Safety and efficacy of hemp products in broiler production

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Animal Science

University of Manitoba

Winnipeg, Manitoba, Canada

April 2018

ABSTRACT

The overall aim of the research presented in this thesis was to evaluate production performance parameters, and the fatty acid and tetrahydrocannabinol (THC) contents of breast and thigh muscles of fast-growing broiler chickens consuming diets containing hemp (*Cannabis sativa L.*) products. To address this aim, two experiments were conducted in growing broiler chickens to assess performance (gain, feed intake, feed efficiency) and muscle lipid and cannabinoid levels, in birds consuming diets containing whole hemp seed, hemp oil or hemp meal. In general, the use of hemp products, when included at isonitrogenous and isocaloric substitution levels, led to similar performance in broilers as corn-soybean meal-fed control birds. Additionally, given high levels of alpha-linolenic acid, an omega-3 fatty acid, in hemp products, muscle tissues were enriched in this fatty acid. Finally, levels of THC were below limits of detection in edible broiler tissues. The results provide evidence of the safety and efficacy of hemp product inclusion for use in diets of broiler chickens.

ACKNOWLEDGMENTS

Foremost, I would like to express my gratitude to my advisor Dr House for his continuous support throughout my M. Sc. program. His encouragement, advice, enthusiasm, and knowledge were very helpful and motivating through the program, and I feel very fortunate to have had the opportunity to work alongside him. I would also like to thank my committee members: Dr. Aliani and Dr. Slominiski for serving on my committee and providing insightful comments and suggestions.

I am grateful to the organizations that provided funding, including the Canadian Hemp Trade Alliance and the University of Manitoba – Faculty of Agricultural and Food Sciences.

I thank my parents for their continued support and confidence in me. From them, I have learned to work hard and to take pride in a job well done. I also thankful to my lab members, including Neijat, Erin and Shusheng for their support of my research work, and to Jason Neufeld and Dennis Labossiere, for their technical support.

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Abbreviations

AA	arachidonic acid
ALA	alpha-linolenic acid
EPA	eicosapentaenoic acid
ETA	eicosatetraenoic acid
FA	fatty acid
FO	flaxseed oil
GLA	gamma-linolenic acid
HDL	high density lipoprotein
HO	hempseed oil
HPLC	high-performance liquid chromatography
HS	hempseed
HM	hemp meal
HO	hemp oil
LA	linoleic acid
LC	liquid chromatography
LCPUFA	long-chain polyunsaturated fatty acids
MS	mass spectrometry
MUFA	monounsaturated fatty acids
n-3	n-3
n-6	omega-6
NS	not significant
OA	oleic acid

PA	phosphoric acid
PALM	palmitic acid
PALMO	palmitoleic acid
PUFA	polyunsaturated fatty acids
SA	stearic acid
SD	standard deviation
SDA	stearidonic acid
SE	standard error of the mean
SFA	saturated fatty acids
THC	tetrahydrocannabinol

1.0 GENERAL INTRODUCTION

Cannabis sativa L. is an herbaceous plant belonging to the family *cannabinaceae* (Turner et al., 1978). The varieties primarily grown for industrial purposes, including feed and fiber, are collectively known as hemp. Traditionally, hemp is grown for its fiber which is used for the production of specialty paper products, including cigarette paper, bank notes and tea bags. The outer portion, or cambium, represents the stem tissue and contains fiber (17-20% lignin; 80-83% cellulose) that is used for such purposes as the production of biocomposites and specialty paper. The legal cultivation, via license, of industrial hemp was permitted by the Canadian government in 1998 (Health Canada, 2016) following a long period of prohibition. In 1937, the Canadian government banned industrial hemp production due to confusion with other types of cannabis which contain the psychoactive compound THC. With the move to repermit the production of hemp under license, Health Canada established guidelines that require industrial hemp to have less than 0.3% THC in the plant, leaves and flowering heads. Production in Canada, in 2008-2009, was restricted to approximately 20,000 hectares, with 90% of the land located in the Eastern Prairie regions (Manitoba and Saskatchewan). Hemp production in other regions is also subjected to regulatory oversight. In Europe, varieties of hemp which contain less than 0.2% of THC level on a dry matter basis can be grown. In Australia, hemp was legalized by the Queensland state government in 2002. In the United States, hemp cultivation is banned, but the usage of hemp products is not. In some countries, like, Russuia, China and Hungary hemp productions was nevever banned.

Beyond its role in providing fibre, the seeds of the hemp plant contain 30-35% oil (Callaway, 2004), 25 % crude protein (CP), 34% carbohydrate and 32% neutral detergent fiber (NDF; House et al., 2010) and thus could serve as an althernative feed ingredient for livestock or

as a human food ingredient. By expelling oil from the hempseed, the resultant hemp meal has a higher protein content and a lower energy value as compared to whole hemp seed, due to removal of the dilution effect of the oil. The crude protein and fibre values for the hemp meal are approximately 1.7 times that of hemp seed (40 and 10%, respectively; House et al., 2010). Hemp oil has between 75 to 80% polyunsaturated fatty acids (PUFA), with 17-19 % α-linolenic acid (ALA) and 60% linoleic acid (LA) (Parker et al., 2003). Other vegetable oils, with the exception of flax, typically having less than 9% ALA.

