Evaluation of Hempseed (*Cannabis sativa* L.) Presscake and Oil on Production Performance, Amelioration of Fatty Liver Disease, and Feather Pecking in Laying Hens (*Gallus gallus domesticus*)

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2024

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ABSTRACT

As the Canadian poultry industry transitions to alternative housing systems by 2036, larger group sizes may increase feather pecking (FP), leading to mortality, and reduced egg production. Hempseeds are rich in protein and fatty acids, which may support egg production and feather growth, and the CBD may provide a calming effect. Hemp's antioxidant and anti-inflammatory properties may reduce fatty liver disease (FLD), a prominent cause of mortality in cage systems. Two 14-week studies tested hempseed presscake (HP at 10, 20%) and hempseed oil (HO at 3 and 6%) as feed ingredients in laying hen diets. Study 1 involved Lohmann LSL-Lite White and Lohmann Brown-Lite hens in conventional housing; study 2 used Lohmann LSL-Lite White in a single-tier system. Results showed that hemp products improved egg quality, bone strength, and feather condition, with potential benefits for FLD. Findings support hemp's inclusion in poultry diets, with applications under review for CFIA approval.

LIST OF ABBREVIATIONS USED

ACUC	Animal Care and Use Committee
ALA	α-Linolenic acid
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
CAT	Catalase
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBG	Cannabigerol
CBN	Cannabinol
CCAC	Canadian Council on Animal Care
CF	Crude fat
CFIA	Canadian Food Inspection Agency
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FCR	Feed conversion ratio
FLD	Fatty liver disease

FLHS	Fatty liver hemorrhagic syndrome
FP	Feather pecking
GCN2	General controlled non-repressed 2 kinase
GE	Gross energy
GGT	Gamma-glutamyl transferase
GLA	Gamma linolenic
GSH	Glutathione
HO	Hempseed oil
HP	Hempseed presscake
IL-1	Interleukin-1
IL-1β	Interleukin 1 beta
IL-6	Interleukin-6
LA	Linoleic acid
MDA	Malondialdehyde
iNOS2	Inducible nitric oxide synthase
NAFLD	Non-alcoholic fatty liver disease
Nrf2	Nuclear factor erythroid 2–related factor 2
PUFAs	Polyunsaturated fatty acids
SAAL1	Serum amyloid A-like 1
SOD	Superoxide dismutase
THC	Delta 9-tetrahydrocannabinol
TNF-α	Tumor necrosis factor alpha
VLDL	Very low-density lipoprotein

ACKNOWELDGEMENTS

I would like to express my gratitude to my supervisor, Dr. Stephanie Collins, for her invaluable guidance, support and encouragement throughout this research. Her expertise has been instrumental in shaping this thesis and in advancing my skills as a researcher. I am deeply grateful for the time, effort, and feedback she provided towards the successful completion of this research.

I would also like to extend my appreciation to my supervisory committee, Dr. Bruce Rathgeber and Dr. Stefanie Colombo, for their insightful feedback and continuous support. Their input and recommendations have been crucial in enhancing the quality of this work.

Special thanks to Janice MacIsaac for her exceptional assistance with data collection and analysis, scheduling, and ordering the necessary supplies. I would also like to extend my gratitude to Xujie Li, who contributed greatly to the completion of the statistical analyses crucial to the success of this work.

I am incredibly grateful to the Atlantic Poultry Research Center and its dedicated staff, including Michael McConkey, Sarah MacPherson and Krista Budgell, for providing their expertise in feeding, egg collection, sampling and general management of the birds. Special thanks to Jamie Fraser who greatly contributed to this research by assisting with the production of the diets for these trials. Additional thanks to Vicki Mackintosh and Sasha Di Stefano-Pitre for their significant contributions to data collection. I would also like to thank Jing Lu, Guoyu Hu, Abigail Black, Sarah Purcell, Samantha Gardner, and Effie Burke for their assistance with data collection and sampling throughout the trial.

This research would not have been possible without the generous financial support provided by Egg Farmers of Canada, Mitacs, the Atlantic Poultry Research Institute, Egg

Farmers of New Brunswick, and the New Brunswick Government Sustainable Canadian Agriculture Partnership. Their financial contributions were greatly appreciated and have significantly facilitated the progress and success of this research.

Lastly, I am forever grateful for the unconditional support from my friends, family, and loved ones throughout this journey.

CHAPTER 1: INTRODUCTION

In response to consumer preferences for a cage-free egg product, Canada's egg industry is transitioning away from conventional cage housing of hens towards alternative housing systems such as single-tier or multi-tier aviary systems. Conventional cage housing typically involves small wire cages with limited space available for hen movement and performance of natural behaviors (Hartcher and Jones, 2017). Alternative housing systems provide increased space for movement and enrichments including nest boxes, perches, scratch pads and foraging/dust bathing material promoting the performance of natural behaviors (Donaldson and O'Connell, 2012; Rodenburg et al., 2010). Both conventional and alternative rearing systems pose bird welfare concerns. In conventional cage systems, the most prominent cause of bird mortality is fatty liver hemorrhagic syndrome (FLHS; Shini et al., 2019). In alternative housing systems, feather pecking (FP) and cannibalism are the most common critical welfare concerns (Decina et al., 2019a; Kittelsen et al., 2022). During the industry transition, many hens in Canada will be housed in conventional cage systems until they are completely phased out by 2036, and FLHS can still occur in alternative housing systems. Management practices, including nutritional management, that decrease the risk of severe FP and cannibalism in laying hens and reduce the incidence of fatty liver disease (FLD) prove vital to egg production as the industry moves towards group housing systems. The nutritional benefits of hemp and the calming effects of cannabidiol (CBD) may have the potential to reduce FLD and FP behavior (Iffland and Grotenhermen, 2017).

FLD is a metabolic disorder characterized by excess fat accumulation in the liver and abdomen. The disorder can lead to blood clots and rupture of the liver causing sudden mortality by FLHS reaching up to 5% mortality during a laying cycle (Hamid et al., 2019; Lin et al., 2021). Shini et al. (2019) reported that 74% of mortality in caged laying hens was diagnosed as FLHS. The main factors thought to contribute to FLD include lack of exercise and restricted movement, high environmental temperatures, high stress, and physiological factors such as excess abdominal fat or high levels of plasma estrogens and hen strain type (Hartcher and Jones, 2017; Lin et al., 2021). Although reduced in non-caged birds, FLD can still occur in hens reared in alternative housing systems, as heavier, well-nourished free-range birds weighing 2.08 kg can have a higher prevalence of FLD totaling 15.2% of the flock (Sibanda et al., 2020). This suggests hens may suffer from sub-acute and chronic FLHS, with the chronic form causing little or no mortality with a sudden drop in egg production (Chen et al., 2006). Hens affected by this disease have reduced egg production, egg weights and reduced feed conversion ratios (FCR's). If FLD can be prevented by dietary changes, it will result in improved chicken health, improved feed conversion efficiency, and reduction of the loss of animals to FLHS. The anti-inflammatory properties of hemp may reduce the incidence of FLD in commercial laying hen flocks by targeting underlying inflammatory processes, thereby contributing to improved bird health and welfare (Kaushal et al., 2020).

Severe FP in hens has been documented in all types of housing systems, but laying hens housed in aviaries and community cages exhibit increased instances of FP and cannibalism compared to those housed in conventional cages (Fossum et al., 2009; Lambton et al., 2015; Decina et al., 2019a). Once FP occurs in a single-tier aviary, multi-tier aviary, free range or free run system, there is a greater tendency for the behavior to spread throughout the flock as the birds are housed in larger group sizes. Once pecking behavior starts, it is difficult to control (Petek and McKinstry, 2010; Hartcher and Jones, 2017; Cronin and Glatz, 2021). It is a serious welfare concern because severe FP, whereby hens vigorously peck at and pull out the feathers of other birds, causes pain, stress, injuries, and susceptibility to disease. The loss of feather coverage leaves bare areas which may progress to tissue pecking and mortality due to cannibalism (Petek and McKinstry, 2010; Decina et al., 2019a). There are many causes of mortality in hens during the laying period, but FP and cannibalism behaviors are considered the primary cause of death (Weitzenburger et al., 2005). It is of great economic concern to the producer as FP can lead to a reduction in egg production and pecked hens compensate for the loss of insulation from the loss of feathers through higher feed consumption, which leads to higher feed costs (Petek and McKinstry, 2010). Critical factors include hen strain or genetic traits, overcrowding, excessive light, high temperatures, and insufficient or improperly placed feeders and drinkers (Petek and Mckinstry, 2010; Hartcher and Jones, 2017). Nutritional imbalances, such as mineral deficiencies, feed type, high-energy diets heavy in corn or low in fiber, along with bird stress, lack of enrichment, litter type, and other environmental factors from an early age and onwards, also play a role (Petek and Mckinstry, 2010; Hartcher and Jones, 2017).

In Canada, white hen strains are more often associated with conventional cage and furnished cage systems, while brown hen strains are often reared in floor or non-cage systems (Petrik et al., 2015; Van Staaveren et al., 2018). As laying hen housing systems shift from caged housing to alternative housing systems, fearfulness or reactive fear responses may increase due to hens being housed in larger group sizes (Rentsch et al., 2023). The larger group sizes may increase risk of injury and collision with other birds, or the housing environment resulting in keel bone fractures leading to an overall reduction in bone strength and the reactive fear response may increase feather pecking behaviour (Nelson et al., 2020; Rentsch et al., 2023). White hen stains are known to be more fearful than brown hen strains (Rentsch et al., 2023). While brown hens are considered more dominant, with more aggressive and territorial behaviour, white hens tend to be more fearful and more susceptible to feather pecking (Odén et al., 2002; De Haas et al., 2013). Brown hen strains are also more susceptible to FLD compared to white hen strains. Zhang et al. (2018) reported that dwarf Jaingxing-Huang were more susceptible to FLD than white leghorn laying hens and a local Beijing-You breed. Stake et al. (1980) observed that Rhode Island Red hens were more susceptible to FLHS than the White Leghorn. This justifies the use of both brown and white laying hen strains in the current study, as understanding the differences in behavior and health between brown and white hens under varying housing conditions can inform better management practices.

Nutritional management that decreases the risk of severe FP and cannibalism in laying hens prove vital to egg production as the industry moves towards group housing systems. The nutritional benefits of hemp and the calming effects of cannabidiol (CBD) may reduce instances of FP and cannibalism (Iffland and Grotenhermen, 2017). Recent research incorporating hempseed and hempseed oil in the diets of laying hens has been directed at enriching eggs with n-3 fatty acids while offering a more balanced ratio of n-3/n-6 fatty acids (Neijat et al., 2016; Taaifi et al., 2023b). Hemp's antioxidant and anti-inflammatory properties have been reported to reduce oxidative stress and reduce levels of pro-inflammatory cytokines, which may ameliorate fatty liver disease (Fallahi et al., 2022). The tocopherol content of hemp may also enrich eggs with higher levels of antioxidants adding to their nutritional value for consumers (Taaifi et al., 2023b). A common approach to enriching eggs with n-3 fatty acids is the inclusion of flaxseed and fish oil in livestock feed (Kralik et al., 2021; Lee et al., 2021). Currently, the exploration of novel and alternative sources of proteins and natural plant resources for feed production is of increasing interest.

CHAPTER 2: LITERATURE REVIEW

2.1: Aspects of hemp for nutrition in chickens

Hemp (*Cannabis sativa* L.) is a multiuse annual herbaceous crop. Its fibers are used to produce paper and clothing materials (Klir et al., 2019; Müssig, 2010). The edible seeds can be food for both humans and animals, and they are also utilized in other industrial processes, such as cosmetics (Klir et al., 2019; Müssig, 2010). Additionally, the flowers of the hemp plant are harvested for cannabinoids, which are used in pharmaceuticals (Klir et al., 2019; Müssig, 2010). In Canada, following the legalization of the cultivation of industrial hemp in 1998, licensed growers have increased the production of hemp and hempseed (Gakhar et al., 2012; Jing et al., 2017). With the increase in availability of hempseed and hempseed products, there has been an increase in opportunity for its use as a replacement of soybean as a source of protein and fat for livestock feed (Gakhar et al., 2012; Klir et al., 2019).

Hemp can be utilized in various aspects of livestock rearing. Hempseed and HP, which is obtained after the hempseed oil (HO) is cold-pressed from the seed and contains various amounts of remaining fats, is appropriate for use as feed ingredients for animals. The whole plant, including the stalk and leaves, can be fed to ruminants and the hemp hurds, which are the woody core of hemp fibers can be used as bedding for animal enrichment (Clarke and Merlin, 2013; Klir et al., 2019: Müssig, 2010). HO extracted from the seed can provide a rich source of essential fatty acids and the seed and hempseed cake can provide a rich source of fat and protein for animal diets (Klir et al., 2019).

Hempseeds generally contain over 30% oil, approximately 25% protein, 30% carbohydrates, 28% fiber and trace amounts of vitamins and minerals with a gross energy

(GE) content of 5258 kcal/kg (Callaway, 2004; Shahid et al., 2015; Stastnik et al., 2020). It also contains a small amount of delta-9-tetrehydrocannabinol, a strong fat-soluble antioxidant, which stimulates appetite (Shahid et al., 2015; Stastnik et al., 2020). The percentage of fats in a dehulled seed is around 42- 47% (Stastnik et al., 2020). Hemp oil, obtained from the seed after pressing, consists of 75-80% polyunsaturated fatty acids (PUFA), including 15-25% α-linolenic acid (ALA, 18:3n-3), 3-6% gamma linolenic acid (GLA, 18:3n-6) and 53-60% linoleic acid (LA, 18:2n-6). HO is also a rich source of tocopherols, containing 1500 mg/kg, and there are two readily digestible proteins, edestin and albumin, that are most abundant and contain all essential amino acids (Shahid et al., 2015; Stastnik et al., 2020).

North American laying hen diets typically consist of cereal grains and fat or oil sources, which provide high levels of n-6 PUFA, predominantly LA and low levels of n-3 PUFAs (Gakhar et al., 2012; Neijat et al., 2016). This results in conventional eggs that contain low levels of n-3 PUFAs, specifically ALA, docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA, 22:6n-3), and negligible levels of eicosapentaenoic acid (EPA, 20:5n-3; Neijat et al., 2016). For humans to balance antioxidant activities in the body, it is important to consume antioxidant-rich foods, this can include eggs enriched with vitamin E or n-3 PUFAs which can have both antioxidant and pro-oxidant effects depending on specific conditions (Oppedisano et al., 2020). This can be achieved by incorporating nutricines into laying hen feed mixtures, as n-3 PUFAs in the hen diet are incorporated into egg yolks (Kralik et al., 2020). Oilseeds and oils from oilseeds, such as flax, canola and acai are a good source of n-3 PUFAs, but hempseed and HO are rich in both n-3 PUFAs and n-6 PUFAs, providing an additional option as a feed ingredient to enrich eggs (Gakhar et al., 2012; Jing et al., 2017; Neijat et al., 2016). The gamma-linolenic acid (GLA) found in HO acts as an intermediate in the production of eicosanoids, which may have similar anti-inflammatory and anti-proliferative properties as EPA and DHA (Jing et al., 2017).

2.2: Cannabinoids in hemp

Prior to the legalization and regulation of industrial hemp in 1998, hemp cultivation was prohibited in Canada in 1938 under the Opium and Narcotic Drug Act due to its high cannabinoid content (Heath Canada, 2018). Delta 9-tetrahydrocannabinol (THC) was the cannabinoid of concern due to its psychoactive properties (Health Canada, 2018). The Canadian Food Inspection Agency (CFIA) only allows for hemp to be grown with less than 0.3% THC. However, there is no limit on the amount of CBD in industrial hemp (Health Canada, 2020). Hempseeds generally contain little to no CBD unless it is extracted from the flower or leaves of the hemp plant, in which case the CBD content is between 12-18% (VanDolah et al., 2019). Currently, hemp products (HP, HO, hempseed) are not approved for use as livestock feed ingredients in Canada (CFIA, 2023). Hemp requires approval for use in poultry diets. This requires ensuring that the feed is safe for poultry and efficient for its intended use. THC and CBD are the most abundant and well-understood phytocannabinoids found in marijuana and hemp strains, respectively (VanDolah et al., 2019). In *C. sativa* plants and freshly harvested tissues, cannabinoids exist predominantly in their carboxylic acid forms (CFIA, 2021). For example, approximately 95% of CBD is found in its acidic form, cannabidiolic acid (CBDA; Fallahi et al., 2022). Cannabinoids can be converted to their neutral counterpart by decarboxylation when there is exposure to heat, light, or alkaline conditions (Fallahi et al., 2022). The extraction method for the HO can also affect the cannabinoid content. Extraction methods include cold-pressing, steam distillation, solvent extraction, CO_2 extraction, microwave, or ultrasound-assisted processing (Burton et al., 2022). The major cannabinoids include CBD, cannabichromene (CBC), cannabigerol (CBG), THC, and cannabinol (CBN; Kanabus et al., 2021). Unlike THC, CBD has no narcotic properties, but does have antioxidant, anti-inflammatory, antipsychotic, anxiolytic, and anticonvulsant properties (Fallahi et al., 2022). CBC does not have psychoactive effects, but it is associated with the presence of THC and is present in all cannabis varieties (Kanabus et al., 2021). The properties of CBC are not fully understood. CBG also has no psychoactive properties and its precursor cannabigerolic acid (CBGA) is a biosynthetic precursor for CBD and THC (Fallahi et al., 2022). CBN is a degradation product of THC and possesses weaker psychoactive properties and antioxidant properties (Zhang et al., 2024). CBN binds to cannabinoid receptors in the body with a higher affinity for cannabinoid receptor 2 (CB2) and is weak when bound to cannabinoid receptor 1 (CB1; Kanabus et al., 2021). CB1 receptors of the endocannabinoid system, which is a complex signaling pathway in the body, have been located in the central nervous system, digestive tract, liver, fatty tissue, kidneys, muscles and heart (Kanabus et al., 2021; Zhang et al., 2024). CB2 receptors are present on immune cells and in peripheral tissues (spleen, blood cells, and bone), and activation of those receptors stimulates the release of inflammatory cytokines (Kanabus et al., 2021; Sparks et al., 2022; Zhang et al., 2024). CBD modulates the activation of CB1 and CB2 receptors (Kanabus et al., 2021). CBD is the active cannabinoid compound in hemp (Skřivan et al., 2019). The anti-inflammatory and antioxidant properties of CBD may reduce fatty lever disease, and the calming effect of CBD may reduce FP. CBD has also been reported to activate CB2 receptors in bone tissue to regulate pro-osteogenic functions (Sparks et al., 2022).

2.3: Fatty liver disease in laying hens

Fatty liver disease (FLD) is a metabolic disorder characterized by excess accumulation of fat in the liver. Bird mortality occurs only when FLD escalates to FLHS, whereby massive hemorrhaging of the liver is present along with abdominal bleeding (Navarro-Villa et al., 2019; Zhu et al., 2020; Lin et al., 2021). Excessive fat deposition in the abdominal cavity is also observed (Zhu et al., 2020). FLD is induced by an unbalanced nutrient intake and has many other predisposing factors such as increased feed intake, lack of exercise, environmental factors such as high temperature, increased circulating oestrogen concentrations, and genetics (Navarro-Villa et al., 2019; Zhu et al., 2020). A study in Bejing, China, showed that dwarf Jaingxing-Huang chicken were more susceptible to FLD than the laying White Leghorn and local Beijing-You breeds (Zhang et al., 2018). The progression to FLHS is associated with the production of inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) Interleukin-1 (IL-1), serum amyloid A-like1 (SAAL1), and inducible nitric oxide synthase (iNOS2; Xing et al., 2020). These cytokines are involved in the pathogenesis of FLHS (Xing et al., 2020). The inflammatory responses they trigger further exacerbate the condition, contributing to the severe outcomes observed in FLHS (Xing et al., 2020).

FLD typically occurs in caged laying hens at peak production and the condition is difficult to identify in the initial stages because there are no clinical symptoms (Gao et al., 2019; Navarro- Villa et al., 2019; Zhu et al., 2020). FLHS generally becomes evident by a sudden drop in egg production and may drop by 15% over 11 weeks (Miao et al., 2021).

Diagnosis can only be confirmed during post-mortem evaluation (Zhu et al., 2020). Post-mortem evaluations also indicate hyperlipidemia, which is the presence of above normal lipid levels in the blood, mainly triglycerides (Navarro-Villa et al., 2019; Lin et al., 2021). Elevated plasma dipeptidyl peptidase 4, an enzyme related to non-alcoholic fatty liver disease (NAFLD) in humans, has been identified in laying hens with FLHS. FLHS is similar to NAFLD because they share the pathologic feature of having excess lipid deposition in the liver (Zhu et al., 2020; Lin et al., 2021; Meng et al., 2021). Fat deposition in the liver is considered FLD when the liver fat fraction is 5% or higher and in FLHS-affected hens, the liver fat fraction can exceed 40% and go up to 70% (Schwimmer et al., 2009; Navarro-Villa et al., 2019). The pathology of FLHS is associated with inflammation, lipid disorder, oxidative stress, autophagy, and an imbalance of gut microbiota (Gao et al., 2019; Xing et al., 2020; Lin et al., 2021). The etiology of FLHS in chickens, however, is still largely unknown.

The liver is a multi-purpose organ involved in the metabolism of fat, carbohydrates, protein, vitamins and minerals, removal of waste products and detoxification. It is the major organ involved in lipid synthesis and metabolism in birds and in humans (Lin et al., 2021). Changes in lipid homeostasis, such as hepatic lipid accumulation, transportation, and metabolism are the basis for FLD (Zhu et al., 2020; Lin et al., 2021). Hens with FLHS may have excess fatty acid supply or inhibited oxidation in the liver resulting in an increase in triglyceride synthesis and elevated blood low-density lipoprotein cholesterol (Dong and Tong, 2019; Zhu et al., 2020). Enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been used as indicators of liver damage and are increased in hens with FLHS (Hamid et al., 2019; Gao et al., 2019).

FLD in chickens, induced by a high-energy, low-protein diet, can be reversed with appropriate dietary interventions. Adding genistein to laying hen diets has been shown to improve lipid metabolism and reduce liver inflammation, effectively alleviating FLD. (Gao et al., 2021). An antioxidant such as resveratrol, when added to the diet of laying hens at 400 mg/kg could ameliorate FLHS (Wang et al., 2020). In rats, the n-6 and n-3 PUFAs ratio of hempseed and its anti-inflammatory and antioxidant properties ameliorated FLD when oxidative stress and inflammation were key mediators (Kaushal et al., 2020).

2.4: Attempts to address fatty liver disease in hens using nutritional strategies

2.4.1: Fatty Acids

Polyunsaturated fatty acids (PUFAs), specifically n-3 PUFAs have been reported to have significant hepatoprotective properties (Parker et al., 2012). Flaxseed (*Linum usitatissimum* L.) is a common fiber crop used in poultry feed for enrichment of n-3 fatty acid content in eggs (Moghadam and Cherian, 2017). Flaxseed is an oil seed that is rich in omega 3 PUFAs with the whole flax seed containing an average of approximately 23% α-linolenic acid (18-3 n-3, ALA) and flaxseed oil containing 40-50% ALA (Davis et al., 2016; Moghadam and Cherian, 2017). Terrestrial sources of ALA include oil seeds and their oils and leafy green vegetables, but it can also be sourced as long chain 20 and 22 carbon n-3 fatty acids from marine oils (Moghadam and Cherian, 2017). The mechanisms of how n-3 PUFAs ameliorate FLD when included in the diet of laying hens has not been fully elucidated, but it is proposed to improve hepatic lipid, oxidative and inflammatory homeostasis (Davis et al., 2016).

Davis et al. (2016) tested whole flaxseed, defatted flaxseed and flaxseed oil supplemented into the diet of laying hens and observed that hens fed a whole flax diet had reduced steatosis and hepatocellular ballooning. Serum AST concentration was reduced by 80% in hens fed the whole flaxseed and defatted flaxseed diet compared to the control diet. Hepatic n-3 PUFA enrichment was improved with all three flaxseed treatment diets (Davis et al., 2016). The enrichment of the liver with EPA and DHA has been associated with reduced steatosis and hepatocellular injury, although the mechanism behind this remains unclear, it may be due to EPA's antihyperlipidemic and antioxidative properties (Hirotani et al., 2015; Davis et al., 2016). Additionally, the transcript abundance of genes associated with FLD were downregulated in the hens fed the flaxseed oil treatment. These down-regulated transcripts are involved in cholesterol metabolism, fatty acid metabolism, insulin signaling and inflammatory signaling (Davis et al., 2016). However, the underlying mechanisms contributing to the modification of hepatic transcript abundance is still unclear. A possible factor contributing to the flaxseed oil treatment group having greater modification of transcript abundance is the difference in bioavailability of ALA between the treatment diets. Flaxseed oil exhibits greater bioavailability of ALA than that exhibited by flaxseed meal (Patenaude et al., 2009).

2.4.2: Antioxidants

Natural antioxidants such as polyphenols, carotenoids and flavonoids are derived from plants and can be added to the diet of laying hens to enhance laying performance and egg quality by improving resistance to oxidative stress (Li et al., 2022). Resveratrol is an example of a natural plant polyphenol found in grapes and red wine (Xing et al., 2020). The betatrophin and antioxidant properties of resveratrol can protect the liver against damage through regulation of cell death pathways and amelioration of hepatic lipid accumulation (Wang et al., 2020). Dietary resveratrol can also significantly increase egg production rate, egg quality and intestinal health of laying hens (Ding et al., 2022). Widely accepted biological markers of oxidative stress include abnormal levels of malondialdehyde (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD; Xing et al., 2020). MDA is a product of lipid peroxidation. Xing et al. (2020) reported an increase in MDA and a decrease in GSH, CAT and SOD levels in the liver of hens with FLD. The decrease in GSH, CAT and SOD in the liver of FLD affected hens suggests that the antioxidant system was damaged by free radicals as these compounds play a role in scavenging free radicals and protecting cells from oxidative stress (Xing et al., 2020). The antioxidant gene nuclear factor erythroid 2–related factor 2 (Nrf2) plays a role in the transcription of enzymes (heme oxygenase-1 and SOD-1) that regulate intracellular reactive oxygen species levels (Zhang et al., 2013). Nrf2, heme oxygenase-1 and SOD-1 were significantly increased in the ovaries of FLD affected hens, indicating that oxidative stress may damage the ovaries of FLD affected hens (Xing et al., 2020).

Xing et al. (2020) observed that the addition of resveratrol at a dose of 400 mg/kg in in the diet of laying hens with FLD reduced lipid vacuoles, increased laying rate, decreased levels of MDA and increased SOD, CAT and GSH levels. Resveratrol also reduced activation of widely accepted inflammatory genes (nuclear factor-κB, TNF-α, Interleukin-1 beta (IL-1β), and IL-6; Xing et al., 2020)).

Ding et al. (2022) observed that dietary resveratrol at dose of 600 mg/kg in the diet of laying hens with oxidative stress induced by intraperitoneal injection of tert-butyl hydroperoxide, increased egg laying rate, feed intake, levels of jejunal SOD, Glutathione peroxidase and total antioxidant capacity and reduced MDA and inflammatory gene expression. Ding et al. (2022) also observed an increase in jejunal-barrier related proteins and ovarian hormone receptors. While tert-butyl hydroperoxide does not induced FLD in laying hens, it does induce oxidative stress which plays an important role in the pathogenesis of FLD. Resveratrol can also improve hepatic steatosis partially by triggering beneficial autophagy and by influencing apoptosis of different cell types (Wang et al., 2020). Research on the optimal dosage of resveratrol in the diet of laying hens to ameliorate FLD is limited with no definitive guidelines.

2.4.3: Amino Acids

Dietary valine, an essential amino acid in the diet of laying hens, participates in growth, and the synthesis of proteins and acts a as precursor for other amino acids (Jian et al., 2021). Valine is particularly important during egg formation, where it plays a significant role in protein synthesis (Jian et al., 2021). A deficiency in valine reduces feed efficiency, and high levels of leucine can exacerbate the effect of valine deficiency. Synthetic L-valine supplementation has been shown to ameliorate gut health by enhancing intestinal villi morphology, strengthening the intestinal barrier, and reducing caecum pathogenic abundances of *Fusobacteriota* and *Deferribacterota* (Jian et al., 2021). However, Jian et al. (2021) concluded that 0.74% and 0.79% dietary synthetic L-valine in the diet of laying hens could accelerate the occurrence of FLD by promoting hepatic lipogenesis and creating an imbalance in the inhibition of oxidative and inflammatory responses. Although dietary valine ameliorated gut health and inhibited intestinal inflammatory response of general controlled non-repressed 2 kinase (GCN2), it also had adverse effects on liver health (Jian et al., 2021). GCN2 is a crucial enzyme that regulates intestinal inflammation and hepatic fatty acid homeostasis when amino acids are deficient, and when GCN2 is deficient, intestinal inflammation is enhanced and hepatic steatosis develops (Jian et al., 2021). The effects of valine involve complex interactions between metabolism, oxidative stress, and inflammation. Jian et al. (2021) suggested reducing dietary valine as an approach to preventing FLD in laying hens. However, the effect of valine on the gut-liver axis and relation to lipid metabolism in the liver of laying hens during peak production is not well understood and more research is necessary.

Glycine is typically referred to as a non-essential amino acid for poultry (Nam et al., 2023). However, glycine acts a precursor molecule for purine, GSH, bile salt, uric acid, and creatine synthesis in poultry (Nam et al., 2023). With selective breeding and increased metabolic demand, researchers suggest glycine be considered essential to the poultry diet. Nam et al. (2023) tested glycine at 0.341% and 0.683% in the diet of laying hens reared under heat stress conditions of 31.4 ± 1.17°C for 8 hours / day and 26.7± 1.10°C for the remaining time. It was observed that 0.683% glycine in the diet of hens resulted in an anti-fatty liver effect and should be considered as a preventative measure for FLD in hens under heat stress (Nam et al., 2023). Dietary glycine reduced corticosterone and heterophil-lymphocyte ratio, decreased hemorrhagic score and total fat concentrations. This was the first study to report anti-fatty liver effects of glycine dietary supplementation in laying hens exposed to heat stress. However, the results of this study could have been due to the close metabolic relationship of glycine to methionine, choline, and betaine which are reported to prevent hepatic fat accumulation in poultry (Kidd et al., 1997; Choi et al., 2012; Beheshti Moghadam et al., 2021). Further studies should evaluate the relationship between methionine, choline, betaine, and glycine in laying hens, especially under heat stress conditions.

Methionine is a precursor to S-adenosylmethionine, which serves as a methyl donor in biological reactions and is involved in gene expression, lipid metabolism and synthesis of phosphatidylcholine (Liu et al., 2019; Lin et al., 2021). Phosphatidylcholine is an essential component for formation of very low-density lipoprotein (VLDL), which is responsible for transporting lipids from the liver (Liu et al., 2019; Lin et al., 2021). Disruptions in the conversion of choline to phosphatidylcholine can impair VLDL formation and lipid export from the liver, leading to an accumulation of lipids and contributing to fatty liver disease (FLD; Lin et al., 2021).

Lysine and methionine serve as carnitine precursors, which are crucial for transporting long-chain fatty acids into mitochondria, where they undergo β-oxidation (Lin et al., 2021). Inadequate intake of essential amino acids, especially methionine and lysine, leads to reduced carnitine levels, potentially causing FLD due to compromised fatty acid metabolism (Lin et al., 2021). Therefore, supplementation of methionine or lysine in the diet could ameliorate FLD in laying hens, but further studies should be conducted to evaluate this as there are limited studies involving the effects of dietary supplementation of these compounds for ameliorating FLD in laying hens.

Taurine is a sulfur-containing amino acid derived from methionine and cysteine metabolism and exerts many physiological effects (Han et al., 2023). Taurine is involved in cell membrane stabilization, bile acid conjugation, calcium homeostasis, anti-inflammation, anti-oxidation and immunomodulation (Han et al., 2023; San et al., 2023). Taurine supplementation at a dose of 2.5 or 5g/kg decreased hepatic fat and serum ALT, AST and gamma-glutamyl transferase (GGT) and decreased MDA concentration in the liver (Han et al., 2023). However, taurine at 10g/kg adversely increased serum GGT (Han et al., 2023). No clear reasoning was reported as to why this occurred. Serum AST, ALT and GGT are reported as biomarkers of FLD and oxidative stress is recognized as a cause and result of FLD (Han et al., 2023). Taurine has antioxidative properties and can directly scavenge free radicals to inhibit an imbalance of oxygen reactive species. This is evident by the decrease in MDA (which is a product of lipid peroxidation), which occurs with dietary taurine, as MDA is a marker for oxidative stress (Han et al., 2023).

Taurine has also been shown to influence bile acid homeostasis as taurine is involved in bile acid synthesis in the liver which plays a role in absorption and digestion of dietary fats (Han et al., 2023). More research is needed to investigate the effect of dietary supplementation of taurine and bile acid synthesis on laying hen liver health. San et al. (2023) tested a corn soybean meal diet for laying hens supplemented with 0.05% and 0.3% taurine. They found that taurine protects mitochondria in hepatocytes from lipid accumulation and free fatty acids. This protection occurs by upregulating the expression of proteins involved in reducing mitochondrial swelling and improving the mitochondrial integrity (San et al., 2023). Taurine also enhances mitochondrial adenosine triphosphate (ATP) production and regulates mitochondrial autophagy which plays a crucial role in regulating lipid droplet accumulation in FLD afflicted laying hens. Reducing mitochondrial damage and maintaining mitochondrial homeostasis are essential to ensuring normal fat metabolism as oxidative stress is a key factor of mitochondrial damage (San et al., 2023). The results offer a potential target and a scientific reasoning for utilizing taurine as a preventive measure against FLD and diseases related to mitochondria. Further research should be conducted to explore the pathophysiological roles and actions of taurine in hepatocytes as the relationship could be more complex.

2.5: Feather pecking and cannibalism behavior in laying hens

Feather pecking (FP), the behavior whereby hens peck, pull or pluck and sometimes eat the feathers of conspecifics, is a welfare issue for commercial laying hen farms (Lambton et al., 2015; Decina et al., 2019b;). As Canada's egg industry transitions from conventional cage housing systems to alternative housing systems, such as enriched cages, single-tier floor systems, multi-tier aviaries, and free-range systems, there is a greater risk of FP behavior resulting in feather damage when a pecking bird has access to a larger number of pecking victims (Zeltner et al., 2000). The act of FP spreads within a flock through social transmission and because of this, it is more challenging to control FP in alternative housing systems than in conventional systems through identification and removal of the initial feather pecker (Zeltner et al., 2000; Rodenburg et al., 2013).

FP is distinguished as six different types of pecking behavior: (1) gentle FP without feather removal, (2) severe FP leading to loss of feathers, (3) aggressive pecking typically directed at the head or neck to establish dominance within hierarchy, (4) injurious tissue pecking and cannibalism, (5) vent pecking and (6) toe pecking (Savory, 1995; Jung and Knierim, 2017). Factors contributing to prevalence of FP include strain of hen or genetic traits, overcrowding, excessive light, and high temperatures (Petek and McKinstry, 2010; Hartcher and Jones, 2017). Additionally, insufficient or improperly placed feeders or drinkers, nutritional imbalances such as mineral deficiencies, high-energy or low-fiber diets, and the type of feed used can contribute to FP (Petek and McKinstry, 2010; Hartcher and Jones, 2017). Lack of enrichment, litter type and other environmental factors from an early age and onwards also play a role (Petek and McKinstry, 2010; Hartcher and Jones, 2017). Stress-inducing factors further influence FP behaviour (Petek and McKinstry, 2010; Hartcher and Jones, 2017).

In 2017, 122 farms with hens housed in alternative housing systems across Canada received survey packages on housing and management and were asked to conduct feather scoring on 50 hens from their flock (Decina et al., 2019a). Of the 122 farms invited, 65 farms responded to the survey. An average 26% of birds had moderate or severe feather damage due to FP in the 39 flocks housed in non-cage systems, of which 17 flocks were housed in single-tier floor systems and 22 flocks were housed in multi-tier aviary systems (Decina et al., 2019a). The 26 furnished cage housing systems had an average feather damage prevalence of 22% (Decina et al., 2019b). An epidemiological study in the UK of 62 free-range and organic farms, with 119 individual flocks, observed vent pecking in 19.5% of free-range flocks and 29.9% of organic flocks (Lambton et al., 2015). Each farm was observed on two occasions and cannibalism was reported in 26.6% of farm visits (Lambton et al., 2015). In France, 79 furnished cage flocks on 56 farms had a severe feather pecking prevalence of 32.9% and a cannibalism prevalence of 2.5%, and 80 free-range flocks on 75 farms had a severe feather pecking prevalence of 23.8% and a cannibalism prevalence of 8.8% (Coton et al., 2019).

Low protein diets are associated with increased prevalence of FP and mortality attributable to cannibalism (Cain et al., 1984; Ambrosen and Petersen, 1997). Chickens fed diets high in fiber, especially insoluble fiber had reduced cannibalism mortality from 13.2 to 3.9% compared to a commercial diet in hens 17-20 weeks of age and from 28.9 to 14.3% in hens 21-24 weeks of age (Hartini et al., 2002). Hempseed contains 25% protein and 28%

fiber, which makes it an interesting feed ingredient to test for the reduction of FP and cannibalism (Callaway, 2004; Shahid et al., 2015; Stastnik et al., 2020).

The objectives of this study are to:

1. Analyze production performance and feed efficiency by determining egg quality, egg yolk fatty acid profiles, FP behavior and incidence of cannibalism and mortality rate.
2. Assess the impact of HO and HP on the bone by measuring breaking strength and mineral composition and on the liver by determining incidence of FLD via color scoring, histological evidence of fat globule infiltration, and size and signs of hemorrhage in the liver.

The hypothesis is that HP at 10% and 20% and HO at 3% and 6% inclusion in the laying hen diet will reduce the incidence of FLD and FP, while increasing production performance, bone strength and egg quality in both a conventional cage and single-tier housing system. The null hypothesis for the study is that the reduction of FLD and FP and increase in production performance, bone strength and egg quality in laying hens is independent of dietary HP inclusion in the layer diet at 10 and 20% and HO at 3 and 6% in both the conventional cage system and single-tier system. The alternative hypothesis is that the reduction of FLD and FP and increase in production performance, bone strength and egg quality in laying hens is dependent of dietary HP inclusion in the layer diet at 10 and 20% and HO at 3 and 6% in both the conventional cage system and single-tier system.

