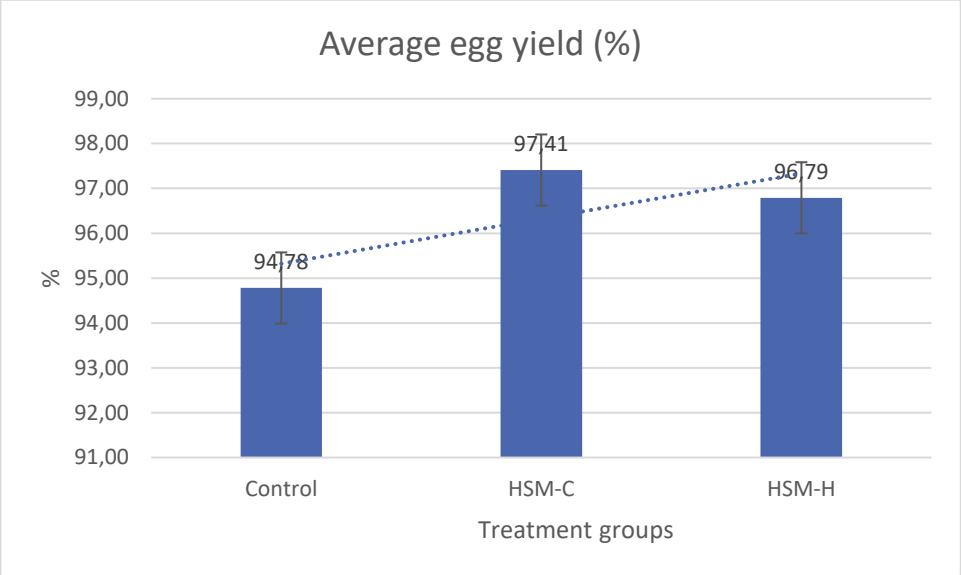


Figure 3.2. Effects of addition of HSM-C and HSM-H on average egg yield, %

Effects of addition of HSM-C and HSM-H on the egg of weight of laying hens are shown in Table 3.5. There was no significant difference in egg weight between the groups in all periods except period 2 and 3. In the 2nd period, HSM-H group's egg weight was significantly higher than C group; however, there was no significant difference between the HSM-C and HSM-H group's egg weight of laying hens ($P<0.05$). on the other hand HSM-H group's egg weight at period 3 significantly higher than HSM-C group ($P<0.01$), and no difference observed between C and HSM-H group in this period.

Effects of HSM-C and HSM-H group supplementation in diets of laying hens on the egg mass values was given in Table 3.6. There was no significant difference in terms of egg mass among the groups in all periods ($P>0.05$).

Table 3.4. Effects of addition of cold and hot processed hempseed meal to diets on egg weight (g/day) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	62.47±0.78	61.96±0.73	62.73±0.60	0.731
2	62.15±0.92	64.24±0.77	64.76±0.60	0.056
3	63.14±0.66[ab]	61.83±0.81[b]	65.06±0.63[a]	0.007**
4	63.93±0.81	64.37±0.76	63.63±0.57	0.346
5	64.54±0.69	64.58±0.62	65.47±0.68	0.540
6	64.30±0.71	64.73±0.89	65.06±0.71	0.791
7	63.66±0.75	65.07±0.68	64.68±0.75	0.370
8	63.68±0.70	64.55±0.81	65.41±0.74	0.273
Overall	63.22±0.45	63.38±0.53	63.70±0.47	0.314

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, *:P<0.05, **: P<0.01.

Table 3.5. Effects of addition of cold and hot processed hempseed meal to diets on egg mass (g/day) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	59.79±0.97	60.44±1.42	60.85±2.06	0.884
2	59.08±1.36	61.21±2.29	60.47±1.43	0.683
3	59.98±1.53	60.30±1.44	61.55±1.25	0.721
4	59.21±2.14	63.61±1.83	62.42±1.32	0.221
5	60.92±1.36	64.88±1.46	61.58±1.21	0.102
6	62.21±0.94	65.39±1.48	65.26±0.90	0.105
7	59.36±1.56	62.18±1.76	59.75±0.90	0.353
8	59.88±1.42	60.83±1.54	59.23±1.16	0.726
Overall	60.05±0.75	62.36±0.72	61.39±0.66	0.088

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability.

Effects of the addition of HSM-C and HSM-H on egg albumen height of laying hens are given in Table 3.7. There were significant effects on egg albumen height in the 1st, 2nd, and 6th periods; nevertheless, there were no significant differences between groups in all

other periods. In the first period, the HSM-C and HSM-H group's Hmm values were significantly higher than the control group (P<0.01). In period 2, in the HSM-H group, Hmm values were lower than the control and HSM-H added groups, and in period 6, the HSM-C group's Hmm values were higher than those of the C and HSM-H groups (P<0.05). No significant difference was observed between the groups in the overall period.

Table 3.6. Effects of addition of cold and hot processed hempseed meal to diets on egg albumen height (Hmm, mm) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	3.50±0.20[b]	4.39±0.27[a]	4.84±0.20 [a]	0.001**
2	4.14±0.16 [a]	4.31±0.14 [a]	3.64±0.19 [b]	0.014*
3	4.28±0.12	4.42±0.15	4.04±0.17	0.182
4	4.78±0.15	4.57±0.22	5.00±0.11	0.192
5	4.25±0.28	4.80±0.25	4.68±0.19	0.260
6	4.76±0.18[b]	5.46±0.17 [a]	4.83±0.19 [b]	0.013*
7	4.74±0.20	4.55±0.22	4.54±0.18	0.720
8	5.47±0.22	5.48±0.17	4.91±0.22	0.086
Overall	4.50±0.09	4.75±0.08	4.56±0.07	0.086

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

Effects of the addition of HSM-C and HSM-H on the Haugh unit of laying hens are given in Table 3.8. There was no significant difference in HU between treatment groups in all periods and overall periods except periods 2, 3, and 6. In period 2, the Haugh unit of the HSM-H group was higher than the control and HSM-H groups (P<0.05). However, there was no significant statistically difference between the control and HSM-C groups in terms of HU of eggs. Also, in the 3rd period, C and HSM-C group's HU values were significantly higher than the HSM-H group; however, there was no significant difference between the C and HSM-C groups. In the 6th period, the HSM-C group's HU value was significantly higher than the C and HSM-H group, and there was no significant difference between the C and HSM-H groups in this period (P<0.05).

Table 3.7. Effects of addition of cold and hot processed hempseed meal to diets on Haugh unit (HU) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	53.14±2.70	55.46±3.29	57.73±2.04	0.495
2	59.59±1.46^{b}	59.33±1.55^{b}	53.92±2.02^{a}	0.032*
3	61.16±1.29^{a}	62.25±1.24^{a}	56.38±2.07^{b}	0.024*
4	64.54±1.69	60.86±2.57	66.69±1.00	0.089
5	54.41±3.20	62.11±2.75	61.24±2.43	0.112
6	63.26±2.24^{b}	70.18±1.53^{a}	63.14±2.43^{b}	0.029*
7	63.65±2.34	60.17±2.70	60.39±2.21	0.528
8	69.58±2.57	70.70±1.65	65.83±2.12	0.249
Overall	61.17±1.09	62.63±0.93	60.67±0.77	0.310

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, *: P<0.05, **: P<0.01.

Effects of the addition of HSM-C and HSM-H on the Roche Color scale (RCS) value of laying hens are given in Table 3.9. There were significant differences in terms of RCS values in all periods and the overall period (P<0.05 without periods 1, and 7. The HSM-C group's RCS values were higher than the C group in all significantly affected periods (P<0.05 and P<0.01), and also HSM-C group's RCS values were higher than the HSM-H group's RCS values at period 6 (P<0.01). There were no significant differences between the HSM-C group and the HSM-H group's RCV values in general, but, mostly HSM-H group's RCS values were higher than the C group. Moreover, RCS values of the HSM-H and HSM-C groups were higher than the C group in the overall period (P<0.01).