While the nutrient content of hempseed holds promise for both human and livestock consumption, the presence of cannabinoids within the seed or seed derivative remains a potential issue. Cannabinoids in hemp plant are produced in glandular organs known as trichomes and the cannabinoids spread all through the plant surface, with the exception of roots and seeds. Glandular organs are heavily present along leave veins and side of the leaves. Delta-9-tetrahydrocannabinol (THC) is the main psychoactive substance present in hemp plants and forms the basis of the regulatory oversight. More than 90% of the cannabinoid content consists of delta-9-tetrahydrocannabinol (THC). Additionally, there are approximately 60 other forms of cannabinoids, and these have yet to be fully characterized. Of those that have been characterized, cannabinol (CBN) and cannabidiol (CBD) represent two cannabinoids. While the psychoactive properties of THC are well documented, additional side effects that have been potentially linked to cannabis users include changes in pre-cancerous cells and chronic inflammatory responses in airways (Kalant, 2004). Cannabinoids have been used in different pathological conditions in humans and animals such as pain, loss of appetite, antiemetic (Kalant, 2001) with cancer chemotherapy, Parkinson disease, epilepsy (Robson, 2014; Devinsky et al., 2014) and multiple sclerosis (Wade et al., 2010; Flachenecker et al., 2014; Paolicelli et al., 2016). Cannabinoids are

not metabolically produced by the hemp seed itself, however they could be present in the hemp oil due to residual contamination (Ross et al., 2000). Cannabinoids are present in resin and can be transferred from leaves and flowers to the seeds. With processing, they could be transferred into the hemp oil. Cannabinoids in the cannabis plant are synthesized and accumulated in the form of cannabinoid acid (DeMeijer et al., 2003). Cannabinoid acid does not possess psychotropic properties. For psychotropic properties to be realized, cannabinoid acid must be decarboxylated to phenol, e.g. by smoking plant dried matter. Cannabinoid acid can be transformed into various cannabinoids by the process of decarboxylation at high temperature where the conversion rate is rapid. Additinally, they can also form at room temperature, but this transformation tends to be very slow. The impact of heat on the transformation places considerable importance on analytical procedures where heat is involved, including standard gas chromatography methods. When describing total cannabinoids after analysis, it is generally positioned as cannabinoids plus cannabinoid acid-derived cannabinoids.

Given the nutritional profile of hempseed, opportunities exist to use hemp products in poultry rations. For example, given its high content of ALA, flaxseed is currently used in laying hen rations for the production of omega 3-enriched eggs (Cherian and Sim, 1991; Gonzalez-Esquerra and Lesson, 2001; Cherian and Quezada, 2016). Both ALA and LA cannot be synthesized in animals, so they must be provided in the diet. In animal cells, long chain polyunsaturated fatty acids (LC-PUFA) are made through the metabolism of ALA and LA. Hemp, canola, soybean and sunflower are edible oils which contain reasonably good amounts of omega-6 fatty acids. However, not all vegetable oils provide significant levels of n-3 fatty acids. Hemp oil provides a 3 to 1 ratio of omega-6 to n-3 fatty acids, one that is considered favorable for human health. Too high a ratio may lead to asthma, depression, autoimmune diseases, diabetes, obesity, cancer and

cardiovascular diseases (Simopoulos, 2006). As such, hempseed and its derived products may prove useful feed ingredients in poultry diets to increase the ALA and total n-3 intake, with potential benefical effects on both the health of the birds and the resultant animal-derived food products destined for human consumption.

2.0 LITERATURE REVIEW

2.1 Overview of the chicken meat industry

2.1.1 Global chicken meat industry

Chicken meat is one of best sources of quality protein for the human diet. Globally poultry meat production has increased by 11 fold (9 million tons to 105 million tons) from 1960 to 2012 (Speedy, 2003; FAOSTAT 2012). This increase in production is primarily due to three factors: (i) better understanding of the nutritional needs of the birds; (ii) improvements with in flock disease control; and (iii) improvements in the genetic selection progress in genetics (Ravindran 2013). Feed is single major production cost (Davis et al. 2013) and can go up to 70 % of total production cost. The price of poultry meat can be decreased by increasing the efficiency of feed utilization and finding alternative, cheaper feed ingredients.

2.1.2 Canadian Chicken industry

Canada produced $2.4 billion worth of chicken products in 2015. There were 2690 registered chicken producers in 2015 in Canada. Canada produced almost 1.10 billion kilograms of chicken meat in 2015. In Canada, on average farms produced 555,496 kg (live weight) of chicken meat. In 2015, per capita chicken meat consumption in Canada was recorded to be 31.86 kg. In comparing the location of procurement, approximately 61% (686 million kg) of Canada's total chicken consumption was purchased from retail stores, 24% (269 million kg) was used in fast

food service establishments, 10% (114 million kg) was used in restaurant services and 5% (61 million kg) was consumed in hotels and other institutions. As a supply managed commodity, international trade is subject to certain control measures, including production quotas and tariffs. In 2014, the United States imported 97% of Canadian chick exports, worth approximately $15 million. Canada also exported chicken edible by products and meat to 63 countries, worth over $460.7 million. (Agriculture and Agri-Food Canada, 2016).

2.2 Nutrient requirements of Broiler chicken

Main nutrient require for broiler maintenance and growth are crude protein, carbohydrates, fat, vitamins, minerals and water (Cheeke, 2005). Dietary carbohydrates from cereal grains like corn, grain sorghum, barley and wheat are important source of energy for poultry. Corn and soybean meal are most commonly used ingrideints in poultry diet. With the increase of price of corn and soybean meal increased the interest in alternative ingridients. Hemp due to high protein quality high unsaturated fatty acid proportion and low presence of anti-nutritional factors (Kalmendal, 2008) could be used as alternative feed ingredient in commercial poultry feed.