CHAPTER 3: MATERIALS AND METHODS

3.1: Birds and housing

3.1.1: Trial 1 – Conventional cage housing system

One hundred Lohmann LSL-Lite White and one hundred Lohmann Brown-Lite commercial laying hens at 59-weeks of age were used in this 14-week production trial. The hens were housed and managed at the Atlantic Poultry Research Center following the Dalhousie University Faculty of Agriculture's Animal Care and Use Committee (ACUC) guidelines (ACUC file# 2022-035), which follow the Canadian Council on Animal Care (CCAC) codes of practice (2009). All birds were randomly assigned to conventional wire cages at five birds/cage, in the center tier of a two-sided, three-tiered battery conventional cage system with a density of 600cm^2/bird.

Hens were identified at the start of the trial using leg bands and the initial feather condition was scored. Feed consumption, body weight, egg production and egg quality (specific gravity, albumen height, egg yolk color, egg breaking strength and eggshell thickness) were determined for every 14-day period. Fatty acid (ACOS, 2007; method Ce 1j-07) and cannabinoid (AC-090) content of the eggs was also determined.

On day 0, prior to allocating the birds to cages, 10 hens were randomly selected to be euthanized by cervical dislocation to collect baseline liver tissue, tibias, and breast tissue samples. On week 14, at the end of the trial, two birds from each cage were euthanized to collect livers, tibias, and breast tissue samples for further analysis. Livers were analyzed for weight, color score, fat content, and histological analysis to assess fatty pathological changes in the liver tissue. Tibias were collected to quantify bone strength and breast tissue was collected to analyze cannabinoid content in the breast.

3.1.2: Trial 2 – Single-tier housing system

One hundred and twenty Lohmann LSL-Lite White commercial laying hens at 59-weeks of age were used in this 14-week production trial. The hens were housed at the APRC following the Dalhousie University Faculty of Agriculture ACUC guidelines (ACUC# 2022-036) that follow the CCAC codes of practice (2009). The birds were housed in one room in a single-tier system consisting of 12 floor pens with a density of 1900 cm^2 / bird and 10 birds / pen. Each pen included enrichments; a perch, 15.5cm perch space / bird, a nesting box with three individual nesting areas with a minimum nest space area of 83.2 cm^2 which was enclosed on three sides, and foraging materials such as wood chips. At the start of the trial, the hens were leg-banded for identification and instances of cannibalism and FP was recorded. Their initial feather condition was scored according to Dennis et al. (2009). On day 0, 10 birds were randomly selected to be euthanized by cervical dislocation for collection of baseline tissues as described for the conventional cage trial. On week 12, two birds from each floor pen in the single-tier trial were tested for tonic immobility, according to Shi et al. (2019). At the end of the 14-week trial, five birds from each pen were euthanized by cervical dislocation for collection of final samples.

3.2: Ingredients and experimental approach

The test ingredients in the hen diets were expeller-pressed (cold-pressed) HP (Hemp Oil Canada, St. Agathe, MB, Canada) and HO (Hemp Oil Canada, St. Agathe, MB, Canada) extracted from the process of cold-pressing the seeds to produce the presscake. The presscake was ground using a hammer mill prior to its addition to the layer diets. A high-energy control diet was fed to ensure FLD is induced. All diets were made isocaloric and isonitrogenous to ensure the observations were due to the experimental treatment. The conventional cage trial evaluation of hemp products incorporated into a high-energy diet of layers utilized five diets (a high-energy soybean control, 10% HP, 20% HP, 3% HO and 6% HO) and was fed to two separate strains of hen (Lohmann LSL-Lite White and Lohmann Brown-Lite). The single-tier trial evaluation of hemp products incorporated into a high-energy soybean meal diet of layers was arranged utilizing three of these diets: the high-energy soybean meal control diet with 0% hemp, the 20% HP diet and the 6% HO diet which were fed to Lohmann LSL-Lite White hens.

3.3: Chemical analysis

Standard proximate analysis procedures for nutrient analysis of the ingredients, diets and egg yolks were as follows: dry matter (DM; 100-moisture; AOAC, 2005; method no. 934.01), CP (AOAC, 2005; method no. 990.03; Leco protein/N analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA)), and GE. To determine the GE content of the samples, a Parr adiabatic bomb calorimeter was used (Parr Adiabatic Calorimeter, Model 6300, Parr Instrument Co., Moline, IL, USA) (Model 6520A, Parr Instrument Co., Moline, IL, USA)). The Nova Scotia Department of Agriculture (Truro, NS, Canada) determined the crude fat (CF) level of the HP and HO (AOCS, 2005; method AM 5–04; ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY, USA)), the nitrogen content (Dumas method; Ebeling, 1968) which was multiplied by 6.25 to calculate CP, and the mineral analysis which was conducted using an Inductively Coupled Argon Plasma analyzer (Varian 725-ES, Agilent Technologies, Inc., Santa Clara, Cal, USA; AOAC, 2023; method no. 968.08). The amino acid profiles of the HP ingredient and the experimental diets were analyzed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia, within the College of Agriculture, Food and Natural Resources

(Columbia, MO, USA), using AOAC method 982 E (a,b,c), chapter 453.05 (AOAC, 2006). Four egg yolk samples (each consisting of three pooled yoks) and one breast tissue sample (randomly chosen from the single-tier housing system) were freeze-dried and ground, then sent to Innotech Alberta (Vegreville, AB, Canada) for analysis of cannabinoid content using their internal standard operating procedure (AC-090) based on the Industrial Hemp Technical Manual-Standard Operation Procedures for Sampling, Testing and Processing Methodology. The samples were extracted using a SPEX Geno/Grinder and analyzed using liquid chromatography / mass spectrometry with results reported without moisture correction. Vitamin E analysis of four egg yolk samples (each consisting of three pooled yoks) was run by MasterLab Canada (St-Hyacinthe, QC, Canada) using their internal reference (MA#13; AOAC International, 1995; method 992.04; Epler et al., 1993; AOAC International, 1999; pg 288-297; AOAC International, 2008; pg 1070-1082) analyzing the samples by liquid chromatography and assayed using a UV detector. Fatty acid (AOCS, 2007; method Ce 1j-07) content of the egg yolks was analysed by University of Missouri (Columbia MO, USA). Mineral composition of the tibias was determined using (Inductively Coupled Argon Plasma analyzer (Varian 725-ES, Agilent Technologies, Inc., Santa Clara, Cal, USA) (AOAC, 2023; method no. 968.08).

3.4: Production performance

For each replicate group in both the conventional cage and single-tier trial, total feed consumption and egg production was measured daily, whereas hen body weight (g) was recorded for every 14-day period. Feed conversion ratio (FCR) was expressed as grams of feed consumed / average grams of egg weight and / total number of eggs produced. All production parameters were summarized for every 14-day period.

3.5: Collection and analysis of liver samples

Livers were collected for baseline assessment from 10 birds euthanized at the beginning of each trial and from two birds / cage and five birds / floor pen at the end of each trial (week 14). Liver weight was determined upon collection and a HunterLab MiniScan XE™ (model 45/0-L, Hunters Associates Laboratory, Inc. Reston, VA, USA) was used to determine the color score of each liver. Samples were also collected for liver fat content histological assessment. All histology samples were taken from the left side of the liver and stored in formalin. Samples taken for fat composition were freeze-dried using a Thermo-Fisher Scientific freeze-dryer (ModulY0D-0115, Ashville, NC, US) for 48 hours at -40^{o}C.

3.5.1: Liver - Fat composition

DM of samples collected for fat composition was determined according to method no. 934.01 of AOAC (2005). An ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY, USA) was used to measure the CF content (AOCS, 2005; method Am 5-04) of the livers.

3.5.2: Liver - Histology

Formalin-fixed liver samples were wax-fixed and stained with hematoxylin and eosin staining according to the procedure described by Bullerwell et al. (2016) and these formalin fixed samples were prepared by the Animal Health Laboratory, Agriculture and Food Operations Branch (Nova Scotia Department of Agriculture, Truro, NS, Canada). The samples were dehydrated using a series of graded alcohol baths and the alcohol was then effaced from the samples using xylene and embedded in paraffin wax via Tissue-Tek VIP (Sakura Finetek USA inc., Torrance, CA, USA). A 5um cross-section was cut from the sample and placed in a water bath of 35.5°C. The sample was then mounted to a slide and stained with hematoxylin and eosin.

The slides were then assessed for accumulation of fat vacuoles within hepatocytes using a Leica DM750™ microscope at 40x magnification, a digital microscope camera Leica ICC50™ and LAS EZ software (version 3.4.0) to capture a digital image of three randomly chosen areas for each liver section. Lipid accumulation is represented by macrovesicular fatty changes which occur when large fat vacuoles displace the nucleus and cytoplasm to the side of the hepatocyte, and by microvesicular changes where multiple smaller vacuoles accumulate in the cytoplasm with the nucleus centrally located. Image J software (version 1.53) was used to analyze and quantify lipid accumulation by identifying non-staining areas of cytoplasm as fat vacuoles (Figure 1).

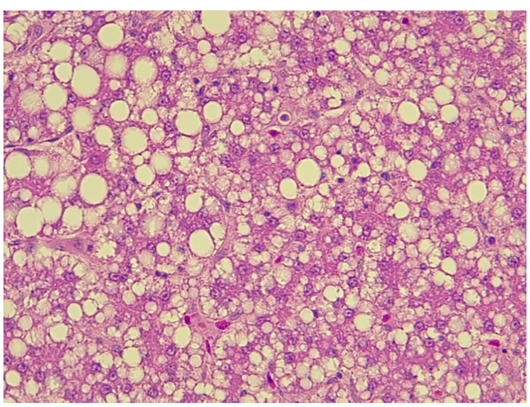
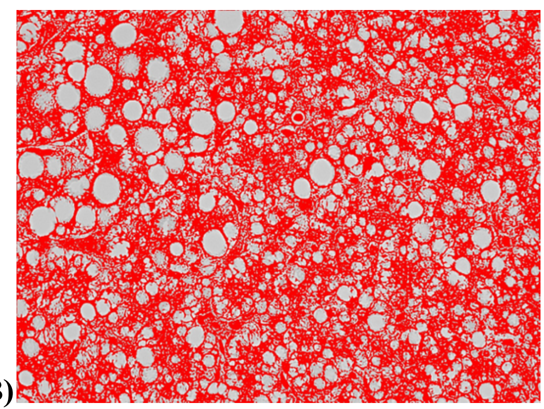
(A)  **(B)** 

Figure 1. (A) Liver histology image of fat vacuole infiltration within hepatocytes for bird 2 in cage 1-90, (image replicate #2) which was the fed 20% HP. Liver sample was stained with hematoxylin and eosin. (B) Liver sample image analyzed using image J software where the percent area that was non-stained was subtracted from the stained area (colored red) to get the percent fat of the liver.

3.6: Egg quality

Eggs were collected twice a week on Tuesdays and Thursdays for egg quality analysis. Cracked eggs were removed prior to testing. Eggs were analyzed for specific gravity, egg breaking strength, weight, albumen height, egg yolk color, and eggshell thickness.

3.6.1: Egg - Specific gravity

This was measured by flotation of the eggs in a graded series of saline solutions ranging from 1.066 to 1.098 in increments of 0.004 as described by Hamilton (1982).

3.6.2: Egg - Albumen height

A tripod micrometer (product QCH with QCH-CB, Technical Services and Supplies, York, UK) and an electric height gauge (product QCD, Technical Services and Supplies, York, UK) was used to measure the albumen height of each egg. Individual eggs were broken onto an egg breakout table (product QCA-P, Technical Services and Supplies, York, UK) and the measurement of albumen height was conducted according to Keener et al. (2006).

3.6.3: Egg - Yolk color

The egg yolk was separated from the albumen and homogenized to determine the yolk pigmentation using a HunterLab MiniScan XE™ (model 45/0-L, Hunters Associates Laboratory, Inc. Reston, VA, USA), which generates reflectance values using the daylight illuminant setting (D65). Egg yolk pigmentation was measured in duplicate for each egg yolk and the average was reported. Yolk scores were recorded using a L* a* b* system, which provides color scores for lightness, green/red, and blue/yellow, respectively.

3.6.4: Egg - Breaking strength

Shell static compression strength of each egg was measured using a TA.XTplus texture analyzer (Texture Technologies Corp, New York, NY, USA; Jones et al., 2014). A 30-kg load-cell was used.

3.6.5: Eggshell thickness

Shell thickness was determined using a TA.XTplus texture analyzer (Texture Technologies Corp, New York, NY, USA). Two measurements were taken; one from the equatorial region and one from the apical region of each egg and the average was reported.

3.6.6: Egg yolk fatty acid, vitamin E and cannabinoid analysis

At the end of each trial, eggs were collected from each cage and each floor pen for the analysis of fatty acid, vitamin E and cannabinoid content in the yolks as per the procedures described in section 3.3. For each analysis, three eggs / cage and three eggs / pen were cracked and separated from the albumen and pooled for testing.

3.7: Feather scoring

The feather condition of each bird was scored for every 14-day period on a scale of 0 to 5, with 0 being the best with a full smooth plumage and 5 being the worst with completely bare areas and injury to the skin (Dennis et al., 2009). Each birds' feathers condition was scored at 7 body regions: head, neck, abdomen, breast, tail, back and wings. An average of the 7 body regions was taken as the total body feather condition for each bird.

3.8: Bone strength

The right and left tibia were removed from the 10 birds euthanized at the start of each trial and from each of the two birds euthanized / cage and five birds euthanized / pen at the end of each 14-week production period. All tissue was removed from each bone and each bone was placed in a -20°C freezer prior to being air-dried and weighed. Length of bones and diameter at the midpoint was measured using a caliper and recorded. A 30-kg load cell, a three-point bend rig and a standard sheer plate was used to measure breaking strength with a TA.XTplus texture analyzer (Texture Technologies Corp., Scarsdale, New York, USA) with Exponent Stable Micro Systems software (version 6.1.7.0). Each bone was placed with the same facial plane on a flat surface. The sheer plate descended perpendicular to the bone at a speed of 0.5 mm/sec for a distance of 20 mm to break the bone at its midpoint.

3.9: Tonic immobility

Tonic immobility (TI) was tested on two birds / floor pen at the beginning and end of the single-tier trial. The hens tested were identified by their leg bands prior to the TI test, which was carried out according to Shi et al. (2019). During testing, a single hen was placed on its back on top of a towel laid out on a wooden cradle. TI was induced after gently restraining the hen for 15s, with one hand on its sternum. The experimenter then slowly removed their hands from the hen and stepped back to observe the hen from a distance. If the bird were to right itself within 15s, the induction was to be repeated up to 5 times until TI occurred. The duration of TI was a measure of the time until the bird rights itself within a maximum time frame of 5 minutes. The number of inductions and the latency from induction until the first head movement (i.e., the hen is alert and performing scanning movements rather than postural or reflexive changes), and the number of head movements until termination of TI were recorded for each hen. If TI was not induced in the hens within the 15s restraint period after 5 attempts, then 0 minutes was recorded for the duration of TI and the latency until the first head movement and the number of inductions will be scored as 5 minutes. If a bird remains in TI and does not right itself during the maximum time frame of 5 minutes, then a score of 5 minutes was given for the duration of TI.

3.10: Statistical analysis

Each of the trials were arranged as a completely randomized design. The main factors for the conventional cage trial were hemp by-product (a high-energy soybean meal control with 0% hemp, 10% HP, 20% HP, 3% HO, 6% HO) and strain of hen (Lohmann Brown-Lite and Lohmann LSL-Lite White), with each strain analyzed separately. The single-tier trial was analyzed as a one-way analysis with the main factors being hemp by-product (a high-energy soybean meal control with 0% hemp, and 20% presscake and 6% HO) and strain of hen (Lohmann LSL-Lite White). Data from both trials was subjected to the Proc Mixed procedure of SAS. The level for significance was $P \leq 0.05$. Normality was determined using four tests: Shapiro-Wilks, Camera von Mises, Kolmogirov Smirnov, and Anderson-Darling ($P > 0.05$). Outliers were removed by transforming the data using log 10 and square root 10 transformations. Average feather condition was subjected to a chi-square test to determine any significant effects the diet had on the presence of feather damage ($P \leq 0.05$). All data was also analyzed using factorial analysis to compare strain, treatment and housing system using IBM SPSS (version 29).

CHAPTER 4: RESULTS

4.1: Proximate nutrients, amino acids, and fatty acids in ingredients and diets

Table 4.1.1 presents the analyzed chemical composition of the hemp by-products (HP and HO) on an as fed basis. Diets outlined in Table 4.1.2 were formulated to meet or exceed the nutrient requirement of laying hens according to National Research Council guidelines (NRC, 1994). The amino acid and fatty acid profile of HP and the dietary treatments are provided in Table 4.1.3 and Table 4.1.4, respectively.

Table 4.1.1. Composition of experimental ingredients (hempseed presscake and hempseed oil).

Ingredient	HP		HO
	As Fed	Dry	As Fed
Dry matter (%)	93.60	-	-
Crude protein (%)	37.70	40.28	-
Crude fat (%)	9.05	9.67	-
Gross energy (Kcal kg^{-1})	4685.19	5005.55	-
Calcium (%)	0.14	0.15	-
Potassium (%)	0.93	0.99	-
Magnesium (%)	0.53	0.57	-
Phosphorous (%)	1.15	1.23	-
Sodium (%)	<0.02	ND	-
Copper (ppm)	18.09	19.33	-
Manganese (ppm)	109.34	116.82	-
Zinc (ppm)	79.75	85.21	-
Vitamin D (D3) (μg/100g)	ND	ND	ND
Vitamin E (mg/100g)	0.60	0.60	6.30
Vitamin D (D3) (IU/100g)	ND	ND	ND
Fat (GC/FID)	6.85	7.32	99.40
Acid detergent fiber (%)	33.18	35.54	-
Neutral detergent fiber (%)	37.69	40.37	-
Vitamin E	0.90	1.00	9.40
Cannabinoid content			
Cannabidiol (CBD) (μg/g)	1.00	1.10	4.50
Cannabidiolic acid (CBDA) (μg/g)	3.10	3.30	27.90
CBD Potency (%)	0.00	0.00	0.00
d9-Tetrahydrocannabinol (d9-THC) (μg/g)	0.10	0.10	1.00
Tetrahydrocannabinolic acid A (THCA-A) (μg/g)	0.20	0.20	2.80
THC Potency (%)	0.00	0.00	0.00

Vitamin D reportable detection limit is 0.25 μg/100g and 20 IU/100g.
ND = not detected.
HP = hempseed presscake.
HO = hempseed oil.

Table 4.1.2. Composition of treatment diets containing hempseed presscake and hempseed oil (based on 110 g hen^{-1}day^{-1}).

	Treatment				
Ingredient	**Control**	**10% HP**	**20% HP**	**3% HO**	**6% HO**
Corn	28.90	27.06	25.04	28.65	28.40
Wheat	10.00	10.00	10.00	10.00	10.00
Barley	20.00	20.00	20.00	20.00	20.00
Soybean meal	23.13	15.06	7.01	23.18	23.23
Hempseed presscake	0.00	10.00	20.00	0.00	0.00
Hempseed oil	0.00	0.00	0.00	3.00	6.00
Soybean oil	5.59	5.44	5.34	2.80	0.00
Limestone	4.90	4.93	4.96	4.90	4.90
Shell mix^{Z}	2.45	2.46	2.48	2.45	2.45
Oyster shell	2.45	2.46	2.48	2.45	2.45
Dicalcium phosphate	1.18	1.09	1.01	1.18	1.18
Methionine	0.50	0.53	0.53	0.54	0.54
Lysine HCl	0.00	0.11	0.30	0.00	0.00
Iodized salt	0.35	0.36	0.36	0.35	0.35
Vitamin/mineral premix^{Y}	0.50	0.50	0.50	0.50	0.50
Calculated Analysis					
Metabolizable energy (Kcal kg^{-1})	2942.00	2942.00	2942.00	2942.00	2942.00
Crude protein (%)	15.91	15.91	15.91	15.91	15.91
Crude fat (%)	7.48	8.04	8.64	7.71	7.93
Calcium (%)	4.00	4.00	4.00	4.00	4.00
Available phosphorus (%)	0.37	0.37	0.37	0.37	0.37
Lysine (%)	0.90	0.83	0.83	0.90	0.90
Methionine & Cystine (%)	0.75	0.75	0.75	0.75	0.75
Tryptophan (%)	0.25	0.22	0.19	0.25	0.25
Sodium (%)	0.16	0.16	0.16	0.16	0.16
Determined Analysis					
Dry matter (%)	89.45	89.88	90.49	88.88	89.78
Crude protein (%)	16.90	17.40	18.25	17.25	18.20
Crude fat (%)	8.45	7.67	8.27	7.22	8.20
Gross energy (Kcal kg^{-1})	3786.71	3735.01	3895.64	3931.44	3725.38
Calcium (%)	4.90	4.37	3.75	4.19	3.77
Potassium (%)	0.67	0.64	0.61	0.68	0.78
Magnesium (%)	0.15	0.18	0.22	0.14	0.18
Phosphorous (%)	0.52	0.61	0.70	0.52	0.59
Sodium (%)	0.18	0.19	0.19	0.17	0.19
Copper (ppm)	40.92	28.37	41.75	22.42	28.84
Manganese (ppm)	112.44	111.78	126.83	95.44	96.35
Zinc (ppm)	109.07	117.47	143.51	119.74	110.91

Table 4.1.2. Composition of treatment diets containing hempseed presscake and hempseed oil (based on 110 g hen^{-1}day^{-1}), continued.

	Treatment				
Determined Analysis	**Control**	**10% HP**	**20% HP**	**3% HO**	**6% HO**
Acid detergent fiber (%)	4.61	7.63	9.53	3.81	4.19
Neutral detergent fiber (%)	18.86	16.39	17.98	9.62	11.28
Vitamin E (mg/kg)	31.80	29.50	28.20	28.00	30.60
Cannabinoid content					
Cannabidiol (CBD) (μg/g)	0.00	0.10	0.20	0.20	0.30
Cannabidiolic acid (CBDA) (μg/g)	0.00	0.40	0.70	0.90	1.70
CBD Potency (%)	0.00	0.00	0.00	0.00	0.00
d9-Tetrahydrocannabinol (d9-THC) (μg/g)	0.00	0.00	0.00	0.00	0.00
Tetrahydrocannabinolic acid A (THCA-A) (μg/g)	0.00	0.00	0.00	0.10	0.10
THC Potency (%)	0.00	0.00	0.00	0.00	0.00

HP =hempseed presscake, HO = hempseed oil.

[Z]Graymont (QC) Inc. Boucherville, QC. Calcium carbonate (1.40-3.35 mm).

[Y] Supplied / kg diet; vitamin A, 8000 IU; vitamin D_3, 2500 IU; vitamin E, 60 mg; vitamin K, 2.97 mg; 7.6 mg; DL Ca-pantothenate, 7.2 mg; vitamin B_{12}, 0.012 mg; niacin, 30.7 mg; folic acid, 0.66 mg; choline chloride, 641 mg; biotin, 0.16 mg; pyridoxine, 4.0 mg; thiamine, 1.9 mg; manganous oxide, 70.2 mg; zinc oxide, 80 mg; copper sulphate, 25 mg; selenium 0.15 mg; ethoxyquin, 50 mg; corn, 2572 mg; ground limestone, 500 mg.

Table 4.1.3. Amino acid profile of ingredients (hempseed presscake) and treatment diets (0% hemp / high soybean meal control, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Treatments					
	HP	Control	10% HP	20% HP	3% HO	6% HO
Alanine	1.40	0.75	0.77	0.71	0.81	0.74
Arginine	3.85	1.01	1.27	1.33	1.08	1.03
Aspartic Acid	3.41	1.56	1.63	1.47	1.69	1.56
Cysteine	0.57	0.29	0.31	0.29	0.27	0.27
Glutamic Acid	5.65	3.16	3.12	2.96	3.29	3.11
Glycine	1.44	0.67	0.70	0.67	0.71	0.67
Histidine	0.90	0.44	0.46	0.44	0.47	0.44
Hydroxylysine	0.00	0.00	0.00	0.00	0.00	0.00
Hydroxyproline	0.07	0.03	0.03	0.03	0.02	0.03
Isoleucine	1.37	0.72	0.72	0.67	0.76	0.71
Lanthionine §	0.02	0.01	0.01	0.01	0.01	0.01
Leucine	2.19	1.31	1.30	1.17	1.40	1.29
Lysine	1.55	0.91	1.01	1.01	1.00	0.93
Methionine	0.78	0.46	0.61	0.58	0.48	0.42
Ornithine §	0.02	0.01	0.01	0.01	0.01	0.01
Phenylalanine	1.54	0.82	0.83	0.76	0.87	0.81
Proline	1.36	1.09	1.02	0.96	1.12	1.07
Serine	1.54	0.74	0.73	0.66	0.80	0.69
Taurine §	0.09	0.17	0.15	0.14	0.16	0.16
Threonine	1.13	0.59	0.61	0.55	0.64	0.60
Tryptophan	0.39	0.21	0.20	0.18	0.22	0.21
Tyrosine	0.94	0.53	0.53	0.46	0.55	0.52
Valine	1.70	0.80	0.83	0.80	0.84	0.79
Total	31.91	16.28	16.85	15.86	17.20	16.07
Crude protein	36.40	15.81	17.82	16.67	18.08	16.55

HP = hempseed presscake. HO = hempseed oil.

Table 4.1.4. Fatty Acid profile of ingredients (hempseed presscake, hempseed oil) and treatment diets (0% hemp / high soybean meal control, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

			Treatment				
	HP	HO	Control	10% HP	20% HP	3% HO	6% HO
Crude fat (W/W%)	6.50	>98.5	4.88	6.13	5.60	3.74	3.34
C14:0	0.05	0.04	0.08	0.10	0.09	0.07	0.06
Myristoleic (9c-14:1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C15:0	0.03	0.02	0.02	0.03	0.04	0.03	0.03
C15:1n5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Palmitic (16:0)	7.53	5.41	12.38	12.30	11.49	10.21	8.45
Palmitoleic (9c-16:1)	0.11	0.10	0.10	0.10	0.10	0.10	0.11
Margaric (17:0)	0.10	0.07	0.11	0.12	0.12	0.10	0.08
10c-17:1	0.00	0.04	0.00	0.01	0.02	0.00	0.00
Stearic (18:0)	2.81	2.42	3.49	3.46	3.44	2.98	2.38
Elaidic (9t-18:1)	0.00	0.02	0.02	0.03	0.01	0.00	0.00
Oleic (9c-18:1)	9.10	9.03	19.80	18.52	17.73	15.06	12.43
Vaccenic (11c-18:1)	0.92	0.73	1.27	1.22	1.20	1.03	0.85
Linolelaidic (18:2t)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Linoleic (18:2n6)	53.64	55.25	53.87	53.48	53.85	54.50	55.22
Linolenic (18:3n3)	16.85	19.31	6.62	7.73	8.52	11.39	14.30
g-Linolenic [C18:3n6]	3.47	3.68	0.02	0.36	0.60	1.42	2.45
Stearidonic (18:4n3)	1.18	1.51	0.04	0.13	0.20	0.57	0.97
Arachidic (20:0)	1.00	0.82	0.35	0.39	0.48	0.55	0.70
Gondoic (20:1n9)	0.46	0.01	0.24	0.24	0.28	0.32	0.41
C20:2	0.05	0.08	0.02	0.03	0.03	0.03	0.07
Homo-g-linolenic [C20:3n6]	0.11	0.00	0.00	0.04	0.05	0.00	0.00
Homo-a-linolenic (20:3n3)	0.02	0.02	0.00	0.00	0.00	0.00	0.01
Arachidonic [20:4n6]	0.07	0.00	0.05	0.00	0.05	0.05	0.05
3n-Arachidonic (20:4n3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EPA (20:5n3)	0.00	0.00	0.00	0.01	0.00	0.00	0.00
C21:0	0.04	0.02	0.04	0.02	0.05	0.03	0.02
Behenic (22:0)	0.47	0.32	0.35	0.34	0.38	0.34	0.32
Erucic [22:1n9]	0.08	0.02	0.02	0.03	0.05	0.04	0.04
C22:2n6	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Adrenic [C22:4n6]	0.03	0.00	0.02	0.02	0.02	0.02	0.02
Clupanodonic (22:5n3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DHA (22:6n3)	0.00	0.02	0.00	0.00	0.00	0.00	0.00
C23:0	0.00	0.04	0.00	0.05	0.02	0.00	0.00
Lignoceric (24:0)	0.26	0.14	0.18	0.16	0.18	0.18	0.19
Nervonic (24:1n9)	0.09	0.01	0.05	0.06	0.08	0.08	0.07

HP = hempseed presscake. HO = hempseed oil.

Fatty acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a “as is” basis.

4.2: Lohmann Brown-Lite hens reared in the conventional cage system

Table 4.2.1 gives an overview of production data for Lohmann Brown-Lite hens reared in the conventional cage housing system and fed five dietary treatments. Brown hens fed 10% HP had a significantly heavier ($P \leq 0.05$) body weight than those fed 6% HO in period 2 (2241 g vs. 2059 g), period 3 (2272 g vs. 2075 g) and period 6 (2264 g vs. 2035 g). Feed consumption was significantly higher ($P \leq 0.05$) in brown hens fed 10% HP in periods 1, 3, 4, 5 and 6 than in brown hens fed the 6% HO diet. Egg production of brown hens fed 3% HO diet was lower ($P \leq 0.05$) than those fed the control diet in period 5 and 10% HP in period 6, respectively. Brown hens fed the 3% HO and 6% HO diets had an improved ($P \leq 0.05$) feed conversion than those fed 10% HP diet in period 4. However, the 6% HO diet resulted in the poorest ($P \leq 0.05$) feed conversion among dietary treatments.

Table 4.2.1. Average production performance (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Average weight (g)						
I	2237±39^{A}	2200±39^{AB}	2122±39^{AB}	2189±39^{AB}	2037±39^{B}	0.0164
P 1	2215±40	2208±40	2132±40	2191±40	2073±40	0.1100
P 2	2229±41^{AB}	2241±41^{A}	2133±41^{AB}	2229±41^{AB}	2059±41^{B}	0.0345
P 3	2275±39^{A}	2277±39^{A}	2162±39^{AB}	2200±39^{AB}	2075±39^{B}	0.0164
P 4	2255±48	2272±48	2162±48	2187±48	2064±48	0.0519
P 5	2248±51	2271±51	2153±51	2179±51	2054±51	0.0626
P 6	2251±47^{A}	2264±47^{A}	2125±47^{AB}	2201±47^{AB}	2035±47^{B}	0.0170
Feed consumption (g/bird/day)						
P 1	115.0±2.0^{AB}	117.2±2.0^{AB}	122.1±2.0^{A}	109.4±2.0^{B}	111.0±2.0^{B}	0.0028
P 2	110.6±2.8	109.4±2.8	116.3±2.8	104.5±2.8	105.2±2.8	0.0551
P 3	104.4±2.9^{AB}	107.8±2.9^{AB}	109.1±2.9^{A}	97.7±2.9^{AB}	96.0±2.9^{B}	0.0168
P 4	104.4±2.9^{AB}	107.8±2.9^{AB}	109.1±2.9^{A}	97.7±2.9^{AB}	96.0±2.9^{B}	0.0168
P 5	104.4±2.9^{AB}	107.8±2.9^{AB}	109.1±2.9^{A}	97.7±2.9^{AB}	96.0±2.9^{B}	0.0168
P 6	104.4±2.9^{AB}	107.8±2.9^{AB}	109.1±2.9^{A}	97.7±2.9^{AB}	96.0±2.9^{B}	0.0168
Overall	108.5±3.2	108.5±3.2	107.8±3.2	100.5±3.2	98.9±3.2	0.1218
Hen day egg production (%)						
P 1	92.1±3.9	87.9±3.9	93.9±3.9	88.9±3.9	83.6±3.9	0.4025
P 2	92.5±2.6	91.1±2.6	92.5±2.6	92.1±2.6	91.4±2.6	0.9921
P 3	91.9±2.1	87.9±2.1	91.4±2.1	91.8±2.1	88.2±2.1	0.4790
P 4	96.2±2.3	88.6±2.3	91.4±2.3	90.0±2.3	87.9±2.3	0.1263
P 5	94.3±2.6^{A}	85.7±3.0^{AB}	91.4±2.6^{AB}	82.1±2.6^{B}	82.9±2.6^{AB}	0.0211
P 6	92.5±1.7^{AB}	96.3±2.4^{A}	90.7±1.7^{AB}	86.9±1.7^{B}	73.3±1.9^{C}	0.0001
Feed conversion						
P 1	2.10±0.11	2.19±0.11	2.07±0.11	1.98±0.11	2.24±0.11	0.5753
P 2	2.04±0.07	2.00±0.07	2.03±0.07	1.89±0.07	1.87±0.07	0.3514
P 3	1.88±0.05	1.98±0.05	1.86±0.05	1.77±0.05	1.79±0.05	0.0843
P 4	1.82±0.05^{AB}	1.97±0.06^{A}	1.88±0.06^{AB}	1.72±0.05^{B}	1.80±0.05^{B}	0.0368
P 5	1.88±0.07	1.92±0.07	1.95±0.07	1.87±0.07	1.77±0.07	0.4008
P 6	1.78±0.06^{B}	1.90±0.06^{B}	1.86±0.06^{B}	1.70±0.06^{B}	2.20±0.06^{A}	0.0002
Overall	1.92±0.05	1.99±0.05	1.94±0.05	1.82±0.05	1.96±0.05	0.1759

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A\text{-}C}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05)

Table 4.2.2 shows the influence of experimental diets on egg quality measures on brown hens. Neither egg weight nor shell weight was different (P>0.05) among dietary treatments. Egg specific gravity was significantly higher (P≤0.05) in brown hens fed 3% HO diet than those fed 20% HP and control diets in period 1. Eggs from brown hens fed 3% HO also had a significantly stronger (P≤0.05) shell breaking strength than brown hens fed 10% HP. Albumen height was significantly higher in brown hens fed 10% HP, 20% HP and 3% HO than the control group in period 2 (6.2 mm, 6.5 mm, 6.2mm vs. 5.1 mm). While in period 6, brown hens fed 6% HO had a significantly higher (P≤0.05) albumen height (6.9 mm) than brown hens fed the control (5.7 mm), 10% HP (6.1 mm) and 3% HO (5.7 mm) diets. Shell thickness was significantly higher in brown hens fed the control (period 3, 0.44 mm) than those fed 20% HP (period 3, 0.40 mm). Egg yolk L* score (lightness) was significantly higher (P≤0.05) in brown hens fed 3% HO than in brown hens fed 10% HP and 20% HP in period 1. While in period 2, L* score was significantly higher (P≤0.05) in brown hens fed the control, 3% HO, and 6% HO than in brown hens fed 10% HP. Generally, brown hens fed 20% HP produced eggs with higher redness (a* score) and yellowness (b* score) values compared with those fed 3% HO and 6% HO diets. In period 1, egg yolk a* score was significantly higher (P≤0.05) in brown hens fed 20% HP (12.8) than in brown hens fed 3% HO (9.2) and 6% HO (8.8). Similarly, significantly higher (P≤0.05) a* score values were found in eggs produced by brown hens fed 10% HP (11.9) and 20% HP (11.4) than those in the control (8.9), 3% HO (8.5) and 6% HO (9.3) groups in period 2. In period 4 and 5, the 10% HP and 20% HP resulted in a higher (P≤0.05) a* score in egg yolk than those brown hens fed control diet. Both control and HP diets had a higher (P≤0.05) yolk a* score than those fed HO diets. Brown hens fed 20% HP (12.2) had a significant higher yolk a* score than those fed the control and HO diets and the a* score of yolk produced by brown hen fed 10% HP was higher than those fed 3% HO diet in period 6. Egg yolk b* score was significantly higher ($P \leq 0.05$) produced by brown hens fed 20% HP (75.4) than brown hens fed 3% HO (68.3) and 6% HO (68.3) in period 1. In periods 4, 5 and 6, both 10% and 20% HP diets resulted in a significantly higher ($P \leq 0.05$) b* score than in brown hens fed 3% HO and 6% HO.