Table 3.8. Effects of addition of cold and hot processed hempseed meal to diets on Roche Color scale (intensity) value of laying hens

Periods	Control	HSM-C	HSM-H	P
1	8.63±0.16	8.77±0.10	8.87±0.13	0.461
2	8.70±0.12^{b}	9.03±0.08^{a}	8.90±0.07^{ab}	0.04*
3	8.57±0.15^{b}	8.93±0.07^{a}	8.93±0.07^{a}	0.016*
4	8.63±0.17^{b}	9.10±0.09^{a}	9.00±0.07^{a}	0.015*
5	8.80±0.22	9.00±0.05	9.07±0.07	0.345
6	8.63±0.19^{b}	9.30±0.11^{a}	8.83±0.14^{b}	0.008**
7	8.73±0.10	8.67±0.19	8.97±0.06	0.231
8	8.67±0.16^{b}	9.10±0.09^{a}	8.93±0.11^{ab}	0.047*
Overall	8.67±0.06^{b}	8.99±0.04^{a}	8.94±0.04^{a}	0.001**

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, **: P<0.01.

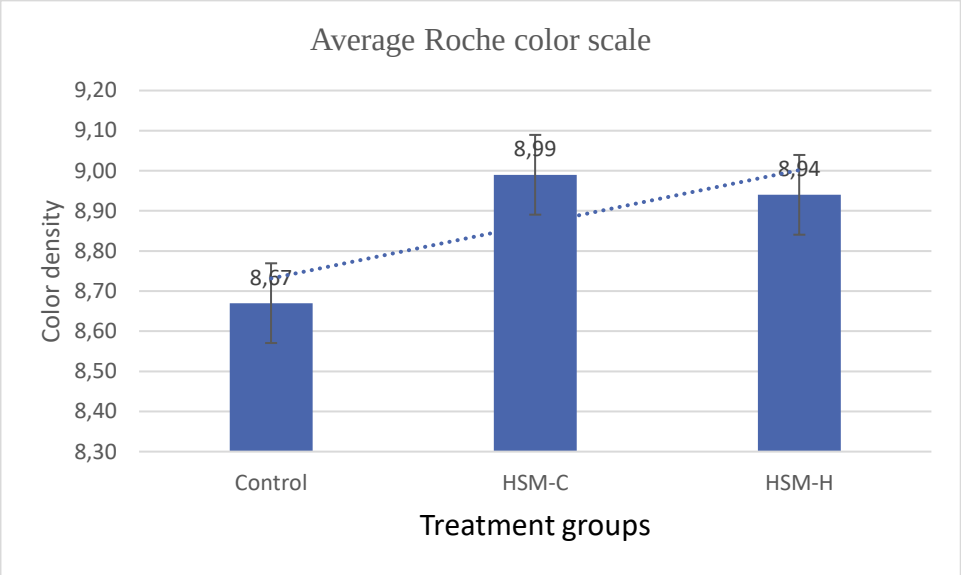


Figure 3.3. Effects of addition of HSM-C and HSM-H on average Roche color scale values

Effects of addition of HSM-C and HSM-H on egg yolk brightness of laying hens is given in Table 3.10. There was no significant difference in egg yolk brightness between treatment groups at period 3, 4, 5, and 8 and the overall duration. Egg yolk L*values in HSM-H group was higher than C group (P<0.01) but, similar HSM-C group at period 1. The HSM-C and HSM-H group's yolk L* values were lower than C group in period 2, and 7 (P<0.01). In period 6, HSM-H group's L* values were lower than the C and HSM-C groups (P<0.01).

Table 3.9. Effects of addition of cold and hot processed hempseed meal to diets on egg yolk lightness (L*) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	49.62±0.41[b]	51.85±0.75[ab]	58.28±0.43[a]	0.000**
2	58.63±0.55[a]	56.00±0.47[b]	55.35±0.63[b]	0.000**
3	56.77±0.40	56.44±0.32	55.98±0.40	0.338
4	56.42±0.40	55.66±0.38	55.65±0.33	0.250
5	56.34±0.42	56.01±0.41	55.77±0.38b	0.601
6	55.33±0.35[a]	54.68±0.42[a]	51.89±0.51[b]	0.000**
7	56.84±0.36[a]	55.11±0.42[b]	55.60±0.34[b]	0.005**
8	54.93±0.32	54.65±0.37	53.67±0.50	0.075
Overall	56.05±0.20	55.46±0.22	55.55±0.19	0.095

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

Effects of addition of HSM-C and HSM-H on egg yolk redness of laying hens is given in Table 3.11. There was no significant difference in egg yolk brightness between treatment groups in all periods except the period 1 and 8. The egg yolk a* values in the C group was lower than those of HSM-C and HSM-H group in period 1 and 8 (P<0.01). However, there were no significant differences between the groups in terms of egg yolk a* values in the other periods and overall duration.

Table 3.10. Effects of addition of cold and hot processed hempseed meal to diets on egg yolk redness (a*) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	0.44±0.12**[b]**	1.09±0.11**[a]**	0.81±0.14 **[a]**	0.002**
2	0.78±0.15	1.35±0.14	2.87±1.72	0.316
3	0.72±0.09	1.51±0.39	0.92±0.10	0.057
4	1.42±0.15	1.55±0.16	1.32±0.14	0.554
5	1.42±0.16	1.46±0.14	1.03±0.11	0.059
6	1.02±0.11	1.23±0.16	1.40±0.10	0.109
7	0.84±0.13	0.59±0.13	0.90±0.16	0.250
8	0.67±0.15**[b]**	1.35±0.14**[a]**	1.53±0.16**[a]**	0.001**
Overall	0.91±0.06	1.26±0.09	1.34±0.24	0.107

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

Effects of addition of HSM-C and HSM-H on egg yolk yellowness (b*) of laying hens are given in Table 3.12. Egg yolk b* values in HSM-C and HSM-H supplemented groups in the first and overall periods were higher than C group (P<0.05 and P<0.01). Again, b* value of HSM-H group was significantly lower than HSM-C group in period 1. However, b* value of HSM-H group was significantly lower than C and HSM-C groups and b* value of HSM-C group was higher than C group in period 5 (P<0.01).

Table 3.11. Effects of addition of cold and hot processed hempseed meal to diets on egg yolk yellowness (b*) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	23.89±0.26^{c}	30.40±1.63^{b}	43.21±0.75^{a}	0.001**
2	40.75±1.00	39.90±0.71	40.00±0.74	0.728
3	39.82±0.81	38.53±0.51	39.90±0.58	0.250
4	37.08±0.66^{b}	39.31±0.62^{a}	38.84±0.52^{a}	0.027*
5	37.30±0.62^{b}	39.75±0.63^{a}	34.76±0.98^{c}	0.001**
6	36.72±0.50	36.86±0.78	36.49±0.67	0.921
7	39.04±0.89	39.15±0.99	39.38±0.70	0.959
8	37.47±0.66	36.63±0.77	36.27±0.66	0.464
Overall	36.35±0.33^{b}	37.74±0.40^{a}	38.53±0.32^{a}	0.001**