Table 2.1 Nutrient requirements of Broilers

Nutrients	0 to 3 weeks	3 to 6 weeks
Crude protein (%)	23.00	20.00
ME (Kcal/Kg)	3200	3200
Ca (%)	1.00	0.90
Available P (%)	0.45	0.35
Linoleic acid (%)	1.00	1.00
Methionine (%)	0.50	0.38
Lysine	1.10	1.00
Arginine	1.25	1.10

Source NRC 1994

2.3 Fats

Triglycerides and phospholipids both belong to a group of compounds called fats or lipids. Triglycerides are sometimes known as visible fats, as they make up the bulk of visible adipose tissue/fat stores in meats. They are structurally distinct from phospholipids (invisible fats) which have different roles. Phospholipids play pivotal roles in the structure and function of cell membranes, whereas triglycerides predominantly serve as a storage form of energy or fatty acids destined for other metabolic functions. Triglycerides consist of three fatty acids attached to a molecule of glycerol, whereas phospholipids consist of two fatty acids attached to glycerol molecule (James et al, 2000). The constituent fatty acids can be classified based on their degree of saturation and the locationa of double bonds (Defilippis and Sperling, 2006).

Stearic acid is a saturated fatty acids (SFA; no double bond) and is labeled as 18:0. The latter nomenclature indicates that stearic acid has 18 carbons with no double bond. Other saturated fatty acids include butyric (4:0), caproic (6:0), caprylic (8:0), capric (10:0), lauric (12:0), myristic(14:0), and palmitic (16:0). The main sources of saturated fats in livestock diets are animal fats and coconut oils. Saturated fats are less susceptible to oxidation and thus have greater stability than polyunsaturated fats. However, from a perspective of human nutrition, saturated fat consumption can increase serum LDL-cholesterol levels which increase the risk for adverse cardiovascular events (James et al., 2000). The typical North American diet provides approximately 10% of the energy in the form of saturated fats (Wright et al., 2000).

Monounsaturated fatty acids (MUFA), such as palmitoleic acid (16:1) and oleic acid (18:1), have one double bond. Monounsaturated fats have been shown to lead to decreases in plasma LDL concentrations (Kris-Etherton et al., 1999), and may represent good replacements for saturated fatty acids. Additinally, MUFAs do not hinder the metabolism n-3 fatty acids and therefore

represent good replacements for n-6 fats, when considering overall human health outcomes (James et al., 2000). The Canadian adult diet gets approximately 12-13% of energy from monounsaturated fats (Health Canada, 2009). Dietary monounsaturated fats, especially when used as replacements for unsaturated fats can help in decreasing cardiovascular diseases and metabolic syndrome (Gillingham et al., 2011)

Polyunsaturated fatty acids (PUFA) contain two or more double bonds. PUFA can be further classified on the basis of the location of the first double bond, including n-3, n-6 and n-9 series. Polyunsaturated fatty acids having double bonds after the third carbon are known as n-3 PUFA and PUFA which contain double bonds after the sixth carbon are called n-6 PUFA (and so on). Fatty acids within the n-3 and n-6 PUFA classes must be derived from dietary precursors, the essential fatty acids LA (n-6) and ALA (n-3), as they cannot be synthesized in body (Covington, 2004).

2.5 Fats in poultry nutrition

Fats posses highest caloric density of all nutrients. Fats (twice energy valuethan carbohydrates and proteins) are preffered ingrideints in fulfilling the fast growing bird requirement. Hemp seed oil contains 1to 3 ratio of linolenic acid (17 to 19%) and linoleic acid (60%) (Parker et al., 2003). Due to its perfect unsaturated fatty acid ratio as compare to corn , soybean and conola oil it could be used as alternative feed ingredient in poultry feed.

Table 2.2 Recommended Versus Current Fatty Acid Intakes

Dietary Fatty Acids	Recommended Intake[a] (%of energy)	Current population Intake[c]	Change in Population Intake
Total fat	20-35%	34%	34%
Saturated Fat	<10% <7%[b]	11%	6%
Trans fat	<1%	2%	<1%
Monounsaturated Fat	<25%	12%	17%
Polyunsaturated Fat	<10%	7%	7%

6% decrease in LDL-C ⇩ 6-12% decrease in CVD

a American Dietetic Association and Dietitians of Canada recommendation for fatty acid intakes
b American Heart Association recommendation for cardiovascular disease prevention
c What We Eat in America, NHANES 2007-2008 (mean of US male and females (ages 20-59)

2.4 Metabolism of n-3 and n-6 Fatty acids

ALA is an 18 carbon, short chain n-3 fatty acid. Dietary sources of ALA n-3 fatty acids include hemp seed oil, flaxseed and their oil, canola oil, soy, chia seed oil, camelina oil and walnuts. Within the n-3 series (n-3), eicosapentaenoic acid (20:5 n-3; EPA) and docosahexaenoic acid (22:6 n-3; DHA) are derived from the essential fatty acid ALA. Within the omega-6 (n-6 series), arachidonic acid (20:4 n-6; AA) is derived from the dietary essential fatty acid LA. It can be hard to get appropriate quantities of EPA and DHA through diet, although marine animals and plants such including algae contain EPA and DHA. Commonly eaten land plants are rich in the short chain n-3 and omega-6 fatty acids, but do not provide the n-3 or n-6 PUFA. Animal cells can convert ALA into EPA and DHA via the sequential action of desaturase and elongase enzymes. The same enzymes are used to convert LA into AA (Neff et al., 2011). Different studies have shown different percentages of conversion of ALA to EPA and DHA. One study showed only 2-10% ALA converts to EPA and DHA in human beings (Chiu et al., 2008). Other study showed only 7 % of ALA converted to EPA and 0.013% into DHA in 21 to 63years humans from from (Goyens et al., 2005). Hussein et al reported 0.3% conversion of ALA into EPA and <0.01% into DHA (Hussein et al., 2005). Some studies showed that less than 1% of ALA converted into EPA and DHA (Covington, 2004; Singh, 2005). Metabolic path way LA and ALA is shown in figure 2.1. The conversion of ALA into EPA and DHA require desaturation and elongation in animals. Chicken as compared to other animals may produce more DPA through to tetracosapentaenoic acid, precursor of DHA (Gregory et al ., 2013)