Table 4.2.2. Egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Egg weight (g)						
I	59.7±1.3	61.3±1.3	62.9±1.3	62.4±1.3	60.7±1.3	0.4246
P 1	58.7±1.3	61.8±1.3	62.2±1.3	60.3±1.3	61.6±1.3	0.3580
P 2	60.5±1.2	62.0±1.2	64.2±1.2	60.1±1.2	61.0±1.2	0.1652
P 3	61.2±1.3	61.5±1.3	64.6±1.3	64.1±1.3	63.8±1.3	0.2531
P 4	60.9±1.1	63.2±1.1	61.7±1.1	63.1±1.1	64.0±1.1	0.3291
P 5	64.4±1.3	61.7±1.3	63.4±1.3	65.3±1.3	60.2±1.3	0.0948
P 6	64.6±1.4	63.8±1.4	63.6±1.4	63.5±1.4	63.1±1.4	0.9577
Egg specific gravity						
I	1.087±0.001	1.082±0.001	1.087±0.001	1.089±0.001	1.087±0.001	0.0533
P 1	1.087±0.001^{B}	1.088±0.001^{AB}	1.087±0.001^{B}	1.090±0.001^{A}	1.087±0.001^{AB}	0.0122
P 2	1.088±0.001	1.089±0.001	1.089±0.001	1.089±0.001	1.090±0.001	0.7318
P 3	1.090±0.001	1.089±0.001	1.090±0.001	1.089±0.001	1.091±0.001	0.7539
P 4	1.088±0.001	1.086±0.001	1.086±0.001	1.089±0.001	1.085±0.001	0.1195
P 5	1.088±0.001	1.088±0.001	1.087±0.001	1.087±0.001	1.084±0.001	0.2687
P 6	1.089±0.002	1.089±0.002	1.088±0.002	1.089±0.002	1.084±0.002	0.1011
Egg breaking strength (g/force)						
I	6252.7±613.2	5090.5±613.2	5517.9±613.2	6261.1±613.2	4694.0±613.29	0.3172
P 1	5910.1±351.2	5663.8±351.2	5910.8±351.2	5615.3±351.2	5557.1±351.2	0.9183
P 2	5574.2±401.3	5874.3±401.3	6101.5±401.3	5816.8±401.3	6352.6±401.3	0.7070
P 3	5312.2±302.6^{AB}	4227.2±302.6^{B}	5513.9±302.6^{AB}	5657.4±302.6^{A}	5419.8±302.6^{AB}	0.0306
P 4	5534.6±340.4	5591.5±340.4	5324.2±340.4	5749.0±340.4	5261.5±340.4	0.8446
P 5	5123.0±432.5	5410.1±432.5	5838.5±432.5	5241.7±432.5	4783.5±432.5	0.5416
P 6	5468.5±239.2	5295.4±239.2	5805.2±239.2	5687.7±239.2	6422.3±276.3	0.0735
Albumen height (mm)						
I	6.5±0.3	5.9±0.3	6.5±0.3	6.1±0.3	5.8±0.3	0.4061
P 1	5.6±0.3	5.8±0.3	6.2±0.3	5.5±0.3	5.9±0.3	0.6389
P 2	5.1±0.2^{B}	6.2±0.2^{A}	6.5±0.2^{A}	6.2±0.2^{A}	5.6±0.2^{AB}	0.0074
P 3	5.8±0.2	6.4±0.2	5.9±0.2	5.8±0.2	6.5±0.2	0.1491
P 4	4.8±0.3	5.5±0.3	5.4±0.3	5.2±0.3	5.7±0.3	0.1999
P 5	5.6±0.3	5.2±0.3	5.6±0.3	6.4±0.3	5.7±0.3	0.2103
P 6	5.7±0.3^{B}	5.8±0.3^{B}	6.1±0.3^{AB}	5.7±0.3^{B}	6.9±0.3^{A}	0.0156
Shell weight (g)						
I	6.0±0.1	6.0±0.1	6.5±0.1	6.5±0.1	6.3±0.1	0.0636
P 1	6.0±0.2	5.8±0.2	6.2±0.2	5.6±0.2	6.1±0.2	0.4217
P 2	6.0±0.1	6.2±0.1	6.3±0.1	6.0±0.1	6.3±0.1	0.4822
P 3	6.1±0.2	6.0±0.2	6.2±0.2	6.1±0.2	6.4±0.2	0.5763
P 4	6.0±0.2	6.2±0.2	6.1±0.2	6.4±0.2	6.3±0.2	0.3944
P 5	6.4±0.3	6.0±0.3	6.2±0.3	6.4±0.3	5.6±0.3	0.1719
P 6	6.5±0.2	6.3±0.2	6.2±0.2	6.4±0.2	5.7±0.2	0.0861
Shell Thickness (mm)						
I	0.49±0.01	0.49±0.01	0.50±0.01	0.52±0.01	0.51±0.01	0.1144
P 1	0.42±0.01	0.42±0.01	0.41±0.01	0.43±0.01	0.43±0.01	0.9054
P 2	0.44±0.01	0.46±0.01	0.42±0.01	0.43±0.01	0.44±0.01	0.1368
P 3	0.44±0.01^{A}	0.42±0.01^{AB}	0.40±0.01^{B}	0.42±0.01^{AB}	0.41±0.01^{AB}	0.0436
P 4	0.42±0.01	0.40±0.01	0.40±0.01	0.41±0.01	0.40±0.01	0.6726
P 5	0.42±0.02	0.42±0.02	0.45±0.02	0.41±0.02	0.38±0.02	0.1592
P 6	0.44±0.01	0.44±0.01	0.42±0.01	0.43±0.01	0.39±0.01	0.1168
L*						
I	62.1±0.4	60.2±0.4	61.8±0.4	61.6±0.4	61.8±0.4	0.0622

Table 4.2.2. Egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
P 1	60.5±0.4^{AB}	59.9±0.4^{B}	59.6±0.4^{B}	61.8±0.4^{A}	60.7±0.4^{AB}	0.0158
P 2	62.3±0.5^{A}	58.9±0.5^{B}	60.6±0.5^{AB}	62.2±0.5^{A}	61.2±0.5^{A}	0.0006
P 3	61.5±0.7	59.8±0.7	61.8±0.7	62.2±0.7	60.6±0.7	0.1399
P 4	61.7±0.5	61.3±0.5	60.3±0.5	61.1±0.5	61.7±0.5	0.3283
P 5	63.0±0.5	62.1±0.5	61.5±0.5	63.2±0.5	63.2±0.5	0.1069
P 6	61.7±0.6	60.0±0.6	60.6±0.6	61.9±0.6	61.2±0.6	0.1974
a*						
I	11.6±0.4	12.5±0.4	12.2±0.4	11.9±0.4	11.7±0.4	0.5202
P 1	10.1±0.3^{BC}	11.3±0.3^{AB}	12.8±0.3^{A}	9.2±0.3^{C}	8.8±0.3^{C}	0.0001
P 2	8.9±0.5^{B}	11.9±0.5^{A}	11.4±0.5^{A}	8.5±0.5^{B}	9.3±0.5^{B}	0.0002
P 3	9.0±1.0	11.1±1.0	8.6±1.0	9.2±1.0	11.1±1.0	0.2419
P 4	9.4±0.3^{B}	10.3±0.3^{B}	12.0±0.3^{A}	7.7±0.3^{C}	6.2±0.3^{D}	0.0001
P 5	9.6±0.4^{B}	11.1±0.4^{B}	13.0±0.4^{A}	7.8±0.4^{C}	6.7±0.4^{C}	0.0001
P 6	9.9±0.4^{B}	10.6±0.4^{AB}	12.2±0.4^{A}	7.6±0.4^{C}	9.0±0.4^{BC}	0.0001
b*						
I	73.1±1.2	74.0±1.2	74.8±1.2	73.5±1.2	73.2±1.2	0.8512
P 1	70.2±1.3^{AB}	72.9±1.3^{AB}	75.4±1.3^{A}	68.3±1.3^{B}	68.3±1.3^{B}	0.0043
P 2	67.0±1.3	71.0±1.3	69.6±1.3	66.7±1.3	67.4±1.3	0.1453
P 3	67.0±2.5	70.3±2.5	65.0±2.5	65.6±2.5	71.8±2.5	0.2593
P 4	69.3±1.8^{AB}	72.3±1.8^{A}	74.9±1.8^{A}	64.2±1.8^{B}	61.7±1.8^{B}	0.0006
P 5	71.4±1.4^{BC}	76.8±1.4^{AB}	80.6±1.4^{A}	67.3±1.4^{CD}	64.3±1.4^{D}	0.0001
P 6	70.3±1.3^{AB}	72.3±1.3^{A}	73.7±1.3^{A}	65.1±1.3^{BC}	61.5±1.3^{C}	0.0001

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A\text{-}D}$Least-squared means±SEM (standard error of the mean within rows with different letters differ significantly (P≤0.05).

Table 4.2.3 shows the fatty acid composition of egg yolk from Lohmann Brown-Lite hens. Yolk palmitic acid content in eggs from brown hens fed 3% HO were significant higher ($P \leq 0.05$) than those fed 10% HP and 6% HO. The palmitoleic acid content in egg yolks was significantly higher ($P \leq 0.05$) in brown hens fed 3% HO than those fed control, 10% HP and 20% HP, and the content of palmitoleic acid within yolk was intermediate in the 6% HO group. The highest ($P \leq 0.05$) values of stearic acid, stearidonic acid, linolenic acid, g-linolenic acid, homo-g-linolenic acid, homo-a-linolenic acid, and EPA were found in egg yolks from brown hens fed the 6% HO diet. Following the 6% HO treatment, the 3% HO inclusion level had a higher ($P \leq 0.05$) content of those fatty acids than the control group. The amount of arachidic acid in egg yolk produced by brown hens fed 6% HO is significantly lower ($P \leq 0.05$) than all other dietary treatments. The contents of C21:0, C22:0 or C24:0 were not affected ($P > 0.05$) by the dietary treatments.

Table 4.2.3. Fatty acid content of egg yolks from Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Treatment					
	Control	**10% HP**	**20% HP**	**3% HO**	**6% HO**	**P-Value**
Crude fat (W/W%)	52.81±0.27	53.52±0.33	53.10±0.23	53.13±0.30	53.45±0.59	0.654
C14:0	0.22±0.01	0.23±0.00	0.24±0.02	0.27±0.01	0.25±0.01	0.073
Myristoleic (9c-14:1)	$0.02±0.00^{AB}$	$0.02±0.00^{B}$	$0.02±0.00^{AB}$	$0.03±0.00^{A}$	$0.03±0.00^{AB}$	0.034
C15:0	0.05±0.01	0.05±0.00	0.05±0.01	0.06±0.00	0.06±0.00	0.176
C15:1n5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Palmitic (16:0)	$21.63±0.20^{AB}$	$21.59±0.11^{B}$	$22.33±0.70^{AB}$	$23.38±0.66^{A}$	$21.48±0.07^{B}$	0.043
Palmitoleic (9c-16:1)	$1.44±0.05^{B}$	$1.33±0.01^{B}$	$1.40±0.09^{B}$	$1.68±0.02^{A}$	$1.49±0.05^{AB}$	0.003
Margaric (17:0)	0.22±0.01	0.22±0.01	0.22±0.01	0.20±0.01	0.22±0.01	0.519
10c-17:1	0.09±0.03	0.06±0.03	0.09±0.03	0.04±0.03	0.03±0.03	0.386
Stearic (18:0)	$8.65±0.14^{B}$	$9.08±0.14^{AB}$	$8.67±0.15^{B}$	$8.72±0.13^{B}$	$9.5±0.19^{A}$	0.004
Elaidic (9t-18:1)	0.09±0.00	0.09±0.01	0.09±0.00	0.09±0.00	0.08±0.00	0.236
Oleic (9c-18:1)	$34.16±0.96^{A}$	$34.07±0.65^{A}$	$32.39±1.22^{AB}$	$32.74±0.36^{A}$	$30.05±0.52^{B}$	0.003
Vaccenic (11c-18:1)	2.33±0.27	1.91±0.28	2.17±0.26	1.62±0.24	1.48±0.24	0.162
Linolelaidic (18:2t)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Linoleic (18:2n6)	23.82±1.14	23.83±0.18	24.81±0.59	23.13±0.30	25.45±0.35	0.118
Linolenic (18:3n3)	$1.49±0.10^{D}$	$1.71±0.03^{C}$	$1.92±0.06^{C}$	$2.35±0.09^{B}$	$3.60±0.08^{A}$	<0.001
g-Linolenic [C18:3n6]	$0.13±0.01^{C}$	$0.14±0.01^{BC}$	$0.15±0.01^{BC}$	$0.17±0.01^{B}$	$0.22±0.02^{A}$	<0.001
Stearidonic (18:4n3)	$0.01±0.00^{C}$	$0.02±0.00^{C}$	$0.02±0.00^{BC}$	$0.04±0.01^{AB}$	$0.05±0.01^{A}$	<0.001
Arachidic (20:0)	$0.03±0.00^{A}$	$0.02±0.00^{A}$	$0.03±0.00^{A}$	$0.03±0.00^{A}$	$0.04±0.00^{B}$	<0.001
Gondoic (20:1n9)	0.16±0.01	0.16±0.00	0.14±0.01	0.14±0.01	0.14±0.01	0.212
C20:2	0.21±0.02	0.21±0.00	0.20±0.02	0.18±0.01	0.21±0.01	0.427
Homo-g-linolenic [C20:3n6]	$0.14±0.01^{B}$	$0.16±0.01^{B}$	$0.16±0.01^{B}$	$0.18±0.02^{AB}$	$0.22±0.01^{A}$	0.002
Homo-a-linolenic (20:3n3)	$0.02±0.00^{B}$	$0.03±0.00^{BC}$	$0.03±0.00^{BC}$	$0.04±0.00^{B}$	$0.06±0.00^{A}$	<0.001
Arachidonic [20:4n6]	1.81±0.02	1.81±0.04	1.70±0.09	1.68±0.09	1.82±0.03	0.334

Table 4.2.3. Fatty acid content of egg yolks from Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Treatment					
	Control	**10% HP**	**20% HP**	**3% HO**	**6% HO**	**P-Value**
3n-Arachidonic (20:4n3)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	-
EPA (20:5n3)	0.02±0.00^{C}	0.03±0.00^{BC}	0.03±0.00^{BC}	0.04±0.00^{B}	0.06±0.01^{A}	<0.001
C21:0	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.496
Behenic (22:0)	0.02±0.01	0.02±0.00	0.03±0.02	0.03±0.01	0.03±0.01	0.864
Erucic [22:1n9]	0.00±0.00	0.01±0.00	0.00±0.00	0.01±0.00	0.01±0.00	0.657
C22:2n6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Adrenic [C22:4n6]	0.13±0.01	0.12±0.00	0.10±0.01	0.11±0.01	0.11±0.00	0.071
Clupanodonic (22:5n3)	0.14±0.02	0.15±0.00	0.14±0.03	0.14±0.02	0.20±0.01	0.140
DHA (22:6n3)	1.26±0.06	1.33±0.02	1.25±0.15	1.40±0.16	1.59±0.03	0.164
C23:0	0.01±0.01	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.01	0.876
Lignoceric (24:0)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.675
Nervonic (24:1n9)	0.01±0.00	0.01±0.00	0.0±0.00	0.01±0.00	0.01±0.00	0.252

HP= hempseed presscake. HO= hempseed oil.

Fatty Acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis.

$^{A-D}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05)

Table 4.2.4 shows the average feather scoring condition for 7 body regions on Lohmann Brown-Lite hens fed five treatment diets. No differences in feather scores were found among dietary treatments for brown hens reared in a conventional cage system. Table 4.2.5 shows the total body feather condition of Lohmann Brown-Lite hens fed five treatment diets. Chi-square analysis showed no differences in total body feather condition among dietary treatments for brown hens reared in a conventional cage system.

Table 4.2.4. Average feather score (means ± standard error) for 7 body regions (head, neck, abdomen, breast, tail, back and wing) of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Head						
I	1.9±0.1	1.8±0.1	2.0±0.1	1.9±0.1	1.9±0.1	0.8762
P 1	2.1±0.1	1.9±0.1	1.9±0.1	1.9±0.1	1.8±0.1	0.8100
P 2	2.1±0.1	2.0±0.1	2.1±0.1	1.9±0.1	2.0±0.1	0.9554
P 3	2.1±0.2	2.1±0.2	2.1±0.1	2.1±0.1	2.0±0.1	0.9663
P 4	2.2±0.1	2.0±0.1	2.2±1.3	2.3±0.1	2.2±0.1	0.6556
P 5	2.4±0.2	2.1±0.2	2.2±0.2	2.3±0.2	2.0±0.2	0.7390
P 6	1.5±0.06	1.5±0.6	1.5±0.6	1.5±0.6	1.4±0.6	0.9744
Neck						
I	3.1±0.1	2.9±0.1	3.2±0.1	2.9±0.1	2.9±0.1	0.1296
P 1	3.0±0.1	3.1±0.1	3.3±0.1	3.0±0.1	3.1±0.1	0.5732
P 2	3.5±0.1	3.5±0.1	3.4±0.1	3.5±0.1	3.3±0.1	0.5320
P 3	3.3±0.1	3.2±0.1	3.3±0.1	3.3±0.1	3.2±0.1	0.9315
P 4	3.2±0.1	3.3±0.1	3.3±0.1	3.2±0.1	3.1±0.1	0.8325
P 5	3.3±0.1	3.1±0.1	3.2±0.1	3.3±0.1	3.2±0.1	0.6761
P 6	3.5±0.1	3.3±0.1	3.3±0.1	3.5±0.1	3.3±0.1	0.5592
Abdomen						
I	2.9±0.1	2.9±0.1	2.9±0.1	2.7±0.1	2.6±0.1	0.1520
P 1	3.1±0.1	3.0±0.1	3.0±0.1	3.1±0.1	2.9±0.1	0.1943
P 2	3.2±0.1	3.0±0.1	3.1±0.1	3.1±0.1	2.9±0.1	0.0889
P 3	3.2±0.1	3.1±0.1	3.1±0.1	3.1±0.1	2.9±0.1	0.4380
P 4	3.1±0.1	3.1±0.1	3.1±0.1	3.3±0.1	3.1±0.1	0.4490
P 5	3.5±0.1	3.2±0.1	3.3±0.1	3.3±0.1	3.4±0.1	0.5424
P 6	3.4±0.1	3.1±0.1	3.0±0.1	3.1±0.1	3.2±0.1	0.4298
Breast						
I	3.2±0.1	3.0±0.1	3.1±0.1	3.1±0.1	2.9±0.1	0.1633
P 1	3.1±0.1	3.0±0.1	3.1±0.1	3.1±0.1	3.0±0.1	0.6500
P 2	3.5±0.1	3.1±0.1	3.2±0.1	3.4±0.1	3.1±0.1	0.4243
P 3	3.5±0.2	3.2±0.2	3.3±0.2	3.3±0.2	3.1±0.2	0.6301
P 4	3.4±0.1	3.3±0.1	3.3±0.1	3.4±0.1	3.1±0.1	0.4187
P 5	3.3±0.1	3.1±0.1	3.1±0.1	3.2±0.1	3.3±0.1	0.6684
P 6	3.7±0.2	3.4±0.2	3.3±0.2	3.7±0.2	3.5±0.2	0.3762
Tail						
I	1.9±0.3	2.1±0.3	2.3±0.3	1.9±0.3	1.9±0.3	0.8893
P 1	2.0±0.3	2.0±0.3	2.1±0.3	2.0±0.3	1.9±0.3	0.9863
P 2	1.6±0.3	1.7±0.3	1.7±0.3	1.6±0.3	1.6±0.3	0.9963
P 3	1.8±0.3	1.6±0.3	1.7±0.3	1.7±0.3	1.6±0.3	0.9882
P 4	2.0±0.3	1.9±0.3	2.1±0.3	1.9±0.3	1.8±0.3	0.9876
P 5	2.6±0.2	2.5±0.2	2.7±0.2	2.6±0.2	2.4±0.2	0.8617
P 6	2.2±0.3	2.0±0.3	2.2±0.3	1.8±0.3	1.8±0.3	0.7447
Back						
I	1.9±0.5	2.0±0.5	2.7±0.5	1.3±0.5	2.1±0.5	0.5249
P 1	2.9±0.3	2.3±0.3	2.5±0.3	2.4±0.3	2.7±0.3	0.6627
P 2	3.0±0.2	2.7±0.2	2.7±0.2	2.4±0.2	2.5±0.2	0.3985
P 3	2.5±0.3	2.4±0.3	2.7±0.3	2.1±0.3	2.5±0.3	0.6352
P 4	2.7±0.3	2.6±0.3	2.9±0.3	2.4±0.3	2.7±0.3	0.8184
P 5	3.0±0.2	2.6±0.2	2.9±0.2	2.8±0.2	2.6±0.2	0.7227

Table 4.2.4. Average feather score (means ± standard error) for 7 body regions (head, neck, abdomen, breast, tail, back and wing) of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
P 6	2.9±0.4	2.7±0.4	2.8±0.4	2.5±0.4	2.4±0.4	0.8684
Wing						
I	2.1±0.2	2.1±0.2	2.5±0.2	2.1±0.2	2.4±0.2	0.1660
P 1	2.7±0.2	2.5±0.2	2.7±0.2	2.5±0.2	2.5±0.2	0.6759
P 2	2.3±0.2	2.4±0.2	2.5±0.2	2.3±0.2	2.5±0.2	0.9565
P 3	2.5±0.2	2.3±0.2	2.5±0.2	2.3±0.2	2.5±0.2	0.8376
P 4	2.6±0.2	2.7±0.2	2.9±0.2	2.6±0.2	2.8±0.2	0.7682
P 5	3.0±0.1	2.9±0.1	3.0±0.1	3.1±0.1	2.9±0.1	0.3056
P 6	2.7±0.2	2.6±0.2	2.9±0.2	2.5±0.2	2.5±0.2	0.6684

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.
Least squared means±SEM (standard error of the mean) within rows.

Table 4.2.5.Total body feather condition score of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, analyzed using chi-square.

Feather Score	Control	10% HP	20% HP	3% HO	6% HO	P-Value	Chi-square
I							
0	0	0	0	0	0	0.9618	10.3272
1	0	0	0	0	0		
2	11	13	5	15	12		
3	9	7	14	5	8		
4	0	0	1	0	0		
5	0	0	0	0	0		
P 1							
0	0	0	0	0	0	1.0000	1.7582
1	0	0	0	0	0		
2	6	8	5	8	8		
3	14	12	15	12	12		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 2							
0	0	0	0	0	0	0.9999	4.2228
1	0	0	0	0	0		
2	4	7	6	7	10		
3	16	12	14	13	10		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 3							
0	0	0	0	0	0	0.9630	10.2699
1	0	0	0	0	1		
2	6	9	6	10	8		
3	13	10	13	10	11		
4	0	0	1	0	0		
5	0	0	0	0	0		
P 4							
0	0	0	0	0	0	0.3333	22.1327
1	0	0	0	0	1		
2	6	7	4	9	8		
3	13	11	15	11	10		
4	0	1	1	0	1		
5	0	0	0	0	0		
P 5							
0	0	0	0	0	0	0.9464	10.9931
1	0	0	0	0	0		
2	1	5	3	1	4		
3	17	14	17	19	14		
4	1	0	0	0	2		
5	0	0	0	0	0		
P 6							
0	0	0	0	0	0	0.9098	12.1792
1	0	0	0	0	1		
2	3	6	3	7	8		
3	15	13	17	11	10		
4	1	0	0	1	1		
5	0	0	0	0	0		

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

Tibia parameters and mineral analysis are presented in Table 4.2.6. Of the measured tibia quality variables, only tibia length was affected by the dietary treatments. Brown hens fed 3% HO had a longer ($P \leq 0.05$) tibia than those fed 10% HP and 20% HP, and the tibia length of hens in the 6% HO and control groups were intermediate. No differences ($P > 0.05$) in liver weight, hepato-somatic index, fat content, liver color L* score, or a* score were found among dietary treatments. However, the liver of brown hens fed 20% HP had higher yellowness (b* score) values than those fed 6% HO and control diets (Table 4.2.7).

Table 4.2.6. Average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Body weight (g)	2217±77.6	2130±77.6	2134±77.6	2208±77.6	2105±77.6	0.7869
Tibia weight (g)	10.0±0.3	9.4±0.3	9.5±0.3	10.3±0.3	9.8±0.3	0.2192
Tibia length (mm)	121.6±1.0^{AB}	119.5±1.0^{B}	119.3±1.0^{B}	125.1±1.1^{A}	121.3±1.0^{AB}	0.0098
Tibia width (mm)	6.6±0.1	6.5±0.1	6.7±0.1	6.7±0.1	6.7±0.1	0.6053
Tibia breaking strength (g/force)	17777±1894.8	19259±1894.8	17534±1894.8	16739±1894.8	16723±1894.8	0.8738
Dry matter (%)	86.1±0.7	86.4±0.7	85.4±0.7	86.9±0.7	86.7±0.7	0.5299
Ash (%)	45.3±1.2	45.7±1.2	44.1±1.2	42.4±1.2	43.1±1.2	0.3047
Calcium (mg/g)	731.02±42.10	659.47±42.10	661.27±42.10	717.34±42.10	700.98±42.10	0.6708
Phosphorous (mg/g)	334.35±21.14	309.25±21.14	309.02±21.14	332.58±21.14	335.08±21.14	0.7938
Magnesium (mg/g)	10.35±0.52	9.69±0.52	9.95±0.52	10.30±0.52	10.13±0.52	0.3653
Zinc (mg/g)	1.04±0.07	0.90±0.07	0.85±0.07	0.96±0.07	0.99±0.07	0.3653
Iron (mg/g)	0.45±0.03	0.35±0.03	0.41±0.03	0.36±0.03	0.37±0.03	0.1171
Potassium (mg/g)	7.38±0.48	6.48±0.48	6.87±0.48	6.72±0.48	7.25±0.48	0.6700
Sodium (mg/g)	20.57±1.04	17.91±1.04	18.00±1.04	20.46±1.04	18.93±1.04	0.2418
Sulphate (mg/g)	9.15±0.74	7.93±0.74	7.90±0.74	8.98±0.74	8.61±0.74	0.6604

HP = hempseed presscake. HO = hempseed oil.
Initial body weight (n=3) = 1948g. Initial tibia weight (n=3) = 9.2g. Initial tibia length (n=3) = 118.8mm. Initial tibia width (n=3) = 6.2mm. Initial tibia breaking strength (n=3) = 18950 g/force.
$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.2.7. Average liver analysis (means ± standard error) from period 6 of Lohmann Brown-Lite hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Body weight (g)	2217±77.6	2130±77.6	2134±77.6	2208±77.6	2105±77.6	0.7869
Liver weight (g)	41.2±2.5	44.1±2.5	45.0±2.5	42.2±2.5	43.3±2.5	0.8292
Hepato-somatic index (%)	1.87±0.09	2.06±0.09	2.11±0.09	1.92±0.09	2.07±0.09	0.3048
Histological liver fat (%)	34.4±1.1	31.8±1.1	32.3±1.1	32.2±1.1	31.2±1.1	0.3813
Proximate liver fat (%)	25.9±1.6	22.7±1.9	21.2±1.6	21.0±1.6	23.2±1.6	0.2391
L*	38.8±0.9	40.7±0.9	39.9±0.9	39.5±0.9	40.8±0.9	0.4975
a*	14.6±0.5	13.5±0.5	14.1±0.5	13.1±0.5	13.2±0.5	0.3241
b*	19.5±0.8^{B}	21.6±0.8^{AB}	23.5±0.8^{A}	20.1±1.0^{AB}	18.7±0.8^{B}	0.0078

HP = hempseed presscake. HO= hempseed oil.
Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.
Initial body weight (n=3) = 1948g. Initial liver weight (n=3) = 37.7g. Initial average (2 replicates) L* score (n=3) =30.3. Initial average (2 replicates) a* score (n=3) = 13.0. Initial average (2 replicates) b* score (n=3) = 16.6.
$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

4.3: Lohmann LSL-Lite White hens reared in the conventional cage system

An overview of the production performance and egg quality for Lohmann LSL-Lite White hens reared in a conventional cage system fed all five treatment diets are shown in Table 4.3.1 and Table 4.3.2. White hens consumed significantly more (P≤0.05) feed when fed the control (period 5, 108.3 g/bird/day) than white hens fed 3% HO (period 5, 98.5 g/bird/day) and 6% HO (period 5, 98.3 g/bird/day). Egg production was significantly higher (P≤0.05) in period 5 when white hens were fed the control (95.7%), 10% HP (96.3%), 20% HP (95.2%) and 3% HO (93.6%) compared to hens fed 6% HO (84.3%). Feed conversion was significantly improved (P≤0.05) in period 2 for white hens fed 3% HO (1.68) compared to white hens fed 20% HP (1.95). Period 4 showed a better feed conversion in white hens fed 3% HO (1.54) and 6% HO (1.59) than those fed 20% HP

(1.81). In period 5, white hens fed 3% HO had a better (P≤0.05) feed conversion than those fed 6% HO diet. White hens in period 6 had significantly improved feed conversion when fed 10% HP (1.54) compared to 6% HO (1.77). The average feed conversion between period 1 and 6 was significantly better in white hens fed 10% HP (1.68) and 3% HO (1.64) than white hens fed 20% HP (1.84).

No differences (P>0.05) in egg weight, specific gravity, shell strength, albumen height or shell weight were found among dietary treatments (Table 4.3.2). Lohmann LSL-Lite White hens fed a 3% HO diet produced eggs with significantly thicker (P≤0.05) eggshells than those fed 20% HP. Egg yolk L* score was significantly higher (P≤0.05) in white hens fed 3% HO (62.7) than in white hens fed 20% HP (60.6) in period 1. In periods 2, 3 and 5, L* score was significantly higher (P≤0.05) in white hens fed the control (period 2, 62.6; period 3 62.9; period 5, 64.6), 3% HO (period 2, 63.4; period 3, 63.0; period 5, 64.7) and 6% HO (period 2, 63.1; period 3, 62.3; period 5, 64.8) diets than in white hens fed 20% HP (period 2, 60.8; period 3, 60.8; period 5, 62.2). In period 6, L* score was significantly higher (P≤0.05) in white hens fed 3% HO (63.8) than in white hens fed 10% HP (62.2) and 20% HP (60.0). Egg yolk a* score in period 1 was significantly higher (P≤0.05) in white hens fed 20% HP (11.3) than in white hens fed the control (9.2), 3% HO (8.2) and 6% HO (8.0). In period 2, a* score was significantly higher (P≤0.05) in white hens fed 20% HP (12.5) compared to control (9.0), 10% HP (10.7), 3% HO (7.5) and 6% HO (6.8). Yolk a* score was also significantly higher (P≤0.05) in white hens fed 20% HP from periods 3 to 6. Egg yolk b* score in periods 1 and 2 was significantly higher (P≤0.05) in white hens fed 20% HP compared to control, 10% HP, 3% HO and 6% HO. The b* scores of yolks produced by white hens fed 10% HP or 20% HP were higher ($P \leq 0.05$) than other dietary treatments from periods 3 to 6 (Table 4.3.2).

Table 4.3.1. Average production performance (means ± standard error) from period 0 to period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Average weight (g)						
I	1908±50	1959±50	1971±50	1913±50	2009±50	0.5891
P 1	1903±54	1952±54	1958±54	1910±54	1996±54	0.7381
P 2	1926±53	1978±53	1991±53	1918±53	1999±53	0.7230
P 3	1945±49	1986±49	1987±49	1935±49	2026±49	0.6961
P 4	1943±49	1969±49	1954±49	1942±49	2014±49	0.8243
P 5	1941±48	1976±48	1977±48	1945±48	2004±48	0.8742
P 6	1982±46	1965±46	2004±46	1943±46	2015±46	0.7990
Feed consumption (g/bird/day)						
P 1	116.6±2.6	118.2±2.6	120.0±2.6	110.1±2.6	112.8±2.7	0.1014
P 2	112.3±2.9	111.9±2.9	114.3±2.9	105.4±2.9	107.2±2.9	0.2100
P 3	107.1±2.5	104.2±2.5	105.9±2.5	98.9±2.5	101.1±2.5	0.1838
P 4	107±2.9	104.1±2.9	104.7±2.9	101.1±2.9	101.9±2.9	0.6317
P 5	108.3±2.2^{A}	103.2±2.2^{AB}	107.8±2.2^{AB}	98.5±2.2^{B}	98.3±2.2^{B}	0.0119
P 6	105.7±2.9	99.6±2.9	103.8±2.9	97.4±2.9	97.1±2.9	0.1802
Overall	109.5±2.5	106.9±2.5	109.4±2.5	101.9±2.5	103.1±2.5	0.1370
Hen day egg production (%)						
P 1	93.9±1.5	98.2±1.5	97.6±1.7	94.3±1.5	95.7±1.5	0.2143
P 2	96.1±1.1	96.4±1.1	96.7±1.3	96.1±1.1	96.4±1.1	0.9946
P 3	95.0±1.5	97.5±1.5	93.8±1.7	97.5±1.5	96.4±1.5	0.4353
P 4	95.7±1.2	94.3±1.2	94.8±1.3	97.9±1.2	94.6±1.2	0.2423
P 5	95.7±1.7^{A}	96.3±1.7^{A}	95.2±2.0^{A}	93.6±1.7^{A}	84.3±1.7^{B}	0.0012
P 6	94.6±2.5	96.3±2.5	94.8±2.9	91.1±2.5	85.4±2.5	0.0585
Feed conversion						
P 1	1.92±0.06	1.82±0.06	2.06±0.06	1.82±0.06	1.86±0.06	0.0847
P 2	1.83±0.05^{AB}	1.77±0.05^{AB}	1.95±0.05^{A}	1.68±0.05^{B}	1.76±0.05^{AB}	0.0098
P 3	1.71±0.07	1.63±0.07	1.80±0.07	1.57±0.07	1.64±0.07	0.2167
P 4	1.65±0.05^{AB}	1.69±0.05^{AB}	1.81±0.05^{A}	1.54±0.05^{B}	1.59±0.05^{B}	0.0128
P 5	1.69±0.05^{AB}	1.61±0.05^{AB}	1.76±0.05^{AB}	1.59±0.05^{B}	1.78±0.05^{A}	0.0303
P 6	1.56±0.05^{AB}	1.54±0.05^{B}	1.68±0.05^{AB}	1.62±0.05^{AB}	1.77±0.05^{A}	0.0363
Overall	1.73±0.04^{AB}	1.68±0.04^{B}	1.84±0.04^{A}	1.64±0.04^{B}	1.73±0.04^{AB}	0.0176

I = Initial, P = Period. HP =hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.3.2. Egg quality (means ± standard error) from period 0 to period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Egg weight (g)						
I	64.9±0.9	66.3±0.9	64.6±0.9	64.3±0.9	63.4±0.9	0.3286
P 1	63.8±1.1	65.5±1.1	65.1±1.1	65.4±1.1	63.1±1.1	0.4416
P 2	66.0±1.2	66.0±1.2	67.1±1.2	65.0±1.2	64.0±1.2	0.4533
P 3	67.9±1.1	65.4±1.1	65.3±1.1	67.3±1.1	69.9±1.1	0.3167
P 4	67.0±1.3	66.9±1.3	65.8±1.3	66.4±1.3	65.7±1.3	0.9189
P 5	71.5±1.5	67.1±1.5	67.9±1.5	66.2±1.5	64.7±1.5	0.0640
P 6	67.8±1.3	64.8±1.3	66.7±1.3	65.0±1.3	64.7±1.3	0.4016
Egg specific gravity						
I	1.084±0.001	1.083±0.001	1.085±0.001	1.084±0.001	1.084±0.001	0.8866
P 1	1.085±0.001	1.085±0.001	1.085±0.001	1.086±0.001	1.086±0.001	0.4832
P 2	1.085±0.001	1.085±0.001	1.084±0.001	1.085±0.001	1.086±0.001	0.6368
P 3	1.088±0.001	1.088±0.001	1.087±0.001	1.088±0.001	1.087±0.001	0.9189
P 4	1.083±0.001	1.083±0.001	1.083±0.001	1.083±0.001	1.084±0.001	0.8537
P 5	1.083±0.001	1.083±0.001	1.084±0.001	1.083±0.001	1.085±0.001	0.2457
P 6	1.086±0.001	1.087±0.001	1.085±0.001	1.087±0.001	1.088±0.001	0.3366
Egg breaking strength (g/force)						
I	4846.4±712.6	5235.5±712.6	4774.9±712.6	4394.5±712.	4562.4±712.6	0.9344
P 1	5177.0±369.7	5097.9±369.7	5403.2±369.7	5223.8±369.7	5317.4±369.7	0.9793
P 2	5398.6±371.0	5368.9±371.0	5291.9±371.0	4772.9±371.0	5723.0±371.0	0.5128
P 3	5079.6±391.0	5030.6±391.0	5587.8±391.0	4884.3±391.0	5057.7±391.0	0.7570
P 4	4550.7±346.5	4445.1±346.5	5563.2±346.5	4993.0±346.5	5336.6±346.5	0.1539
P 5	3819.9±340.2	4933.4±340.2	5147.5±340.2	5034.7±340.2	4841.8±340.2	0.0889
P 6	4540.2±274.6	4888.3±274.6	5230.3±274.6	4996.2±274.6	4752.0±274.6	0.4946
Albumen height (mm)						
I	7.4±0.2	7.2±0.2	7.3±0.2	7.0±0.2	7.2±0.2	0.7677
P 1	7.5±0.2	7.4±0.2	7.0±0.2	7.9±0.2	7.2±0.2	0.1473
P 2	7.2±0.3	6.9±0.3	7.2±0.3	7.0±0.3	6.8±0.3	0.7072
P 3	7.3±0.3	7.5±0.3	7.3±0.3	7.1±0.3	6.8±0.3	0.3602
P 4	6.2±0.2	5.9±0.2	6.4±0.2	6.2±0.2	5.9±0.2	0.5102
P 5	6.7±0.3	6.2±0.3	6.8±0.3	6.5±0.3	6.6±0.3	0.6099
P 6	7.0±0.2	7.0±0.2	6.5±0.2	6.9±0.2	7.1±0.2	0.3541
Shell weight (g)						
I	6.4±0.1	6.5±0.1	6.4±0.1	6.4±0.1	6.3±0.1	0.9739
P 1	6.2±0.1	6.3±0.1	6.4±0.1	6.4±0.1	6.2±0.1	0.4495
P 2	6.4±0.1	6.3±0.1	6.4±0.1	6.2±0.1	6.2±0.1	0.8535
P 3	6.5±0.1	6.2±0.1	6.2±0.1	6.4±0.1	6.6±0.1	0.1294
P 4	6.4±0.1	6.4±0.1	6.2±0.1	6.4±0.1	6.3±0.1	0.8229
P 5	6.8±0.1	6.4±0.1	6.5±0.1	6.3±0.1	6.2±0.1	0.1057
P 6	6.40±0.1	6.2±0.1	6.2±0.1	6.2±0.1	6.2±0.1	0.7108
Shell Thickness (mm)						
I	0.49±0.01	0.49±0.01	0.50±0.01	0.50±0.01	0.50±0.01	0.6853
P 1	0.40±0.01	0.41±0.01	0.42±0.01	0.42±0.01	0.44±0.01	0.1147
P 2	$0.42±0.01^{AB}$	$0.43±0.01^{AB}$	$0.42±0.01^{B}$	$0.45±0.01^{A}$	$0.44±0.01^{AB}$	0.0322
P 3	0.42±0.01	0.41±0.01	0.40±0.01	0.41±0.01	0.42±0.01	0.3149
P 4	0.39±0.01	0.39±0.01	0.39±0.01	0.40±0.01	0.39±0.01	0.9520
P 5	0.41±0.02	0.41±0.02	0.41±0.02	0.39±0.02	0.39±0.02	0.8300
P 6	0.43±0.01	0.43±0.01	0.42±0.01	0.43±0.01	0.43±0.01	0.9754

Table 4.3.2. Egg quality (means ± standard error) from period 0 to period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
L*						
I	62.9±0.5	62.7±0.5	62.4±0.5	62.8±0.5	62.9±0.5	0.8329
P 1	62.0±0.4^{AB}	61.8±0.4^{AB}	60.6±0.4^{B}	62.7±0.4^{A}	62.3±0.4^{AB}	0.0221
P 2	62.6±0.4^{A}	61.7±0.4^{AB}	60.8±0.4^{B}	63.4±0.4^{A}	63.1±0.4^{A}	0.0014
P 3	62.9±0.3^{A}	62.0±0.3^{AB}	60.8±0.3^{B}	63.0±0.3^{A}	62.3±0.3^{A}	0.0023
P 4	63.5±0.6	63.1±0.6	61.3±0.6	63.7±0.6	63.3±0.6	0.0622
P 5	64.6±0.4^{A}	63.7±0.4^{AB}	62.2±0.4^{B}	64.7±0.4^{A}	64.8±0.4^{A}	0.0009
P 6	63.0±0.4^{AB}	62.2±0.4^{B}	60.0±0.4^{C}	63.8±0.4^{A}	62.6±0.4^{AB}	0.0001
a*						
I	10.9±0.4	11.2±0.4	10.9±0.4	10.6±0.4	10.2±0.4	0.4179
P 1	9.2±0.4^{BC}	10.4±0.4^{AB}	11.3±0.4^{A}	8.2±0.4^{C}	8.0±0.4^{C}	0.0000
P 2	9.0±0.3^{C}	10.7±0.3^{B}	12.5±0.3^{A}	7.5±0.3^{D}	6.8±0.3^{D}	0.0000
P 3	8.7±0.3^{C}	10.1±0.3^{B}	11.8±0.3^{A}	7.6±0.3^{C}	7.6±0.3^{C}	0.0000
P 4	8.3±0.4^{B}	9.4±0.4^{B}	12.3±0.4^{A}	7.8±0.4^{B}	5.9±0.4^{C}	0.0001
P 5	8.8±0.4^{B}	10.3±0.4^{B}	12.0±0.4^{A}	7.1±0.4^{C}	6.0±0.4^{C}	0.0001
P 6	8.6±0.2^{C}	9.6±0.2^{B}	11.9±0.2^{A}	7.0±0.2^{D}	8.9±0.2^{BC}	0.0001
b*						
I	71.3±1.2	73.3±1.2	70.5±1.2	71.6±1.2	71.4±1.2	0.6014
P 1	68.8±1.5^{AB}	71.5±1.5^{AB}	74.0±1.5^{A}	65.6±1.5^{B}	66.8±1.5^{B}	0.0096
P 2	69.3±1.3^{BC}	74.8±1.3^{AB}	75.4±1.3^{A}	64.3±1.3^{CD}	59.7±1.3^{D}	0.0000
P 3	68.4±1.3^{AB}	71.8±1.3^{A}	73.6±1.3^{A}	64.1±1.3^{BC}	62.4±1.3^{C}	0.0000
P 4	67.7±1.2^{B}	73.4±1.2^{A}	77.8±1.2^{A}	63.2±1.2^{BC}	59.3±1.2^{C}	0.0001
P 5	69.9±1.2^{B}	76.8±1.2^{A}	80.4±1.2^{A}	64.2±1.2^{C}	63.3±1.2^{C}	0.0001
P 6	67.2±1.0^{B}	72.9±1.0^{A}	74.0±1.0^{A}	61.8±1.0^{C}	60.6±1.0^{C}	0.0001

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.
Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.
$^{A\text{-}D}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.3.3 provides the fatty acid composition of egg yolks produced by Lohmann LSL-Lite White hens. The amount of oleic acid in egg yolks significantly decreased (P≤0.05) with the addition of hemp by-products and the 6% HO diet resulted in the yolk containing the least (P≤0.05) amount of oleic acid. Moreover, white hens fed 6% HO had a significantly increased (P≤0.05) amount of saturated fatty acids (myristic acid, stearic acid and arachidic acid) and unsaturated fatty acids (linolenic acid, g-linolenic acid, homo-g-linolenic acid, homo-a-linolenic acid, clupanodonic acid and DHA).