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

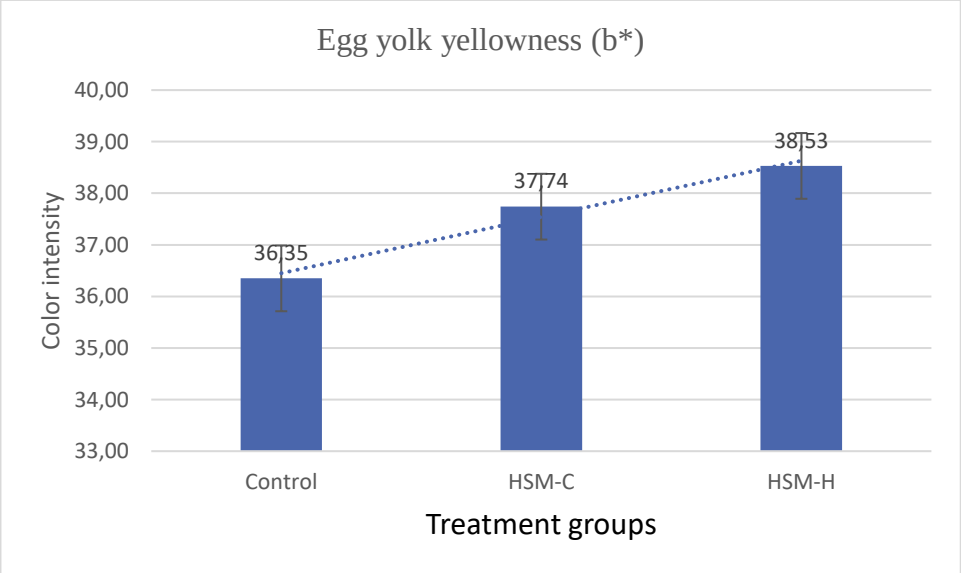


Figure 3.4. Effects of addition of HSM-C and HSM-H on average egg yolk yellowness of laying hens

Effects of the addition of HSM-C and HSM-H on the eggshell weight of laying hens are given in Table 3.13. The eggshell weight values of the HSM-H group were significantly higher than the C group in all periods except periods 4 and 8 (P<0.01). In the first period, the eggshell weight of the HSM-C group was significantly higher than the C group (P<0.01). In period 4, there was no statistical difference between the control group and HSM-C and HSM-H groups, while ESW value of HSM-H group was found to be statistically higher than HSM-C group (P<0.01). Differences between groups were not statistically significant in the period 8 (P>0.05).

Table 3.12. Effects of addition of cold and hot processed hempseed meal to diets on the eggshell weight (g) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	5.13±0.07^{c}	5.63±0.10^{b}	6.25±0.08^{a}	0.001**
2	5.91±0.11^{b}	5.86±0.08^{b}	6.33±0.07^{a}	0.001**
3	6.09±0.10^{b}	5.99±0.08^{b}	6.42±0.08^{a}	0.001**
4	6.18±0.07^{ab}	6.11±0.07^{b}	6.38±0.08^{a}	0.033*
5	5.87±0.10^{b}	5.97±0.08^{b}	6.47±0.06^{a}	0.001**
6	6.24±0.09^{b}	6.09±0.10^{b}	6.49±0.04^{a}	0.002**
7	6.03±0.09^{b}	6.27±0.08^{b}	6.57±0.10^{a}	0.001**
8	5.98±0.09	5.98±0.11	6.13±0.08	0.438
Overall	5.93±0.04^{b}	5.99±0.03^{b}	6.38±0.03^{a}	0.001**

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, *: P<0.05, **: P<0.01.

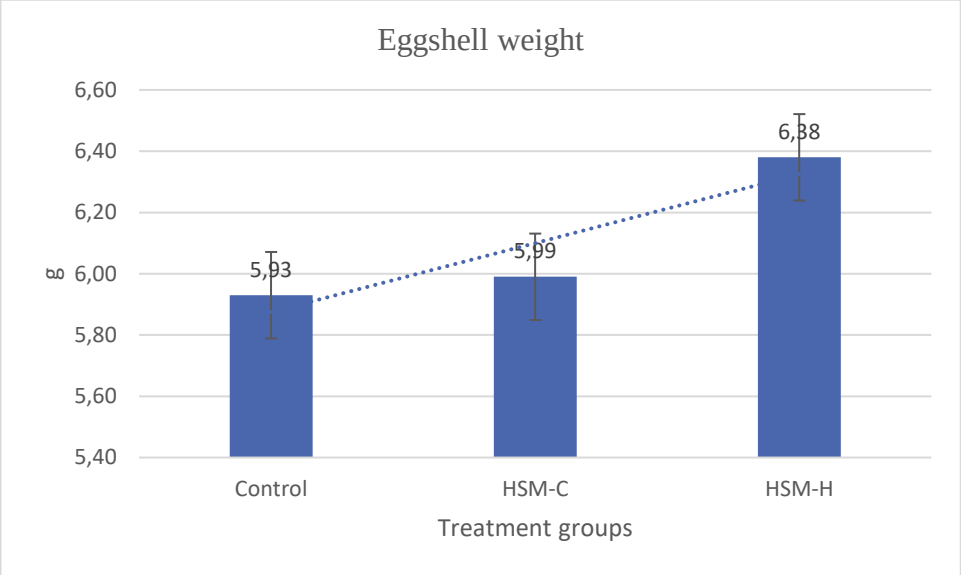


Figure 3.5. The effects of addition of HSM-C and HSM-H on eggshell weight

The effects of addition of HSM-C and HSM-H on eggshell weight percentage of laying hens is given in Table 3.14 and Figure 3.4.

In the first period, egg shell ratio of HSM-H group was significantly higher than C and HSM-C groups and egg shell ratio of the HSM-C group was found to be significantly higher than C group ($P<0.01$). In the 2nd, 4th and 6th periods, egg shell ratio of HSM-H group was found to be significantly higher than HSM-C group ($P<0.05$, $P<0.01$), but the differences between C group and both groups were not found to be significant. In the 5th, 7th and overall periods, egg shell ratio of HSM-H group was found to be statistically significantly higher than C and HSM-C groups ($P<0.01$).

Table 3.13. Effects of addition of cold and hot processed hempseed meal to diets on the eggshell ratio (%) of laying hens.

Periods	Control	HSM-C	HSM-H	P
1	8.26±0.13^{c}	9.01±0.19^{b}	9.74±0.12^{a}	0.001**
2	9.53±0.18^{ab}	9.17±0.17^{b}	9.80±0.14^{a}	0.025*
3	9.65±0.11	9.71±0.13	9.87±0.08	0.327
4	9.69±0.12^{ab}	9.54±0.16^{b}	10.04±0.12^{a}	0.034*
5	9.12±0.16^{b}	9.25±0.12^{b}	9.91±0.14^{a}	0.001**
6	9.69±0.11^{ab}	9.42±0.13^{b}	10.01±0.10^{a}	0.002**
7	9.49±0.13^{b}	9.64±0.10^{b}	10.18±0.16^{a}	0.001**
8	9.41±0.15	9.31±0.21	9.52±0.17	0.714
Overall	9.35±0.06^{b}	9.38±0.08^{b}	9.88±0.06^{a}	0.001**

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

Effects of the addition of HSM-C and HSM-H on the eggshell thickness of laying hens are given in Table 3.15. In the first period, the eggshell thickness of the C and HSM-H groups was higher than the HSM-C group. In the third period, the eggshell thickness of the HSM-H group was higher than the C group but similar to the HSM-C group. In the periods 5, 6, and 7, the eggshell thickness value of the HSM-H group was significantly higher than the C and HSM-C groups (P<0.01). In the overall period, the eggshell thickness of the HSM-H group was higher than the HSM-C group (P<0,01) and similar to the C group. No statistical difference was found between the groups in terms of eggshell thickness in the 2nd and 4th periods.