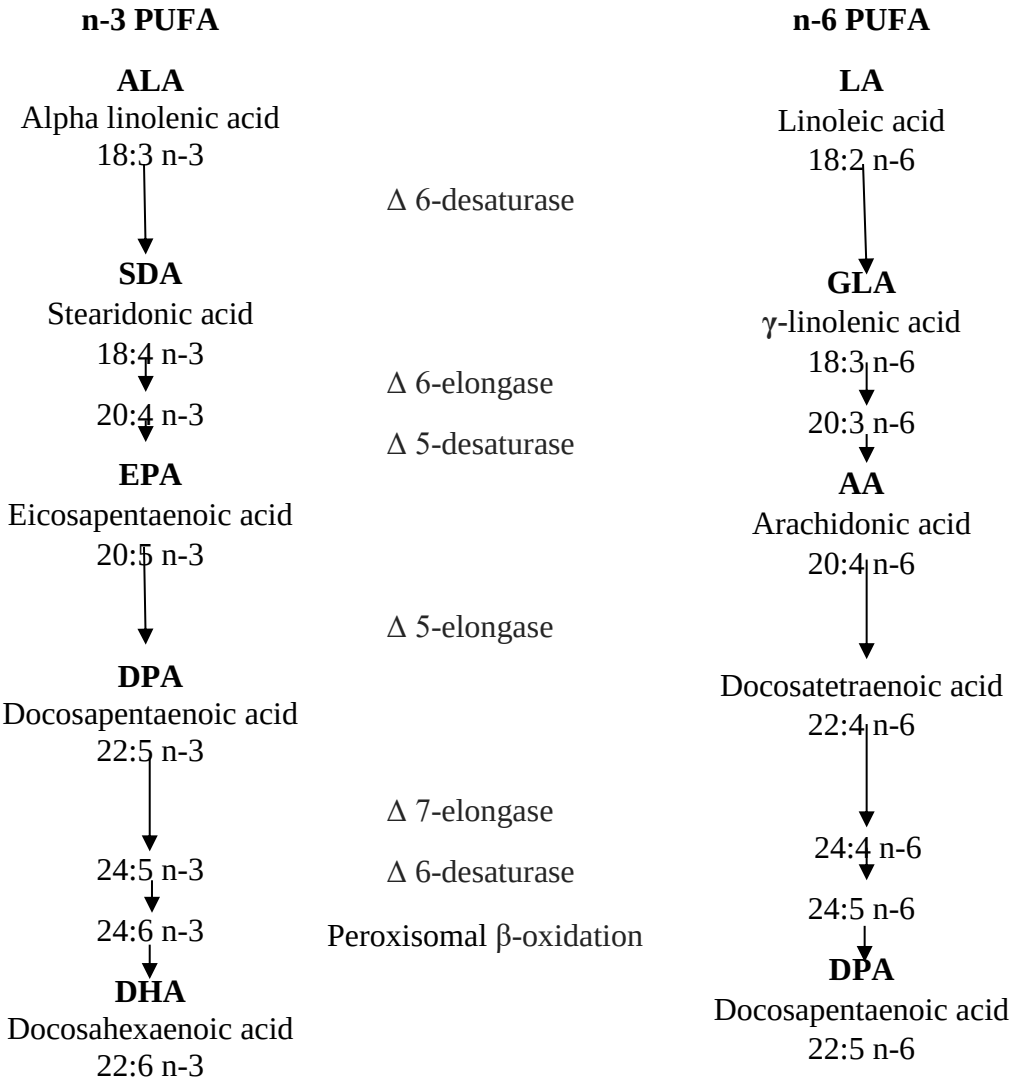


Figure 2.1 The metabolic pathway of n-6 and n-3 fatty acids
(Adapted from Whelan and Rust, 2006)

2.6 Fatty acid deposition in broiler chicken meat

Typically, fat in broiler meat consists of 32% polyunsaturated fatty acids, 30.5% of unsaturated fatty acids and 33.5% of saturated fatty acids (Ratnayake et al., 1993). This compares to the contents observed in ruminant meats, which typically contain high levels of saturates due to biohydrogenation of PUFA by rumen microbes (Bourre et al., 2005). The distribution of fatty acids in beef meat cuts consist of 40% saturated fatty acids, 40% monounsaturated fatty acids and 20% polyunsaturated fatty acids (Ratnayake et al., 1993). Broiler chickens tend to deposit fat within tissues in a similar proportion to that which is observed in the diet. Therefore, by changing the ratio of these fatty acids in broiler diets, improvements in the nutritional quality of broiler meat could be realized. Poultry diets have a direct influence on the lipid profile of chicken meat (Lopez-Ferrer et al., 2001; Howe et al., 2002). EPA, DPA and DHA was inceased in broiler breast and thigh meat was increased 4 to 9 times when dietary ALA was increased as compared to control diet (Kartikasari et al., 2012).