Table 4.3.3. Fatty acid content of egg yolks from of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Treatment					
	Control	**10% HP**	**20% HP**	**3% HO**	**6% HO**	**P-Value**
Crude fat (W/W%)	52.22±0.44	52.71±0.18	53.00±0.09	52.37±0.27	52.79±0.31	0.328
C14:0	0.26±0.01^{AB}	0.25±0.01^{AB}	0.24±0.01^{B}	0.27±0.01^{A}	0.28±0.01^{A}	0.015
Myristoleic (9c-14:1)	0.03±0.00^{AB}	0.02±0.00^{B}	0.02±0.00^{AB}	0.03±0.00^{A}	0.03±0.00^{AB}	0.011
C15:0	0.05±0.00	0.05±0.01	0.04±0.00	0.05±0.00	0.05±0.00	0.454
C15:1n5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Palmitic (16:0)	23.61±0.12	23.55±0.41	23.24±0.21	24.21±0.43	23.74±0.20	0.289
Palmitoleic (9c-16:1)	1.27±0.25	0.76±0.21	0.72±0.18	1.00±0.35	1.27±0.27	0.414
Margaric (17:0)	0.20±0.00	0.26±0.05	0.22±0.01	0.19±0.01	0.21±0.01	0.179
10c-17:1	0.10±0.00	0.12±0.02	0.10±0.00	0.10±0.00	0.09±0.00	0.240
Stearic (18:0)	10.11±0.03^{BC}	9.91±0.14^{C}	10.76±0.29^{BC}	10.54±0.17^{ABC}	11.02±0.17^{A}	0.003
Elaidic (9t-18:1)	0.15±0.03	0.19±0.06	0.14±0.03	0.14±0.03	0.09±0.00	0.479
Oleic (9c-18:1)	33.49±0.49^{A}	31.29±0.64^{B}	31.25±0.70^{B}	31.17±0.54^{B}	28.83±0.32^{C}	<0.001
Vaccenic (11c-18:1)	1.61±0.23	2.01±0.23	1.93±0.22	1.67±0.25	1.29±0.21	0.240
Linolelaidic (18:2t)	0.00±0.00	0.05±0.03	0.00±0.00	0.05±0.02	0.00±0.00	0.137
Linoleic (18:2n6)	22.06±0.19	23.37±0.60	23.35±1.23	21.95±0.28	23.69±0.32	0.228
Linolenic (18:3n3)	1.27±0.03^{D}	1.54±0.08^{CD}	1.66±0.12^{C}	2.12±0.04^{B}	3.14±0.09^{A}	<0.001
g-Linolenic [C18:3n6]	0.12±0.00^{D}	0.14±0.01^{CD}	0.16±0.01^{BC}	0.18±0.01^{B}	0.23±0.02^{A}	<0.001
Stearidonic (18:4n3)	0.02±0.01	0.02±0.01	0.02±0.00	0.04±0.01	0.05±0.01	0.093
Arachidic (20:0)	0.03±0.00^{B}	0.04±0.01^{AB}	0.04±0.00^{B}	0.04±0.00^{AB}	0.05±0.0^{A}	0.017
Gondoic (20:1n9)	0.15±0.03	0.07±0.03	0.07±0.04	0.11±0.04	0.12±0.03	0.393
C20:2	0.21±0.01	0.22±0.01	0.21±0.01	0.20±0.01	0.20±0.01	0.580
Homo-g-linolenic [C20:3n6]	0.17±0.01^{C}	0.19±0.01^{BC}	0.20±0.01^{BC}	0.22±0.01^{B}	0.26±0.01^{A}	<0.001
Homo-a-linolenic (20:3n3)	0.02±0.01^{C}	0.03±0.00^{BC}	0.04±0.00^{BC}	0.04±0.00^{AB}	0.06±0.00^{A}	<0.001
Arachidonic [20:4n6]	1.87±0.01	1.83±0.03	1.88±0.03	1.88±0.04	1.89±0.05	0.792

Table 4.3.3. Fatty acid content of egg yolks from of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Treatment					
	Control	**10% HP**	**20% HP**	**3% HO**	**6% HO**	**P-Value**
3n-Arachidonic (20:4n3)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
EPA (20:5n3)	0.02±0.00	0.02±0.00	0.03±0.00	0.04±0.00	0.06±0.00	-
C21:0	0.02±0.01	0.03±0.01	0.02±0.00	0.02±0.00	0.01±0.00	0.318
Behenic (22:0)	0.03±0.01	0.03±0.01	0.02±0.00	0.03±0.01	0.04±0.01	0.773
Erucic [22:1n9]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.657
C22:2n6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Adrenic [C22:4n6]	0.11±0.00	0.08±0.02	0.11±0.00	0.10±0.00	0.10±0.00	0.192
Clupanodonic (22:5n3)	$0.08±0.00^{C}$	$0.10±0.01^{C}$	$0.13±0.01^{AB}$	$0.11±0.01^{BC}$	$0.14±0.01^{A}$	<0.001
DHA (22:6n3)	$1.16±0.02^{C}$	$1.27±0.04^{C}$	$1.39±0.03^{B}$	$1.42±0.04^{B}$	$1.57±0.02^{A}$	<0.001
C23:0	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.00	0.01±0.00	0.848
Lignoceric (24:0)	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.252
Nervonic (24:1n9)	0.01±0.00	0.01±0.00	0.1±0.00	0.01±0.00	0.01±0.00	-

HP = hempseed presscake. HO = hempseed oil.
Fatty acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis.
$^{A\text{-}C}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Dietary treatments did not affect (P>0.05) the average feather scoring condition for 7 body regions on Lohmann LSL-Lite White hens when reared in a conventional cage system (Table 4.3.4). Table 4.3.5 shows the total body feather condition of Lohmann LSL-Lite White hens fed five treatment diets. Chi-square analysis showed no differences in total body feather condition among dietary treatments for white hens reared in a conventional cage system.

Table 4.3.4. Average feather score (means ± standard error) for 7 body regions (head, neck, abdomen, breast, tail, back and wing) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Head						
I	1.1±0.2	1.1±0.2	1.1±0.2	1.1±0.2	0.9±0.2	0.8493
P 1	2.1±0.1	1.9±0.1	2.0±0.1	1.8±0.1	1.7±0.1	0.5832
P 2	2.2±0.1	2.0±0.1	2.1±0.1	2.1±0.1	2.0±0.1	0.1268
P 3	2.1±0.1	2.1±0.1	2.1±0.1	2.1±0.1	2.1±0.1	0.8734
P 4	2.2±0.5	2.1±0.5	3.0±0.5	2.1±0.5	2.1±0.5	0.6419
P 5	2.4±0.2	2.1±0.2	2.0±0.2	2.2±0.2	1.9±0.2	0.3465
P 6	2.2±0.1	2.1±0.1	2.1±0.1	2.2±0.1	2.1±0.1	0.6571
Neck						
I	2.3±0.2	2.5±0.2	2.3±0.2	2.5±0.2	2.3±0.2	0.7984
P 1	2.9±0.1	3.1±0.1	2.9±0.1	3.1±0.1	2.9±0.1	0.2051
P 2	2.9±0.	2.9±0.1	2.9±0.1	3.1±0.1	3.0±0.1	0.6684
P 3	3.0±0.1	3.0±0.1	2.9±0.1	2.9±0.1	2.9±0.1	0.3129
P 4	2.9±0.1	3.0±0.1	2.9±0.1	2.9±0.1	2.9±0.1	0.6838
P 5	2.9±0.1	2.9±0.1	2.8±0.1	3.1±0.1	3.0±0.1	0.1005
P 6	2.9±0.1	3.0±0.1	3.0±0.1	3.0±0.1	2.9±0.1	0.6982
Abdomen						
I	2.1±0.2	2.0±0.2	2.1±0.2	2.3±0.2	2.1±0.2	0.9457
P 1	2.9±0.1	3.0±0.1	3.1±0.1	3.0±0.1	3.0±0.1	0.7117
P 2	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.1±0.0	0.3324
P 3	3.1±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	0.4380
P 4	3.0±0.1	3.0±0.1	3.0±0.1	3.0±0.1	2.9±0.1	0.6795
P 5	3.1±0.1	3.1±0.1	3.0±0.1	3.1±0.1	3.1±0.1	0.8229
P 6	3.0±0.0	3.1±0.0	3.0±0.0	3.0±0.0	3.0±0.0	0.8988
Breast						
I	2.5±0.1	2.3±0.1	2.1±0.1	2.3±0.2	2.5±0.2	0.6630
P 1	3.0±0.0	3.0±0.0	3.1±0.0	3.1±0.0	3.0±0.0	0.1543
P 2	3.1±0.1	3.1±0.1	3.0±0.1	3.1±0.1	3.1±0.1	0.6838
P 3	3.1±0.1	3.0±0.1	3.0±0.1	3.1±0.1	2.9±0.1	0.3324
P 4	3.0±0.1	3.0±0.1	3.0±0.1	3.1±0.1	3.0±0.1	0.6684
P 5	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	0.7362
P 6	3.1±0.1	3.2±0.1	3.0±0.1	3.1±0.1	3.1±0.0	0.8319
Tail						
I	1.7±0.1	1.7±0.1	1.5±0.1	1.7±0.1	1.5±0.1	0.7209
P 1	2.2±0.2	2.1±0.2	2.2±0.2	1.9±0.2	2.1±0.2.	0.7824
P 2	1.3±0.2	1.1±0.2	1.1±0.2	1.3±0.2	1.0±0.2	0.6539
P 3	1.5±0.1^{A}	1.2±0.1^{B}	1.1±0.1^{B}	1.1±0.1^{B}	1.0±0.1^{B}	0.0013
P 4	2.5±0.2	2.2±0.2	2.0±0.2	2.3±0.2	1.9±0.2	0.2199
P 5	2.5±0.2	2.1±0.2	2.0±0.2	2.1±0.2	1.9±0.2	0.3850
P 6	1.6±0.2	1.5±0.2	1.6±0.2	1.5±0.2	1.4±0.2	0.9237
Back						
I	1.4±0.2	1.2±0.2	1.2±0.2	1.3±0.2	1.1±0.2	0.7573
P 1	2.1±0.2	1.9±0.2	2.0±0.2	2.3±0.2	2.1±0.2	0.7727
P 2	2.1±0.2	1.9±0.2	1.9±0.2	2.3±0.2	1.7±0.2	0.1643
P 3	2.1±0.2	2.1±0.2	2.1±0.2	2.3±0.2	2.2±0.2	0.9618
P 4	2.5±0.2	2.2±0.2	2.2±0.2	2.4±0.2	2.1±0.2	0.6508
P 5	2.6±0.2	2.2±0.2	2.1±0.2	2.5±0.2	2.1±0.2	0.4465

Table 4.3.4. Average feather score (means ± standard error) for 7 body regions (head, neck, abdomen, breast, tail, back and wing) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
P 6	2.1±0.2	1.8±0.2	1.9±0.2	2.2±0.2	1.9±0.2	0.6514
Wing						
I	1.7±0.2	1.3±0.2	1.5±0.2	1.7±0.2	1.3±0.2	0.2546
P 1	2.1±0.1	2.1±0.1	1.8±0.1	2.1±0.1	1.9±0.1	0.3347
P 2	1.5±0.1	1.3±0.1	1.3±0.1	1.3±0.1	1.1±0.1	0.7438
P 3	1.9±0.1	1.6±0.1	1.5±0.1	1.6±0.1	1.3±0.1	0.0931
P 4	2.5±0.2	2.3±0.2	2.2±0.2	2.7±0.2	2.3±0.2	0.4251
P 5	2.5±0.2	2.0±0.2	2.2±0.2	2.5±0.2	2.2±0.2	0.4500
P 6	1.9±0.2	1.8±0.2	1.7±0.2	1.8±0.2	1.6±0.2	0.9063

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.3.5. Total body feather condition score of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, analyzed using chi-square.

Feather Score	Control	10% HP	20% HP	3% HO	6% HO	P-Value	Chi-square
I							
0	0	0	0	0	0	0.9990	5.9041
1	4	7	5	3	6		
2	15	12	15	17	14		
3	1	1	0	0	0		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 1							
0	0	0	0	0	0	1.0000	2.0202
1	0	0	0	0	0		
2	10	12	13	9	11		
3	10	8	7	11	9		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 2							
0	0	0	0	0	0	0.9931	7.8073
1	0	0	0	0	0		
2	14	18	19	16	19		
3	6	2	1	4	1		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 3							
0	0	0	0	0	0	0.9999	4.0672
1	0	0	0	0	0		
2	15	19	18	18	17		
3	5	1	2	2	3		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 4							
0	0	0	0	0	0	0.8922	12.6410
1	0	0	0	0	0		
2	4	9	10	5	11		
3	16	11	9	15	9		
4	0	0	0	0	0		
5	0	0	1	0	0		
P 5							
0	0	0	0	0	0	0.9999	3.6912
1	0	0	0	0	0		
2	7	10	10	6	10		
3	13	9	9	14	10		
4	0	0	0	0	0		
5	0	0	0	0	0		
P 6							
0	0	0	0	0	0	1.0000	2.0998
1	0	0	0	0	0		
2	15	14	15	12	15		
3	5	5	4	8	5		
4	0	0	0	0	0		
5	0	0	0	0	0		

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

In the conventional cage system, white hens fed 6% HO had a significantly greater ($P \leq 0.05$) tibia length compared to hens fed 10% HP, and the tibia length of hens fed 6% HO and the control, 20% HP and 3% HO diets were intermediate (Table 4.3.6). Tibia bones were significantly stronger ($P \leq 0.05$) in white hens fed the control and 20% HP than those fed 10% HP, but there was no statistical difference between hens fed the control and 20% treatment groups and the 3% HO and 6% HO treatment groups. Bone strength in hens fed 10% HP did not statistically differ from the 3% HO and 6% HO treatment groups. Ash content was significantly higher ($P \leq 0.05$) in hens fed 20% HP than in hens fed 10% HP, and 6% HO diets (Table 4.3.6).

Table 4.3.6. Average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Body weight (g)	1925±78.6	1863±78.6	2055±78.6	2044±78.6	2036±78.6	0.3521
Tibia weight (g)	7.3±0.2	7.0±0.2	7.2±0.2	7.3±0.2	7.4±0.2	0.5462
Tibia length (mm)	117.6±0.6^{AB}	116.1±0.6^{B}	118.2±0.6^{AB}	118.3±0.6^{AB}	119.6±0.6^{A}	0.0186
Tibia width (mm)	5.9±0.1	5.9±0.1	5.7±0.1	5.8±0.1	5.8±0.1	0.6894
Tibia breaking strength (g/force)	21159±1307.5^{A}	13657±1307.5^{B}	20069±1307.5^{A}	17823±1307.5^{AB}	18715±1307.5^{AB}	0.0102
Dry matter (%)	84.0±0.8	84.0±0.8	83.9±0.8	84.8±0.8	85.2±0.8	0.6689
Ash (%)	49.6±0.7^{AB}	46.4±0.7^{C}	50.2±0.7^{A}	47.8±0.7^{ABC}	46.5±0.7^{BC}	0.0048
Calcium (mg/g)	510.15±28.96	489.93±28.96	494.47±28.96	544.45±28.96	496.27±28.96	0.6756
Phosphorous (mg/g)	240.81±14.01	232.14±14.01	234.96±14.01	258.04±14.01	232.71±14.01	0.6731
Magnesium (mg/g)	7.14±0.33	6.89±0.33	7.05±0.33	7.36±0.33	6.92±0.33	0.8594
Zinc (mg/g)	0.65±0.04	0.59±0.04	0.56±0.04	0.70±0.04	0.60±0.04	0.2025
Iron (mg/g)	0.30±0.03	0.29±0.03	0.26±0.03	0.31±0.03	0.33±0.03	0.4155
Potassium (mg/g)	6.29±0.45	6.33±0.45	6.24±0.45	6.57±0.45	5.71±0.45	0.7467
Sodium (mg/g)	13.64±0.80	13.05±0.80	13.24±0.80	14.26±0.80	13.33±0.80	0.8391
Sulphate (mg/g)	5.45±0.43	5.01±0.43	5.21±0.43	5.58±0.43	5.07±0.43	0.8539

HP = hempseed presscake. HO = hempseed oil.

Initial body weight (n=3) = 1957g. Initial tibia weight (n=3) = 8.1g. Initial tibia length (n=3) = 121.5mm. Initial tibia width (n=3) = 6.1mm. Initial tibia breaking strength (n=3) = 20311 g/force.

$^{A-C}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly ($P \leq 0.05$).

No differences (P>0.05) in body weight, liver weight, hepato-somatic index, and percent fat were observed among five dietary treatments. The liver of white hens fed 20% HP had higher redness and yellowness pigmentation of the liver with a higher (P≤0.05) a* score value than the 3% HO and 6% HO groups, and a higher (P≤0.05) b* value than the other four dietary treatments (Table 4.3.7).

Table 4.3.7. Average liver analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	P-Value
Body weight (g)	1925±78.6	1863±78.6	2055±78.6	2044±78.6	2036±78.6	0.3521
Liver weight (g)	44.8±3.0	40.7±3.0	50.6±3.0	48.4±3.0	45.3±3.0	0.2412
Hepato-somatic index (%)	2.3±0.1	2.2±0.1	2.4±0.1	2.4±0.1	2.2±0.1	0.3819
Histological liver fat (%)	30.0±1.4	26.5±1.4	32.2±1.4	29.3±1.4	30.4±1.4	0.1061
Proximate liver fat (%)	32.0±3.5	24.3±3.5	29.3±4.1	25.4±4.1	28.8±3.5	0.5859
L*	41.8±1.4	39.1±1.4	42.5±1.4	43.0±1.4	42.6±1.4	0.3131
a*	13.3±0.5^{AB}	13.6±0.5^{AB}	15.4±0.5^{A}	12.8±0.5^{B}	12.8±0.5^{B}	0.0190
b*	21.7±1.8^{B}	19.9±1.8^{B}	29.9±1.8^{A}	19.7±1.8^{B}	20.1±1.8^{B}	0.0046

HP = hempseed presscake. HO = hempseed oil.
Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.
Initial body weight (n=3) = 1957g. Initial liver weight (n=3) = 40.9g. Initial average (2 replicates) L* score (n=3) = 40.2. Initial average (2 replicates) a* score (n=3) = 12.2. Initial average (2 replicates) b* score (n=3) =22.6.
$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

4.4: Lohmann LSL-Lite White hens reared in the single-tier system

Table 4.4.1 gives an overview of production data for Lohmann LSL-Lite White hens reared in the single-tier housing system and fed the control, 20% HP and 6% HP diets. In period 2, feed consumption was significantly higher ($P \leq 0.05$) in white hens fed the control (111.3 g/bird/day) than those fed 6% HO (102.0 g/bird/day) with no statistical difference from hens fed the 20% HP diet. Feed consumption in period 5 was significantly higher ($P \leq 0.05$) in white hens fed the 20% HP (107.7 g/bird/day) than white hens fed the control (95.3 g/bird/day) with no statistical difference from the hens fed the 6% HO diet. No differences ($P > 0.05$) in body weight, egg production or feed conversion were found among dietary treatments.

Table 4.4.1. Average production performance (means ± standard error) from period 0 to period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Average weight (g)				
I	1852±21	1821±21	1877±21	0.2217
P 1	1889±23	1874±23	1913±23	0.5214
P 2	1916±24	1905±24	1916±24	0.9294
P 3	1921±27	1903±27	1924±27	0.8367
P 4	1925±28	1906±28	1891±28	0.6950
P 5	1912±23	1898±23	1902±23	0.9012
P 6	1924±25	1918±25	1916±25	0.9711
Feed consumption (g/bird/day)				
P 1	120.3±2.2	116.4±2.2	114.9±2.2	0.2309
P 2	111.3±2.2^{A}	109.3±2.2^{AB}	102.0±2.2^{B}	0.0375
P 3	102.7±2.2	110.7±2.2	102.8±2.2	0.0504
P 4	100.1±2.2	106.8±2.2	98.8±2.2	0.0685
P 5	95.3±2.7^{B}	107.7±2.7^{A}	97.0±2.7^{AB}	0.0213
P 6	96.1±3.1	102.1±3.1	94.3±3.1	0.2274
Overall	104.3±2.2	108.8±2.2	101.6±2.2	0.1122
Hen day egg production (%)				
P 1	96.4±2.0	90.9±2.0	93.9±2.0	0.1917
P 2	97.4±1.9	95.5±1.9	95.2±1.9	0.6759
P 3	94.1±2.1	96.1±2.1	94.1±2.1	0.7632
P 4	90.6±2.8	93.6±2.8	90.9±2.8	0.7294
P 5	90.3±2.3	96.1±2.3	89.9±2.3	0.1665
P 6	91.5±3.1	93.2±3.1	81.8±3.1	0.0585
Feed conversion				
P 1	2.00±0.10	2.00±0.10	1.90±0.10	0.6055
P 2	1.78±0.04	1.83±0.04	1.68±0.04	0.0549
P 3	1.70±0.10	1.80±0.10	1.70±0.10	0.5287
P 4	1.70±0.10	1.80±0.10	1.70±0.10	0.4571
P 5	1.61±0.05	1.73±0.05	1.67±0.05	0.2742
P 6	1.62±0.05	1.70±0.05	1.79±0.05	0.0972
Overall	1.73±0.04	1.80±0.04	1.74±0.04	0.4386

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

The influence of experimental diets on egg quality measures on white hens in the single-tier system is shown in Table 4.4.2. Egg weight, specific gravity, albumen height, shell weight or shell thickness were not different (P>0.05) among dietary treatments. Eggs had a significantly stronger (P≤0.05) shell breaking strength when white hens were fed 20% HP (5262.9 kg) than those produced by white hens fed the control (4514.8 kg), with no significant difference from hens fed 6% HO. Egg yolk L* score was the highest (P≤0.05) in white hens fed the control and 6% HO treatment diets than white hens fed 20% HP for periods 1,2,3,4 and 5. In period 6, L* score was significantly higher (P≤0.05) in white hens fed the control (63.1) than in white hens fed 20% HP (61.3) and 6% HO (62.1). Egg yolk a* score was significantly higher (P≤0.05) when white hens were fed 20% HP than when white hens were fed the control or 6% HO in periods 1, 2, 3, 4 and 5. In period 6, egg yolk a* score was significantly higher (P≤0.05) in white hens fed 20% HP than in other three dietary treatments. In period 1, egg yolk b* score was significantly higher (P≤0.05) in white hens fed 20% HP than white hens fed other treatment diets. Egg yolk b* score was significantly higher (P≤0.05) in white hens fed 20% HP than in white hens fed the control and 6% HO for periods 2, 3 ,4 5 and 6.

Table 4.4.2. Egg quality (means ± standard error) from period 0 to period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Egg weight (g)				
I	64.0±0.7	63.6±0.7	63.4±0.7	0.8691
P 1	64.2±0.7	62.6±0.7	64.0±0.7	0.2722
P 2	64.2±0.8	64.7±0.8	64.2±0.8	0.8770
P 3	65.5±0.4	64.9±0.4	65.7±0.4	0.4041
P 4	65.6±0.8	65.0±0.8	64.5±0.8	0.6640
P 5	65.0±0.6	64.7±0.6	64.5±0.6	0.8198
P 6	65.4±0.7	64.9±0.7	64.4±0.7	0.5894
Egg specific gravity				
I	1.083±0.001	1.084±0.001	1.085±0.001	0.3947
P 1	1.086±0.001	1.085±0.001	1.086±0.001	0.9273
P 2	1.085±0.001	1.085±0.001	1.085±0.001	0.9250
P 3	1.084±0.001	1.085±0.001	1.086±0.001	0.6360
P 4	1.083±0.001	1.084±0.001	1.082±0.001	0.6758
P 5	1.084±0.0004	1.085±0.0004	1.083±0.0004	0.2832
P 6	1.086±0.002	1.087±0.002	1.088±0.002	0.8352
Egg breaking strength (g/force)				
I	4713.2±311.9	4639.3±311.9	5064.8±311.9	0.6052
P 1	4462.5±348.7	5340.3±348.7	5343.7±348.7	0.1760
P 2	4834.5±212.3	5007.0±212.3	4935.8±212.3	0.8489
P 3	4514.8±139.1^{B}	5262.9±139.1^{A}	4820±139.1^{AB}	0.0130
P 4	5515.0±118.1	5261.8±118.1	5212.1±118.1	0.2058
P 5	4749.9±132.7	4929.7±132.7	4579.5±132.7	0.2293
P 6	4405.7±239.8	5085.2±239.8	4700.8±239.8	0.1885
Albumen height (mm)				
I	6.4±0.2	6.3±0.2	6.3±0.2	0.9756
P 1	6.6±0.2	6.8±0.2	6.8±0.2	0.6930
P 2	6.5±0.1	6.5±0.1	6.5±0.1	0.9310
P 3	6.0±0.1	6.3±0.1	6.3±0.1	0.3103
P 4	5.9±0.2	6.2±0.2	6.0±0.2	0.4801
P 5	6.3±0.2	6.6±0.2	6.5±0.2	0.5303
P 6	6.3±0.2	6.7±0.2	6.3±0.2	0.2484
Shell weight (g)				
I	6.3±0.1	6.6±0.1	6.4±0.1	0.5531
P 1	6.3±0.1	6.1±0.1	6.2±0.1	0.4284
P 2	6.2±0.1	6.3±0.1	6.2±0.1	0.5014
P 3	6.2±0.1^{A}	6.3±0.1^{A}	6.3±0.1^{A}	0.6888
P 4	6.4±0.1	6.3±0.1	6.3±0.1	0.8246
P 5	6.2±0.1	6.2±0.1	6.0±0.1	0.0907
P 6	6.2±0.1	6.3±0.1	6.2±0.1	0.6903
Shell Thickness (mm)				
I	0.49±0.01	0.50±0.01	0.50±0.01	0.3177
P 1	0.47±0.01	0.46±0.01	0.46±0.01	0.3936
P 2	0.46±0.01	0.46±0.01	0.46±0.01	0.8570
P 3	0.46±0.01	0.47±0.01	0.46±0.01	0.6275
P 4	0.40±0.01	0.40±0.01	0.40±0.01	0.9289
P 5	0.44±0.01	0.42±0.01	0.41±0.01	0.2932

Table 4.4.2. Egg quality (means ± standard error) from period 0 to period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil, continued.

	Control	20% HP	6% HO	P-Value
P 6	0.44±0.01	0.44±0.01	0.44±0.01	0.5365
L*				
I	62.9±0.2	63.1±0.2	62.8±0.2	0.6771
P 1	62.2±0.3^{A}	61.1±0.3^{B}	62.7±0.3^{A}	0.0042
P 2	63.4±0.3^{A}	61.8±0.3^{B}	63.4±0.3^{A}	0.0045
P 3	62.9±0.2^{A}	61.0±0.2^{B}	63.0±0.2^{A}	0.0003
P 4	63.2±0.3^{A}	61.9±0.3^{B}	64.5±0.3^{A}	0.0012
P 5	63.8±0.1^{A}	61.9±0.1^{B}	63.9±0.1^{A}	0.0001
P 6	63.1±0.2^{A}	61.3±0.2^{C}	62.1±0.2^{B}	0.0001
a*				
I	10.6±0.2	10.7±0.2	10.7±0.2	0.9382
P 1	9.1±0.2^{B}	11.5±0.2^{A}	8.2±0.3^{C}	0.0000
P 2	8.7±0.2^{B}	11.5±0.2^{A}	7.3±0.2^{C}	0.0000
P 3	8.2±0.2^{B}	10.9±0.2^{A}	6.9±0.2^{C}	0.0001
P 4	8.1±0.2^{B}	10.8±0.2^{A}	6.4±0.2^{C}	0.0001
P 5	7.9±0.3^{B}	10.7±0.3^{A}	5.8±0.3^{C}	0.0001
P 6	7.5±0.3^{B}	10.4±0.3^{A}	8.2±0.3^{B}	0.0001
b*				
I	70.2±0.7	70.2±0.7	69.8±0.7	0.9157
P 1	69.0±0.7^{B}	73.7±0.7^{A}	66.9±0.7^{B}	0.0002
P 2	67.3±0.7^{B}	74.0±0.7^{A}	63.5±0.7^{C}	0.0001
P 3	64.9±0.7^{B}	70.2±0.7^{A}	61.8±0.7^{C}	0.0001
P 4	65.9±0.8^{B}	72.1±0.8^{A}	61.0±0.8^{C}	0.0001
P 5	66.7±0.8^{B}	74.4±0.8^{A}	60.4±0.8^{C}	0.0001
P 6	62.0±0.7^{B}	70.5±0.7^{A}	57.0±0.7^{C}	0.0001

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A\text{-}C}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05)

The fatty acid composition of egg yolk produced by Lohmann LSL-Lite White hens in the single-tier system is presented in Table 4.4.3. Oleic acid in the egg yolks was significantly decreased ($P \leq 0.05$) with the addition of hemp products and the 6% HO diet resulted in the yolk containing the least ($P \leq 0.05$) amount of oleic acid. Moreover, white hens fed 6% HO significantly increased ($P \leq 0.05$) the amount of saturated fatty acids (arachidic acid) and unsaturated fatty acids (linolenic acid, g-linolenic acid, homo-g-linolenic acid, homo-a-linolenic acid, EPA, clupanodonic acid and DHA). However, stearic acid was significantly higher ($P \leq 0.05$) in white hens fed 20% HP than in hens fed the control with the intermediate being hens fed 6% HO diet. Clupanodonic acid was significantly higher ($P \leq 0.05$) in white hens fed 6% HO than those fed the control with the 20% HP treatment group being the intermediate.

Table 4.4.3. Fatty acid content of egg yolks from Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Treatment			
	Control	**20% HP**	**6% HO**	**P-Value**
Crude fat (W/W%)	52.86±0.31	52.85±0.31	52.75±0.81	0.979
C14:0	0.24±0.01	0.25±0.01	0.26±0.01	0.498
Myristoleic (9c-14:1)	0.03±0.00	0.02±0.01	0.03±0.01	0.440
C15:0	0.05±0.01	0.05±0.00	0.05±0.00	0.100
C15:1n5	0.00±0.00	0.00±0.00	0.00±0.00	-
Palmitic (16:0)	23.66±0.28	23.587±0.23	23.54±0.83	0.960
Palmitoleic (9c-16:1)	1.01±0.29	0.91±0.27	0.93±0.30	0.964
Margaric (17:0)	0.21±0.00	0.22±0.00	0.22±0.01	0.053
10c-17:1	0.10±0.00	0.10±0.00	0.09±0.01	0.274
Stearic (18:0)	9.96±0.10^{B}	10.87±0.19^{A}	10.23±0.30^{AB}	0.039
Elaidic (9t-18:1)	0.10±0.00	0.09±0.00	0.09±0.00	0.178
Oleic (9c-18:1)	34.10±0.64^{A}	31.11±0.70^{B}	29.48±0.23^{B}	<0.001
Vaccenic (11c-18:1)	1.88±0.29	1.71±0.25	1.56±0.24	0.690
Linolelaidic (18:2t)	0.00±0.00	0.00±0.00	0.00±0.01	0.405
Linoleic (18:2n6)	21.51±0.33^{B}	24.03±0.70^{A}	23.74±0.60^{A}	0.022
Linolenic (18:3n3)	1.22±0.02^{C}	1.77±0.10^{B}	2.87±0.12^{A}	<0.001
g-Linolenic [C18:3n6]	0.13±0.01^{B}	0.14±0.01^{B}	0.21±0.01^{A}	<0.001
Stearidonic (18:4n3)	0.01±0.00	0.02±0.01	0.03±0.01	0.254
Arachidic (20:0)	0.03±0.00^{B}	0.04±0.00^{B}	0.05±0.00^{A}	<0.001
Gondoic (20:1n9)	0.12±0.04	0.10±0.04	0.10±0.04	0.911
C20:2	0.21±0.01	0.23±0.02	0.21±0.01	0.709
Homo-g-linolenic [C20:3n6]	0.19±0.01^{B}	0.21±0.01^{B}	0.28±0.03^{A}	0.013
Homo-a-linolenic (20:3n3)	0.03±0.00^{B}	0.03±0.00^{B}	0.06±0.01^{A}	0.003
Arachidonic [20:4n6]	1.85±0.03	1.88±0.05	1.86±0.03	0.826
3n-Arachidonic (20:4n3)	0.00±0.00	0.00±0.00	0.00±0.00	-
EPA (20:5n3)	0.02±0.00^{C}	0.03±0.00^{B}	0.05±0.00^{A}	<0.001
C21:0	0.02±0.01	0.02±0.01	0.01±0.00	0.435
Behenic (22:0)	0.02±0.00	0.02±0.00	0.02±0.00	-
Erucic [22:1n9]	0.00±0.00	0.00±0.01	0.00±0.01	0.622
C22:2n6	0.00±0.00	0.00±0.00	0.00±0.00	-
Adrenic [C22:4n6]	0.10±0.00	0.11±0.01	0.10±0.01	0.176
Clupanodonic (22:5n3)	0.08±0.01^{B}	0.10±0.01^{AB}	0.12±0.01^{A}	0.036
DHA (22:6n3)	1.18±0.03^{C}	1.25±0.02^{B}	1.41±0.02^{A}	<0.001
C23:0	0.01±0.00	0.01±0.00	0.01±0.01	0.698
Lignoceric (24:0)	0.01±0.00	0.02±0.00	0.02±0.00	0.274
Nervonic (24:1n9)	0.01±0.00	0.01±0.00	0.01±0.00	-

HP= hempseed presscake. HO = hempseed oil. Fatty acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis. $^{A\text{-}C}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

The vitamin E content of egg yolks and the cannabinoid content of egg yolks and breast tissue from Lohmann LSL-Lite White hens in the single-tier system are presented in Table 4.4.4. Vitamin E content in egg yolks was significantly higher ($P \leq 0.05$) from hens fed control and 20% HP diet than hens fed the 6% HO. No cannabinoids were detected in the eggs or breast tissue.

Table 4.4.4. Vitamin E content of egg yolks and cannabidiol content of egg yolks and breast tissue of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Vitamin E (mg/kg) (n=4)	110.2±5.7^{A}	109.5±3.9^{A}	85.3±1.7^{B}	0.003
Cannabinoid content of yolk (n=4)				
Cannabidiol (CBD) (μg/g)	0.00	0.00	0.00	-
Cannabidiolic acid (CBDA) (μg/g)	0.00	0.00	0.00	-
CBD Potency (%)	0.00	0.00	0.00	-
d9-Tetrahydrocannabinol (d9-THC) (μg/g)	0.00	0.00	0.00	-
Tetrahydrocannabinolic acid A (THCA-A) (μg/g)	0.00	0.00	0.00	-
THC Potency (%)	0.00	0.00	0.00	-
Cannabinoid content of breast (n=1)				
Cannabidiol (CBD) (μg/g)	0.00	0.00	0.00	-
Cannabidiolic acid (CBDA) (μg/g)	0.00	0.00	0.00	-
CBD Potency (%)	0.00	0.00	0.00	-
d9-Tetrahydrocannabinol (d9-THC) (μg/g)	0.00	0.00	0.00	-
Tetrahydrocannabinolic acid A (THCA-A) (μg/g)	0.00	0.00	0.00	-
THC Potency (%)	0.00	0.00	0.00	-

HP = hempseed presscake. HO = hempseed oil.
$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly ($P \leq 0.05$).

Table 4.4.5 shows the average feather scoring condition for 7 body regions on white hens reared in the single-tier system fed the control, 20% HP and 6% HO diets. Tail score was significantly better ($P \leq 0.05$) in white hens fed the control than those fed the 6% HO diet, and the tail score of white hens fed 20% HP was intermediate in periods 1 and 2. The back score was significantly better ($P \leq 0.05$) in the white hens fed the 20% HP than those fed the 6% HO diet with the intermediate being the control treatment group. All other body regions had no statistical difference ($P > 0.05$) among treatments. Table 4.4.6 shows the total body feather condition for Lohmann LSL-Lite White hens fed three treatment diets. Chi-square analysis showed no differences in total body feather condition among dietary treatments for white hens reared in a single-tier system. Dietary treatments did not affect ($P > 0.05$) the tonic immobility of Lohmann LSL-Lite White hens when reared in a single-tier system (Table 4.4.7).