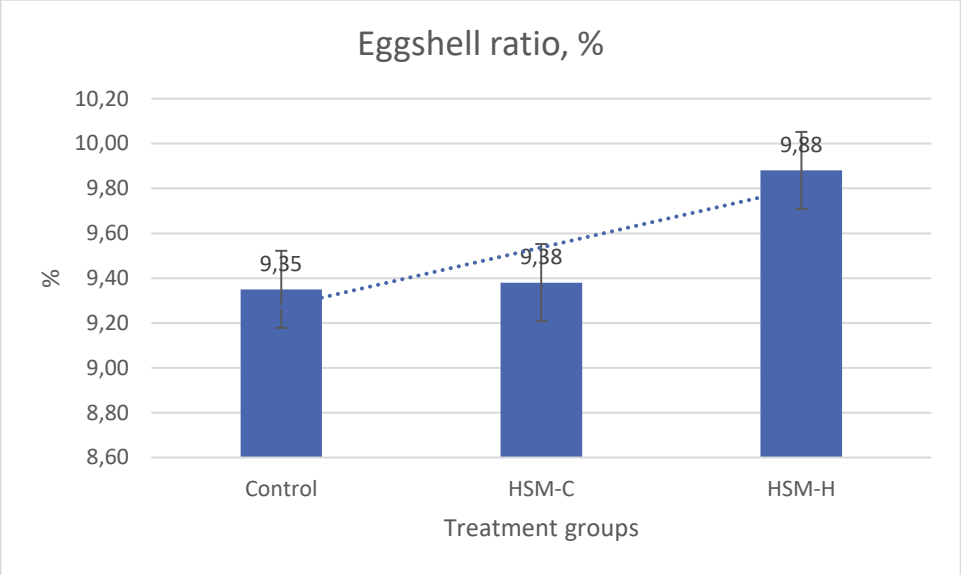


Figure 3.6. The effects of the addition of HSM-C and HSM-H on eggshell ratio, %

Table 3.14. Effects of addition of cold and hot processed hempseed meal to diets on eggshell thickness (mm) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	36.39+0.58^{a}	32.04+0.31^{c}	34.95+0.19^{b}	0.00
2	36.42±0.42^{ab}	35.40±0.34^{b}	36.63±0.38^{a}	0.053
3	36.45±0.43^{a}	35.80±0.47^{ab}	34.64±0.35^{b}	0.011
4	35.40±0.43	35.08±0.42	35.04±0.56	0.844
5	33.76±0.41^{b}	33.67±0.30^{b}	35.41±0.40^{a}	0.002
6	34.89±0.44^{b}	34.40±0.50^{b}	36.47±0.45^{a}	0.006
7	33.21±0.47^{c}	35.08±0.51^{b}	37.65±0.35^{a}	0.001
8	34.37±0.51	34.83±0.60	34.11±0.50	0.631
Overall	35.11±0.19^{ab}	34.57±0.22^{b}	35.63±0.20^{a}	0.002

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

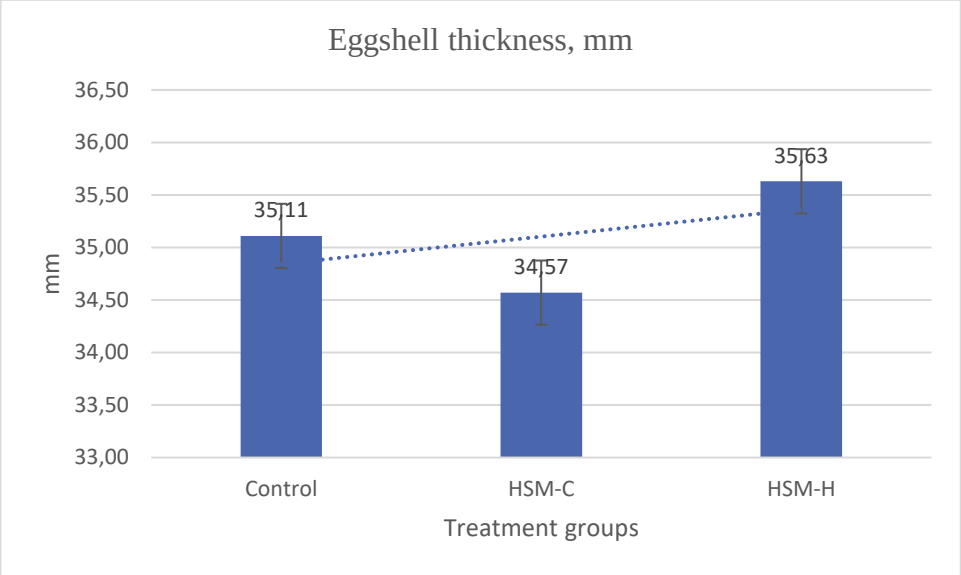


Figure 3.7. Effects of the addition of HSM-C and HSM-H on the eggshell on eggshell thickness (mm) of laying hens

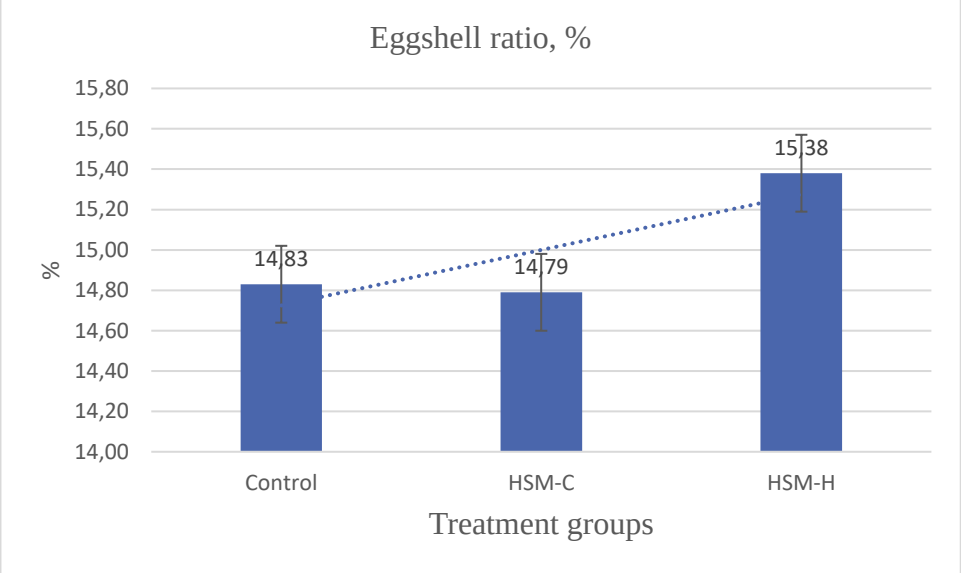


Figure 3.8. Effects of the addition of HSM-C and HSM-H on the eggshell ratio (%) of laying hens

Effects of the addition of HSM-C and HSM-H on the eggshell surface area of laying hens are given in Table 3.16. There was no significant difference in eggshell surface area between treatment groups in all periods except the second and third periods. In the 2nd period, the eggshell surface area of the HSM-H group was significantly higher than the C group but similar to the HSM-C group ($P<0.05$). In the 3rd period, the eggshell surface

area of the HSM-H group was statistically higher than the HSM-C group but similar to the control group (P<0.01).

Table 3.15. Effects of addition of cold and hot processed hempseed meal to diets on the eggshell surface area (ESSA, mm) of laying hens.

Periods	Control	HSM-C	HSM-H	P
1	73.26±0.62	73.27±0.64	74.31±0.44	0.333
2	73.26±0.77^{b}	75.01±0.64^{ab}	75.45±0.50^{a}	0.044*
3	74.10±0.55^{ab}	73.00±0.68^{b}	75.69±0.52^{a}	0.007**
4	74.74±0.67	75.11±0.63	74.51±0.47	0.776
5	75.26±0.57	75.29±0.51	76.02±0.56	0.544
6	75.16±0.58	75.40±0.74	75.68±0.59	0.847
7	74.52±0.62	75.70±0.56	75.37±0.62	0.367
8	74.54±0.58	75.26±0.67	75.34±0.58	0.600
Overall	74.36±0.35	74.75±0.35	75.30±0.31	0.149

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

Effects of the addition of HSM-C and HSM-H on eggshell weight per unit (ESWPU) of laying hens is given in Table 3.17. There was a significant difference in all periods except period 8. The ESWPU in the HSM-C group was statistically higher than the HSM-H group in all periods (P<0.01). At the same time, the ESWPU of the C group was statistically higher than the HSM-H group in the 1, 3, 5, 6, 7, and overall periods (P<0.01). The differences between the C group and HSM-C and between the C and HSM-H were not significant in the 2nd and 4th periods.