An alternative strategy to enriching poultry meat with n-3 PUFA, particularly the long chain PUFA EPA and DHA, is to include marine oils in the broiler diets. The inclusion of 1.0 or 1.25% tuna oil into broiler diets (0 to 49 days) significantly increased the n-3 PUFA (especially EPA and DHA) in breast and thigh muscle. EPA and DHA was increased 4 times as compared to control diet in breast muscles. EPA in thigh muscles was increased 10 times and in DHA in thiugh muscles increased 6 times as comapared to control (Morales-Barrera et al., 2013).Many studies have shown that fish oil inclusion in broiler diets can also increase the level of n-3 PUFA in broiler meat (Bou et al., 2005; Chekani-Azar et al., 2008). Increasing the n-3 PUFA of broiler meat via dietary enrichment with fish oil can produce negative impacts on meat sensory properties (Chekani-Azar et al., 2008), including bad odours and taste (Bou et al., 2005). The use of shorter

chain n-3 fatty acids (ALA) may provide a balance between enrichment and organoleptic properties. As flaxseed is also good source of n-3 PUFA, namely ALA, as well as moderate levels of monounsaturated fatty acids and low level of saturated fatty acids, its inclusion into broiler chicken diets can also increase n-3 PUFA levels in broiler meat. The use of flax seed to increase the level of n-3 fatty acid in chicken meat was first reported by Phetteplace and Watkins in 1989.

Table 3.3 Effect of dietary PUFA on depostion of PUFA in chicken meat

Authors	Animals	Experimental feed	Inclusion period	Performance	Fatty acid deposition
Poureslami et al., (2010)	400 1-day-old Ross-308 chickens	Basal Diet: containing 5% Palm fat Dietary treatments: diets supplemented with 3% of soybean oil, linseed oil and fish oil	From 7 to 42 days	Data not shown	Linseed diet fed chicken meat contain n-3 PUFA 11 times greater than average of other dietary treatments. However n-3 LCPUFA was 5 times higher in fish fed diets as compared to other diets.
Kartikasari et al., (2012)	60 1-day-old cobb 500 birds	Six dietary treatments rtanging 5 to 10% fat. Basal diet supplemented with blended flaxseed, macadamia and canola oil. ALA level varied 0.3% (Control) to 8% (Diet 5) of total energy.	From 2 to 28 Days	No statistical difference in weight of bird	Total n-3 PUFA in breast increased from 2.1% (Control) to 25.5% (Diet 5) and thigh muscle from 1.8% (Control) to 25.9% (Diet 5) increased with th the increase in ALA in feed.

Continued

Authors	Animals	Experimental feed	Inclusion period	Performance	Fatty acid deposition
Konieczka et al., (2017)	160 one-day-old Ross 308 female broiler	Basal Diet: Lard was used as supplementary fat. In experimental diets Fatty acids composition was modified by using fish oil combined with either lard, flax seed or rape seed	From 15 to 36 Days	Growth performance among all treatments were same	EPA in breast muscles was 1.35% to 1.63% of total fatty acids in experimental group as compared to control there was traceable amount of EPA present. The ALA, LA, PUFAn-3 and PUFA n-6 was hifgher in chickens breast muscles fed fish oil with flaxseed.Similar type of results were recorded in thigh muscles.
Kanakri et al., (2017)	240 0ne-day-old cobb 500 male broiler	Three types of diets were fed.1). Control. 2). Low ALA (macadamia oil 3% w/w) 3). High ALA (Flaxseedoil 3% w/w)	Upto 42 days	All growth performance parameters were not significantaly different	n-3 LCPUFA in breast and thigh was significantaly high in group fed high ALA for whole 6 weeks. But not signficantaly different from group fed first two week low ALA and then 4 weeks high ALA.

Authors	Animals	Experimental feed	Inclusion period	Performance	Fatty acid deposition
Azcona et al., (2008)	300 1-day-old cobb 500 broiler chicks half male half female	Basal diet: consist corn-soybean Experimental diets were added either flaxseed, rapeseed, chia seed or chia meal	From day 22 to day 46	Broiler weight, weight gain and FCR were less in flaxseed fed chickens. Feed consumption high in flaxdiet.	n-3 LCPUFA was significantaly high in chia seed and chia meal. N-6 to n-3 ratio was lowest in chia seed fed chickens.
Maroufyan et al., (2012)	300 one-day-old male brolier	Four different levels T1 (0.5%), T2 (8.0%), T3 (11.5%), T4 (16.5%) of n-3 PUFA by using the combination of sunflower oil and tuna oil	Upto 42 days	Feed conversion ratio of T2 group was significantaly better as compared to other groups.	n-3 PUFA of breast muscles was significantaly higher in T3 and T4 groups as compared to control.N-6 to n-3 ratio was also significantaly less in T4 group

Continued

Authors	Animals	Experimental feed	Inclusion period	Performance	Fatty acid deposition
Mandal et al., (2014)	300 one-day-old Cobb 400 unsexed broiler chickens	Basal diet: consist of corn-soybean meal with 1% oil in starter and 2% in finisher diet (22 to 39 days). Linseed oil and barn oil was used to get low (5:1), medium (10:1) and high (>20:1) n-6 to n-3	1 to 39 days	Average daily gain, average feed intake and feed conversion ratio was similar among all treatments	In breast muscles, n-3 LCPUFA was significantaly higher in low n-6 to n-3 ratio fed chickens. Similar type of results showed in thigh muscles
Mirshekar et al., 2015	336 1-day-old cobb 500 unsexed chikens	Control diet: contains soybean oil fed all 42 days. Experimental diets: Flaxseed was offer for 7, 14 , 28, 35 and 42 days.	1 to 42 days	All growth performance parameters were same among all the treatments.	Total PUFA were highest and MUFAwas lowest in group fed flaxseed for 35 days. N-6 to n-3 ratio was lowest in group fed flaxseed for 42 days.