Table 4.4.5. Average feather score (means ± standard error) for 7 body regions (head, neck, abdomen, breast, tail, back and wing) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Head				
I	2.0±0.1	1.9±0.1	2.1±0.1	0.5247
P 1	2.1±0.0	2.0±0.0	2.1±0.0	0.4208
P 2	2.1±0.0	2.1±0.0	2.1±0.0	0.6760
P 3	2.1±0.1	2.1±0.1	2.1±0.1	0.6531
P 4	2.2±0.1	2.2±0.1	2.2±0.1	0.9122
P 5	2.3±0.1	2.2±0.1	2.4±0.1	0.5076
P 6	2.2±0.1	2.5±0.1	2.6±0.1	0.0793
Neck				
I	2.5±0.1	2.6±0.1	2.6±0.1	0.7351
P 1	2.5±0.1	2.5±0.1	2.5±0.1	0.9106
P 2	2.5±0.1	2.5±0.1	2.5±0.1	0.9684
P 3	2.3±0.2	2.3±0.2	2.1±0.2	0.7558
P 4	2.3±0.1	2.4±0.1	2.2±0.1	0.6911
P 5	2.3±0.1	2.3±0.1	2.3±0.1	0.9295
P 6	2.2±0.1	2.4±0.1	2.3±0.1	0.3634
Abdomen				
I	3.0±0.05	3.1±0.05	2.9±0.05	0.0859
P 1	3.0±0.1	3.1±0.1	3.1±0.1	0.4959
P 2	3.1±0.04	3.1±0.04	3.1±0.04	1.000
P 3	3.3±0.1	3.7±0.1	3.5±0.1	0.1217
P 4	3.4±0.1	3.8±0.1	3.3±0.1	0.0585
P 5	3.6±0.1	3.6±0.1	3.6±0.1	0.9678
P 6	3.4±0.1	3.7±0.1	3.9±0.1	0.1326
Breast				
I	2.9±0.0	2.9±0.0	2.9±0.0	0.7674
P 1	3.0±0.0	3.0±0.0	3.0±0.0	0.5694
P 2	3.1±0.1	3.1±0.1	3.1±0.1	0.6932
P 3	3.0±0.0	2.9±0.0	2.9±0.0	0.3006
P 4	3.08±0.04	2.98±0.04	3.00±0.04	0.2243
P 5	3.03±0.03	3.03±0.03	3.00±0.03	0.7997
P 6	3.2±0.1	3.1±0.1	3.1±0.1	0.7289
Tail				
I	1.9±0.2	1.7±0.2	2.0±0.2	0.4696
P 1	1.5±0.1^{B}	1.5±0.1^{AB}	1.8±0.1^{A}	0.0361
P 2	1.2±0.1^{B}	1.3±0.1^{AB}	1.5±0.1^{A}	0.0358
P 3	1.2±0.1	1.3±0.1	1.3±0.1	0.8005
P 4	1.4±0.1	1.3±0.1	1.5±0.1	0.4418
P 5	1.4±0.1	1.4±0.1	1.4±0.1	0.8901
P 6	1.2±0.1	1.3±0.1	1.3±0.1	0.5354
Back				
I	1.5±0.2	1.3±0.2	1.3±0.2	0.6697
P 1	1.5±0.1^{AB}	1.2±0.1^{B}	1.7±0.1^{A}	0.0352
P 2	1.7±0.1	1.5±0.1	1.8±0.1	0.3020
P 3	1.6±0.1	1.3±0.1	1.7±0.1	0.0593
P 4	1.7±0.1	1.6±0.1	1.8±0.1	0.4693
P 5	1.9±0.1	1.7±0.1	1.8±0.1	0.4691

Table 4.4.5. Average feather score (means ± standard error) for 7 body regions (head, neck, abdomen, breast, tail, back and wing) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil, continued.

	Control	20% HP	6% HO	P-Value
Period 6	1.7±0.1	1.7±0.1	1.7±0.1	0.9432
Wing				
I	1.3±0.1	1.3±0.1	1.3±0.1	0.9841
P 1	1.2±0.1	1.1±0.1	1.2±0.1	0.8563
P 2	1.3±0.1	1.2±0.1	1.3±0.1	0.8582
P 3	1.3±0.1	1.1±0.1	1.3±0.1	0.6077
P 4	1.4±0.1	1.3±0.1	1.4±0.1	0.8633
P 5	1.4±0.1	1.5±0.1	1.4±0.1	0.6150
P 6	1.4±0.1	1.5±0.1	1.5±0.1	0.5580

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.4.6. Total body feather condition of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil, analyzed using chi-square.

Feather Score	Control	20% HP	6% HO	P-Value	Chi-square
I					
0	0	0	0	0.7796	6.4118
1	0	0	1		
2	33	38	31		
3	7	2	8		
4	0	0	0		
5	0	0	0		
P 1					
0	0	0	0	0.9941	2.2430
1	0	0	0		
2	38	35	34		
3	2	5	6		
4	0	0	0		
5	0	0	0		
P 2					
0	0	0	0	0.9992	1.4012
1	0	0	0		
2	38	36	35		
3	2	4	5		
4	0	0	0		
5	0	0	0		
P 3					
0	0	0	0	0.9397	4.1630
1	0	1	0		
2	37	34	34		
3	2	5	6		
4	0	0	0		
5	0	0	0		
P 4					
0	0	0	0	0.9980	1.7322
1	0	0	0		
2	31	33	36		
3	8	7	4		
4	0	0	0		
5	0	0	0		
P 5					
0	0	0	0	1.0000	0.0941
1	0	0	0		
2	32	33	32		
3	7	7	8		
4	0	0	0		
5	0	0	0		
P 6					
0	0	0	0	0.6195	8.0960
1	0	0	0		
2	35	28	25		
3	4	12	15		
4	0	0	0		
5	0	0	0		

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

Table 4.4.7. Tonic immobility (means ± standard error) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Duration (sec)				
I	69.9±26.9	132.3±26.9	71.4±26.9	0.2273
P 6	106.9±34.7	121.8±34.7	93.1±34.7	0.8461
Head movement latency				
I	35.8±19.6	35.8±19.6	34.8±19.6	0.9991
P 6	27.3±18.7	43.5±18.7	34.6±18.7	0.8308
Data below this row was not normalized by transformation				
Induction				
I	2.0± 0.3	2.0±0.3	2.0±0.3	0.7674
P 6	1.3±0.2	1.8±0.2	1.0±0.2	0.0751
Head movement count				
I	1.3±0.7	1.8±0.7	1.3±0.7	0.8345
P 6	1.0±2.8	1.8±2.8	6.0±2.8	0.3758

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.
Least-squared means±SEM (standard error of the mean) within rows.

In the single-tier system, white hens fed the 6% HO had significantly stronger (P≤0.05) tibia bones than hens fed 20% HP, with no statistical difference (P>0.05) from the control (Table 4.4.8). DM was significantly higher (P≤0.05) in tibias from hens fed 6% HO than hens fed 20% HP with the control treatment being intermediate. Zinc content was significantly higher (P≤0.05) in hens fed the control than hens fed 20% HP with no statistical difference from the 6% HO treatment group (Table 4.4.8). No differences (P>0.05) in body weight, liver weight, hepato-somatic index, percent fat and liver L* and a* were observed among the three dietary treatments. The liver of white hens had a significant higher (P≤0.05) b* score when fed the 20% HP, indicating the liver was more yellow than when hens were fed other dietary treatments (Table 4.4.9).

Table 4.4.8. Average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann LSL White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Body weight (g)	1902±46.7	1913±46.7	1902±46.7	0.9829
Tibia weight (g)	7.0±0.1	6.8±0.1	7.1±0.1	0.4324
Tibia length (mm)	117.7±0.7	116.8±0.7	117.8±0.7	0.5730
Tibia width (mm)	5.7±0.1	5.8±0.1	5.8±0.1	0.8231
Tibia breaking strength (g/force)	19970±1096.3^{AB}	16885±1096.3^{B}	21255±1096.3^{A}	0.0515
Dry matter (%)	86.1±0.3^{AB}	85.9±0.3^{B}	87.2±0.3^{A}	0.0366
Ash (%)	50.1±0.9	48.6±0.9	49.8±0.9	0.5370
Calcium (mg/g)	480.75±13.17	477.72±13.17	507.46±13.17	0.2652
Phosphorous (mg/g)	225.42±6.32	226.95±6.32	239.42±6.32	0.2786
Magnesium (mg/g)	6.70±0.19	6.53±0.19	6.83±0.19	0.5518
Zinc (mg/g)	0.69±0.02^{A}	0.60±0.02^{B}	0.68±0.02^{AB}	0.0266
Iron (mg/g)	0.30±0.02	0.30±0.02	0.30±0.02	0.9789
Potassium (mg/g)	5.42±0.17	5.84±0.17	5.54±0.17	0.2661
Sodium (mg/g)	13.02±0.55	14.29±0.55	14.59±0.55	0.1565
Sulphate (mg/g)	4.61±0.18	4.73±0.18	5.15±0.18	0.1430

HP = hempseed presscake. HO = hempseed oil.
Initial body weight (n=4) = 1922g. Initial tibia weight (n=4) =7.3g. Initial tibia length (n=4) = 118.0mm. Initial tibia width (n=4) =5.7mm. Initial tibia breaking strength (n=4) = 20321 g/force.
$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.4.9. Average liver analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a single-tier housing system, fed a hemp (*Cannabis sativa* L.) by product diet at an inclusion rate of 0% hemp, 20% hempseed presscake and 6% hempseed oil.

	Control	20% HP	6% HO	P-Value
Body weight (g)	1902±46.7	1913±46.7	1902±46.7	0.9829
Liver weight (g)	42.6±1.5	42.4±1.5	42.5±1.5	0.9940
Hepato-somatic index (%)	2.2±0.1	2.2±0.1	2.2±0.1	0.9346
Histological liver fat (%)	33.7±2.1	34.3±2.1	31.7±2.1	0.6692
Proximate liver fat (%)	25.05±2.1	29.6±2.1	24.1±2.1	0.1808
L*	40.5±0.6	40.1±0.6	39.2±0.6	0.3240
a*	12.5±0.4	13.3±0.4	12.1±0.4	0.1277
b*	21.5±0.9^{B}	27.0±0.9^{A}	19.0±0.9^{B}	0.0005

HP = hempseed presscake. HO = hempseed oil.
Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value. Initial body weight (n=4) = 1922g. Initial liver weight (n=4) =47.9g. Initial average (2 replicates) L* score (n=4) = 39.7. Initial average (2 replicates) a* score (n=4) = 13.3. Initial average (2 replicates) b* score = 19.9.
$^{A-B}$Least-squared means±SEM (standard error of the mean) within rows with different letters differ significantly (P≤0.05).

4.5: Comparison of strains and housing systems

Comparisons of production performance between Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier system showed that brown hens had a significantly higher (P≤0.05) body weight than white hens (Table 4.5.1). Hens reared in the conventional cage system also had significantly higher (P≤0.05) body weight than hens reared in the single-tier system (Table 4.5.1). Hens fed 10% HP had a significant increase (P≤0.05) in body weight for all periods with an interaction between treatment and strain in the initial period, period 3 and 6 (Table 4.5.2). Feed conversion was most improved (P≤0.05) in the white hens versus brown hens in all periods and in the single-tier system compared to the conventional cage system for periods 2 and 5 (Table 4.5.1). Period 6 had an interaction effect (P≤0.05) between treatment and strain. Hen day egg production was significantly higher (P≤0.05) in white hens than in brown hens. No significant (P>0.05) comparisons were made for the remaining production parameters.

Table 4.5.1. Comparison of average production performance (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
Average weight (g)									
I	2155.31±24.32^{A}	1896.49±13.42^{B}	13.69	<0.001	2053.70±17.00^{A}	1850.16±17.82^{B}	13.69	<0.001	-
P 1	2163.99±24.84^{A}	1915.70±13.13^{B}	13.52	<0.001	2053.96±17.59^{A}	1892.18±17.50^{B}	13.52	<0.001	-
P 2	2170.70±24.78^{A}	1935.13±13.00^{B}	13.28	<0.001	2065.94±17.27^{A}	1912.54±17.50^{B}	13.28	<0.001	-
P 3	2197.28±24.64^{A}	1942.84±13.14^{B}	13.55	<0.001	2085.47±17.34^{A}	1915.04±17.93^{B}	13.55	<0.001	-
P 4	2185.58±24.36^{A}	1932.80±13.53^{B}	13.67	<0.001	2073.79±17.39^{A}	1906.39±18.54^{B}	13.67	<0.001	-
P 5	2180.91±23.39^{A}	1932.51±13.30^{B}	13.36	<0.001	2074.43±16.93^{A}	1903.33±17.95^{B}	13.36	<0.001	-
P 6	2175.55±23.35^{A}	1947.20±12.76^{B}	12.84	<0.001	2078.23±16.37^{A}	1918.63±17.25^{B}	12.84	<0.001	-
Feed consumption (g/bird/day)									
P 1	114.97±1.29	116.14±0.97	0.77	0.467	115.24±0.92	117.19±1.32	0.77	0.293	-
P 2	109.21±1.47	109.19±1.07	0.86	0.991	109.70±1.00	107.53±1.67	0.86	0.293	-
P 3	103.01±1.66	104.17±0.97	0.87	0.519	103.22±1.02	105.40±1.62	0.87	0.295	-
P 4	104.13±1.71	103.05±0.97	0.88	0.558	103.93±1.04	101.91±1.58	0.88	0.338	-
P 5	102.83±2.02	102.00±1.18	1.05	0.706	103.02±1.20	99.98±2.19	1.05	0.227	-
P 6	100.38±1.95	99.51±1.14	1.02	0.681	100.55±1.18	97.48±1.90	1.02	0.206	-
Hen day egg production (%)									
P 1	89.29±1.75^{B}	94.31±0.98^{A}	0.95	0.009	91.96±1.18	93.75±1.23	0.95	0.436	-
P 2	92.11±1.13^{B}	95.48±0.86^{A}	0.72	0.020	93.63±0.86	96.04±1.03	0.72	0.156	-
P 3	90.23±0.93^{B}	94.91±0.93^{A}	0.74	0.001	92.62±0.89	94.77±1.15	0.74	0.224	-
P 4	90.80±1.12	93.32±1.01	0.77	0.114	92.54±0.90	91.70±1.54	0.77	0.649	-
P 5	87.82±1.56^{B}	92.41±0.94^{A}	0.88	0.010	90.22±1.06	92.06±1.49	0.88	0.385	-
P 6	85.36±2.39^{B}	90.63±1.23^{A}	1.23	0.036	88.54±1.47	88.8±2.22	1.23	0.923	-
Feed conversion									
P 1	2.11±0.05^{A}	1.92±0.02^{B}	0.03	<0.001	2.00±0.03	1.97±0.04	0.03	0.571	-
P 2	1.96±0.03^{A}	1.78±0.02^{B}	0.02	<0.001	1.88±0.02^{A}	1.76±0.03^{B}	0.02	0.016	-
P 3	1.86±0.03^{A}	1.69±0.02^{B}	0.02	<0.001	1.76±0.03	1.73±0.03	0.02	0.516	-
P 4	1.82±0.03^{A}	1.67±0.02^{B}	0.02	<0.001	1.74±0.02	1.70±0.03	0.02	0.476	-

Table 4.5.1. Comparison of average production performance (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products, continued.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
P 5	1.88 ± 0.03^{A}	1.68 ± 0.02^{B}	0.02	<0.001	1.78 ± 0.02^{A}	1.67 ± 0.02^{B}	0.02	0.026	-
P 6	1.89 ± 0.05^{A}	1.66 ± 0.02^{B}	0.03	<0.001	1.76±0.03	1.70±0.03	0.03	0.335	-

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.2. Comparison of average production performance (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing	Treatment *Strain
Average weight (g)									
I	1962.35±28.79^{B}	2079.45±38.61^{A}	1931.25±25.68^{B}	2051.23±41.39^{AB}	1950.54±24.69^{B}	13.69	0.005	0.484	0.032
P 1	1974.15±28.64^{AB}	2080.25±40.52^{A}	1959.54±24.36^{B}	2050.92±41.73^{AB}	1973.89±24.68^{B}	13.52	0.039	0.743	0.169
P 2	1996.91±28.33^{AB}	2106.69±41.38^{A}	1983.41±23.37^{B}	2055.38±40.03^{AB}	1972.83±24.19^{B}	13.28	0.024	0.755	0.104
P 3	2013.90±29.29^{AB}	2127.77±40.79^{A}	1988.57±24.26^{B}	2067.50±41.85^{AB}	1987.01±23.93^{B}	13.55	0.015	0.670	0.039
P 4	2011.29±28.83^{AB}	2116.67±42.74^{A}	1978.36±25.21^{B}	2064.60±37.93^{AB}	1964.82±24.96^{B}	13.67	0.008	0.905	0.109
P 5	2002.85±29.37^{B}	2123.63±39.35^{A}	1980.58±25.66^{B}	2062.00±38.21^{AB}	1965.22±22.25^{B}	13.36	0.005	0.715	0.064
P 6	2019.76±28.40^{AB}	2115.50±39.33^{A}	1990.47±22.97^{B}	2070.79±36.44^{AB}	1970.41±22.68^{B}	12.84	0.008	0.454	0.014
Feed consumption (g/bird/day)									
P 1	117.32±1.32^{AB}	117.72±1.46^{A}	119.48±1.64^{A}	109.77±1.04^{C}	112.89±1.24^{BC}	0.77	<0.001	0.043	0.392
P 2	111.38±1.42^{A}	110.61±2.17^{AB}	113.28±1.50^{A}	104.96±1.57^{B}	104.82±1.63^{B}	0.86	<0.001	0.401	0.650
P 3	104.72±1.39^{AB}	106.00±2.41^{A}	108.55±1.19^{A}	98.32±1.69^{C}	99.99±1.60^{BC}	0.87	<0.001	0.217	0.374
P 4	104.68±1.51^{AB}	105.56±2.73^{A}	107.33±1.58^{A}	100.21±1.38^{AB}	99.16±1.67^{B}	0.88	0.006	0.355	0.414
P 5	103.79±2.26^{AB}	105.24±2.97^{A}	108.31±1.14^{A}	97.30±1.16^{BC}	96.27±1.68^{C}	1.05	<0.001	0.012	0.343
P 6	102.59±1.85^{A}	100.89±2.57^{AB}	104.32±1.69^{A}	96.49±0.89^{AB}	94.16±2.27^{B}	1.02	0.002	0.179	0.386
Hen day egg production (%)									
P 1	94.17±0.85	93.04±2.62	91.96±2.25	91.61±2.23	91.07±2.64	0.954	0.845	0.596	0.096
P 2	95.33±1.04	94.20±2.45	92.92±2.10	94.11±1.41	94.35±1.07	0.716	0.867	0.838	0.906
P 3	93.67±1.17	92.68±2.28	92.02±2.12	94.64±1.52	92.92±1.30	0.741	0.863	0.490	0.329
P 4	94.17±1.47	91.43±2.24	91.31±2.02	93.93±1.81	91.1±1.20	0.771	0.548	0.208	0.172
P 5	93.43±1.46^{A}	92.32±2.29^{AB}	93.60±1.34^{A}	87.86±2.88^{AB}	85.65±1.25^{B}	0.882	0.003	0.060	0.087
P 6	92.87±0.99^{A}	92.05±3.18^{A}	91.70±1.57^{A}	88.97±2.03^{A}	78.70±2.96^{B}	1.231	<0.001	0.559	0.081
Feed conversion									
P 1	1.99±0.04	2.00±0.08	2.05±0.05	1.90±0.06	2.00±0.08	0.03	0.598	0.913	0.177
P 2	1.88±0.04^{AB}	1.88±0.06^{AB}	1.93±0.03^{A}	1.78±0.06^{AB}	1.77±0.04^{B}	0.02	0.045	0.987	0.830
P 3	1.77±0.04	1.80±0.07	1.82±0.04	1.67±0.06	1.71±0.04	0.02	0.181	0.821	0.128

Table 4.5.2. Comparison of average production performance (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing	Treatment *Strain
P 4	1.72±0.04^{AB}	1.83±0.06^{A}	1.82±0.03^{A}	1.63±0.05^{B}	1.66±0.04^{B}	0.02	0.004	0.776	0.348
P 5	1.73±0.04	1.76±0.06	1.81±0.04	1.73±0.07	1.74±0.04	0.02	0.664	0.841	0.081
P 6	1.66±0.04^{B}	1.72±0.08^{B}	1.75±0.03^{B}	1.66±0.05^{B}	1.92±0.07^{A}	0.03	0.004	0.579	0.004

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A\text{-}C}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

The comparison of Lohmann LSL-Lite White hens reared in the conventional cage and single-tier system, fed five treatment diets, showed a significantly higher ($P \leq 0.05$) body weight for hens in the conventional cage system (Table 4.5.3) and no effect ($P > 0.05$) of treatment on body weight (Table 4.5.4). Feed consumption was significantly higher ($P \leq 0.05$) in every period, except period 4 with white hens consuming the most feed in the control and 10% HP group for periods 1 and 2 and in the 20% HP group for periods 3 and 5. Feed consumption had a significant ($P \leq 0.05$) interaction between treatment and housing in period 5 (Table 4.5.4). Henday egg production for white hens was significantly higher ($P \leq 0.05$) in period 5 and 6 when they were fed the 10% HP diet with an interaction between treatment and housing in period 5 (Table 4.5.4). Feed conversion in white hens was significantly better ($P \leq 0.05$) when they were fed 3% HO (Table 4.5.4).

Table 4.5.3. Comparison of average production performance (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Cage	Floor	SEM	P-Value
Average weight (g)				
I	1952.08±19.00^{A}	1850.16±17.82^{B}	13.42	<0.001
P 1	1943.92±19.55	1892.18±17.50	13.13	0.050
P 2	1962.24±19.15	1912.54±17.50	13.00	0.057
P 3	1975.91±18.87^{A}	1915.04±17.93^{B}	13.14	0.021
P 4	1964.23±19.41^{A}	1906.39±18.54^{B}	13.53	0.033
P 5	1967.95±19.29^{A}	1903.33±17.95^{B}	13.30	0.015
P 6	1981.90±18.44^{A}	1918.63±17.25^{B}	12.76	0.013
Feed consumption (g/bird/day)				
P 1	115.52±1.34	117.19±1.32	0.97	0.411
P 2	110.19±1.38	107.53±1.67	1.07	0.237
P 3	103.44±1.21	105.40±1.62	0.97	0.336
P 4	103.74±1.24	101.91±1.58	0.97	0.370
P 5	103.22±1.33	99.98±2.19	1.18	0.189
P 6	100.73±1.39	97.48±1.90	1.14	0.171
Hen day egg production (%)				
P 1	94.64±1.40	93.75±1.23	0.98	0.666
P 2	95.14±1.25	96.04±1.03	0.86	0.620
P 3	95.00±1.34	94.77±1.15	0.93	0.906
P 4	94.29±1.32	91.70±1.54	1.01	0.223
P 5	92.63±1.24	92.06±1.49	0.94	0.778
P 6	91.71±1.45	88.82±2.22	1.23	0.264
Feed conversion				
P 1	1.90±0.03	1.97±0.04	0.02	0.163
P 2	1.80±0.03	1.76±0.03	0.02	0.387
P 3	1.67±0.03	1.73±0.03	0.02	0.227
P 4	1.66±0.03	1.70±0.03	0.02	0.285
P 5	1.68±0.03	1.67±0.03	0.02	0.742
P 6	1.64±0.03	1.70±0.03	0.02	0.133

I = Initial, P = Period. $^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.4. Comparison of average production performance (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
Average weight (g)								
I	1870.90±21.50	1958.80±45.91	1870.85±27.73	1913±44.43	1921.43±26.91	13.42	0.285	0.411
P 1	1893.98±21.67	1952.35±46.55	1901.95±25.58	1910.10±47.17	1940.82±26.85	13.13	0.601	0.565
P 2	1919.43±21.67	1977.70±46.55	1933.55±24.68	1917.50±46.37	1944.10±27.20	13.00	0.798	0.510
P 3	1927.83±21.89	1985.80±43.98	1930.82±25.82	1935.15±46.09	1957.85±27.25	13.14	0.749	0.567
P 4	1929.97±22.03	1968.65±45.88	1921.65±27.64	1941.90±42.38	1931.75±28.39	13.53	0.929	0.382
P 5	1920.95±23.90	1978.32±39.91	1922.03±28.52	1944.95±45.00	1935.53±24.80	13.30	0.828	0.628
P 6	1942.41±22.48	1967.89±39.68	1945.02±25.98	1942.80±43.17	1948.98±25.68	12.76	0.991	0.856
Feed consumption (g/bird/day)								
P 1	118.45±1.76^{A}	118.22±2.17^{A}	118.17±2.08^{AB}	110.08±1.49^{B}	113.81±1.63^{AB}	0.97	0.038	0.311
P 2	111.79±2.07^{A}	111.85±2.58^{A}	111.75±1.88^{AB}	105.38±1.86^{AB}	104.61±1.99^{B}	1.07	0.032	0.667
P 3	104.88±1.92^{AB}	104.20±1.81^{AB}	108.27±1.58^{A}	98.93±2.43^{B}	101.99±1.85^{AB}	0.97	0.037	0.172
P 4	103.55±2.10	104.05±1.88	105.74±2.13	101.11±1.70	100.34±1.97	0.97	0.348	0.256
P 5	101.79±3.10^{AB}	103.24±2.41^{AB}	107.74±1.54^{A}	98.47±1.18^{AB}	97.62±1.67^{B}	1.18	0.020	0.026
P 6	100.90±2.53	99.57±2.42	102.95±2.35	97.40±1.20	95.70±2.26	1.14	0.209	0.372
Hen day egg production (%)								
P 1	95.18±1.10	98.21±1.07	90.98±3.38	94.29±1.75	94.82±0.75	0.98	0.279	0.750
P 2	96.75±1.17	96.43±1.24	93.13±3.04	96.07±1.07	95.80±0.91	0.86	0.643	0.497
P 3	94.56±1.61	97.50±0.68	92.32±3.05	97.50±0.90	95.27±1.07	0.93	0.430	0.136
P 4	93.17±2.14	94.29±1.17	91.25±3.01	97.86±0.92	92.77±1.38	1.01	0.464	0.200
P 5	93.01±2.04^{A}	96.25±1.22^{A}	94.69±1.42^{A}	93.57±2.44^{A}	87.05±1.38^{B}	0.94	0.009	0.036
P 6	93.06±1.41^{A}	96.34±1.86^{A}	92.19±2.32^{A}	91.07±3.27^{AB}	83.57±2.44^{B}	1.23	0.007	0.585
Feed conversion								
P 1	1.93±0.03^{AB}	1.82±0.02^{B}	2.04±0.06^{A}	1.82±0.05^{B}	1.90±0.04^{AB}	0.02	0.023	0.664
P 2	1.81±0.03^{AB}	1.77±0.02^{AB}	1.89±0.04^{A}	1.68±0.03^{B}	1.72±0.04^{B}	0.02	0.003	0.708
P 3	1.71±0.04	1.63±0.03	1.79±0.05	1.57±0.07	1.67±0.04	0.02	0.052	0.798

Table 4.5.4. Comparison of average production performance (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
P 4	1.67±0.04^{AB}	1.69±0.02^{AB}	1.79±0.03^{A}	1.54±0.05^{B}	1.62±0.04^{B}	0.02	0.005	0.482
P 5	1.65±0.02^{AB}	1.61±0.04^{AB}	1.74±0.03^{A}	1.59±0.06^{B}	1.73±0.04^{AB}	0.02	0.031	0.733
P 6	1.59±0.03^{B}	1.54±0.05^{B}	1.69±0.03^{AB}	1.62±0.07^{B}	1.78±0.03^{A}	0.02	0.001	0.923

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

$^{A\text{-}B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.5 gives an overview of the comparison of egg quality between Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier system. Table 4.5.6 shows the effect of the five treatments on egg quality for the two strains of hens in the two housing systems. White hens produced a heavier ($P \leq 0.05$) egg than brown hens with no effect ($P > 0.05$) of the housing system or treatment on egg weight. Brown hens produced a significantly denser ($P \leq 0.05$) egg than white hens in all periods and eggs were significantly denser ($P \leq 0.05$) from hens reared in the conventional cage system for all periods after the initial period. There was no effect ($P > 0.05$) of treatment on egg specific gravity, except in period 3 where hens fed 3% HO had a significantly denser egg. However, there was a significant interaction ($P \leq 0.05$) between treatment and strain in period 5. Eggs were significantly stronger ($P \leq 0.05$) in all periods except period 3 and 4 with eggs from brown hens having a stronger breaking strength than eggs from white hens and eggs from conventional cage system having a stronger breaking strength ($P \leq 0.05$) than eggs from the single-tier system in periods 1, 2 and 6. Treatment had a significant ($P \leq 0.05$) effect on egg breaking strength in period 5 with hens consuming 10% HP, 20% HP and 3% HO having the highest ($P \leq 0.05$) egg breaking strength compared to the control and hens fed 6% HO were the intermediate. In period 6, egg breaking strength was strongest ($P \leq 0.05$) in hens fed 20% HP and 3% HO and intermediate in hens fed 10% HP and 6% HO.

Albumen height was highest ($P \leq 0.05$) in eggs from white hens in periods 1 to 5. In the initial period and 3, albumen height was significantly higher ($P \leq 0.05$) in eggs from hens reared in the conventional cage system. In periods 4 and 5, albumen height was significantly higher ($P \leq 0.05$) in egg from hens reared in the single-tier system. Treatment had a significant effect (P≤0.05) on albumen height in period 5 with hens fed 20% HP, 3% HO and 6% HO and having the highest (P≤0.05) albumen height and the control was intermediate. Shell weight was significantly higher (P≤0.05) in period 1 and 4 for eggs from white hens compared to brown hens. In period 5, shell weight was highest (P≤0.05) when hens were fed the control, 20% HP, and 3% HO diets, lowest in eggs from hens fed 6% HO and intermediate in hens fed 10% HP. In periods 1, 2, 3 and 6, eggs from white hens had a significantly thicker (P≤0.05) eggshell compared to eggs from brown hens. Eggshells were significantly thicker (P≤0.05) in eggs produced from hens reared in the single-tier system for all periods except the initial period and period 4. Treatment had significant effect (P≤0.05) on eggshell thickness in all periods except period 2, 4 and 6. Hens fed 3% HO had eggs with significantly thicker (P≤0.05) eggshells in the initial period. For period 1, eggshell thickness significantly increased (P≤0.05) in hens fed the control, 20% HP and 6% HO diets, was intermediate in hens fed 3% HO and lowest in hens fed 10% HP. Hens fed the control and 6% HO diet produced an egg with a significantly thicker eggshell in period 3 and in hens fed the control in period 5. There was a significant interaction (P≤0.05) between treatment and housing in the initial period and periods 1, 3 and 5 and a significant interaction (P≤0.05) between treatment and strain in periods 1 and 3.

Egg yolks were significantly lighter (P≤0.05) in eggs produced by white hens in all periods and egg yolk lightness was highest (P≤0.05) in eggs from hens reared in single-tier system for all periods except periods 5 and 6. Egg yolk lightness was generally significantly lighter (P≤0.05) in eggs from hens fed the control, 3% HO and 6% HO diets. In period 2, egg yolk lightness had a significant interaction (P≤0.05) between treatment and housing and treatment and strain. Egg yolk red pigmentation was significantly increased ($P \le 0.05$) in the initial period and periods 1, 2, 5 and 6 for eggs from brown hens. Egg yolks had increased ($P \le 0.05$) red pigmentation for eggs produced by hens reared in the conventional cage system in the initial period and periods 3, 5 and 6. Treatment had a significant effect on egg yolk red pigmentation with the highest ($P \le 0.05$) color score generally found in egg yolks from hens fed 10% HP and 20% HP. Egg yolk red pigmentation had a significant interaction ($P \le 0.05$) between treatment and housing in periods 2, 4 and 5 and a significant interaction ($P \le 0.05$) between treatment and strain in period 2. Egg yolk yellow pigmentation was significantly higher ($P \le 0.05$) in brown in hens for the initial period and periods 1, 5 and 6. Eggs from hens reared in the conventional cage system had a significantly higher yellow pigmentation of the egg yolk in the initial period and periods 3, 5 and 6. Treatment had a significant effect on egg yolk yellow pigmentation with hens fed 10% HP and 20% HP generally having the highest score.

Table 4.5.5. Comparison of average egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
Egg weight (g)									
I	61.42±0.49^{B}	64.02±0.33^{A}	0.29	<0.001	63.04±0.39	63.67±0.42	0.29	0.273	-
P 1	60.94±0.50^{B}	63.96±0.33^{A}	0.29	<0.001	62.78±0.39	63.61±0.43	0.29	0.153	-
P 2	61.47±0.48^{B}	64.80±0.31^{A}	0.28	<0.001	63.53±0.39	64.39±0.38	0.28	0.121	-
P 3	62.82±0.57^{B}	65.87±0.35^{A}	0.31	<0.001	64.80±0.42	65.37±0.45	0.31	0.360	-
P 4	62.58±0.50^{B}	65.46±0.37^{A}	0.31	<0.001	64.36±0.40	65.04±0.48	0.31	0.276	-
P 5	62.89±0.61^{B}	65.76±0.37^{A}	0.33	<0.001	65.26±0.50	64.73±0.41	0.33	0.419	-
P 6	63.78±0.57	65.17±0.39	0.33	0.064	64.81±0.42	64.82±0.51	0.33	0.984	-
Egg specific gravity									
I	1.087±0.0007^{A}	1.084±0.0003^{B}	0.0003	<0.001	1.085±0.0005	1.084±0.0004	0.0003	0.053	-
P 1	1.088±0.0004^{A}	1.086±0.0003^{B}	0.0002	<0.001	1.087±0.0003^{A}	1.086±0.0003^{B}	0.0002	0.019	-
P 2	1.089±0.0005^{A}	1.085±0.0003^{B}	0.0003	<0.001	1.087±0.0004^{A}	1.085±0.0003^{B}	0.0003	<0.001	-
P 3	1.090±0.0006^{A}	1.086±0.0004^{B}	0.0004	<0.001	1.089±0.0004^{A}	1.085±0.0005^{B}	0.0004	<0.001	-
P 4	1.087±0.0006^{A}	1.084±0.0003^{B}	0.0003	<0.001	1.085±0.0004^{A}	1.084±0.0003^{B}	0.0003	0.015	-
P 5	1.087±0.0006^{A}	1.084±0.0002^{B}	0.0003	<0.001	1.085±0.0004^{A}	1.084±0.0003^{B}	0.0003	0.023	-
P 6	1.088±0.0006^{A}	1.086±0.0003^{B}	0.0003	0.005	1.087±0.0004^{A}	1.086±0.0004^{B}	0.0003	0.010	-
Egg breaking strength (g/force)									
I	5560.14±263.29^{A}	4799.03±155.44^{B}	135.38	0.014	5151.22±179.64	4819.00±203.56	135.38	0.221	-
P 1	5732.71±159.59^{A}	5192.17±85.68^{B}	78.23	0.002	5482.01±108.6^{A}	5152.93±105.60^{B}	78.23	0.039	-
P 2	5946.80±172.50^{A}	5064.97±88.39^{B}	83.07	<0.001	5630.05±119.43^{A}	4932.97±105.39^{B}	83.07	<0.001	-
P 3	5251.13±187.17	4969.38±91.95	84.13	0.138	5189.56±122.18	4877.87±112.24	84.13	0.065	-
P 4	5492.17±176.85	5192.86±95.00	84.31	0.118	5220.15±127.68	5326.85±108.88	84.31	0.528	-
P 5	5263.92±167.45^{A}	4756.95±82.55^{B}	76.42	0.003	5009.21±115.46	4755.87±96.06	76.42	0.098	-
P 6	5588.68±143.60^{A}	4767.09±88.02^{B}	78.90	<0.001	5192.16±99.00^{A}	4715.99±118.29^{B}	78.90	0.002	-
Albumen height (mm)									
I	6.12±0.14^{B}	6.64±0.07^{A}	0.06	<0.001	6.67±0.10^{A}	6.33±0.08^{B}	0.06	0.008	-

Table 4.5.5. Comparison of average egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
P 1	5.82±0.16^{B}	6.98±0.08^{A}	0.08	<0.001	6.63±0.12	6.74±0.09	0.08	0.448	-
P 2	5.91±0.16^{B}	6.65±0.07^{A}	0.07	<0.001	6.46±0.11	6.46±0.09	0.07	0.977	-
P 3	5.98±0.16^{B}	6.57±0.07^{A}	0.07	<0.001	6.59±0.11^{A}	6.21±0.07^{B}	0.07	0.006	-
P 4	5.32±0.12^{B}	6.06±0.06^{A}	0.06	<0.001	5.70±0.09^{B}	6.05±0.08^{A}	0.06	0.003	-
P 5	5.68±0.17^{B}	6.52±0.07^{A}	0.07	<0.001	6.13±0.11^{B}	6.49±0.08^{A}	0.07	0.009	-
P 6	6.03±0.16	6.95±0.36	0.27	0.139	6.47±0.10	6.98±0.56	0.27	0.349	-
Shell weight (g)									
I	6.27±0.08	6.39±0.04	0.04	0.132	6.34±0.05	6.38±0.05	0.04	0.581	-
P 1	5.97±0.10^{B}	6.24±0.04^{A}	0.04	0.002	6.14±0.06	6.20±0.05	0.04	0.439	-
P 2	6.17±0.06	6.27±0.03	0.03	0.104	6.23±0.04	6.26±0.04	0.03	0.602	-
P 3	6.17±0.08	6.31±0.04	0.03	0.067	6.28±0.05	6.27±0.04	0.03	0.811	-
P 4	6.18±0.07^{B}	6.33±0.04^{A}	0.03	0.044	6.26±0.05	6.33±0.05	0.03	0.298	-
P 5	6.10±0.10	6.25±0.04	0.04	0.074	6.27±0.06	6.15±0.04	0.04	0.120	-
P 6	6.23±0.08	6.21±0.04	0.04	0.852	6.24±0.05	6.19±0.05	0.04	0.419	-
Shell thickness (mm)									
I	0.503±0.005	0.494±0.002	0.002	0.053	0.499±0.003	0.493±0.003	0.002	0.140	-
P 1	0.422±0.005^{B}	0.447±0.003^{A}	0.003	<0.001	0.420±0.003^{B}	0.462±0.003^{A}	0.003	<0.001	-
P 2	0.439±0.006^{B}	0.451±0.003^{A}	0.003	0.043	0.436±0.004^{B}	0.461±0.002^{A}	0.003	<0.001	-
P 3	0.419±0.005^{B}	0.445±0.003^{A}	0.003	<0.001	0.416±0.003^{B}	0.463±0.003^{A}	0.003	<0.001	-
P 4	0.406±0.005	0.399±0.005	0.004	0.425	0.399±0.003	0.404±0.007	0.004	0.488	-
P 5	0.413±0.007	0.416±0.004	0.004	0.653	0.406±0.005^{B}	0.426±0.005^{A}	0.004	0.005	-
P 6	0.425±0.006^{B}	0.438±0.003^{A}	0.003	0.043	0.429±0.004^{B}	0.441±0.004^{A}	0.003	0.029	-
L*									
I	61.57±0.23^{B}	62.84±0.10^{A}	0.10	<0.001	62.11±0.15^{B}	62.94±0.11^{A}	0.10	<0.001	-
P 1	60.53±0.20^{B}	62.04±0.12^{A}	0.11	<0.001	61.28±0.16^{B}	62.07±0.15^{A}	0.11	<0.001	-
P 2	61.12±0.24^{B}	62.73±0.14^{A}	0.13	<0.001	61.82±0.18^{B}	62.86±0.19^{A}	0.13	<0.001	-
P 3	61.21±0.28^{B}	62.28±0.13^{A}	0.12	<0.001	61.78±0.17^{B}	62.29±0.17^{A}	0.12	0.034	-

Table 4.5.5. Comparison of average egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
P 4	61.17±0.25^{B}	63.15±0.16^{A}	0.15	<0.001	62.11±0.20^{B}	63.20±0.20^{A}	0.15	<0.001	-
P 5	62.57±0.20^{B}	63.46±0.14^{A}	0.12	<0.001	63.29±0.16	63.14±0.17	0.12	0.512	-
P 6	61.06±0.22^{B}	62.19±0.13^{A}	0.11	<0.001	61.71±0.17	62.13±0.15	0.11	0.069	-
a*									
I	12.00±0.21^{A}	10.67±0.10^{B}	0.10	<0.001	11.37±0.14^{A}	10.63±0.13^{B}	0.10	<0.001	-
P 1	10.55±0.29^{A}	9.54±0.15^{B}	0.14	0.001	10.02±0.19	9.56±0.20	0.14	0.095	-
P 2	10.06±0.31^{A}	9.21±0.18^{B}	0.16	0.019	9.63±0.22	9.19±0.22	0.16	0.161	-
P 3	9.41±0.34	8.83±0.17	0.15	0.098	9.27±0.20^{A}	8.63±0.22^{B}	0.15	0.035	-
P 4	9.13±0.34	8.43±0.19	0.17	0.069	8.83±0.25	8.38±0.22	0.17	0.188	-
P 5	9.60±0.35^{A}	8.43±0.19^{B}	0.17	0.002	9.19±0.24^{A}	8.23±0.24^{B}	0.17	0.005	-
P 6	9.84±0.26^{A}	8.86±0.15^{B}	0.13	<0.001	9.48±0.18^{A}	8.69±0.18^{B}	0.13	0.002	-
b*									
I	73.66±0.56^{A}	70.54±0.30^{B}	0.28	<0.001	72.60±0.40^{A}	69.98±0.35^{B}	0.28	<0.001	-
P1	71.26±0.72^{A}	69.61±0.42^{B}	0.36	0.048	70.32±0.51	69.72±0.52	0.36	0.407	-
P2	68.52±0.73	68.42±0.54	0.45	0.926	68.46±0.63	68.43±0.63	0.45	0.978	-
P3	66.84±0.92	66.44±0.45	0.41	0.677	67.45±0.57^{A}	65.53±0.57^{B}	0.41	0.019	-
P4	68.50±1.01	66.75±0.59	0.51	0.132	68.09±0.74	66.27±0.69	0.51	0.074	-
P5	72.04±0.97^{A}	68.64±0.61^{B}	0.53	0.004	71.42±0.71^{A}	67.41±0.73^{B}	0.53	<0.001	-
P6	68.59±0.76^{A}	64.81±0.57^{B}	0.48	<0.001	67.97±0.58^{A}	63.28±0.71^{B}	0.48	<0.001	-

I = Initial, P = Period.

Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.6. Comparison of average egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% H0	SEM	P-Value	Treatment *Housing	Treatment *Strain
Egg weight (g)									
I	63.35±0.58	63.79±0.95	63.64±0.59	63.35±0.80	62.92±0.52	0.29	0.892	0.467	0.273
P 1	63.01±0.59	63.77±0.87	63.03±0.60	62.85±0.93	63.39±0.52	0.29	0.929	0.043	0.139
P 2	63.86±0.55	63.88±0.80	64.93±0.55	61.90±0.79	63.85±0.52	0.28	0.053	0.564	0.303
P 3	65.74±0.68	64.25±0.84	64.26±0.62	64.56±0.87	65.68±0.55	0.31	0.281	0.327	0.501
P 4	64.89±0.55	64.91±0.85	64.45±0.80	64.74±0.82	64.62±0.52	0.31	0.988	0.498	0.365
P 5	66.27±0.71	64.36±1.04	65.09±0.59	65.60±0.96	63.71±0.59	0.33	0.071	0.026	0.398
P 6	65.68±0.65	64.30±0.98	64.97±0.70	64.37±0.99	64.12±0.58	0.33	0.489	0.850	0.998
Egg specific gravity									
I	1.084±0.0005	1.083±0.0011	1.085±0.0006	1.086±0.0010	1.085±0.0007	0.0003	0.098	0.510	0.243
P 1	1.086±0.0004	1.087±0.0007	1.086±0.0005	1.088±0.0008	1.086±0.0005	0.0002	0.147	0.800	0.416
P 2	1.086±0.0004	1.087±0.0009	1.086±0.0005	1.087±0.0008	1.086±0.0005	0.0003	0.338	0.801	0.675
P 3	1.086±0.0008^{BC}	1.089±0.0009^{AB}	1.086±0.0008^{C}	1.090±0.0008^{A}	1.087±0.0006^{BC}	0.0004	0.005	0.586	0.902
P 4	1.085±0.0005	1.085±0.0009	1.084±0.0005	1.086±0.0010	1.084±0.0005	0.0003	0.301	0.819	0.138
P 5	1.085±0.0005	1.085±0.0008	1.085±0.0005	1.085±0.0008	1.084±0.0005	0.0003	0.273	0.856	0.039
P 6	1.086±0.0006	1.088±0.0006	1.086±0.0005	1.088±0.0009	1.086±0.0006	0.0003	0.093	0.177	0.007
Egg breaking strength (g/force)									
I	5025.94±254.54	5163.00±363.90	4852.46±325.21	5327.79±387.97	4882.54±240.79	135.38	0.867	0.198	0.128
P 1	5160.81±145.04	5368.31±129.61	5498.34±145.58	5419.56±359.39	5354.80±139.76	78.23	0.636	0.367	0.653
P 2	5182.72±139.60	5680.37±251.29	5266.33±135.74	5714.10±285.20	5115.74±191.60	83.07	0.158	0.769	0.149
P 3	4787.47±179.71	5151.85±212.53	5263.80±145.19	5217.75±278.92	4968.91±168.64	84.13	0.297	0.481	0.465
P 4	5323.64±166.60	5018.31±227.07	5300.92±186.62	5371.01±284.09	5251.51±142.68	84.31	0.873	0.390	0.372
P 5	4630.79±144.5^{B}	5171.72±240.17^{A}	5128.82±165.46^{A}	5134.02±145.86^{A}	4661.40±144.61^{AB}	76.42	0.032	0.114	0.611
P 6	4626.83±134.65^{B}	5097.77±216.54^{AB}	5243.65±157.08^{A}	5244.97±162.92^{A}	4780.56±178.49^{AB}	78.90	0.022	0.880	0.884
Albumen height (mm)									

Table 4.5.6. Comparison of average egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% H0	SEM	P-Value	Treatment *Housing	Treatment *Strain
I	6.58±0.11	6.52±0.24	6.55±0.13	6.55±0.17	6.38±0.13	0.06	0.825	0.481	0.037
P 1	6.60±0.15	6.60±0.29	6.73±0.11	6.81±0.34	6.72±0.14	0.08	0.926	0.886	0.025
P 2	6.31±0.14	6.47±0.25	6.59±0.11	6.69±0.23	6.39±0.16	0.07	0.504	0.287	0.041
P 3	6.22±0.15	6.84±0.21	6.51±0.12	6.53±0.22	6.30±0.14	0.07	0.122	0.379	0.186
P 4	5.73±0.11	5.68±0.21	6.06±0.10	5.72±0.20	5.95±0.12	0.06	0.183	0.941	0.211
P 5	6.25±0.14^{AB}	5.71±0.31^{B}	6.45±0.11^{A}	6.41±0.18^{A}	6.39±0.13^{A}	0.07	0.050	0.835	0.546
P 6	6.29±0.14	6.39±0.26	6.53±0.13	6.38±0.23	7.63±1.06	0.27	0.418	0.783	0.998
Shell weight (g)									
I	6.28±0.07	6.22±0.11	6.45±0.08	6.45±0.09	6.37±0.07	0.04	0.261	0.882	0.155
P 1	6.21±0.07	6.08±0.15	6.19±0.07	6.00±0.17	6.20±0.06	0.04	0.557	0.161	0.058
P 2	6.21±0.05	6.27±0.08	6.34±0.05	6.08±0.10	6.24±0.05	0.03	0.104	0.708	0.623
P 3	6.31±0.07	6.25±0.10	6.15±0.08	6.34±0.10	6.34±0.05	0.03	0.303	0.052	0.458
P 4	6.31±0.06	6.31±0.11	6.26±0.08	6.36±0.09	6.28±0.05	0.03	0.952	0.479	0.338
P 5	6.34±0.09^{A}	6.20±0.11^{AB}	6.26±0.06^{A}	6.35±0.12^{A}	5.99±0.07^{B}	0.04	0.009	0.040	0.100
P 6	6.29±0.06	6.27±0.09	6.23±0.07	6.28±0.12	6.06±0.08	0.04	0.170	0.052	0.031
Shell thickness (mm)									
I	0.488±0.004^{B}	0.490±0.007^{AB}	0.498±0.004^{AB}	0.511±0.005^{A}	0.499±0.004^{AB}	0.002	0.020	0.776	0.861
P 1	0.446±0.005^{A}	0.417±0.006^{B}	0.443±0.005^{A}	0.424±0.007^{AB}	0.448±0.004^{A}	0.003	0.002	0.020	0.030
P 2	0.450±0.004	0.446±0.014	0.447±0.004	0.445±0.008	0.451±0.004	0.003	0.938	0.180	0.041
P 3	0.446±0.005^{A}	0.419±0.008^{BC}	0.439±0.007^{AB}	0.415±0.006^{C}	0.445±0.004^{A}	0.003	0.002	0.011	0.003
P 4	0.413±0.009	0.395±0.008	0.397±0.009	0.404±0.008	0.395±0.005	0.004	0.391	0.929	0.994
P 5	0.428±0.007^{A}	0.413±0.012^{AB}	0.424±0.006^{AB}	0.397±0.008^{B}	0.402±0.007^{B}	0.004	0.021	0.223	0.079
P 6	0.442±0.005	0.438±0.007	0.435±0.006	0.435±0.009	0.425±0.006	0.003	0.315	0.436	0.070
L*									
I	62.68±0.17^{A}	61.54±0.40^{B}	62.68±0.15^{A}	62.22±0.31^{AB}	62.67±0.21^{A}	0.10	0.012	0.540	0.153
P 1	61.89±0.20^{A}	60.86±0.36^{B}	60.81±0.23^{B}	62.35±0.24^{A}	62.32±0.20^{A}	0.11	<0.001	0.921	0.701

Table 4.5.6. Comparison of average egg quality (means ± standard error) from period 0 to period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% H0	SEM	P-Value	Treatment *Housing	Treatment *Strain
P 2	62.97±0.26^{A}	60.71±0.48^{C}	61.84±0.24^{B}	62.32±0.40^{AB}	62.80±0.20^{A}	0.13	<0.001	0.038	0.039
P 3	62.73±0.19^{A}	61.36±0.27^{B}	60.57±0.21^{B}	62.41±0.36^{A}	62.78±0.20^{A}	0.12	<0.001	0.785	0.407
P 4	63.02±0.25^{AB}	62.13±0.39^{BC}	61.44±0.21^{B}	62.40±0.57^{BC}	63.70±0.28^{A}	0.15	<0.001	0.156	0.551
P 5	63.78±0.19^{A}	62.90±0.28^{B}	61.86±0.20^{C}	63.96±0.26^{A}	63.92±0.21^{A}	0.12	<0.001	0.870	0.795
P 6	62.76±0.19^{A}	61.22±0.31^{BC}	60.86±0.18^{C}	62.87±0.35^{A}	62.02±0.21^{B}	0.11	<0.001	0.378	0.142
a*									
I	10.83±0.19^{B}	11.84±0.31^{A}	11.05±0.19^{AB}	11.28±0.30^{AB}	10.76±0.19^{B}	0.10	0.032	0.360	0.893
P 1	9.36±0.22^{B}	10.95±0.31^{A}	11.69±0.21^{A}	8.75±0.31^{BC}	8.34±0.18^{C}	0.14	<0.001	0.759	0.925
P 2	9.08±0.23^{B}	10.88±0.54^{A}	10.75±0.27^{A}	8.82±0.55^{BC}	8.03±0.24^{C}	0.16	<0.001	<0.001	0.042
P 3	8.42±0.18^{B}	10.63±0.26^{A}	11.39±0.19^{A}	7.93±0.26^{B}	7.08±0.18^{C}	0.15	<0.001	0.708	0.530
P 4	8.34±0.20^{C}	9.85±0.26^{B}	11.26±0.19^{A}	7.73±0.54^{C}	6.23±0.20^{D}	0.17	<0.001	0.011	0.215
P 5	8.44±0.22^{B}	10.72±0.28^{A}	11.35±0.19^{A}	7.38±0.26^{C}	6.05±0.17^{D}	0.17	<0.001	0.046	0.180
P 6	8.18±0.19^{C}	9.97±0.21^{B}	11.01±0.20^{A}	7.33±0.25^{D}	8.43±0.20^{C}	0.13	<0.001	0.185	0.160
b*									
I	70.89±0.58^{AB}	73.44±0.96^{A}	71.20±0.52^{AB}	72.46±0.88^{AB}	70.71±0.48^{B}	0.28	0.049	0.993	0.393
P1	69.28±0.59^{B}	72.23±1.06^{A}	74.07±0.63^{A}	67.04±1.03^{C}	67.14±0.55^{C}	0.36	<0.001	0.993	0.973
P2	68.51±0.76^{ABC}	69.52±1.63^{AB}	71.45±0.82^{A}	66.90±1.66^{BC}	65.42±0.72^{C}	0.45	<0.001	<0.001	0.220
P3	65.96±0.52^{B}	72.42±0.77^{A}	70.98±0.76^{A}	63.95±0.91^{BC}	61.88±0.53^{C}	0.41	<0.001	0.343	0.973
P4	66.92±0.70^{B}	72.75±1.03^{A}	73.57±0.77^{A}	63.74±1.28^{BC}	60.68±0.76^{C}	0.51	<0.001	0.076	0.648
P5	68.32±0.66^{B}	76.83±0.70^{A}	76.68±0.66^{A}	65.71±1.13^{B}	61.80±0.63^{C}	0.53	<0.001	0.308	0.404
P6	64.64±0.69^{B}	72.57±0.81^{A}	71.80±0.56^{A}	63.38±0.68^{B}	58.63±0.70^{C}	0.48	<0.001	0.137	0.031

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A-D}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Tables 4.5.7 and Table 4.5.8 give an overview of egg quality comparison between Lohmann LSL-Lite White hens fed dietary hemp and reared in a conventional cage and single-tiered system. Egg weight increased significantly ($P \leq 0.05$) in period 5 for eggs laid by white hens in the conventional cage system. Treatment had no effect ($P > 0.05$) on egg weight but there was a significant interaction ($P \leq 0.05$) in period 5 between treatment and housing. Egg density was highest ($P \leq 0.05$) in period 3 when eggs were produced by white hens in the conventional cage system. Treatment had no effect ($P > 0.05$) on egg specific gravity.

Eggs had a significantly stronger ($P \leq 0.05$) breaking strength when laid by white hens reared in the conventional cage system in period 2. In period 6, egg breaking strength was strongest ($P \leq 0.05$) in white hens fed 20% HP with 10% HP, 3% HO and 6% HO being the intermediate. In the initial period and periods 1, 2 and 3, egg albumen height was highest ($P \leq 0.05$) in eggs produced by white hens reared in the conventional cage system. In period 1, egg albumen height was significantly higher ($P \leq 0.05$) in white hens fed 3% HO and the intermediate was 10% HP. In period 3, hens fed 10% HP had the highest ($P \leq 0.05$) albumen height with 3% HO being the intermediate. Egg shell weight has significantly increased ($P \leq 0.05$) for eggs produced by white hens reared in the conventional cage system in period 5. Eggs produced by white hens reared in single-tier system had significantly thicker ($P \leq 0.05$) eggshells in periods 1, 2, 3 and 5. Eggshells were thickest ($P \leq 0.05$) from hens fed the control, 20% HP and 6% HO in periods 1 and 3. In period 5, eggshell thickness was highest ($P \leq 0.05$) in eggs produced by hens fed the control with the intermediate being 10% HP, 20% HP and 6% HO. In period 1, there was a significant interaction ($P \leq 0.05$) for eggshell thickness between treatment and housing.

Egg yolk lightness was highest ($P \leq 0.05$) in eggs produced by white hens reared in the conventional cage system in period 5. Generally, egg yolk lightness was lighter ($P \leq 0.05$) in eggs from hens fed the control, 3% HO and 6% HO diets in periods 1 to 6. In periods 2 and 6, egg yolk lightness had a significant interaction ($P \leq 0.05$) between the treatment and housing system. Egg yolk red pigmentation was generally higher ($P \leq 0.05$) in eggs produced by white hens fed 20% HP with hens fed 6% HO having the least red pigmentation in the egg yolk. In periods 2 and 4, egg yolk red pigmentation had a significant ($P \leq 0.05$) interaction between treatment and housing. Egg yolk yellow pigmentation was significantly increased ($P \leq 0.05$) in eggs produced by white hens reared in the conventional cage system in the initial period and periods 3, 5 and 6. Generally, egg yolk yellow pigmentation was significantly increased ($P \leq 0.05$) in egg produced by white hens fed 10% HP and 20% HP with hens fed 6% HO having the least yellow pigmentation.

Table 4.5.7. Comparison of average egg quality (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Cage	Floor	SEM	P-Value
Egg weight (g)				
I	64.67±0.53	63.67±0.42	0.33	0.156
P 1	64.59±0.51	63.61±0.43	0.33	0.156
P 2	65.56±0.49	64.39±0.38	0.31	0.067
P 3	66.75±0.51	65.37±0.45	0.35	0.054
P 4	66.30±0.51	65.04±0.48	0.37	0.106
P 5	67.55±0.67^{A}	64.73±0.41^{B}	0.37	<0.001
P 6	65.82±0.60	64.82±0.51	0.39	0.227
Egg specific gravity				
I	1.084±0.0006	1.084±0.0004	0.0003	0.833
P 1	1.085±0.0004	1.086±0.0003	0.0003	0.674
P 2	1.085±0.0005	1.085±0.0003	0.0003	0.772
P 3	1.088±0.0006^{A}	1.085±0.0005^{B}	0.0004	<0.001
P 4	1.083±0.0004	1.084±0.0003	0.0003	0.263
P 5	1.084±0.0003	1.084±0.0003	0.0002	0.461
P 6	1.087±0.0005	1.086±0.0004	0.0003	0.202
Egg breaking strength (g/force)				
I	4762.75±236.57	4819.00±203.56	155.44	0.863
P 1	5243.84±142.45	5152.93±105.60	85.68	0.601
P 2	5313.30±155.98^{A}	4932.97±105.39^{B}	88.39	0.040
P 3	5127.99±158.29	4877.87±112.24	91.95	0.191
P 4	4938.75±178.49	5326.85±108.88	95.00	0.052
P 5	4758.83±153.63	4755.87±96.06	82.55	0.986
P 6	4864.01±121.47	4715.99±118.29	88.02	0.426
Albumen height (mm)				
I	7.23±0.08^{A}	6.33±0.08^{B}	0.07	<0.001
P 1	7.41±0.11^{A}	6.74±0.09^{B}	0.08	<0.001
P 2	7.00±0.12^{A}	6.46±0.09^{B}	0.07	<0.001
P 3	7.20±0.12^{A}	6.21±0.07^{B}	0.07	<0.001
P 4	6.09±0.11	6.05±0.08	0.06	0.760
P 5	6.55±0.13	6.49±0.08	0.07	0.685
P 6	6.90±0.10	6.98±0.56	0.36	0.911
Shell weight (g)				
I	6.41±0.06	6.38±0.05	0.04	0.695
P 1	6.30±0.06	6.20±0.05	0.04	0.168
P 2	6.29±0.06	6.26±0.04	0.03	0.621
P 3	6.39±0.06	6.27±0.04	0.04	0.090
P 4	6.34±0.05	6.33±0.05	0.04	0.858
P 5	6.44±0.07^{A}	6.15±0.04^{B}	0.04	<0.001
P 6	6.26±0.06	6.19±0.05	0.04	0.360
Shell thickness (mm)				
I	0.495±0.003	0.493±0.003	0.002	0.622
P 1	0.419±0.005^{B}	0.462±0.003^{A}	0.003	<0.001
P 2	0.433±0.006^{B}	0.461±0.002^{A}	0.003	<0.001

Table 4.5.7. Comparison of average egg quality (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products, continued.

	Cage	Floor	SEM	P-Value
P 3	0.412±0.004^{B}	0.463±0.003^{A}	0.003	<0.001
P 4	0.390±0.004	0.404±0.007	0.005	0.187
P 5	0.399±0.006^{B}	0.426±0.005^{A}	0.004	0.001
P 6	0.433±0.005	0.441±0.004	0.003	0.169
L*				
I	62.65±0.18	62.94±0.11	0.10	0.148
P 1	61.99±0.21	62.07±0.15	0.12	0.762
P 2	62.49±0.22	62.86±0.19	0.14	0.227
P 3	62.25±0.19	62.29±0.17	0.13	0.871
P 4	63.07±0.27	63.20±0.20	0.16	0.693
P 5	64.02±0.20^{A}	63.14±0.17^{B}	0.14	0.002
P 6	62.31±0.23	62.13±0.15	0.13	0.495
a*				
I	10.76±0.15	10.63±0.13	0.10	0.522
P 1	9.51±0.23	9.56±0.20	0.15	0.873
P 2	9.24±0.31	9.19±0.22	0.18	0.907
P 3	9.15±0.24	8.63±0.22	0.17	0.135
P 4	8.52±0.36	8.38±0.22	0.19	0.736
P 5	8.78±0.32	8.23±0.24	0.19	0.168
P 6	9.15±0.24	8.69±0.18	0.15	0.128
b*				
I	71.56±0.55^{A}	69.98±0.35^{B}	0.30	0.011
P1	69.43±0.69	69.72±0.52	0.42	0.738
P2	68.40±1.03	68.43±0.63	0.54	0.978
P3	67.95±0.71^{A}	65.53±0.57^{B}	0.45	0.009
P4	67.67±1.09	66.27±0.69	0.59	0.261
P5	70.80±1.05^{A}	67.41±0.73^{B}	0.61	0.007
P6	67.40±0.87^{A}	63.28±0.71^{B}	0.57	<0.001

I = Initial, P = Period.

Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.8. Comparison of average egg quality (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% H0	SEM	P-Value	Treatment* Housing
Egg weight (g)								
I	64.21±0.61	66.26±1.29	63.85±0.67	64.26±1.30	63.42±0.59	0.33	0.370	0.840
P 1	64.07±0.63	65.48±1.00	63.22±0.69	65.43±1.11	63.84±0.57	0.33	0.359	0.185
P 2	64.71±0.57	66.01±0.70	65.10±0.64	63.74±1.13	64.54±0.55	0.31	0.659	0.838
P 3	66.12±0.71	65.42±1.11	65.09±0.69	67.29±1.12	66.12±0.60	0.35	0.552	0.530
P 4	65.87±0.55	66.80±1.34	65.16±0.93	66.42±1.17	64.77±0.57	0.37	0.574	0.906
P 5	66.79±0.75	67.07±1.40	65.48±0.65	66.14±1.65	64.61±0.63	0.37	0.209	0.022
P 6	65.94±0.71	64.75±1.55	65.28±0.79	65.15±1.72	64.34±0.65	0.39	0.667	0.735
Egg specific gravity								
I	1.083±0.0006	1.083±0.0011	1.084±0.0006	1.084±0.0011	1.085±0.0007	0.0003	0.592	0.484
P 1	1.086±0.0004	1.085±0.0008	1.085±0.0005	1.086±0.0009	1.086±0.0006	0.0003	0.836	0.736
P 2	1.085±0.0005	1.085±0.0009	1.085±0.0006	1.085±0.0008	1.085±0.0005	0.0003	0.990	0.404
P 3	1.085±0.0009	1.088±0.0009	1.085±0.0008	1.088±0.0010	1.086±0.0006	0.0004	0.221	0.669
P 4	1.084±0.0005	1.083±0.0006	1.084±0.0006	1.083±0.0010	1.084±0.0005	0.0003	0.923	0.414
P 5	1.084±0.0004	1.083±0.0004	1.084±0.0005	1.083±0.0005	1.084±0.0005	0.0002	0.404	0.372
P 6	1.085±0.0006	1.087±0.0007	1.086±0.0006	1.087±0.0010	1.086±0.0006	0.0003	0.671	0.471
Egg breaking strength (g/force)								
I	4750.16±278.77	5235.49±541.52	4672.40±369.04	4394.53±629.33	4948.67±234.96	155.44	0.826	0.746
P 1	4936.03±143.13	5097.89±167.34	5367.95±157.82	5223.83±485.27	5308.87±161.27	85.68	0.366	0.768
P 2	5084.85±137.34	5494.12±349.92	5057.53±143.58	5611.38±328.38	4818.88±198.10	88.39	0.154	0.039
P 3	4682.63±164.97	5030.59±218.32	5345.27±143.17	4884.30±426.43	4890.72±191.20	91.95	0.109	0.834
P 4	5271.98±176.87	4445.13±228.45	5294.97±190.61	4993.00±477.65	5249.01±157.44	95.00	0.253	0.087
P 5	4493.42±148.78	4933.39±308.93	4973.58±165.28	5034.62±118.94	4645.61±161.22	82.55	0.162	0.022
P 6	$4446.71±147.19^{B}$	$4888.28±285.58^{AB}$	$5108.89±180.30^{A}$	$4972.50±199.84^{AB}$	$4637.40±173.52^{AB}$	88.02	0.050	0.937
Albumen height (mm)								
I	6.61±0.12	7.22±0.20	6.58±0.13	7.03±0.18	6.52±0.13	0.07	0.073	0.886
P 1	$6.83±0.15^{B}$	$7.43±0.25^{AB}$	$6.86±0.10^{B}$	$7.88±0.33^{A}$	$6.92±0.15^{B}$	0.08	0.005	0.324

Table 4.5.8. Comparison of average egg quality (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% H0	SEM	P-Value	Treatment* Housing
P 2	6.61±0.10	6.75±0.39	6.60±0.13	7.23±0.25	6.58±0.16	0.07	0.282	0.968
P 3	6.40±0.15^{B}	7.49±0.22^{A}	6.55±0.13^{B}	7.08±0.25^{AB}	6.41±0.11^{B}	0.07	<0.001	0.120
P 4	5.96±0.11	5.88±0.27	6.23±0.11	6.19±0.24	6.02±0.13	0.06	0.445	0.452
P 5	6.42±0.11	6.18±0.42	6.64±0.10	6.49±0.26	6.56±0.14	0.07	0.504	0.849
P 6	6.45±0.13	6.99±0.19	6.64±0.16	6.92±0.20	7.81±1.32	0.36	0.686	0.769
Shell weight (g)								
I	6.34±0.07	6.47±0.14	6.44±0.08	6.38±0.12	6.37±0.08	0.04	0.881	0.792
P 1	6.26±0.07	6.32±0.11	6.18±0.07	6.37±0.12	6.22±0.07	0.04	0.689	0.157
P 2	6.26±0.06	6.32±0.10	6.35±0.06	6.12±0.15	6.23±0.06	0.03	0.362	0.874
P 3	6.31±0.07	6.24±0.11	6.24±0.08	6.43±0.15	6.37±0.05	0.04	0.539	0.113
P 4	6.39±0.05	6.42±0.11	6.31±0.10	6.31±0.13	6.29±0.05	0.04	0.818	0.677
P 5	6.34±0.08	6.37±0.15	6.29±0.06	6.28±0.16	6.10±0.06	0.04	0.114	0.138
P 6	6.23±0.07	6.21±0.10	6.24±0.08	6.21±0.18	6.16±0.07	0.04	0.936	0.566
Shell thickness (mm)								
I	0.487±0.004	0.488±0.006	0.497±0.004	0.501±0.005	0.497±0.004	0.002	0.250	0.776
P 1	0.452±0.006^{A}	0.412±0.007^{B}	0.450±0.005^{A}	0.420±0.011^{B}	0.453±0.005^{A}	0.003	<0.001	0.001
P 2	0.452±0.005	0.430±0.013	0.452±0.005	0.455±0.014	0.455±0.004	0.003	0.287	0.098
P 3	0.449±0.005^{A}	0.414±0.009^{B}	0.449±0.007^{A}	0.409±0.009^{B}	0.453±0.004^{A}	0.003	<0.001	0.081
P 4	0.411±0.011	0.389±0.007	0.396±0.010	0.396±0.014	0.393±0.006	0.005	0.598	0.869
P 5	0.431±0.009^{A}	0.408±0.016^{AB}	0.420±0.006^{AB}	0.382±0.009^{B}	0.409±0.008^{AB}	0.004	0.030	0.797
P 6	0.442±0.005	0.434±0.007	0.439±0.006	0.439±0.012	0.434±0.005	0.003	0.909	0.708
L*								
I	62.79±0.18	62.71±0.40	62.90±0.15	62.81±0.35	62.87±0.20	0.10	0.987	0.281
P 1	62.24±0.18^{A}	61.81±0.43^{AB}	61.05±0.25^{B}	62.81±0.27^{A}	62.66±0.20^{A}	0.12	<0.001	0.836
P 2	63.13±0.30^{A}	62.15±0.62A^{B}	62.14±0.25^{B}	62.26±0.67^{AB}	63.19±0.20^{A}	0.14	0.017	0.002
P 3	62.89±0.17^{A}	62.05±0.37^{A}	60.85±0.19^{B}	63.05±0.36^{A}	62.89±0.22^{A}	0.13	<0.001	0.576

Table 4.5.8. Comparison of average egg quality (means ± standard error) from period 0 to period 6 for Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% H0	SEM	P-Value	Treatment* Housing
P 4	63.30±0.28^{A}	63.12±0.31^{AB}	61.75±0.22^{B}	63.72±0.83^{A}	64.18±0.26^{A}	0.16	<0.001	0.254
P 5	64.01±0.20^{A}	63.69±0.31^{A}	62.00±0.22^{B}	64.63±0.33^{A}	64.09±0.23^{A}	0.14	<0.001	0.664
P 6	63.04±0.19^{A}	62.20±0.22^{B}	60.95±0.20^{C}	63.77±0.40^{A}	62.22±0.21^{B}	0.13	<0.001	0.024
a*								
I	10.64±0.20	11.20±0.36	10.73±0.19	10.57±0.25	10.56±0.20	0.10	0.655	0.256
P 1	9.13±0.22^{B}	10.47±0.38^{A}	11.45±0.23^{A}	8.27±0.21^{BC}	8.22±0.20^{C}	0.15	<0.001	0.921
P 2	9.11±0.25^{B}	9.93±0.95^{AB}	10.56±0.31^{A}	9.29±0.86^{AB}	7.71±0.25^{C}	0.18	<0.001	<0.001
P 3	8.34±0.19^{C}	10.08±0.26^{B}	11.24±0.17^{A}	7.56±0.26^{CD}	7.06±0.21^{D}	0.17	<0.001	0.896
P 4	8.10±0.21^{B}	9.40±0.32^{B}	11.04±0.20^{A}	7.80±0.99^{B}	6.27±0.22^{C}	0.19	<0.001	0.045
P 5	8.09±0.22^{B}	10.32±0.31^{A}	10.99±0.19^{A}	7.05±0.32^{C}	5.92±0.19^{D}	0.19	<0.001	0.277
P 6	7.77±0.17^{CD}	9.55±0.26^{B}	10.74±0.20^{A}	7.00±0.31^{D}	8.29±0.23^{C}	0.15	<0.001	0.595
b*								
I	70.31±0.67	73.23±0.72	70.24±0.51	71.50±1.45	70.15±0.47	0.30	0.120	0.802
P1	68.97±0.65^{B}	71.38±1.64^{AB}	73.81±0.70^{A}	65.68±0.95^{C}	66.81±0.60^{C}	0.42	<0.001	0.974
P2	68.83±0.83^{A}	67.95±2.94^{AB}	71.80±0.94^{A}	67.59±3.10^{AB}	64.77±0.79^{B}	0.54	<0.001	<0.001
P3	65.87±0.56^{B}	72.03±0.98^{A}	71.05±0.75^{A}	63.85±1.14^{BC}	61.98±0.61^{C}	0.45	<0.001	0.343
P4	66.30±0.76^{B}	73.38±1.43^{A}	73.21±0.84^{A}	63.24±1.73^{BC}	60.52±0.85^{C}	0.59	<0.001	0.031
P5	67.45±0.65^{B}	76.82±0.81^{A}	75.79±0.70^{A}	64.28±1.78^{BC}	61.21±0.73^{C}	0.61	<0.001	0.368
P6	63.35±0.68^{B}	72.94±1.28^{A}	71.37±0.63^{A}	61.85±0.83^{B}	57.80±0.77^{C}	0.57	<0.001	0.712

I = Initial, P = Period. HP = hempseed presscake. HO = hempseed oil.

Egg yolk color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A-D}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

A comparison of egg yolk fatty acid composition for Lohmann Brown-Lite hens Lohmann LSL-Lite White hens fed dietary hemp and reared in a conventional cage and single-tier system is shown in Table 4.5.9 and Table 4.5.10. Saturated fatty acids, including C14:0, palmitic acid, stearic acid, arachidic acid, and lignoceric acid, were highest in egg yolks in eggs produced by white hens, while C15:0 was highest ($P \leq 0.05$) in egg yolks from eggs produced by brown hens (Table 4.5.9). Palmitic acid and stearic acid were significantly higher ($P \leq 0.05$) in egg yolks from eggs produced by hens reared in the single-tier system (Table 4.5.9). Stearic acid in egg yolks had a significant interaction ($P \leq 0.05$) between treatment and strain (Table 4.5.10). C14:0 was significantly increased ($P \leq 0.05$) in hens fed 3% HO, while 6% HO was the intermediate (Table 4.5.10). Margaric acid was highest ($P \leq 0.05$) in eggs from hens fed 10% HP with the control, 20% HP and 6% HO being the intermediate. Arachidic acid was highest ($P \leq 0.05$) in eggs from hens fed 6% HO (Table 4.5.10).

Monounsaturated fatty acids such as palmitoleic acid, oleic acid, gondoic acid, and erucic acid were highest ($P \leq 0.05$) in egg yolks from eggs produced by brown hens, while 10c-17:1, elaidic acid, and nervonic acid were highest ($P \leq 0.05$) in egg yolks from eggs produced by white hens (Table 4.5.9). Nervonic acid in egg yolks had a significant interaction ($P \leq 0.05$) between treatment and strain (Table 4.5.10). Myristoleic acid was highest in eggs from hens fed 3% HO and lowest in hens fed 10% HP (Table 4.5.10). Oleic acid decreased ($P \leq 0.05$) for every treatment group with 6% HO having the least (Table 4.5.10).

Polyunsaturated fatty acids, including LA, 3n-arachidonic acid, adrenic acid and clupanodonic acid, were highest ($P \leq 0.05$) in egg yolks from eggs produced by brown hens, while homo-g-linolenic acid and arachidonic acid were highest ($P \leq 0.05$) in egg yolks produced by white hens (Table 4.5.9). Homo-g-linolenic was significantly higher ($P \leq 0.05$) in egg yolks from eggs produced by hens reared in the single-tier system, while 3n-arachidonic acid and clupanodonic acid were highest ($P \leq 0.05$) in eggs yolks from eggs produced by hens reared in the conventional cage system (Table 4.5.9). PUFAs including LA, linolenic acid, g-linolenic acid, stearidonic acid, homo-g-linolenic acid, homo-a-linolenic acid, EPA, clupanodonic acid, and DHA was significantly increased ($P \leq 0.05$) in eggs from hens fed the 6% HO diet (Table 4.5.10). Linolelaidic acid, LA, and adrenic acid in eggs had significant interaction ($P \leq 0.05$) between treatment and strain (Table 4.5.10). Linolenic acid and EPA in eggs had a significant interaction ($P \leq 0.05$) between treatment and housing system (Table 4.5.10).