Table 3.16. Effects of addition of cold and hot processed hempseed meal to diets on eggshell per unit area weight (mg) of laying hens

Periods	Control	HSM-C	HSM-H	P
1	14.39±0.22^{a}	13.25±0.25^{b}	12.10±0.14^{c}	0.001**
2	12.51±0.22^{ab}	12.87±0.22^{a}	11.95±0.15^{b}	0.006**
3	12.23±0.15^{a}	12.23±0.14^{a}	11.82±0.10^{b}	0.047*
4	12.12±0.13^{ab}	12.34±0.18^{a}	11.72±0.13^{b}	0.016*
5	12.92±0.25^{a}	12.67±0.17^{a}	11.78±0.13^{b}	0.001**
6	12.09±0.13^{a}	12.45±0.17^{a}	11.66±0.09^{b}	0.001**
7	12.42±0.17^{a}	12.12±0.13^{a}	11.54±0.17^{b}	0.001**
8	12.54±0.18	12.71±0.27	12.36±0.21	0.550
Overall	12.65±0.08^{a}	12.58±0.09^{a}	11.87±0.07^{b}	0.001**

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, *:P>0.05, **: P<0.01.

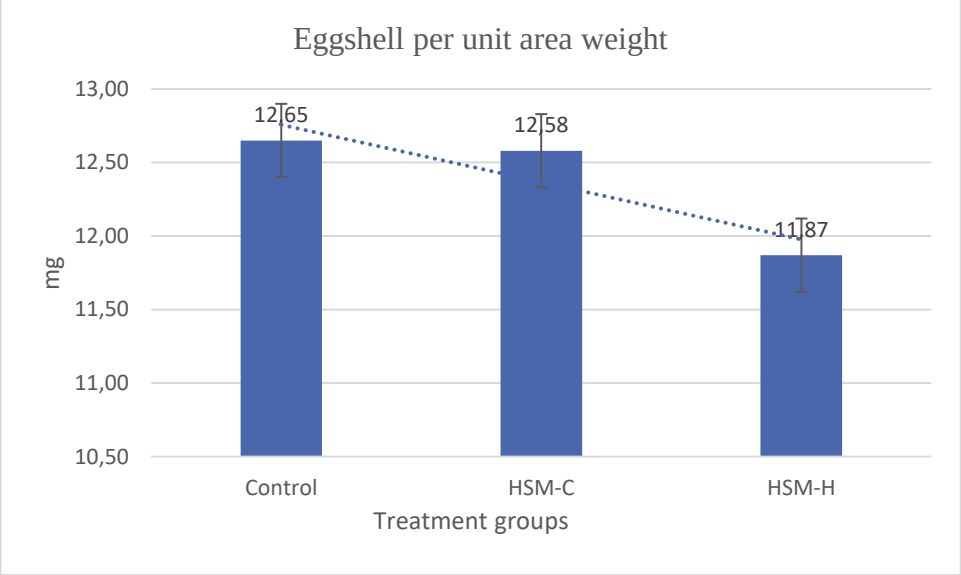


Figure 3.9. Effects of the addition of HSM-C and HSM-H on the eggshell per unit area (mg) of laying hens

3.3. Effect of treatments on egg fatty acid composition

The effects of the addition of HSM-C and HSM-H on egg yolk fatty acid composition on d 42 are presented in Table 3.18. No statistical difference was found between the groups in terms of egg yolk fatty acids.

Table 3.17. Effects of addition of cold and hot processed hempseed meal to diets on egg yolk fatty acid ratio (%) on d 42

Fatty acids	Control	HSM-C	HSM-H	P
(C14:0) Myristic Acid	1.03±0.42	0.82±0.33	1.22±0.70	0.855
(C16:0) Palmitic Acid	26.92±0.44	25.95±0.71	27.32±0.96	0.425
(C16:1) Palmitoleic Acid	2.92±0.12	2.28±0.30	2.59±0.04	0.094
(C18:0) Stearic Acid	9.37±3.00	10.86±2.52	8.34±0.47	0.739
(C18:1n9c) Oleic Acid	38.05±7.31	32.94±7.63	39.38±1.10	0.741
(C18:2n6c) Linoleic Acid	17.47±7.11	24.97±5.77	18.65±1.39	0.580
(C18:3n3) a-Linolenic Acid	3.43±2.81	1.20±0.10	1.49±0.26	0.589
(C20:4n6) Arachidonic Acid	0.62±0.12	0.67±0.05	0.67±0.08	0.888
(C20:5n3) Eicosapentaenoic Acid	0.09±0.02	0.04±0.01	0.05±0.01	0.086
(C22:6n3) Docosahexaenoic Acid	0.11±0.02	0.26±0.07	0.28±0.06	0.091

HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability.

Effects of addition of HSM-C and HSM-H on egg yolk fatty acid composition at period 84 is shown in Table 3.19. The differences were obtained in terms of n-6 and n-3 fatty acids in egg yolk fatty acids. The omega 6 and n-3 ratio in the HSM-C and HSM-H supplemented groups was found to be statistically significantly higher than in the C group ($P<0.01$). No statistically significant difference was found between treatments for other fatty acids.

Table 3.18. Effects of addition of cold and hot processed hempseed meal to diets on egg yolk fatty acid ratio (%) on d 84

Fatty acids	Control	HSM-C	HSM-H	P
(C14:0) Myristic Acid	6.23±5.52	0.98±0.42	0.57±0.12	0.406
(C16:0) Palmitic Acid	24.04±5.15	27.90±0.76	26.86±1.14	0.665
(C16:1) Palmitoleic Acid	3.13±0.66	2.57±0.23	2.52±0.21	0.543
(C18:0) Stearic Acid	8.10±0.37	10.07±1.20	8.66±0.10	0.186
(C18:1n9c) Oleic Acid	43.26±0.40	38.35±1.95	40.13±0.53	0.038
(C18:2n6c) Linoleic Acid	14.02±0.45^{b}	17.15±0.95^{a}	18.76±0.89^{a}	0.004**
(C18:3n3) a-Linolenic Acid	0.45±0.07^{b}	1.10±0.11^{a}	1.19±0.14^{a}	0.001**
(C20:4n6) Arachidonic Acid	0.64±0.05	1.22±0.57	0.87±0.01	0.483
(C20:5n3) Eicosapentaenoic Acid	0.06±0.01	0.07±0.03	0.06±0.01	0.757
(C22:6n3) Docosahexaenoic Acid	0.07±0.01	0.58±0.32	0.38±0.04	0.193

^{a,b}: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability, **: P<0.01.

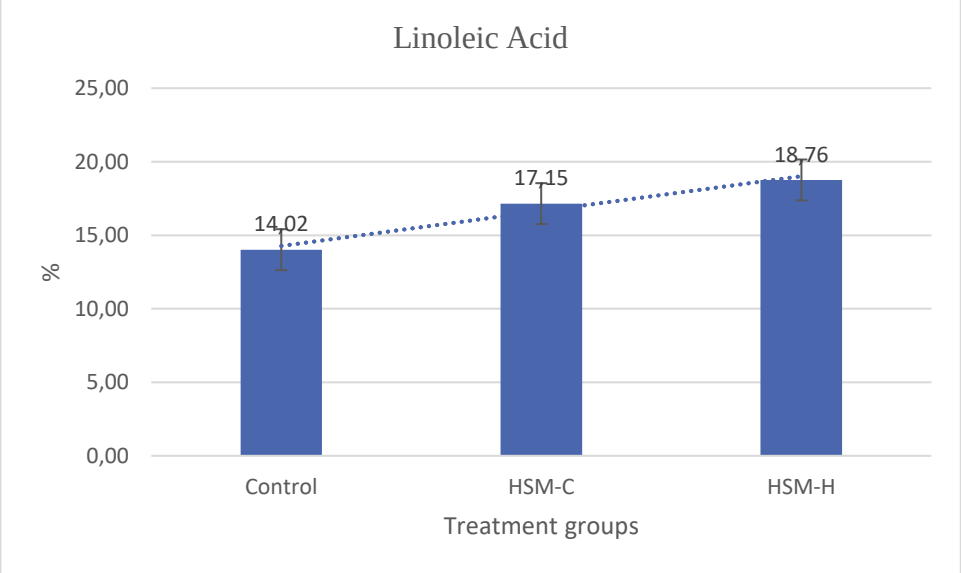


Figure 3.10. The effects of addition of HSM-C and HSM-H on egg yolk Omega-6 fatty acid