Continued

Authors	Animals	Experimental feed	Inclusion period	Performance	Fatty acid deposition
Marco et al., (2013)	300 day-old Cobb broiler chickens	Control diet contains coconut oil. 25%, 50%, 75% and 100% replacement of coconut oil with omega-3 fatty acid enriched feed supplement (FAS).	From 7 to 42 days	No significant difference in growth parameters among all treatments	In thigh muscels with increase in replacement of coconut oil lead to significantaly decrease in saturated fatty acids and increase in n-3 fatty acids.
Elkin et al., (2016)	60 one-day-old female Ross broilers	Diets consist of either conventional soybean (CON) or stearidonic acid–enriched soybean oil (SDASOY).	From day 28 to day 42	Body weight, body weight gain, feed intake and feed conversion ratio was similar between two treatments.	Total n-3 LCPUFA was almost three times higher SDASOY fed group as compared to CON soybean fed group.

2.7 Benefits of essential fatty acids on human health

Fatty acids which cannot be produced by the body and have to be supplemented in the diet are called essential fatty acids. The n-3 ALA and the omega-6 LA are the two dietary essential fatty acids for animals. In recent years research has focused on these EFAs due to their positive role in the prevention of diseases. N-3 fatty acids play important roles in fighting against cardiovascular diseases and in ensuring normal brain functioning (Wang et al., 2006). Saturated and trans fatty acids along with cholesterol are main dietary components linked to atherosclerosis in humans. In contrast, n-3 PUFAs can decrease serum cholesterol levels and are purported to be beneficial in reducing the incidence and severity of myocardial infarction (Zyriax and Windler, 2000). Hypertriglyceridemia (increased blood serum triglycerides) is another cause of cardiovascular diseases which can be decreased by adding n-3 fatty acids to the diet (Hau et al., 1996). The essential fatty acids and their long chain derivatives become incorporated into cell membranes and play important roles in cell membrane viscosity and anti-inflammatory processes (Smith et al., 2011; Conquer et al., 2000). EPA and DHA are also required for fetal development and healthy aging (Dunstan et al., 2007). For example, the brain consists of 60% by weight of phospholipids. Increases in phospholipid can help in treatment of schizophrenia (Peet and Horrobin , 2002). N-3 PUFAs might serve to decrease the incidence of depression by controlling the antiphospholipid antibodies and t-cell over-activity (Colin et al., 2002).

2.8 Dietary n-6 to n-3 ratios

Enriching animal products, including meat, milk and eggs, with functional compounds has received considerable interest as consumer awareness is increasing about the linkages between diet

and health. Increasing the n-3 fatty acid concentration in animal meat and eggs is one such approach that has been taken (Palmquist, 2009). The long chain PUFA (LCPUFA) especially DHA; C22:6n-3) and eicosapenaenoic acid (EPA; C20:5n-3) is synthesized internally in body from α-linolenic acid, but in limited quantity (Simopoulous, 2000). Dietary unsaturated linoleic acid (LA; C18:3n-6) and α-linolenic acid (ALA; C18:3n-3) are desaturated and elongated to respective C20 or C22 polyunsaturated fatty acids through chain elongation enzymes and microsomal Δ5- and Δ6-desaturases (Palmquist, 2009) as shown in fig 1. The first step is Δ6-desaturation by enzyme Δ6-desaturases. Δ6-desaturases enzyme is shared between LA and ALA. Although Δ6-desaturases enzyme has more affinity towards ALA as compared to LA, higher concentrations of LA in the diet can lead to more production of n-6 PUFA. Δ6-desaturation is rate limiting, therefore a higher proportion n-6 PUFA in the diet also reduces the conversion of C18:3n-3 to DHA and EPA. Thus, it is very important to keep a proper n-6 to n-3 ratio (Simpoulous, 2000; Wijendran and Hayes, 2004; Palmquist, 2009).

Previous studies have shown that the beneficial EPA and DHA could be increased in chicken muscles by supplementing chicken diet with oils that have a good quantity of C18:3n-3 and C18:3n-6 fatty acids (López-Ferrer et al., 2001; Newman et al., 2002; Kitessa and Young, 2011). The ALA concentration could be increased two to three folds by using the 5:1(n-6 to n-3) ratio as compared to 25:1 (n-6 to n-3) ratio (Mandal et al., 2014). For better conversion of ALA to n-3 LCPUFA, the ratio of LA to ALA should be 4:1(Gonzalez-Esquerra and Leeson, 2001). The main aim in decreasing the ratio in chicken meat is to get good quality meat for human consumption. ALA plays a positive role in human health. Humans have evolved with a 1 ratio of n-6 to n-3 essential fatty acids in diet. But today, a Western diet typically contains n-6 to n-3 ratio 15:1-16.7:1. The Western diet is excessive in n-6 fatty acid and deficient in n-3 fatty acids

(Simopoulos, 2002). In early 1900, LA consumption was contributing approximately 3% of total energy but now it contributes 5-7% of total energy. This increase is due to more usage of vegetable oils which have more LA (Liou et al., 2007).