Table 4.5.9. Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
Crude fat (W/W%)	53.20±0.16^{A}	52.69±0.11^{B}	0.100	0.010	52.91±0.11	52.82±0.21	0.100	0.692	-
C14:0	0.241±0.007^{B}	0.256±0.003^{A}	0.003	0.035	0.250±0.004	0.251±0.006	0.003	0.921	-
Myristoleic (9c-14:1)	0.024±0.001	0.026±0.001	0.001	0.298	0.025±0.001	0.025±0.002	0.001	1.000	-
C15:0	0.054±0.002^{A}	0.048±0.001^{B}	0.001	0.003	0.051±0.001	0.048±0.001	0.001	0.342	-
C15:1n5	0.000	0.000	0.000	-	0.000	0.000	0.000	-	-
Palmitic (16:0)	22.08±0.24^{B}	23.64±0.10^{A}	0.15	<0.001	22.88±0.19^{B}	23.59±0.16^{A}	0.15	0.048	-
Palmitoleic (9c-16:1)	1.47±0.03^{A}	0.98±0.09^{B}	0.07	<0.001	1.23±0.07	0.95±0.15	0.07	0.069	-
Margaric (17:0)	0.215±0.004	0.215±0.007	0.004	0.972	0.215±0.005	0.216±0.003	0.004	0.896	-
10c-17:1	0.061±0.013^{B}	0.099±0.003^{A}	0.006	0.001	0.081±0.008	0.097±0.002	0.006	0.257	-
Stearic (18:0)	8.92±0.10^{B}	10.42±0.09^{A}	0.12	<0.001	9.70±0.14^{B}	10.35±0.16^{A}	0.12	0.023	-
Elaidic (9t-18:1)	0.088±0.002^{B}	0.122±0.011^{A}	0.007	0.017	0.114±0.009	0.092±0.002	0.007	0.181	-
Oleic (9c-18:1)	32.68±0.43^{A}	31.34±0.35^{B}	0.28	0.018	31.94±0.31	31.56±0.65	0.28	0.574	-
Vaccenic (11c-18:1)	1.90±0.13	1.71±0.08	0.07	0.192	1.80±0.08	1.72±0.14	0.07	0.624	-
Linolelaidic (18:2t)	0.000±0.000	0.013±0.006	0.004	0.079	0.010±0.005	0.001±0.001	0.004	0.281	-
Linoleic (18:2n6)	24.21±0.31^{A}	22.96±0.25^{B}	0.21	0.003	23.54±0.24	23.10±0.45	0.21	0.376	-
Linolenic (18:3n3)	2.21±0.17	1.95±0.12	0.10	0.208	2.08±0.12	1.95±0.21	0.10	0.595	-
g-Linolenic [C18:3n6]	0.160±0.008	0.163±0.007	0.005	0.781	0.163±0.006	0.160±0.011	0.005	0.847	-
Stearidonic (18:4n3)	0.026±0.004	0.022±0.003	0.002	0.500	0.025±0.003	0.018±0.004	0.002	0.238	-
Arachidic (20:0)	0.029±0.002^{B}	0.039±0.002^{A}	0.001	<0.001	0.034±0.002	0.039±0.003	0.001	0.114	-
Gondoic (20:1n9)	0.149±0.003^{A}	0.104±0.012^{B}	0.008	0.005	0.126±0.009	0.107±0.020	0.008	0.326	-
C20:2	0.198±0.006	0.210±0.004	0.003	0.055	0.202±0.003	0.217±0.007	0.003	0.052	-
Homo-g-linolenic [C20:3n6]	0.172±0.008^{B}	0.215±0.007^{A}	0.006	<0.001	0.190±0.006^{B}	0.225±0.014^{A}	0.006	0.014	-
Homo-a-linolenic (20:3n3)	0.035±0.003	0.038±0.003	0.002	0.458	0.036±0.002	0.038±0.005	0.002	0.580	-
Arachidonic [20:4n6]	1.764±0.028^{B}	1.866±0.011^{A}	0.014	<0.001	1.817±0.018	1.861±0.020	0.014	0.200	-
3n-Arachidonic (20:4n3)	0.010±0.000^{A}	0.000±0.000^{B}	0.001	<0.001	0.005±0.001^{A}	0.000±0.000^{B}	0.001	0.001	-

Table 4.5.9. Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products, continued.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing* Strain
EPA (20:5n3)	0.034±0.003	0.033±0.003	0.002	0.929	0.034±0.002	0.031±0.004	0.002	0.670	-
C21:0	0.016±0.001	0.016±0.002	0.001	0.981	0.017±0.001	0.014±0.002	0.001	0.432	-
Behenic (22:0)	0.023±0.004	0.027±0.003	0.002	0.368	0.027±0.003	0.020±0.000	0.002	0.210	-
Erucic [22:1n9]	0.005±0.001^{A}	0.002±0.001^{B}	0.001	0.043	0.003±0.001	0.002±0.001	0.001	0.298	-
C22:2n6	0.000	0.000	0.000	-	0.000	0.000	0.000	-	-
Adrenic [C22:4n6]	0.114±0.004^{A}	0.101±0.003^{B}	0.003	0.015	0.107±0.003	0.103±0.002	0.003	0.480	-
Clupanodonic (22:5n3)	0.154±0.008^{A}	0.107±0.004^{B}	0.005	<0.001	0.132±0.006^{A}	0.102±0.007^{B}	0.005	0.012	-
DHA (22:6n3)	1.366±0.049	1.329±0.025	0.024	0.471	1.36±0.030	1.280±0.031	0.024	0.157	-
C23:0	0.009±0.003	0.008±0.002	0.002	0.906	0.009±0.002	0.007±0.003	0.002	0.569	-
Lignoceric (24:0)	0.011±0.001^{B}	0.013±0.001^{A}	0.001	0.042	0.012±0.001	0.013±0.001	0.001	0.432	-
Nervonic (24:1n9)	0.007±0.001^{B}	0.010±0.000^{A}	0.000	<0.001	0.009±0.001	0.010±	0.000	0.160	-

Fatty acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.10. Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing	Treatment *Strain
Crude fat (W/W%)	52.89±0.20	53.12±0.23	52.98±0.17	52.75±0.23	53.00±0.25	0.100	0.528	0.494	0.781
C14:0	0.238±0.006^{B}	0.241±0.005^{B}	0.243±0.008^{B}	0.273±0.007^{A}	0.260±0.006 ^{AB}	0.003	0.008	0.881	0.289
Myristoleic (9c-14:1)	0.025±0.001^{BC}	0.021±0.001^{C}	0.022±0.001^{BC}	0.031±0.001^{A}	0.026±0.001^{B}	0.001	<0.001	0.625	0.599
C15:0	0.048±0.002	0.051±0.003	0.048±0.002	0.051±0.002	0.053±0.002	0.001	0.360	0.489	0.206
C15:1n5	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-
Palmitic (16:0)	22.97±0.30	22.57±0.42	23.05±0.27	23.79±0.40	22.92±0.34	0.15	0.259	0.964	0.163
Palmitoleic (9c-16:1)	1.24±0.13	1.04±0.15	1.01±0.13	1.34±0.21	1.23±0.14	0.07	0.500	0.765	0.868
Margaric (17:0)	0.206±0.004^{AB}	0.240±0.023^{A}	0.220±0.005^{AB}	0.196±0.005^{B}	0.214±0.003^{AB}	0.004	0.036	0.926	0.177
10c-17:1	0.097±0.008	0.091±0.022	0.096±0.008	0.065±0.017	0.069±0.012	0.006	0.258	0.616	0.183
Stearic (18:0)	9.57±0.20	9.50±0.18	9.88±0.30	9.63±0.36	10.47±0.23	0.12	0.060	0.992	0.044
Elaidic (9t-18:1)	0.112±0.013	0.140±0.034	0.103±0.011	0.115±0.017	0.088±0.002	0.007	0.266	0.739	0.372
Oleic (9c-18:1)	33.92±0.39^{A}	32.68±0.67^{AB}	31.58±0.39^{B}	31.96±0.42^{B}	29.45±0.25^{C}	0.28	<0.001	0.686	0.267
Vaccenic (11c-18:1)	1.94±0.17	1.96±0.17	1.93±0.14	1.64±0.16	1.44±0.13	0.07	0.058	0.511	0.483
Linolelaidic (18:2t)	0.001±0.001	0.025±0.017	0.001±0.001	0.023±0.014	0.001±0.001	0.004	0.056	0.980	0.021
Linoleic (18:2n6)	22.47±0.47^{C}	23.60±0.30^{ABC}	24.06±0.50^{AB}	22.54±0.29^{BC}	24.29±0.34^{A}	0.21	0.005	0.497	0.669
Linolenic (18:3n3)	1.32±0.05^{D}	1.63±0.05^{C}	1.78±0.06^{C}	2.23±0.06^{B}	3.2±0.10^{A}	0.10	<0.001	0.028	0.043
g-Linolenic [C18:3n6]	0.126±0.003^{D}	0.136±0.004^{CD}	0.148±0.005^{C}	0.174±0.005^{B}	0.221±0.006^{A}	0.005	<0.001	0.367	0.643
Stearidonic (18:4n3)	0.014±0.002^{B}	0.015±0.003^{B}	0.017±0.003^{B}	0.030±0.006^{AB}	0.041±0.006^{A}	0.002	<0.001	0.216	0.789

Table 4.5.10. Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing	Treatment *Strain
Arachidic (20:0)	0.028±0.001^{B}	0.031±0.005^{B}	0.033±0.002^{B}	0.033±0.003^{B}	0.048±0.002^{A}	0.001	<0.001	0.840	0.220
Gondoic (20:1n9)	0.143±0.016	0.113±0.023	0.103±0.018	0.128±0.018	0.120±0.015	0.008	0.542	0.816	0.717
C20:2	0.210±0.006	0.211±0.005	0.209±0.009	0.186±0.007	0.207±0.005	0.003	0.124	0.546	0.865
Homo-g-linolenic [C20:3n6]	0.169±0.007^{B}	0.175±0.008^{B}	0.186±0.009^{B}	0.201±0.012^{B}	0.253±0.011^{A}	0.006	<0.001	0.995	0.989
Homo-a-linolenic (20:3n3)	0.023±0.002^{D}	0.030±0.002^{CD}	0.031±0.002^{C}	0.040±0.002^{B}	0.057±0.003^{A}	0.002	<0.001	0.972	0.467
Arachidonic [20:4n6]	1.841±0.013	1.823±0.024	1.819±0.040	1.779±0.060	1.855±0.021	0.014	0.596	0.607	0.132
3n-Arachidonic (20:4n3)	0.003±0.001	0.005±0.002	0.003±0.001	0.005±0.002	0.003±0.001	0.001	0.876	1.000	1.000
EPA (20:5n3)	0.020±0.001^{D}	0.023±0.002^{CD}	0.028±0.002^{C}	0.039±0.001^{B}	0.055±0.003^{A}	0.002	<0.001	0.021	0.268
C21:0	0.018±0.002	0.021±0.006	0.017±0.002	0.015±0.002	0.011±0.001	0.001	0.115	0.948	0.269
Behenic (22:0)	0.022±0.004	0.026±0.007	0.023±0.005	0.026±0.005	0.029±0.005	0.002	0.786	0.681	0.824
Erucic [22:1n9]	0.002±0.001	0.003±0.002	0.003±0.001	0.004±0.002	0.004±0.001	0.001	0.711	0.782	0.380
C22:2n6	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-
Adrenic [C22:4n6]	0.115±0.004	0.099±0.013	0.107±0.004	0.103±0.006	0.103±0.002	0.003	0.373	0.484	0.013
Clupanodonic (22:5n3)	0.103±0.010^{B}	0.121±0.010^{AB}	0.123±0.010^{AB}	0.124±0.012^{AB}	0.153±0.011^{A}	0.005	0.017	0.827	0.421
DHA (22:6n3)	1.198±0.024^{C}	1.299±0.023^{BC}	1.294±0.051^{BC}	1.410±0.074^{AB}	1.523±0.027^{A}	0.024	<0.001	0.440	0.568
C23:0	0.008±0.003	0.006±0.004	0.008±0.004	0.008±0.003	0.011±0.003	0.002	0.914	0.930	0.863
Lignoceric (24:0)	0.010±0.000	0.013±0.002	0.013±0.002	0.010±0.002	0.015±0.002	0.001	0.087	0.802	0.615
Nervonic (24:1n9)	0.009±0.001	0.009±0.001	0.008±0.001	0.009±0.001	0.010±0.00	0.000	0.452	0.394	0.039

HP= hempseed presscake. HO= hempseed oil. Fatty Acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis. $^{A-D}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

A comparison of egg yolk fatty acid composition for Lohmann LSL-Lite White hens fed dietary hemp and reared in a conventional cage and single-tier system is shown in Table 4.5.11 and Table 4.5.12. Saturated fatty acids such as stearic acid and arachidic acid were significantly higher (P≤0.05) in white hens fed 6% HO (Table 4.5.12). C14:0 was highest (P≤0.05) in eggs from hens fed 3% HO with the intermediate being the control, 10% HP and 6% HO treatment groups (Table 4.5.12). Margaric acid increased significantly (P≤0.05) in eggs from hens fed 10% HP with the intermediate being 20% HP and 6% HO (Table 4.5.12).

Myristoleic acid content in egg yolks was highest (P≤0.05) in white hens fed 3% HO, intermediate in the control, 6% HO, and 10% HP groups, and lowest in the 20% HP group (Table 4.5.12). 10c-17:1 content in egg yolks was highest (P≤0.05) in white hens fed 10% HP, intermediate in the control, 20% HP and 3% HO groups (Table 4.5.12). Oleic acid decreased (P≤0.05) in every treatment group compared to the control diet with the 6% HO group having the lowest amount of oleic acid (Table 4.5.12). Elaidic acid was significantly higher (P≤0.05) in eggs yolks from white hens reared in the conventional cage system (Table 4.5.11).

PUFAs including linolenic acid, g-linolenic acid, stearidonic acid, homo-g-linolenic acid, homo-a-linolenic acid, EPA, clupanodonic acid and DHA were highest (P≤0.05) in white hens fed 6% HO (Table 4.5.12). EPA and DHA content in egg yolks from white hens had a significant interaction (P≤0.05) between treatment and housing (Table 4.5.12). LA content in egg yolks was highest (P≤0.05) in white hens fed 20% HP and 6% HO and intermediate in hens fed 10% HP and 3% HO (Table 4.5.12). Adrenic acid was highest (P≤0.05) in eggs from white hens fed 20% HP, lowest in the 10% HP group, and intermediate in the remaining treatment groups (Table 4.5.12). Linolelaidic acid content of egg yolks increased ($P \leq 0.05$) in white hens fed 10% HP and 3% HO (Table 4.5.12).

Table 4.5.11. Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Cage	Floor	SEM	P-Value
Crude fat (W/W%)	52.621±0.130	52.818±0.214	0.113	0.407
C14:0	0.259±0.004	0.251±0.006	0.003	0.258
Myristoleic (9c-14:1)	0.026±0.001	0.025±0.002	0.001	0.635
C15:0	0.048±0.002	0.048±0.001	0.001	0.714
C15:1n5	0.000	0.000	0.000	-
Palmitic (16:0)	23.671±0.140	23.593±0.156	0.104	0.725
Palmitoleic (9c-16:1)	1.002±0.117	0.950±0.152	0.091	0.790
Margaric (17:0)	0.214±0.010	0.216±0.003	0.007	0.895
10c-17:1	0.101±0.005	0.097±0.002	0.003	0.569
Stearic (18:0)	10.467±0.119	10.352±0.160	0.094	0.562
Elaidic (9t-18:1)	0.141±0.016^{A}	0.092±0.002^{B}	0.011	0.027
Oleic (9c-18:1)	31.204±0.403	31.563±0.647	0.345	0.622
Vaccenic (11c-18:1)	1.700±0.108	1.715±0.141	0.084	0.933
Linolelaidic (18:2t)	0.020±0.009	0.001±0.001	0.006	0.100
Linoleic (18:2n6)	22.882±0.306	23.097±0.449	0.251	0.686
Linolenic (18:3n3)	1.947±0.154	1.950±0.212	0.123	0.991
g-Linolenic [C18:3n6]	0.165±0.009	0.160±0.011	0.007	0.742
Stearidonic (18:4n3)	0.025±0.004	0.018±0.004	0.003	0.344
Arachidic (20:0)	0.039±0.002	0.039±0.003	0.002	0.855
Gondoic (20:1n9)	0.102±0.015	0.107±0.020	0.012	0.853
C20:2	0.207±0.004	0.217±0.007	0.004	0.173
Homo-g-linolenic [C20:3n6]	0.209±0.008	0.225±0.014	0.007	0.270
Homo-a-linolenic (20:3n3)	0.037±0.003	0.038±0.005	0.003	0.804
Arachidonic [20:4n6]	1.869±0.014	1.861±0.020	0.011	0.736
3n-Arachidonic (20:4n3)	0.000	0.000	0.000	-
EPA (20:5n3)	0.034±0.003	0.032±0.004	0.003	0.671
C21:0	0.017±0.003	0.014±0.002	0.002	0.466
Behenic (22:0)	0.031±0.004	0.020±0.000	0.003	0.056
Erucic [22:1n9]	0.002±0.001	0.002±0.001	0.001	0.822
C22:2n6	0.000	0.000	0.000	-
Adrenic [C22:4n6]	0.100±0.005	0.103±0.002	0.003	0.713
Clupanodonic (22:5n3)	0.110±0.005	0.102±0.007	0.004	0.345
DHA (22:6n3)	1.36±0.03	1.28±0.03	0.030	0.134
C23:0	0.009±0.003	0.007±0.003	0.002	0.567
Lignoceric (24:0)	0.014±0.001	0.013±0.001	0.001	0.926
Nervonic (24:1n9)	0.010±0.000	0.010±0.000	0.000	-

Fatty acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis.
$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.12.Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
Crude fat (W/W%)	52.540±0.277	52.713±0.177	52.925±0.232	52.373±0.265	52.771±0.237	0.113	0.650	0.457
C14:0	0.250±0.006^{AB}	0.253±0.005^{AB}	0.244±0.006^{B}	0.273±0.010^{A}	0.268±0.007^{AB}	0.003	0.033	0.194
Myristoleic (9c-14:1)	0.028±0.002^{AB}	0.023±0.003^{BC}	0.021±0.001^{C}	0.033±0.003^{A}	0.026±0.002^{ABC}	0.001	0.004	0.587
C15:0	0.046±0.002	0.053±0.006	0.046±0.002	0.048±0.003	0.049±0.001	0.001	0.480	0.273
C15:1n5	0.000	0.000	0.000	0.000	0.000	0.000	-	-
Palmitic (16:0)	23.634±0.141	23.553±0.413	23.410±0.128	24.213±0.430	23.640±0.217	0.104	0.283	0.680
Palmitoleic (9c-16:1)	1.141±0.186	0.755±0.210	0.811±0.155	1.003±0.354	1.098±0.198	0.091	0.615	0.584
Margaric (17:0)	0.200±0.003^{B}	0.263±0.047^{A}	0.219±0.004^{AB}	0.190±0.008^{B}	0.214±0.004^{AB}	0.007	0.031	0.989
10c-17:1	0.099±0.001^{AB}	0.123±0.023^{A}	0.099±0.002^{AB}	0.095±0.003^{AB}	0.090±0.003^{B}	0.003	0.048	0.906
Stearic (18:0)	10.034±0.056^{BC}	9.908±0.144^{C}	10.494±0.218^{ABC}	10.538±0.173^{AB}	10.945±0.122^{A}	0.094	<0.001	0.535
Elaidic (9t-18:1)	0.123±0.018	0.188±0.061	0.111±0.016	0.140±0.031	0.091±0.002	0.011	0.122	0.564
Oleic (9c-18:1)	33.791±0.392^{A}	31.290±0.636^{B}	31.178±0.459^{B}	31.168±0.539^{B}	29.155±0.219^{C}	0.345	<0.001	0.728
Vaccenic (11c-18:1)	1.741±0.179	2.008±0.233	1.819±0.159	1.673±0.248	1.423±0.155	0.084	0.303	0.522
Linolelaidic (18:2t)	0.001±0.001^{B}	0.050±0.031^{A}	0.001±0.001^{B}	0.045±0.023^{A}	0.001±0.001^{B}	0.006	0.005	0.979
Linoleic (18:2n6)	21.788±0.203^{B}	23.368±0.604^{AB}	23.691±0.666^{A}	21.948±0.280^{AB}	23.714±0.314^{A}	0.251	0.007	0.615
Linolenic (18:3n3)	1.244±0.019^{D}	1.543±0.081^{C}	1.714±0.075^{C}	2.118±0.037^{B}	3.005±0.086^{A}	0.123	<0.001	0.095
g-Linolenic [C18:3n6]	0.123±0.003^{D}	0.138±0.005^{CD}	0.150±0.007^{C}	0.180±0.007^{B}	0.221±0.005^{A}	0.007	<0.001	0.055
Stearidonic (18:4n3)	0.015±0.003^{B}	0.015±0.005^{B}	0.016±0.004^{AB}	0.025±0.010^{AB}	0.038±0.009^{A}	0.003	0.045	0.491
Arachidic (20:0)	0.030±0.000^{C}	0.040±0.007^{B}	0.035±0.002^{BC}	0.038±0.003^{BC}	0.051±0.001^{A}	0.002	<0.001	0.901
Gondoic (20:1n9)	0.134±0.024	0.068±0.034	0.083±0.025	0.113±0.036	0.109±0.022	0.012	0.475	0.640
C20:2	0.213±0.004	0.215±0.010	0.216±0.010	0.195±0.005	0.208±0.007	0.004	0.510	0.706
Homo-g-linolenic [C20:3n6]	0.183±0.006^{B}	0.193±0.008^{B}	0.201±0.007^{B}	0.223±0.009^{B}	0.268±0.013^{A}	0.007	<0.001	0.954

Table 4.5.12. Comparison of fatty acid content (means ± standard error) of egg yolks from Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil, continued.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
Homo-a-linolenic (20:3n3)	0.024±0.003^{C}	0.030±0.004^{BC}	0.034±0.002^{BC}	0.043±0.003^{B}	0.056±0.004^{A}	0.003	<0.001	0.781
Arachidonic [20:4n6]	1.855±0.015	1.833±0.031	1.878±0.026	1.880±0.039	1.875±0.027	0.011	0.779	0.911
3n-Arachidonic (20:4n3)	0.000	0.000	0.000	0.000	0.000	0.000	-	-
EPA (20:5n3)	0.019±0.001^{D}	0.020±0.000^{D}	0.030±0.002^{C}	0.040±0.000^{B}	0.054±0.003^{A}	0.003	<0.001	0.008
C21:0	0.018±0.003	0.028±0.011	0.015±0.003	0.015±0.003	0.010±0.000	0.002	0.088	1.000
Behenic (22:0)	0.025±0.005	0.033±0.013	0.021±0.001	0.028±0.008	0.030±0.007	0.003	0.741	0.527
Erucic [22:1n9]	0.001±0.001	0.000±0.000	0.003±0.002	0.000±0.000	0.004±0.002	0.001	0.428	0.781
C22:2n6	0.000	0.000	0.000	0.000	0.000	0.000	-	-
Adrenic [C22:4n6]	0.106±0.002^{AB}	0.078±0.023^{B}	0.110±0.003^{A}	0.100±0.004^{AB}	0.099±0.001^{AB}	0.003	0.038	0.958
Clupanodonic (22:5n3)	0.083±0.003^{C}	0.098±0.006^{BC}	0.113±0.007^{AB}	0.105±0.006^{BC}	0.131±0.008^{A}	0.004	<0.001	0.258
DHA (22:6n3)	1.168±0.016^{C}	1.265±0.035^{BC}	1.318±0.031^{B}	1.418±0.044^{AB}	1.490±0.032^{A}	0.025	<0.001	0.006
C23:0	0.010±0.005	0.008±0.008	0.006±0.004	0.005±0.003	0.010±0.004	0.002	0.915	0.683
Lignoceric (24:0)	0.010±0.000	0.013±0.003	0.015±0.002	0.013±0.003	0.016±0.002	0.001	0.083	0.828
Nervonic (24:1n9)	0.010±0.000	0.010±0.000	0.010±0.000	0.010±0.000	0.010±0.000	0.000	-	-

HP= hempseed presscake. HO= hempseed oil.
Fatty Acid profile expressed as percent of total fat. W/W%= grams / 100 grams of sample. Results are expressed on a "as is" basis.
$^{A-D}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.13 and Table 4.5.14 show the comparison of total body feather condition for Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier housing system. Total body feather condition was significantly better ($P \leq 0.05$) for white hens compared to brown hens in all periods and significantly better ($P \leq 0.05$) in hens reared in the single-tier system compared to the conventional cage system in periods 1 to 6. Feather condition significantly improved ($P \leq 0.05$) in periods 1 and 5 for hens fed the control, 20% HP and 6% HO diets and in period 4 for hens fed 6% HO with hens fed 3% HO showing the least best feather condition.

Table 4.5.15 and Table 4.5.16 show the comparison of the total body feather condition for Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier housing system. In the initial period, total body feather condition was best ($P \leq 0.05$) in white hens reared in the conventional cage system. In periods 1, 4 and 5, total body feather condition was significantly better ($P \leq 0.05$) in the white hens reared in the single-tier system. In the initial period, feather condition was significantly best ($P \leq 0.05$) in white hens fed 10% HP, and intermediate in hens fed 20% HP and 3% HO. In periods 1, 4 and 5, feather condition was significantly better ($P \leq 0.05$) in hens fed the control, 20% HP and 6% HO diets, with white hens fed 3% HO showing the least best feather condition. In period 2, total body feather condition had a significant interaction ($P \leq 0.05$) between treatment and housing.

Table 4.5.13. Total body feather condition score (means ± standard error) of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing *Strain
I	2.45±0.05^{A}	1.97±0.03^{B}	0.03	<0.001	2.11±0.04	2.13±0.03	0.03	0.700	-
P1	2.65±0.05^{A}	2.26±0.03^{B}	0.03	<0.001	2.55±0.04^{A}	2.11±0.03^{B}	0.03	<0.001	-
P2	2.66±0.05^{A}	2.11±0.02^{B}	0.03	<0.001	2.40±0.04^{A}	2.09±0.03^{B}	0.03	<0.001	-
P3	2.59±0.05^{A}	2.11±0.02^{B}	0.03	<0.001	2.36±0.04^{A}	2.10±0.03^{B}	0.03	<0.001	-
P4	2.66±0.06^{A}	2.37±0.04^{B}	0.03	<0.001	2.65±0.04^{A}	2.16±0.03^{B}	0.03	<0.001	-
P5	2.89±0.04^{A}	2.35±0.03^{B}	0.03	<0.001	2.72±0.03^{A}	2.18±0.04^{B}	0.03	<0.001	-
P6	2.73±0.05^{A}	2.27±0.03^{B}	0.03	<0.001	2.50±0.04^{A}	2.26±0.04^{B}	0.03	<0.001	-

I = Initial, P= Period.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.14. Total body feather condition score (means ± standard error) of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment* Housing	Treatment* Strain
I	2.16±0.06	2.03±0.10	2.16±0.06	2.05±0.07	2.11±0.06	0.03	0.544	0.094	0.021
P1	2.33±0.05^{B}	2.50±0.08^{AB}	2.34±0.05^{B}	2.58±0.08^{A}	2.34±0.05^{B}	0.03	0.025	0.434	0.040
P2	2.30±0.05	2.36±0.08	2.24±0.05	2.43±0.08	2.20±0.05	0.03	0.067	0.031	0.287
P3	2.26±0.05	2.28±0.07	2.26±0.06	2.30±0.07	2.24±0.05	0.03	0.966	0.243	0.279
P4	2.47±0.06^{AB}	2.62±0.09^{A}	2.45±0.07^{AB}	2.65±0.08^{A}	2.30±0.06^{B}	0.03	0.004	0.558	0.011
P5	2.50±0.06^{B}	2.61±0.08^{AB}	2.42±0.06^{B}	2.83±0.06^{A}	2.45±0.06^{B}	0.03	<0.001	0.503	0.069
P6	2.33±0.06	2.47±0.08	2.42±0.06	2.54±0.09	2.39±0.06	0.03	0.304	0.025	0.022

I = Initial, P= Period. HP = hempseed presscake. HO =hempseed oil.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.15. Total body feather condition score (means ± standard error) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Cage	Floor	SEM	P-Value
I	1.77±0.05^{B}	2.13±0.03^{A}	0.03	<0.001
P1	2.45±0.05^{A}	2.11±0.03^{B}	0.03	<0.001
P2	2.14±0.04	2.09±0.03	0.02	0.263
P3	2.13±0.03	2.10±0.03	0.02	0.520
P4	2.63±0.05^{A}	2.16±0.03^{B}	0.04	<0.001
P5	2.56±0.05^{A}	2.18±0.04^{B}	0.03	<0.001
P6	2.28±0.05	2.26±0.04	0.03	0.805

I = Initial, P= Period.
$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.16. Total body feather condition score (means ± standard error) of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
I	2.07±0.06^{A}	1.70±0.13^{B}	1.95±0.04^{AB}	1.85±0.08^{AB}	2.02±0.07^{A}	0.03	0.016	0.503
P1	2.20±0.05^{B}	2.40±0.11^{AB}	2.20±0.05^{B}	2.55±0.11^{A}	2.25±0.06^{B}	0.03	0.011	0.355
P2	2.13±0.04	2.10±0.07	2.08±0.04	2.20±0.09	2.10±0.04	0.02	0.667	0.013
P3	2.12±0.04	2.05±0.05	2.10±0.05	2.10±0.07	2.15±0.05	0.02	0.813	0.210
P4	2.41±0.07^{BC}	2.55±0.11^{AB}	2.32±0.07^{BC}	2.75±0.10^{A}	2.22±0.05^{C}	0.04	<0.001	0.364
P5	2.34±0.06^{B}	2.47±0.12^{AB}	2.27±0.06^{B}	2.70±0.11^{A}	2.30±0.06^{B}	0.03	0.006	0.521
P6	2.15±0.05	2.26±0.10	2.27±0.06	2.40±0.11	2.33±0.06	0.03	0.131	0.227

I = Initial, P= Period. HP =hempseed presscake. HO = hempseed oil.
$^{A-C}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Tibia parameters and tibia mineral analysis comparison of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens fed dietary hemp and reared in a conventional cage and single-tier system is provided in Table 4.5.17 and Table 4.5.18. All tibia parameters and minerals were significantly higher ($P \le 0.05$) in brown hens except for ash content of the tibia which was highest ($P \le 0.05$) in white hens. No statistical difference was observed for tibia strength between the strains or between the housing systems ($P > 0.05$). DM and ash content of the tibia was significantly increased ($P \le 0.05$) in hens reared in the single-tier system, while the remaining parameters and minerals were significantly higher in hens reared in the conventional cage system. Tibia weight was significantly heavier ($P \le 0.05$) in hens fed 3% HO and intermediate in hens fed 10% HP and 6% HO. Tibias were significantly longer ($P \le 0.05$) in hens 3% HO and intermediate in hens fed the control and 6% HO diets. No statistical difference was observed for tibia breaking between the treatment diets ($P > 0.05$). Tibia ash content was significantly higher ($P \le 0.05$) in hens fed the control and intermediate in hens fed 10% HP, 20% HP and 6% HO. Calcium, magnesium and zinc content increased significantly ($P \le 0.05$) in hens fed 3% HO and was lowest in hens fed 20% HP with the remaining treatments being intermediate.

Table 4.5.17. Comparison of average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing *Strain
Body weight (g)	2159±30.50^{A}	1937±18.85^{B}	18.10	<0.001	2072±23.50^{A}	1905±23.39^{B}	18.10	<0.001	-
Tibia weight (g)	9.78±0.14^{A}	7.06±0.06^{B}	0.12	<0.001	8.50±0.16^{A}	6.96±0.09^{B}	0.12	<0.001	-
Tibia length (mm)	121.09±0.61^{A}	117.63±0.26^{B}	0.28	<0.001	119.52±0.39^{A}	117.41±0.37^{B}	0.28	<0.001	-
Tibia width (mm)	6.62±0.05^{A}	5.78±0.03^{B}	0.04	<0.001	6.21±0.06^{A}	5.76±0.04^{B}	0.04	<0.001	-
Tibia breaking strength (g/force)	19570±1931.13	20008±992.02	898.72	0.825	20022±1412.66	19670±819.54	898.72	0.848	-
Dry matter (%)	86.41±0.34^{A}	85.59±0.21^{B}	0.18	0.043	85.39±0.25^{B}	86.40±0.25^{A}	0.18	0.006	-
Ash (%)	44.15±0.64^{B}	48.95±0.36^{A}	0.37	<0.001	46.13±0.45^{B}	49.50±0.51^{A}	0.37	<0.001	-
Calcium (mg/g)	694.01±18.00^{A}	496.01±6.40^{B}	10.22	<0.001	600.53±14.97^{A}	488.64±7.25^{B}	10.22	<0.001	-
Phosphorous (mg/g)	324.05±8.69^{A}	234.25±3.09^{B}	4.77	<0.001	281.89±7.00^{A}	230.60±3.49^{B}	4.77	<0.001	-
Magnesium (mg/g)	10.09±0.20^{A}	6.83±0.08^{B}	0.15	<0.001	8.58±0.21^{A}	6.67±0.10^{B}	0.15	<0.001	-
Zinc (mg/g)	0.95±0.03^{A}	0.64±0.01^{B}	0.02	<0.001	0.78±0.03^{A}	0.65±0.01^{B}	0.02	<0.001	-
Iron (mg/g)	0.38±0.01^{A}	0.30±0.01^{B}	0.01	<0.001	0.34±0.01^{A}	0.30±0.01^{B}	0.01	0.003	-
Potassium (mg/g)	6.94±0.21^{A}	5.85±0.10^{B}	0.10	<0.001	6.58±0.14^{A}	5.60±0.11^{B}	0.10	<0.001	-
Sodium (mg/g)	19.17±0.48^{A}	13.78±0.27^{B}	0.31	<0.001	16.34±0.43^{A}	13.97±0.39^{B}	0.31	<0.001	-
Sulphate (mg/g)	8.51±0.31^{A}	5.01±0.11^{B}	0.18	<0.001	6.89±0.25^{A}	4.83±0.15^{B}	0.18	<0.001	-

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.18. Comparison of average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing	Treatment *Strain
Body weight (g)	1977±39.56	1996±53.85	1993±31.20	2126±45.97	1977±36.46	18.10	0.168	0.987	0.605
Tibia weight (g)	7.71±0.23^{B}	8.20±0.33^{AB}	7.48±0.21^{B}	8.78±0.42^{A}	7.75±0.22^{AB}	0.12	0.026	0.979	0.647
Tibia length (mm)	118.56±0.55^{AB}	117.81±0.70^{B}	117.62±0.52^{B}	121.02±1.04^{A}	118.96±0.51^{AB}	0.28	0.012	0.839	0.460
Tibia width (mm)	5.95±0.07	6.17±0.10	5.96±0.09	6.24±0.13	6.00±0.08	0.04	0.163	0.932	0.417
Tibia breaking strength (g/force)	19798±936.59	21930±5124.17	20438±2250.11	17281±1058.23	19683±1099.30	898.72	0.794	0.696	0.500
Dry matter (%)	85.64±0.35	85.18±0.70	85.46±0.34	85.87±0.55	86.66±0.32	0.18	0.087	0.946	0.318
Ash (%)	48.90±0.70^{A}	46.10±0.79^{AB}	47.98±0.58^{AB}	45.11±1.16^{B}	47.60±0.84^{AB}	0.37	0.027	0.163	0.275
Calcium (mg/g)	542.90±22.09^{AB}	574.70±26.82^{AB}	522.23±15.38^{B}	630.90±36.00^{A}	547.98±19.74^{AB}	10.22	0.041	0.603	0.553
Phosphorous (mg/g)	253.05±9.87	270.69±12.53	246.97±7.09	295.31±16.77	259.19±9.85	4.77	0.051	0.709	0.642
Magnesium (mg/g)	7.57±0.31^{AB}	8.30±0.44^{AB}	7.40±0.27^{B}	8.83±0.46^{A}	7.60±0.28^{AB}	0.15	0.047	0.814	0.761
Zinc (mg/g)	0.76±0.03^{AB}	0.74±0.05^{AB}	0.65±0.02^{B}	0.83±0.06^{A}	0.73±0.03^{AB}	0.02	0.018	0.847	0.651
Iron (mg/g)	0.33±0.01	0.32±0.02	0.32±0.02	0.33±0.02	0.32±0.01	0.01	0.930	0.553	0.102
Potassium (mg/g)	6.05±0.24	6.40±0.29	6.16±0.19	6.64±0.26	5.96±0.19	0.10	0.346	0.413	0.052
Sodium (mg/g)	14.84±0.63	15.48±0.81	14.88±0.60	17.36±1.00	15.27±0.61	0.31	0.196	0.186	0.231
Sulphate (mg/g)	5.81±0.37	6.47±0.58	5.54±0.30	7.28±0.60	5.90±0.32	0.18	0.066	0.481	0.499

HP = hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Tibia parameters and tibia mineral analysis comparison of Lohmann LSL-Lite White hens fed dietary hemp and reared in a conventional cage and single-tier system is provided in Table 4.5.19 and Table 4.5.20. Body weight, tibia weight, magnesium content and potassium content of the tibia was highest ($P \leq 0.05$) in white hens reared in the conventional cage system. There was no statistical difference in tibia strength of white hens between the two housing systems or for white hens between the treatment diets ($P > 0.05$). Tibia DM content was highest ($P \leq 0.05$) in white hens reared in the single-tier system. Tibia DM was highest ($P \leq 0.05$) in white hens fed 6% HO and intermediate in hens fed the control and 20% HP diets. Zinc content of the tibia was significantly increased ($P \leq 0.05$) in white hens fed the control and 3% HO diets and intermediate in 10% HP and 6% HO treatment groups.

Table 4.5.19. Comparison of average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Cage	Floor	SEM	P-Value
Body weight (g)	1985 ± 30.31^{A}	1905 ± 23.39^{B}	18.85	0.039
Tibia weight (g)	7.22 ± 0.07^{A}	6.96 ± 0.09^{B}	0.06	0.036
Tibia length (mm)	117.95±0.33	117.41±0.37	0.26	0.311
Tibia width (mm)	5.80±0.04	5.76±0.04	0.03	0.534
Tibia breaking strength (g/force)	20473±2084.48	19670±819.54	992.02	0.691
Dry matter (%)	84.38 ± 0.29^{B}	86.40 ± 0.25^{A}	0.21	<0.001
Ash (%)	48.12±0.47	49.50±0.51	0.36	0.060
Calcium (mg/g)	507.06±11.63	488.64±7.25	6.40	0.160
Phosphorous (mg/g)	239.73±5.65	230.60±3.49	3.09	0.149
Magnesium (mg/g)	7.07 ± 0.14^{A}	6.67 ± 0.10^{B}	0.08	0.020
Zinc (mg/g)	0.62±0.02	0.65±0.01	0.01	0.139
Iron (mg/g)	0.30±0.01	0.30±0.01	0.01	0.867
Potassium (mg/g)	6.23 ± 0.17^{A}	5.60 ± 0.11^{B}	0.10	0.002
Sodium (mg/g)	13.50±0.31	13.97±0.40	0.27	0.398
Sulphate (mg/g)	5.27±0.16	4.83±0.15	0.11	0.055

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly ($P \leq 0.05$).