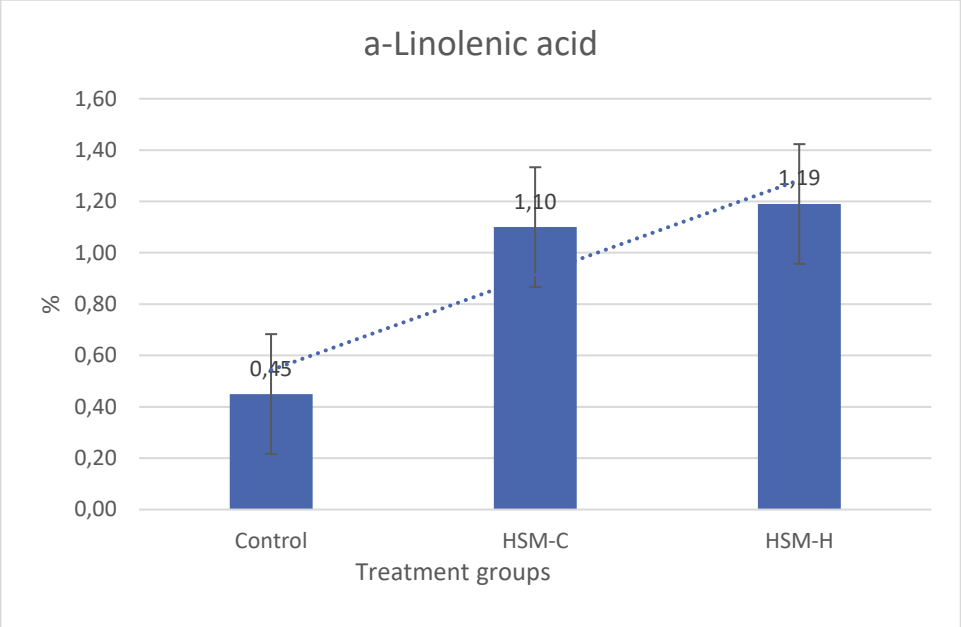


Figure 3.11. The effects of the addition of HSM-C and HSM-H on egg yolk Omega-3 fatty acid

Effects of addition of HSM-C and HSM-H on egg yolk fatty acid composition at the overall period in Table 3.20. No difference was observed between the groups in terms of fatty acids compositions.

Table 3.19. Effects of addition of cold and hot processed hempseed meal to diets on egg yolk fatty acid ratio (%) at overall period

Fatty acids Overall	Control	HSM-C	HSM-H	P
(C14:0) Myristic Acid	3.63±2.69	0.90±0.38	0.90±0.33	0.397
(C16:0) Palmitic Acid	25.48±2.54	26.93±0.63	27.09±0.62	0.729
(C16:1) Palmitoleic Acid	3.03±0.33	2.43±0.20	2.56±0.10	0.193
(C18:0) Stearic Acid	8.73±1.48	10.47±1.40	8.50±0.22	0.463
(C18:1n9c) Oleic Acid	40.65±3.67	35.65±4.00	39.76±0.68	0.509
(C18:2n6c) Linoleic Acid	15.74±3.44	21.06±2.75	18.70±0.45	0.369
(C18:3n3) a-Linolenic Acid	1.94±1.38	1.15±0.07	1.34±0.09	0.772
(C20:4n6) Arachidonic Acid	0.63±0.06	0.95±0.29	0.77±0.05	0.461
(C20:5n3) Eicosapentaenoic Acid	0.07±0.01	0.06±0.02	0.05±0.01	0.383
(C22:6n3) Docosahexaenoic Acid	0.09±0.01	0.42±0.17	0.33±0.04	0.101

HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, P: probability.

3.4. Effect of treatments on blood components

Effects of the addition of HSM-C and HSM-H on blood components are given in Table 3.21. There was no significant difference between the groups in terms of blood glucose, protein, cholesterol, creatinine, AST, ALT, and CBK. However, triglyceride ratios were lower in the HSM-H group than in the control and HSM-C groups (P<0.01), and C and HSM-C groups' triglyceride ratios were similar.

Table 3.201. Effects of addition of cold and hot processed hempseed meal to diets on blood components

Groups	Control	HSM-C	HSM-H	P
Glucose (mg/dL)	238.29±5.60	228.86±3.56	227.14±5.97	0.283
Protein (mg/dL)	5.41±0.21	5.27±0.10	5.28±0.23	0.842
Triglyceride (mg/dL)	1396.29±93.19[a]	1454.43±82.52[a]	913.29±81.22[b]	0.001**
Cholesterol (mg/dL)	135.71±19.60	106.00±5.79	87.57±16.65	0.106
Creatinine (mg/dL)	0.21±0.03	0.19±0.02	0.21±0.03	0.762
Creatinine kinase, IU/L	1647.29±407.30	1915.14±364.40	1712.43±310.49	0.863
AST, IU/L	179.14±10.01	169.14±10.17	189.14±10.30	0.399
ALT, IU/L	469.51±463.42	4.32±0.36	64.45±60.11	0.432

[a,b]: The differences between the averages shown with different superscript letters at the same row are important, HSM-C: hempseed meal cold processed, HSM-H: hempseed meal hot processed, AST: aspartate aminotransferase ALT: Alanin aminotransferase, P: probability, **: P<0.01.

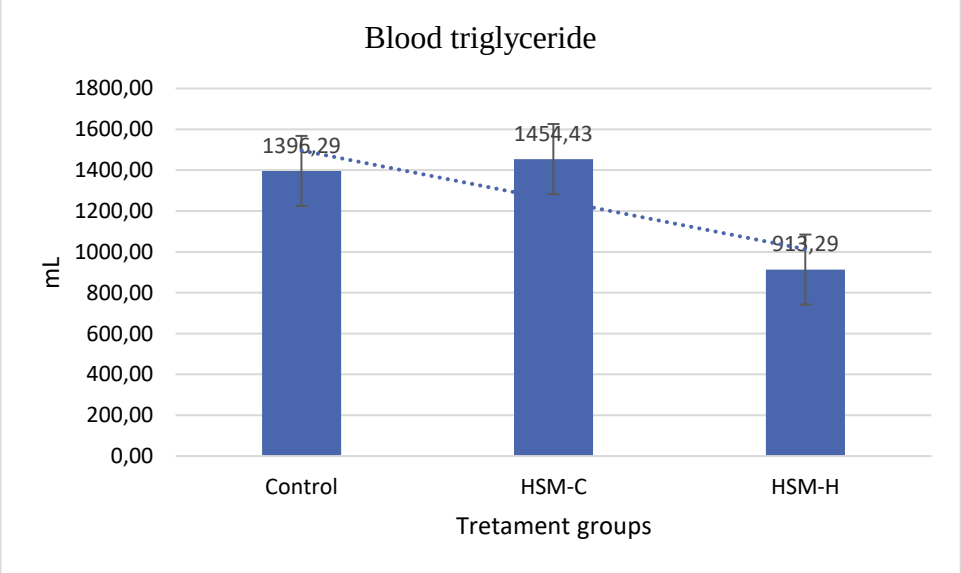


Figure 3.12. Effects of the addition of HSM-C and HSM-H on the blood triglyceride of laying hens

CHAPTER 4

DISCUSSION

There were two purposes in the planning stage of this study. The first purpose was to determine the effects of hempseed meal addition on performance and egg quality in laying hens, and the second one was to determine the effects of heat processing techniques on the usefulness of hempseed meal in laying hen diets and performance, egg quality, fatty acid composition, and blood components. Thermal processing applications are applied to feed some pathogenic microorganisms in feeds, to increase the utilization by bursting the starch, and to inhibit anti-nutritional elements. In pelleting, one of these applications, significant positive results have been obtained today and it has become an important application of the feed industry today. It has been shown in many studies that thermal processing applied in pelleting animal feeds, especially for poultry and pet animals, increases the utilization of the feed.