2.9 Hemp: Potential for use in Poultry Diets

2.9.1 Introduction

Hemp has been used as a source of fiber and food for the last 10,000 years. Hemp oil extracted from hempseeds has been used in dietary supplements, medicinal preparations, cooking, salad dressings, fuels and detergents; The fiber has been used for clothing, paper and ropes. Industrial hemp and marijuana plant both belong to the same species *Cannabis sativa L*. However the content of the psychoactive delta-9 tetrahydrocannabinol (THC) in industrial hemp is very small (0.3%) as compared to the marijuana plant which can contain up to 20% of THC by weight. In 1938, the cultivation of both marijuana and industrial hemp was banned in Canada. Some Canadian universities and few Canadian companies started research on industrial hemp in 1994. After 60 years in 1998 ban was lifted, and industrial hemp production was permitted under strict licensing conditions (Agriculture and Agri-Food Canada).

2.9.2 Nutritive Value of Hemp

With respect to the average proximate composition of hemp seed, it contains (by weight) 30-35% oil (Callaway, 2004), 25 % crude protein, 34% carbohydrate, with a NDF value of 32% (House et al., 2010). Hemp seed also contains insoluble fiber (10-15%), and a good range of minerals (Deferne and Pate, 1996; Pate, 1999). By expelling the oil from the hemp seed, the residual hemp seed meal has a higher protein content and a lower energy value as compared to hemp seed. The crude protein value for the hemp meal is approximatey 40%, roughly 1.7 times that of the hemp

seed (House et al., 2010). Hemp oil is a rich source of polyunsaturated fatty acids (PUFA), with a content between 75-80%. Further evaluation of the PUFA shows that it is comprised of 17-19 % α-linolenic acid (ALA) and 60% linoleic acid (LA) (Parker et al., 2003).. Hemp oil contains three to one (3:1) ratio of linoleic and linolenic polyunsaturated fatty acids that is suggested to be optimal for human nutrition. Due to this balanced ratio, hemp oil is also considered to be an important component of body oils and creams, and is purported to have strong skin penetrative properties (Rausch, 1995).

Hemp seed extraction (mechanical or solvent) produces a hemp meal with a lower oil and higher protein content as compared to hemp seed. On a dry matter basis, hemp meal consists of 96 g/kg ether extract, ash is 89 g/kg, crude fiber is 26.8 g/kg and crude protein is 345 g/kg (Gohl, 1993).

Table 2.4 Typical nutrition profile (%) of hemp seed [a]

	Whole seed	Seed meal
Oil	35.5	11.1
Protein	24.8	33.5
Carbohydrates	27.6	42.6
Moisture	6.5	5.6
Ash	5.6	7.2
Energy (KJ/100g)	2200	1700
Total dietary fiber	27.6	42.6
Digestible fiber	5.4	16.4
Non-digestible fiber	22.2	26.2

Adapted (Callaway, 2004), [a]cv Finola

Table 2.5 Typical values (mg/100g) for vitamins and minerals in hempseed[a]

Vitamin E	90.0
Thiamin (B1)	0.4
Riboflavin (B2)	0.1
Phosphorous	1160
Potassium	859
Magnesium	483
Calcium	145
Iron	14
Sodium	12
Manganese	7
Zinc	7
Copper	2

Adapted (Callaway and Pate 2009), [a]cv Finola

Table 2.6 Typical fatty acid profile (%) of hemp and other seed oils

Seed	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	AL[b] acid	GLA	SDA	%PUFA	n6/n3 ratio
Oil hempseed	5	2	9	56	22	4	2	84	2.5
Fiber hempseed	8	3	11	55	21	1	<1	77	2.7
Black currant	7	1	11	48	13	17	3	81	4.1
Flax (linseed)	6	3	15	15	61	0	0	76	0.2
Evening primrose	6	1	8	76	0	9	0	85	>100
Sunflower	5	11	22	63	<1	0	0	63	>100
Wheat germ	3	17	24	46	5	5	<1	56	10.2
Rape seed	4	<1	60	23	13	0	0	36	1.8
Soy	10	4	23	55	8	0	0	63	6.9
Borrage	12	5	17	42	0	24	0	66	>100
Corn	12	2	25	60	1	0	0	60	60
Olive	15	0	76	8	<1	0	0	8	>100

Adapted (Callaway 2004), [b]AL: alpha-linolenic acid

2.9.3 Hemp in Animal Feed

Fatty acid deposition in meat has a direct relationship with the nature of fatty acids present in feed (Mourot and Hermier, 2001). This relationship is used to modify the fatty acids in meat and inclusion of fatty acids that are good for human health. Mourot and Guillevic (2015), included hemp oil into pig feed to investigate the effect on nutritional quality of meat. In this study, 36 pigs were divided into three equal (12 in each) groups. All the pigs received isolipidic and isoenergetic diet. The first group received palm oil (providing 0.6g of C18:3 n-3 (ALA)/ Kg of feed), the second received rapeseed oil (containing 1.9g of C18:3 n-3 (ALA)/ Kg of feed) and the third group received hemp oil (providing 3.4g of C18:3 n-3 (ALA)/ Kg of feed). There were no significant differences due to hemp oil inclusion on growth performance, body composition or meat quality. However, deposition of ALA in the meat was significantly higher ($p < 0.0001$) in the hemp oil group. Hemp oil may be a good means to improve the ALA level in pig meat.