Table 4.5.20. Comparison of average tibia parameters and tibia mineral analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
Body weight (g)	1909±30.68	1863±70.69	1953±35.12	2044±60.79	1940±40.20	18.85	0.325	0.474
Tibia weight (g)	7.07±0.12	7.00±0.18	6.90±0.11	7.26±0.10	7.18±0.13	0.06	0.421	0.971
Tibia length (mm)	117.69±0.46	116.10±0.63	117.15±0.51	118.27±0.65	118.29±0.53	0.26	0.190	0.403
Tibia width (mm)	5.77±0.04	5.88±0.12	5.74±0.06	5.78±0.07	5.80±0.06	0.03	0.818	0.451
Tibia breaking strength (g/force)	20420±1003.40	24602±10421.83	18225±900.91	17823±1037.06	20529±1262.79	992.02	0.534	0.659
Dry matter (%)	85.52±0.40^{AB}	83.97±0.36^{B}	85.31±0.42^{AB}	84.80±0.77^{B}	86.65±0.35^{A}	0.21	0.008	0.999
Ash (%)	49.93±0.69	46.44±0.98	49.09±0.54	47.77±1.07	48.87±0.83	0.36	0.149	0.064
Calcium (mg/g)	489.15±10.42	489.93±22.45	482.50±10.55	544.45±33.64	504.26±12.59	6.40	0.151	0.545
Phosphorous (mg/g)	229.82±4.96	232.14±10.58	229.24±5.15	258.04±16.72	237.50±6.10	3.09	0.171	0.467
Magnesium (mg/g)	6.78±0.15	6.90±0.28	6.67±0.16	7.36±0.34	6.88±0.16	0.08	0.363	0.557
Zinc (mg/g)	0.68±0.02^{A}	0.59±0.04^{AB}	0.59±0.02^{B}	0.70±0.05^{A}	0.65±0.02^{AB}	0.01	0.006	0.861
Iron (mg/g)	0.30±0.01	0.29±0.02	0.29±0.01	0.31±0.02	0.31±0.01	0.01	0.876	0.128
Potassium (mg/g)	5.67±0.17	6.33±0.46	5.96±0.21	6.57±0.34	5.59±0.16	0.10	0.062	0.466
Sodium (mg/g)	13.20±0.27	13.05±0.64	13.99±0.66	14.26±0.88	14.23±0.56	0.27	0.539	0.435
Sulphate (mg/g)	4.85±0.17	5.01±0.31	4.87±0.24	5.58±0.47	5.13±0.21	0.11	0.493	0.376

HP = hempseed presscake. HO = hempseed oil.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.21 and Table 4.5.22 provide the liver analysis comparison of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier housing system. Body weight was significantly higher ($P \leq 0.05$) in brown hens compared to white hens and highest ($P \leq 0.05$) in hens reared in the conventional cage system versus the single-tier system. Hepatosomatic index and liver proximate fat content significantly decreased ($P \leq 0.05$) in brown hens. Histological liver fat content improved significantly ($P \leq 0.05$) in hens reared in the conventional cage system. Pigmentation of the liver was significantly redder ($P \leq 0.05$) in brown hens compared to white hens and significantly redder ($P \leq 0.05$) in hens reared in the conventional cage system compared to the single-tier system. Liver red pigmentation was significantly increased ($P \leq 0.05$) in hens fed 20% HP than in hens fed 6% HO and the remaining treatments were intermediate. Liver yellow pigmentation was highest ($P \leq 0.05$) in hens fed 20% HP compared to all other treatments.

Table 4.5.21. Comparison of average liver analysis (means ± standard error) from period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (*Cannabis sativa* L.) by-products.

	Brown	White	SEM	P-Value	Cage	Floor	SEM	P-Value	Housing *Strain
Body weight (g)	2159±30.50^{A}	1937±18.85^{B}	18.10	<0.001	2072±23.50^{A}	1905±23.39^{B}	18.10	<0.001	-
Liver weight (g)	43.16±1.07	43.87±0.77	0.63	0.613	44.56±0.86	42.48±0.90	0.63	0.103	-
Hepato-somatic index (%)	2.01±0.05^{B}	2.26±0.03^{A}	0.02	<0.001	2.16±0.04	2.22±0.03	0.02	0.193	-
Histological Liver fat (%)	32.37±0.54	31.01±0.63	0.44	0.135	31.02±0.47^{B}	33.23±1.07^{A}	0.44	0.036	-
Proximate fat (%)	23.36±0.99^{B}	27.63±1.07^{A}	0.82	0.015	26.09±1.08	26.58±1.26	0.82	0.767	-
L*	39.93±0.46	40.72±0.40	0.32	0.261	40.92±0.39	39.93±0.52	0.32	0.122	-
a*	13.69±0.24^{A}	13.05±0.17^{B}	0.14	0.039	13.63±0.19^{A}	12.71±0.18^{B}	0.14	<0.001	-
b*	20.38±0.78	22.38±0.62	0.50	0.070	21.31±0.64	22.47±0.79	0.50	0.249	-

Least-squared means±SEM (standard error of the mean) within rows (P≤0.05)

Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.22. Comparison of average liver analysis (means ± standard error) from period 6 of Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing	Treatment *Strain
Body weight (g)	1977±39.56	1996±53.85	1993±31.2	2126±45.97	1977±36.46	18.10	0.168	0.987	0.605
Liver weight (g)	42.74±1.11	42.41±1.97	44.77±1.32	45.33±1.76	43.32±1.31	0.63	0.620	0.337	0.461
Hepato-somatic index (%)	2.17±0.05	2.12±0.07	2.24±0.04	2.14±0.09	2.19±0.05	0.02	0.661	0.362	0.206
Histological Liver fat (%)	32.67±0.91	29.16±0.94	32.93±1.02	30.74±0.92	31.11±0.94	0.44	0.062	0.906	0.214
Proximate fat (%)	27.25±1.53	25.09±2.86	28.33±1.80	23.19±1.65	25.19±1.53	0.82	0.397	0.438	0.458
L*	40.37±0.54	39.93±0.84	40.60±0.68	41.23±0.79	40.44±0.72	0.32	0.905	0.256	0.291
a*	13.15 ± 0.27^{AB}	13.56 ± 0.34^{AB}	13.92 ± 0.32^{A}	12.94 ± 0.28^{AB}	12.62 ± 0.27^{B}	0.14	0.014	0.494	0.286
b*	21.09 ± 0.68^{B}	20.75 ± 1.07^{B}	26.85 ± 1.14^{A}	19.08 ± 0.96^{B}	19.17 ± 0.81^{B}	0.50	<0.001	0.868	0.445

HP = hempseed presscake. HO= hempseed oil.
Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.
$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.23 and Table 4.5.24 provide the comparison of Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier housing system. Body weight and liver weight were significantly higher (P≤0.05) in white hens reared in the conventional cage system. Histological liver fat content was significantly reduced (P≤0.05) in white hens reared in the conventional cage system. Liver pigmentation scores for lightness and redness were significantly increased (P≤0.05) in white hens reared in the conventional cage system. Histological liver fat content increased (P≤0.05) in hens fed 20% HP and was intermediate in hens fed the control, 3% HO and 6% HO diets. Liver redness and yellow pigmentation was significantly higher (P≤0.05) in hens fed 20% HP.

Table 4.5.23. Comparison of average liver analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system (cage) and a single-tier system (floor), fed dietary hemp (Cannabis sativa L.) by-products.

	Cage	Floor	SEM	P-Value
Body weight (g)	1985 ± 30.31^{A}	1905 ± 23.39^{B}	18.85	0.039
Liver weight (g)	45.96 ± 1.33^{A}	42.48 ± 0.90^{B}	0.77	0.027
Hepato-somatic index (%)	2.31±0.05	2.22±0.03	0.03	0.093
Histological Liver fat (%)	29.67 ± 0.72^{B}	33.23 ± 1.07^{A}	0.63	0.006
Proximate fat (%)	29.36±1.92	26.58±1.26	1.07	0.212
L*	41.91 ± 0.58^{A}	39.93 ± 0.52^{B}	0.40	0.015
a*	13.57 ± 0.30^{A}	12.71 ± 0.18^{B}	0.17	0.010
b*	22.24±1.00	22.47±0.79	0.62	0.854

Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.
$^{A\text{-}B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

Table 4.5.24. Comparison of average liver analysis (means ± standard error) from period 6 of Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed a hemp (*Cannabis sativa* L.) by-product diet at an inclusion rate of 0% hemp, 10% and 20% hempseed presscake and 3% and 6% hempseed oil.

	Control	10% HP	20% HP	3% HO	6% HO	SEM	P-Value	Treatment *Housing
Body weight (g)	1909±30.68	1863±70.69	1953±35.12	2044±60.79	1940±40.20	18.85	0.325	0.474
Liver weight (g)	43.19±1.08	40.73±2.73	44.70±1.60	48.41±2.88	43.33±1.60	0.77	0.316	0.331
Hepato-somatic index (%)	2.26±0.03	2.18±0.07	2.28±0.05	2.37±0.12	2.23±0.05	0.03	0.584	0.294
Histological Liver fat (%)	31.82±1.18^{AB}	26.51±0.78^{B}	33.24±1.43^{A}	29.32±1.44^{AB}	31.05±1.24^{AB}	0.63	0.025	0.761
Proximate fat (%)	27.70±1.80	25.19±4.87	30.93±2.18	25.19±3.15	25.84±1.91	1.07	0.391	0.988
L*	40.83±0.64	39.13±0.98	40.80±0.80	42.96±0.91	40.35±0.91	0.40	0.406	0.521
a*	12.75±0.27^{B}	13.62±0.32^{AB}	13.88±0.37^{A}	12.77±0.51^{AB}	12.45±0.30^{B}	0.17	0.012	0.121
b*	21.54±0.62^{B}	19.88±1.53^{B}	27.79±1.20^{A}	19.66±1.51^{B}	19.30±0.95^{B}	0.62	<0.001	0.679

HP = hempseed presscake. HO= hempseed oil.

Liver color expressed as L* = lightness, a* = green/red value and b* = blue/yellow value.

$^{A-B}$Least-squared means±SEM (Standard error of the mean) within rows with different letters differ significantly (P≤0.05).

4.6: Comparison of liver fat analyses

Table 4.6.1 shows the comparison of histological and proximate methods for the analysis of liver fat content in Lohmann Brown-Lite hens and Lohmann LSL-Lite White hens fed dietary hemp and reared in a conventional cage and single-tier housing system. Histological analysis of hen liver fat content was significantly higher (P≤0.05) than liver fat content by proximate analysis. The comparison of histological and proximate methods for the analysis of liver fat content in Lohmann LSL-Lite White hens fed dietary hemp and reared in the conventional cage and single-tier housing system is shown in Table 4.6.2. Liver fat content significantly increased (P≤0.05) in white hens when analyzed using histological analysis.

Table 4.6.1. Comparison of liver fat content and method of analysis (histology and proximate) of period 6 Lohmann Brown-Lite and Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed dietary hemp (*Cannabis sativa* L.) by-products.

	Liver Fat
Histology	31.53±0.44^{A}
Proximate	26.30±0.82^{B}
SEM	0.513
P-Value	<0.001
Method*Strain	0.005
Method* Treatment	0.895
Method* Housing	0.426
Strain*Treatment	0.203
Strain*Housing	-
Treatment* Housing	0.424
Method*Strain*Treatment	0.890
Method* Strain* Housing	-
Method* Treatment* Housing	0.691
Strain* Treatment* Housing	-
Method* Strain* Treatment* Housing*	-

Least-squared means±SEM (standard error of the mean) within rows (P≤0.05).

Table 4.6.2. Comparison of liver fat content and method of analysis (histology and proximate) of period 6 Lohmann LSL-Lite White hens (*Gallus gallus domesticus*) reared in a conventional cage system and a single-tier system, fed dietary hemp (*Cannabis sativa* L.) by-products.

	Liver Fat
Histology	31.01 ± 0.63^{A}
Proximate	27.63 ± 1.07^{B}
SEM	0.675
P-Value	0.013
Method*Treatment	0.908
Method*Housing	0.022
Treatment*Housing	0.868
Method*Treatment*Housing	0.977

Least-squared means±SEM (standard error of the mean) within rows (P≤0.05).

CHAPTER 5: DISCUSSION

5.1: Production performance

The purpose of this study was to analyze the effect of hempseed by-products in the diet of laying hens on production performance, egg quality, reduction of FP and amelioration of FLD. A general increase in body weight was observed in Lohmann Brown-Lite hens fed 10% HP and significantly lower body weight in hens fed 6% HO. Conversely, Taaifi et al. (2023a) observed a decrease in hen body weight in week 28 and then an increase in body weight after week 36 when a decline in egg production occurred for Lohmann Brown Classic hens fed 10, 20 and 30% hempseed. The variation between body weights between studies may be due to energy consumed, where the energy of the diets in the study by Taaifi et al. (2023a) were standardized at 3000 kcal/kg and the energy of the diets in the current study were 3786.71kcal/kg (control), 3735.01 kcal/kg (10% HP), 3895.64 kcal/kg (20% HP), 3931.44 kcal/kg (3% HO) and 3725.38 kcal/kg (6% HO).

Feed consumption was significantly higher in hens fed 20% HP and significantly lower in hens fed 6% HO. Feed consumption decreased after the first two periods and stabilized afterwards. In contrast, the hens in the study by Taaifi et al. (2023a) showed an increase in feed consumption and then a stability in feed consumption between the last two sampling days when they were fed 10, 20 and 30% hempseed. This variation could be explained by the adaptation of the hens to the treatment diets.

Egg production significantly increased in the final two periods when hens were fed 10% and 20% HP with a significant decrease in egg production in hens fed 3% HO. These results were similar to a study by Taaifi et al. (2023a) where 22-week-old Lohmann Brown Classic hens reared in a semi-automatic cage system were fed 10%, 20% and 30%

hempseed. These hens exhibited the highest egg production when fed the 10% hempseed treatment, which is the same for the current study in the final period. However, in the present study the egg production was only significantly different between the treatment diets in periods 5 and 6 and in period 5 the control group had the highest egg production but not significantly different from the 10% HP, 20% HP and 6% HO treatment groups. It is possible that the results in the final two periods were affected by age of hen since the hens in the current study were 59-weeks of age and older hens would produce less eggs (Tůmová and Gous, 2012).

Production performance for Lohmann LSL-Lite White hens reared in the conventional cage system had no significant difference in body weight between the treatment diets. However, feed consumption significantly increased in period 5 with the control, 10 and 20% HP groups having the highest feed intake and HO groups had the lowest feed intake. Egg production was also significantly increased in period 5 with all treatment groups except 6% HO having the highest egg production. Feed conversion was significantly better in hens fed 20% HP in period 1 and 4, then in period 5 and 6, the 6% HO treatment group had the best feed conversion. With overall feed conversion being significantly improved in the 20% HP treatment group. Conversely, a study by Neijat et al. (2016) found that Lohmann LSL-classic hens reared in individual cages, fed hempseed (10, 20 or 30%) and HO (4.5 or 9%) had no effect on feed intake, egg production and body weight gain and feed conversion was not determined. Kasula et al. (2021b) also observed no difference in feed intake, egg production and feed conversion in Bovan white caged hens (25 hens / cage) fed 10, 20 and 30% HP, but a higher body weight was observed. In the current study, the significant increase in feed consumption egg production and feed conversion in the later periods could be attributed to some cages consuming less feed and laying less due to genetics and age (Liljedahl et al., 1984).

In the present study, Lohmann LSL-Lite White hens reared in the single-tiered system showed no difference between the dietary treatments for body weight, average hen day egg production and feed conversion. Hens consumed more feed when fed the control diet and 20% HP than when hens were fed 6% HO in period 2. Hens consumed the least amount of feed in the control treatment in period 5 (Table 4.19). The findings of Gakhar et al. (2012) reported similar results to the current study with no significant adverse effects on production performance when hemp was fed at dietary inclusion levels of 4, 8 and 12% HO and 10 and 20% hempseed to Bovan white hens reared in an individual metabolic cage system. They also observed lower feed intake in hens fed the 4% HO than hens fed the control diet. Similarly, Jing et al. (2017) fed hemp oil at an inclusion level of 4 and 8% to Lohmann White laying hens in individual metabolic cages and observed no effect on production performance. For the current study, variations in feed intake in periods 2 and 5 could have been due to the pen layout as hens in some pens did not eat from one of two feeders which could have been due to the placement of the feeder being close to the room entrance or social dominance causing a hen to monopolize one feeder (Widowski et al., 2017; Sirovnik et al., 2018). The current study is in agreement with the findings of Kasula et al. (2021a) where no transfer of tetrahydrocannabinol or cannabinoid residues were present in body tissues of Bovan white hens reared in a colony cage system fed 10, 20 and 30% HP diets.

5.2: Egg quality

Egg quality for Lohmann Brown-Lite hens reared in a conventional cage system had no significant difference between the treatment diets for egg weight and shell weight. However, specific gravity was significantly better in hens fed 3% HO in period 1 with no significant difference from the 10% HP and 6% HO treatments. These results did not agree with the findings of Taaifi et al. (2023a), where hemp added to the diet of Lohmann Brown Classic hens in a semi-automatic cage system resulted in a decrease in egg weight from the control to 30% hempseed by an average of 10g after 32 weeks. Taaifi et al. (2023a) also reported an increase in egg weight with age within each treatment group. In the current study, egg weight increased until period 3 and then fluctuated within each treatment group.

In period 1, eggs from the 3% HO group had the highest specific gravity with no significant difference from the 10% HP and 6% HO treatment groups. Konca et al. (2014) also observed an increase in egg specific gravity in laying quail in a cage system fed 10% hempseed. Konca et al. (2019) reported the effects of raw and heat-treated hempseed in the diet of Lohmann Brown hens in a conventional cage system and found that egg specific gravity was not influenced by the treatments. The results of the current study could be explained by the initial adaptation to the treatment diets. Hempseed and HO protein and n-3 fatty acid content may have the potential to influence egg specific gravity (Uddin et al., 1991; Attia et al., 2022).

In period 3 of the current study, egg breaking strength was significantly stronger in hens fed 3% HO with no significant difference from the control, 20% HP and 6% HO treatments while shell thickness was higher in the control with no significant difference from the 10% HP, 3% HO and 6% HO treatment groups. Kasula et al. (2021b) reported an increase in eggshell breaking strength with 10, 20 and 30% HP in the diet of Bovan White colony caged hens and no effect on eggshell thickness. Conversely, Kanbur et al. (2023) reported no effect on eggshell breaking strength and eggshell thickness in white leghorns reared in a conventional cage fed 3.7% HO. Skřivan et al. (2019) found that 3, 6 and 9% hempseed diets fed to Lohmann Brown hens in an enriched cage system decreases eggshell thickness without affecting shell strength. Similarly, Konca et al. (2019) found an increased eggshell thickness when heat treated hempseed was fed to Lohmann Brown hens reared in a conventional cage system, but eggshell breaking strength was not determined. Cufader et al. (2021) also reported a decrease in eggshell thickness when HP (5, 10, 15, 20%) was fed to laying quails and no effect on eggshell breaking strength. The effect of hemp in the diet on eggshell breaking strength and eggshell thickness may vary depending on concentration, form of hemp used, strain of hen and housing type. Further research is needed to elucidate the mechanism behind these effects. Albumen height was highest in brown hens fed 10% HP, 20% HP and 3% HO with no difference from 6% HO. These results disagree with the findings of Konca et al. (2019), where no effect of heat-treated hempseed and raw hempseed was found on albumen height.

The current study showed a significant change in the color parameters of egg yolks from brown caged hens for L* (lightness), a* (redness) and b* (yellowness) values. Generally, the eggs were lighter in the 20% HP, 3% HO and 6% HO treatment groups with a more red pigmented yolk in the 10 and 20% HP group with yolks from the oil treatment group having the least red pigmentation and yolks from the 10 and 20% HP group having a higher yellow pigmentation. Taaifi et al. (2023a) found that Lohmann Brown classic hens in a semi-automatic cage system fed 10, 20 and 30% hempseed had a decrease in egg yolk yellowness. However, Konca et al. (2019) reported similar results to the current study where Lohmann brown hens were fed 15% raw hempseed and 15% heat treated hempseed with an egg yolk Roche color fan value of 10, meaning the yolk had a slightly darker more red pigmentation.

For the Lohmann LSL white hens reared in the conventional cage system egg quality was not significant between the treatment diets for egg weight, specific gravity, egg breaking strength, albumen height, shell weight. For white caged hens the egg yolk pigmentation was generally lighter in hens fed the control, 3 and 6% HO with no difference from the 10% HP, with more red pigmented yolks in the 20% HP group and more yellow pigmentated yolks in the 10 and 20% HP groups. Similarly, Goldberg et al. (2012) studied the effects of hempseed (10% and 20%) and HO (4%, 8%, and 12%) in the diet of individually caged Bovan white hens and reported significant reductions in lightness with significant increases in red and yellow pigmentation with the largest changes observed in the 20% hempseed group.

Gakhar et al. (2012) reported no effect of hempseed (10, 20%) or HO (4, 8, 12%) in the diet of Bovan white hens reared in individual cages on specific gravity, albumen height or eggshell thickness. Silversides and Lefrançois (2005) included HP in the diet of DeKalb Sigma hens at dietary inclusion levels of 0, 5, 10, and 20% and found that it did not significantly affect egg weight and albumen height. Similarly, in the current study, no effect was observed from the inclusion of 20% HP and 6% HO in the diet of Lohmann LSL-Lite White laying hens reared in the single-tier system for egg weight, specific gravity, albumen height, shell weight or shell thickness. However, there was a significant effect on egg breaking strength egg yolk pigmentation. These results agree with the findings of Kasula et al. (2021b), where HP was included in the diet of Bovan White hens reared in a colony cage at 0, 10, 20 and 30% inclusion and an increase in eggshell strength was observed in addition to an increase in egg yolk pigmentation, lutein content and PUFAs such as linolenic acid and LA. Several studies have reported that hemp products have improved yolk fatty acid profile of table eggs (Gakhar et al., 2012; Fabro et al., 2021). Egg yolk pigmentation for white hens reared in the single-tier system was generally lighter in the control and 6% HO group, with more red pigmentation in the 20% HP group and more yellowness in the 20% HP group. Egg yolk vitamin E content increased significantly with hens fed the control 20% HP, this is similar to the findings of Taaifi et al. (2023a) where they observed an increase in vitamin E content with 30% HP in the diet of Lohmann Brown Classic laying hens reared in semi-automatic coup. The current study agrees with Kasula et al. (2021a) where they reported no cannabinoid residues present in the eggs of hens fed dietary HP. An absence of existing research on assessing the effects of dietary hemp fed to white laying hens in alternative housing systems on egg yolk pigmentation resulted in no comparative studies available to validate the findings of the current study regarding egg yolk pigmentation.

5.3: Fatty acid composition

The fatty acid composition of egg yolks from Lohmann Brown-Lite hens reared in the conventional cage system had an increase in n-3 fatty acid content with the addition of dietary hemp. The highest values for linolenic acid, stearidonic acid, homo-a-linolenic acid and EPA were observed in the egg yolks from Lohmann Brown-Lite hens fed the 6% HO diet. For the egg yolks from brown hens fed the HP diet, ALA was higher than in egg yolks from hens fed the control diet, however all other significant n-3 fatty acids mentioned had no significant difference from the control. This result is similar to the findings of Taaifi et al. (2023b) where hempseed at 10, 20 and 30% was fed to Lohmann Brown Classic hens in a semi-automatic cage system and the egg yolk ALA increased with the increase in hempseed and no significant differences were found for EPA or DHA. ALA in animals is converted to DHA and EPA when the ratios of n-6 to n-3 are not too high (Taaifi et al., 2023b). If the ratio of n-6 to n-3 is higher, the conversion efficiency of ALA to DHA and EPA will decrease because of the competition for the same enzymes involved in the metabolic pathways for conversion of ALA to EPA and DHA. Additionally, the production of arachidonic acid from LA will increase due to the higher availability of n-6 fatty acids (Taaifi et al., 2023b). Perhaps oxidative degradation of DHA, EPA and arachidonic acid occurred during the lipid analysis. Conversely, Gakhar et al. (2012) found that n-3 fatty acids including ALA, EPA, DPA, and DHA were increased in egg yolks from Bovan White hens reared in individual cages fed hempseed (10 and 20%) and HO (4, 8 and 12%).

Egg yolk fatty acid content of the Lohmann LSL-Lite White hens in the present study were found to have increased linolenic acid, homo-a-linolenic acid, clupanodonic acid, and DHA which is similar to the white hens in the single-tiered system in the current study. Oleic acid was decreased in the egg yolks from the white hens reared in the single-tier system. Moreover, white hens in the single-tier system had a significantly increased amount of saturated fatty acids (myristic acid, stearic acid and arachidic acid) with the highest amount found in egg yolks from hens fed the 6% HO diet. Conversely, Neijat et al. (2016) observed a decrease in myristic acid in egg yolks from Lohmann LSL-Classic hens reared in a cage system and fed HO at an inclusion level of 4.5% and 9% and an increase in stearic acid in egg yolks was observed from hens fed all treatment diets (Neijat et al., 2016).

The fatty acid composition of egg yolks produced by Lohmann LSL-Lite White hens in the single-tiered system showed that 6% HO and 20% HP increased n-3 PUFAs or the individual linolenic acid, homo-a-linolenic acid, EPA, DHA and clupanodonic acid. Similarly, Jing et al. (2017) reported an increase in n-3 PUFAs; (ALA, EPA, DHA and DPA), in egg yolks from Lohmann White laying hens reared in individual metabolic cages fed hemp oil and HempOmega™ (commercial feed product containing hemp oil, offered by Boreal Technologies) at 4 and 8%. In the current study, n-6 PUFAs including LA, g-linolenic acid and homo-g-linolenic acid were significantly higher in hens fed the hemp diets. Jing et al. (2017) reported no significant difference in egg yolk n-6 fatty acid composition with the addition of dietary hemp oil and HempOmega™. The current study also showed that oleic acid in the egg yolks was reduced with the addition of hemp products, with the 6% HO group having the least amount of oleic acid. Jing et al. (2017) reported similar results of a decrease in oleic acid in egg yolks. Oleic acid is formed from the conversion of palmitic acid (C16:0) to palmitoleic acid (C16:1), which is then converted to stearic acid (C18:0) and subsequently into oleic acid and this conversion involves the enzyme Δ-9 desaturase (Astarita et al., 2011). The enzyme Δ-9 desaturase may be inhibited by n-3 PUFAs, which may have resulted in the reduction in oleic acid. Stearidonic acid would be expected to increase with a decrease in oleic acid, however the current study showed no significant difference in stearidonic acid between the treatment diets. The hempseed diets contained less oleic acid than the control which may explain the decrease in oleic acid in the egg yolks from hens fed dietary hemp.

5.4: Feather scoring and total body feather condition

The current study suggests that the inclusion of hemp products in the diet of laying hens did not have an adverse effect on the feather condition of the 7 body regions or overall total body feather condition. No significant difference between dietary treatments was observed for feather condition in the Lohmann Brown-Lite hens reared in the conventional cage system. For the Lohmann LSL-Lite White hens in the conventional cage system, the tail feather condition was significantly improved in period 3 with the addition of HP and HO compared to the control diet. For the tail and back of white hens in the single tier system, there was a significant difference in feather condition where the 6% HO diet resulted in a poorer feather condition during the early production periods however this could also be attributed to changes in social dominance, age, genetics, or potentially sources of error in determining the condition score. Abraham et al. (2023) reported that the addition of flaxseed oil to the diet of laying hens did improve feather condition across several body regions. Similarly, Baéza et al. (2017) found that linseed oil and microalgae fed to laying ducks reduced FP behavior. Both flaxseed oil and linseed oil are generally higher in n-3 fatty acids, perhaps the more balanced ratio of n-3 and n-6 in hemp oil is slightly lacking in n-3 which may be necessary for maintaining feather condition. Several studies have indicated that stress is associated with increased FP (Vestergaard et al., 1997; El-Lethey et al., 2010; De Haas et al., 2013). Since there were no adverse effects on the feather condition it can be concluded that hemp diets did not increase stress.

5.5: Tonic immobility

In the current study, tonic immobility of the Lohmann LSL-Lite White laying hens reared in a single-tiered system had no significant difference between the treatment diets. A study by Maser et al. (1975) analyzed the potency of injected tetrahydrocannabinol derivatives on tonic immobility in chickens and found that higher potency of delta 9 tetrahydrocannabinol resulted in longer tonic immobility durations. Delta 9 tetrahydrocannabinol potency in the present study was 0%, which explains why there was no significant increase in fear response duration. A study by Sadaka et al. (2021) concluded that CBD at a dose injection of 3μg/g in mice induced a decrease in activity in the neural circuitry controlling stress-related behavior, specifically the ascending reticular activation system in the brain. This suggested that CBD had a calming effect on the body's automatic stress response, since the ascending reticular activation system is involved in the neural circuitry of tonic immobility (Thome et al., 2019; Sadaka et al., 2021). The CBD content of the diets used in the present study was less than 0.3 μg/g, which explains why there was no significant decrease in tonic immobility duration and suggests that using a different cultivar of hemp with a higher CBD content may result in the desired calming effect in the hens.

5.6: Bone strength

Lohmann Brown-Lite hens reared in the conventional cage system had a significantly longer tibia length in the hens fed 3% HO with no difference from those fed 6% HO diet and the control. Brown hens had no significant difference in the breaking strength or mineral composition. While not significant, the breaking strength was lower in the 3% HO and 6% HO treatment groups compared to all other treatments. This contradicted the results of a study by Skřivan et al. (2019), where hempseed was added to the diet of Lohmann Brown hens reared in a three-floor enriched cage system at 3, 6, and 9%. Skřivan et al. (2019) reported that fresh tibia breaking strength increased significantly in all treatment groups compared to the control diet. However, the tibias tested in the current study were dried and not fresh.

In the present study, Lohmann LSL-Lite White hens reared in the conventional cage system had longer tibias in hens fed 6% HO with no difference from the control, 20% HP and 3% HO group and a higher ash content and bone breaking strength in hens fed the 20% HP diet. The inclusion level of 10% HP in the present study had the lowest tibia breaking strength for the white hens reared in the conventional cage system. This could be due to the differences in diet composition between the treatment diets. Bone strength of the Lohmann LSL-Lite White hens reared in the single-tier system was significantly improved when the hens were fed 6% HO. These results agreed with the study by Skřivan et al. (2019) confirming that hempseed and HO does improve bone breaking strength and that CBD content of hemp which is considered the active substance that enhances collagen cross linking in the bone, enhanced fracture healing (Skřivan et al., 2019; Sparks et al., 2022). However, the mechanism of how this occurs is not well understood. In the current study, hens reared in the single-tier system with a stronger bone also had a higher bone zinc content, which is reported to be an essential mineral for bone growth, homeostasis, and regeneration although the mechanisms by which this is promoted are unknown (O'Connor et al., 2020). If a zinc deficiency (10 mg/kg) is present in hens, a decrease in bone formation will occur (Niknia et al., 2023). The increase in zinc in the tibia bone in hens fed 6% HO may be due to the higher zinc content found in hempseed. Hempseeds have been reported to contain the highest levels of iron and zinc compared to other food seeds such as sunflower seed, poppy seed, flaxseed, and sesame seeds (Senila et al., 2020).

However, in hens fed 20% HP, the tibia zinc content was lower than the 6% HO and control group. This may be due to the lower corn and soybean meal content of the 20% HP diet.

5.7: Liver

No significant differences in liver fat by proximate analysis or histological analysis were observed in any dietary treatment in each trial. While not significant, the proximate fat content did decrease slightly with dietary hemp in both strains for the caged hens. The histological fat percentage decreased in the brown hens for every treatment, but for white hens in the conventional cage system, histological fat only decreased when they were fed 10% hempseed and 3% HO. For the white hens reared in the single-tier system, fat content only decreased in hens fed the 6% HO treatment diet. Similarly, Kaushal et al. (2020) reported histological reduction of steatosis in the liver of rats when hempseed lipid fraction (10%) was added to the diet. The hemp also altered the liver coloration where the liver of rats fed hemp had a darker red pigmentation, which indicates a healthy liver color and is similar to the results of this study where we saw a decrease in yellow pigmentation of the liver with the addition of hemp to the diet. In the present study, Lohmann Brown-Lite hens had a significant increase in yellow pigmentation of the liver when they were fed 20% HP and a significant reduction in yellow pigmentation when they were fed the 6% HO diet.

Lohmann LSL-Lite White hens reared in the conventional cage system had an increase in red pigmentation and yellow pigmentation of the liver in hens fed 20% HP and a significant reduction in yellow and red pigmentation in the liver of hens fed all other treatment diets and for the latter in hens fed the 3% HO and 6% HO diet.

Lohmann LSL-Lite White hens reared in the single-tier system had increased yellow pigmentation of the liver when fed the 20% HP diet. This could be an indicator of

FLD as yellow pigmentation has been associated with high liver fat (Rozenboim et al., 2016). Liver color is an important indicator of poultry health and changes in pigmentation may indicate disease. Anene et al. (2023), also observed a higher liver b* score in ISA Brown hens with low feed efficiency, which gave an estimate of higher yellow pigmentation of the liver and corresponded with higher hepatic fat deposition. In the current study, the yellow pigmentation of the liver was significantly reduced in hens fed 6% HO, which could indicate that HO reduced hepatic fat content. However, based on the lack of significant results for all other analyses of the liver, it is difficult to conclude whether hemp ameliorated FLD in laying hens. Future studies in this area of research should include analysis of plasma concentrations of ALT, AST, triglycerides, total cholesterol, VLDL, fatty acid binding protein 4 and lipoprotein lipase, in addition to inflammatory cytokines such as TNF-α, IL-6 IL-1, SAAL1, and iNOS2, which have been indicated as potential biomarkers for the diagnosis of FLD (Xing et al., 2020; Zhu et al., 2020).

5.8: Comparative analysis of laying hen strains and housing systems

Comparative analysis revealed that the inclusion of HP and HO in the diets of Lohmann Brown-Lite and Lohmann LSL-Lite White laying hens significantly influenced production performance and egg quality in certain periods. For example, hens fed 10% and 20% HP had no difference in FCR's compared to the control, but an increase in egg production in period 5 and 6. Hens in period 6 fed 20% HP produced more eggs than hens fed 6% HO. Egg breaking strength improved in the last two periods in all treatment groups compared to the control. Hens fed 20% HP, 3% HO and 6% HO had increased albumen height in period 5, which is perceived by consumers as firmer and fresher (Rizzi, 2021). Yolk pigmentation was most enhanced in hens fed 10% and 20% HP, resulting in a more red and yellow yolk. Hens fed 6% HO had the lightest yolk pigmentation of the hemp treatments. Higher levels of beneficial fatty acids (ALA, EPA, and DHA) were observed with increased hemp inclusion, particularly in hens fed 6% HO. Similar to other studies, including hemp in the diet of laying hens resulted in no adverse effects on production performance and egg quality and increases were observed for n-3 fatty acids (Gakhar et al., 2012; Skřivan et al., 2019).

Strain comparisons showed that white hens had better performance with regards to egg production and feed conversion compared to brown hens. Brown hens had increased egg breaking strength and yolk pigmentation. White hens showed improvement of total body feather condition compared to brown hens with the best feather condition found in hens fed 20% HP and 6% HO. Total body feather condition was best in white hens reared in either system compared with brown hens, but single-tier hens had a better feather condition compared to hens reared in the conventional cage. The absence of significant differences in tibia breaking strength for the comparison between brown and white hens, irrespective of the housing system or dietary treatment, suggests that variations in genetics, diet, and housing, did not influence bone strength under the conditions tested. This finding indicates that dietary inclusion of hemp products does not negatively impact bone strength in either brown or white laying hens, regardless of their housing system. This result contrasts with the findings of Skřivan et al. (2019), who observed an increase in tibia strength when hemp was added to the diet of Lohmann Brown laying hens reared in a three-floor enriched cage system at 10%. The discrepancy might be due to differences in strain response or dietary formulations used in the respective studies. Brown hens had increased mineral composition of the tibia compared to white hens and mineral composition was highest in hens reared in the conventional cage system compared to the single-tier system. Calcium, magnesium and zinc composition of the tibia was highest in hens fed 3% HO compared to hens fed 20% HP. Hepatosomatic index and liver proximate fat content was reduced in brown hens compared to white hens with histological liver fat reduced in hens reared in the conventional cage system. Liver redness and yellow pigmentation was highest in hens fed 20% HP which could be an indication of FLD if the redness was due to blood ruptures. These pigments were reduced in the liver of hens fed all other treatments.

White hens reared in the single-tier system showed a reduction in body weight compared to white hens reared in the conventional cage system, which could indicate improved health and a reduced risk for onset of FLD as the weight was closer to the recommended range (1.7-1.8kg; Lohmann Tierzucht, 2021). An increased eggshell thickness was observed for eggs from white hens reared in the single-tier system compared to the conventional cage system. White hens in the conventional cage system produced eggs with more yellow yolk pigmentation than eggs from white hens reared in the single-tier system. However, albumen height was highest for white hens reared in the conventional cage system. A higher egg breaking strength was found for white hens fed 20% HP in period 6 and shell thickness was highest in white hens fed 20% HP, 6% HO and control diets. No differences were found for white hen tibia strength between the two housing systems or the dietary treatments. White hens fed 3% HO had tibias with more zinc content than hens fed 20% HP. Liver weight was highest in white hens reared in the conventional cage system, while histological liver fat content decreased. Histological analysis showed the lowest liver fat in hens fed 10% HP and the highest liver fat in hens fed 20% HP. White hens reared in the single-tier system had reduced red pigmentation of the liver and were darker pigmentated which may have indicated a healthier liver. Comparison of analyses for liver fat content showed that fat was higher when analyzed by histology. Both fat content analyses should be conducted to analyze FLD in laying hens in future studies in addition to the other tests previously mentioned.

CHAPTER 6: SUMMARY AND CONCLUSION

Based on the current data, dietary inclusion levels of 10% and 20% HP, and 3% and 6% dietary inclusion of HO in laying hen diets had no negative impacts on hen production and egg quality. In the Lohmann Brown-Lite hens housed in conventional cages, there were no perceptible differences among treatments for feather condition. For the Lohmann LSL-Lite White laying hens in the single-tier housing system, feather-pecking incidences did increase slightly (but not significantly), which was expected, based on previous evidence that this behavior is more prevalent in alternative housing systems when compared to conventional cage systems. For hens in the conventional cage, we observed no effect of the dietary hemp treatments on FP behavior. In a single-tier housing system, tail feather scores exhibited the least amount of feather loss in hens fed 20% HP. Tibia strength increased significantly in Lohmann LSL-Lite White hens in the conventional cage fed the 20% HP when compared to the 10% HP diet and the white hens in the single-tier housing systems fed the 6% HO exhibited greater tibia strength than those birds fed the 20% HP diet. There was no carry over of cannabinoids to the eggs or the breast tissue and the eggs were significantly higher in ALA. The current data provides evidence in support of safety and efficacy claims for the use of hempseed products in the diet of laying hens and the potential for these diets to serve as alternative sources of ALA to produce eggs with higher levels of n-3 and n-6 fatty acids. In addition, the decrease in liver fat content although not statistically significant, could be indicative of the potential health benefits associated with hemp product supplementation in the diet of laying hens and the application of hemp to ameliorate FLD, which would prevent huge losses to the industry by increasing egg production and reducing bird mortality.

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