4.1. Effect of treatments on live body weight, feed intake and feed conversion ratio

In this study, the effects of the treatments on live weight change of egg attitudes were not found to be significant. In addition, the FI and FCR of hens were not significantly affected by 15% HSM-C and HSM-H addition to diets. In studies conducted on the addition of HSM to chicken feeds, it was determined that the addition of 5 to 20% hempseed meal did not significantly affect the BW, feed consumption, and FCR values of laying hens (Barani et al., 2017; Cufadar et al., 2021; Silversides and Lefrançois, 2005). Also, lower concentrations of HSM in diets (Halle and Schöne, 2013; Merlita, 2019; Öztürk et al., 2024) did not affect BW, FI and FCR values. In contrast to these results, in some studies, it was determined that 15% HSM addition (Stastnik et al., 2016) decreased BW, but feed consumption and feed utilization were not significantly affected by the treatments. On the other hand, Rajasekhar et al. (2021b) reported 10, 20 and 30% HSM addition to the ration did not affect feed consumption, but increased BW and FCR in hens. The energy values

and amino acid and protein ratios of the feeds consumed by animals can significantly affect the feed consumption of poultry. Therefore, the results obtained in this study cannot be considered to be solely due to hempseed addition to diets. In addition, it is not clear whether the HSM was obtained by hot or cold pressing techniques in different study results reported by these authors. However, in the current study, it was determined that there was no significant difference in the BW, FI and FCR values of the hens because their feeds had very similar energy, protein and amino acid concentrations. The effects of hemp seeds being processed at two different temperatures (60 and 120 °C) were not reflected in the growth and feed utilization values. Hemp seeds contain significant anti-nutritional factors (Russo, 2013b; Zhao et al., 2022) and heat treatment can be effective in reducing the effects of these negative substances (Konca et al., 2019).

4.2. Effect of treatments on egg production and egg quality

In this study, it was determined that HSM addition (both produced by hot and cold pressing methods) significantly increased egg productivity in the first period and overall period compared to the C group. Silversides et al. (2005) reported that the addition of 50, 100, and 200 grams per kilogram of HSM to the diet did not significantly affect egg production. Öztürk et al. (2024) reported that the addition of 4, 8 and 12% HSM to the diet increased egg production, while Cufadar et al. (2021) reported that the addition of 5 to 20% HSM did not significantly affect egg production in laying hens. Anti-nutritional factors in seeds could be reduced by hot treatment, but in this study, cold and hot processing techniques did not affect egg production. As mentioned above, it is thought that the low degree of difference may be related to the sufficient protein and energy intake of the laying hens.

In this study, the effects of HSM obtained by hot and cold pressing on egg weight were found to be significant only in 2 and 3 periods but not in the overall period. The egg weight of the HSM processed by the hot method was significantly higher than others. On the other hand, there were no significant differences between the groups in terms of egg mass in all periods. Egg mass data reflects both egg weight and egg yield's function. Similar observations were noted by others who reported that the addition of hempseeds or HSM did not affect egg weight in hens (Silversides and Lefrançois, 2005; Neijat et al., 2014; Bazdidi et al., 2016; Cufadar et al., 2021). However, in the other reports on HSM

addition (Halle and Schöne, 2013; Mierliţă, 2019; Öztürk et al., 2024) and hempseed addition to diets increased egg weight (Yalcin et al., 2018; Taaifi et al., 2023). In this study, the hot processing technique of hemp seeds had a slight effect on increasing egg weight. The most important factors that increase egg weight are high protein levels and amino acid ratios. In this study, the protein and amino acids needed by the animals were calculated at a level that would meet the needs of the animals. Therefore, no significant effect was observed on egg weight and mass. Cufadar et al. (2021) noted HSM addition to laying hen diets did not affect the egg mass of hens. Besides the addition of hempseed did not affect the egg mass of hens (Goldberg et al., 2012; Bazdidi et al., 2016; Konca et al., 2019b). However, Skřivan et al. (2020) noted that 3, 6% hempseed addition in laying hen diets and Yalcin et al. (2018)) noted that %5, 10, and 20 hempseed addition to quail diets increased egg mass.

Among the egg evaluation criteria, the height of the albumen and Haugh unit are of the egg's internal quality features. The Haugh unit, one of the egg internal quality parameters, is an internal quality parameter calculated as a logarithmic function of the solid albumen height and egg weight. It is also used to reveal meat and blood stains in the albumen. The absence of a significant difference in the Haugh unit values indicates that there is no significant difference in the egg albumen ratio and also the Hmm value as the yolk index, meaning that the internal quality of the egg is not impaired, which has been evaluated as an indicator that hemp seed, used as a new raw material, can be safely used in egg rations. In this study, the height of the egg album was sometimes slightly superior to the control and hot processing methods with the cold processing method, but these differences disappeared when the entire period was evaluated. The Haugh unit values of the eggs were found to be higher in the HSM-H group than in the other groups in 2 periods, lower in 3 periods, higher in 4 periods, and significantly higher in the HSM-C group than in the other groups in 6 periods. An unstable situation was observed between the periods in terms of these values. Some research results showed that hempseed and meal addition increased egg Haugh Unit values of eggs (Bazdidi et al., 2016; Konca et al., 2019b; Skřivan et al., 2020; Öztürk et al., 2024).

In this study, RCS values of the HSM-H and HSM-C groups were mostly higher than the C group in the periods. The C group egg yolk brightness (L*) values were higher than the HSM-C and HSM-H groups. But in contrast to the L* value of the C group, the egg yolk

redness (a*) value was lower than HSM-C and HSM-H groups in the two periods but not significant in the other periods and overall. The egg yolk yellowness (b*) value of HSM added groups was higher than HSM-C and HSM-H groups. Poultry cannot synthesize colorants in their body and have to get them from foods such as xanthophyll pigments and oxycarotenoids which can affect the preferences of consumers (Vuilleumier 1969). In this case, since a* and b* color levels increase in egg yolk color with the use of HSM, it can be said that the use of synthetic colorants can be reduced to some extent. When evaluated in terms of processing technique, the egg yolk RCS and L* values of the HSM-C group were statistically higher than the HSM-H group, but a* and b* values were similar. Yüksel (2017) found that the egg yolk L* value in the control group was higher than the raw and cooked hemp groups and there was no significant difference between the raw and cooked hempseed added groups, but a* and b* values were lower in the control group than the hempseed added groups. In this current study, similar to Yüksel (2017) L* was found to be higher in the control group, while a* and b* values were lower with 15% addition hempseed. Processing the pulp at high temperatures did not increase the usability of colorants. Similarly, Goldberg et al. (2012) reported that hempseed added to laying hen feeds increased the a* and decreased the L* value of egg yolk. In contrast to these findings, Ozturk et al. (2022) and Taaifi et al. (2023) reported that hempseed addition increased L*, a*, and b* values in egg yolk. Goldberg et al. (2012) and Bazdidi et al. (2016) noted that the egg yolk color increased via the addition of a higher level of HS in the hen diets. The color of HS is due to the presence of xanthophylls, carotenoids, and chlorophylls (Latif and Anwar 2009).

In this study, ESW, eggshell ratio, and eggshell thickness were statistically significantly higher in the HSM-H group than in the C and HSM-C groups. Surprisingly, HSM-H positively affected eggshell quality, and this suggests that the degree of digestibility of Ca increased with high temperature (120 °C). Yüksel (2017) (2018) reported that raw and heat-treated hemp seeds in laying hen feeds provided similar shell weight and ratio to the control group and that there was no statistical difference. However, eggshell thickness was found to be higher in the hempseed-added groups than in the control group. It has been claimed that the anti-nutritional factors mentioned in raw hemp, especially phytic acid (Russo, 2013b), do not harm Ca and P metabolism. In a study by Neijat et al. (2014), it was determined that hempseed addition to the diet did not affect blood calcium (Ca)

levels in laying hens. Therefore, it is thought that hemp addition does not negatively affect Ca absorption and does not disrupt Ca homeostasis in the body. On the other hand, in this study, the Ca level in the diet was calculated as similar (% 3.88) and it was determined that there was no problem in terms of Ca balance in the diet and that hemp addition did not cause a negative situation in terms of shell weight. In support of the present results, Gakhar et al. (2012) reported that the effect of hempseed addition to the diet at 10 and 20% on eggshell thickness was insignificant. Bazdidi et al. (2016) reported that the effect of hempseed addition to laying hen feeds on eggshell ratio was insignificant. Halle and Schöne (2013) reported that the effect of hempseed meal used in the diet on eggshell weight was insignificant compared to rapeseed and flaxseed. Eggshell surface area was not statistically significant between the groups. Skřivan et al. (2020) and Taaifi et al. (2023) reported that hempseed addition increased the eggshell thickness of hens. However, (Goldberg et al., 2012; Konca et al., 2019; Öztürk et al., 2024) didn't find a significant effect on eggshell thickness.

4.3. Effect of treatments on egg fatty acid composition

In this study, the composition of egg yolk fatty acids obtained in 3 periods is not statistically significant. However, in the analyses made in 7 periods, it was found that Omega 6 and Omega 3 fatty acids were significantly higher in the groups with HSM added. In the overall period calculated as the average of both groups, no significant difference was found between the groups in terms of fatty acids. A similar result was found by Bazdidi et al. (2016) who noted that the addition of hempseed did not change the fatty acid composition of eggs. However, it was clearly shown that n-3 fatty acids in egg yolk increased by hempseed addition (Konca et al., 2019). For the study, palmitic saturated fatty acid (SFA), which is in dominant in egg yolk, showed a significant decrease in the groups supplemented with hemp seeds compared to the C group. Similar to the results of the present study, Konca et al. (2014), Raza et al. (2016), and Silversides and Lefrançois (2005) reported that hempseed supplementation reduced the palmitic acid content of egg yolk. However, Gakhar et al. (2012), and Goldberg et al. (2012) reported that the palmitic acid content of egg yolk was not significantly affected by hempseed addition. However, Konca et al. (2014) found that the stearic acid ratio in quail eggs was insignificant, while Silversides et al. (2005) and Halle and Schöne (2013) reported that stearic acid decreased. On the other hand, oleic, which is the dominant monounsaturated

fatty acid in egg yolk, and palmitoleic (mono-unsaturated fatty acids = MUFA) which is the second most common monounsaturated fatty acid, showed a significant decrease with the addition of HK and PK in the diet. Another interesting point is that while 22:6 docosahexaenoic acid (DHA) was very low in the control group, it increased in the egg yolk with the addition of HSM-C and HSM-H addition. This situation shows that DHA is synthesized in the hen's body from 18:3 fatty acids and passes into the egg yolk, thus obtaining the desired DHA in foods. Silversides et al. (2005) Gakhar et al. (2012), and Goldberg et al. (2012) reported that oleic acid in egg yolk was not affected by the hempseed ratio in the diet. However, regarding the increase in linoleic ratio with hempseed addition in the present study. Silversides et al. (2005), Halle and Schöne (2013) and Konca et al. (2014), reported that hempseed addition increased the linoleic acid content of egg yolk, while Gakhar et al. (2012) and Goldberg et al. (2012) reported that it was not significantly effective. The α-linolenic acid content of egg yolk at d 84 increased considerably with the addition of HSM-C and HSM-H, which achieved one of the project's most important goals. However, α-linolenic acid content did not increase on d 42 and overall. In contrast to this study's results, the increase in α-linolenic acid content with the addition of hempseed to laying hen diets was reported by Gakhar et al. (2012), Goldberg et al. (2012) and Konca et al. (2019). Similar results were obtained by Shahid et al. (2015) reported that hempseed addition at 15 and 20% did not significantly affect the α-linolenic acid content, while hempseed addition at 25% increased the α-linolenic ratio. In the present study, an increase in EPA and DHA levels was detected (not statistical significant) and similar results were also found by Gakhar et al. (2012), Goldberg et al. (2012) and Konca et al. (2019).

A significant portion of previous studies were conducted with the addition of hemp seeds. However, this study was conducted with hempseed meal, and while the seed contains high amounts of oil, the oil content in the meal is low. Hemp oil is rich in Omega 3 and Omega 6 oils, and when added to feeds, they were transferred to the animal. However, since sunflower oil was used as the oil source in this study, there was no significant increase in the desired fatty acid composition. Heat treatment of feeds is expected to have positive effects on the digestion of proteins and the elimination of anti-nutritional factors. In this study, when the oils were removed with high pressure and heat, it was not expected

that the transfer to the animal and the feed would be high because less oil remained in the HSM.

4.4. Effect of treatments on blood components

In this study, blood analysis showed that glucose, protein, cholesterol, creatinine, AST, and ALT values were not statistically significant. However, the triglyceride rate in the control and cold-processing groups was statistically higher than in the hot-processed HSM group. Konca, et al. (2014) reported that hempseed addition of 5 to 20 % did not affect serum triglyceride, cholesterol, and the high-density lipoprotein, however, low-density lipoprotein (LDL) concentration in HS-supplemented groups was lower than that of the C group. Barani et al. (2017) reported that hempseed supplementation (at 5, 10, 15 and 20 rates) did not affect triglyceride, cholesterol, and HDL rates but decreased LDL rates, while Bazdidi et al. (2016) determined that 10% hempseed supplementation to the diet increased serum triglyceride rates, 5% hempseed supplementation decreased serum LDL rates, but serum cholesterol and HDL levels were not affected by the diets. Mahmoudi et al. (2015) reported that triglyceride, cholesterol HDL and LDL values were insignificant when hempseed supplementation was at 2.5 and 5%, but triglyceride, cholesterol, and LDL rates decreased when hempseed was at 7.5%, and HDL rates increased. On the other hand, it was determined that AST and ALT values used in liver function tests did not change significantly with hemp seeds (Konca et al., 2019). In this case, it is concluded that the consumption of hempseed meal does not have a negative effect on blood values, which indicates that the use of this product in the diet is safe.

CONCLUSION AND RECOMMENDATIONS

Based on the results of this study, the addition of hot and cold-pressed HSM to the diets did not significantly affect egg weight and egg mass performance values. Egg yolk color values were generally positively affected by hemp additions. On the other hand, surprisingly, hot press HSM positively affected eggshell quality. This suggests that the degree of digestibility of Ca increased with increasing temperature.

It is preferred to obtain oil from some medicinal plants by cold method. However, when cold pressing is done, more oil remains in oil seeds and this is an economic loss. During hot pressing, the tightening screw is tightened a little and the temperature increases due to friction. Since tightening the screw creates more friction and tightening pressure, more oil is extracted. High heat treatments cause various anti-nutrition elements in the seeds to be inhibited and digestibility and usefulness increase.

In this study, a similar situation occurred and hot-pressed HSM had positive effects on some parameters. However, since the diets were isocaloric and isonitrogenous, no significant difference was obtained with the control in most of the values. As a result, it can be said that hot-pressed HSM is more beneficial when used as animal feeds.

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