Hemp seed is a potentially valuable feed ingredient. It contains 30-35 g/kg oil which has 80% polyunsaturated fatty acids (Deferne and Pate, 1996). Hemp oil can be used in animal feed, cooking, human food or in industrial products like lubricants, detergents and paints. Solvent or mechanical extraction of hemp seed produces a meal that has low oil and high protein content. Hemp meal contains 345g/kg crude protein (Gohl 1993). Hemp meal could be used as efficient protein source for ruminants because it has relatively higher fiber contents. Mustafa et al. (1999) used meal derived from hemp (*Cannabis Sativa* L) seed to determine the nutritive value of hemp meal for ruminants. Hemp meal and other three meals (canola meal, heated canola meal and borage meal) was offered to two ruminally fistulated cows in a randomized complete block design to investigate *in situ* dry matter and crude protein digestibility. Rumen undegrdable protein available to intestine was measured by pepsin-pancreatin assay.

Total-tract nutrient digestibility of diets was measured by using twenty growing lambs in complete randomized design. In this lamb study, canola meal was replaced by hemp meal at 0, 25%, 50%, 75% and 100% as a protein source. Rumen undegradable protein intestinal digestibility was highest in HM (782.5 g/kg of CP). Sheep fed 200g/Kg hemp meal (in barley based diets) did not show any change in voluntary intake and total –tract nutrient digestibility coefficient. Hemp meal is a good source of rumen undegradable protein (RUP) and also has high post ruminal availability. Hemp meal may be used in ruminant diets as an excellent protein source instead of canola meal without any detrimental effect.

Cereals are usually used as a main source of energy in poultry diets, with oilseed meals serving as protein sources. Fats and oils provide more than double the energy of starch (Lloyd et al., 1978). In feed formulations, fats and oils play important roles in balancing the nutrients. Linoleic acid is present in vegetable oils and has been recorded to be important for getting maximum egg size (March and MacMillan, 1990). Fatty acid composition of egg yolk has direct relation with dietary fatty acids (Eder et al., 1998). Linolenic acid enriched eggs have been produced by using flax. Silversides and Lefrancois, (2005) studied the effect of hemp seed meal on laying hens. Four diets (0, 50, 100 and 200 g/Kg of hemp seed meal) were offered to total 102 DeKalb laying hens (42-weeks old). All the diets were isoenergetic and isonitrogenous. Hens were weighed at the beginning and end of the trial. Feed consumption and egg production (also cracked, broken and soft shelled eggs) were recorded. Eggs were collected after 4 weeks of feeding diets for subsequent fatty acid analysis. There were no significant differences in egg quality, body weight, feed efficiency, feed consumption and egg production between the four diets. However, results showed higher concentrations of LA and ALA and lower concentrations of palmitic acid with increasing inclusion of of hemp seed meal.

Hullar et al., (1999) used different pigeon feeds (hemp, sunflower, lentils, peas, canary seed, sorghum, white and red millet, barley, wheat and corn) to determine digestibility and metabolizable energy contents. All feeds were offered in whole grain form. There were no significant differences between chickens and pigeons in the digestibility of crude protein within the different feeds. The apparent metabolizable energy value in pigeons was slightly higher but not significantly different.

Hemp has potential as alternative poultry feed ingredient. Hemp products are not currently approved as a feed ingredient in Canada, due to a lack of information and data about its safety and efficacy. Neijat et al., (2016) used hempseed (HS) and hemp oil (HO) to increase n-3 polyunsaturated fatty acids, triacylglycerol and phospholipids of egg yolk. In this study, they provided 6 different diets (control, 10% HS, 20% HS, 30% HS, 4.5% HO and 9%HO) to 48 Lohman LSL-Classic hens in completely randomized design. The highest level (30%) of HS and HO (9%) inclusion increased ALA value 12 times and DHA value two to three times. Results showed that the inclusion of hemp seed and hemp oil in laying hen diets helped to increase n-3 fatty acids and decreased the n-6/n-3 ratio. These results were achieved by feeding hemp products for four weeks. Neijat et al., (2014) also used hemp seed and hemp oil to assess the effects on blood plasma chemistry in laying hens. A similar level of hemp seed (10%HS, 20% HS, 30% HS) and hemp oil (4.5% HO and 9%HO) was included in hen diets as utilized previously. Hemp seed and hemp oil did not show any effect on blood chemistry (plasma level of electrolytes and metabolites), hen performance or egg quality. Plasma enzyme concentrations (particularly gamma-glutamyl transferase) was lower in birds consuming the highest concentration of hemp seed (10% HS, 20% HS) and hemp oil (4.5% HO).

Gakhar et al., (2012) included hempseed and hemp seed oil in laying hens diets to investigate their safety and efficacy. Forty eight Bovan White 19-week-old laying hens were fed diets containing no hemp products (control), 10 or 20% hempseed or 4, 8, or 12% hemp oil. There was no change in egg production but egg weight was higher in the 20% HS group as compared to control diet. Feed intake was similar in all groups except in the 4% HO group where feed intake was lower as compared to control. Final body weight was only lower for those hens consuming the 12% HO group. The total α-linolenic acid in egg yolk was directly proportional to dietary n-3 fatty acid intake. The level of docosahexaenoic acid in egg yolk showed a quadratic response to increasing ALA in diet. The expression of key genes (fatty acid desaturase 1 and 2) was significantly lower in HS and HO-fed hens. The inclusion of hemp seed up to 20% and hemp oil up to 12% did not deteriorate the performance of laying hens; however HS and HO inclusion helped to increase n-3 fatty acids in egg yolk.

Khan et al., (2010) studied the effect of feeding *Cannabis Sativa* on broiler carcass quality. The total 160 day-old broiler birds were divided into four groups. *Cannabis sativa* crushed seeds were included in three groups and one group was control. The inclusion level of *Cannabis sativa* seed in feed was 5, 10 and 20%. It was observed that feed intake was significantly lower and weight gain was significantly higher in the 20% seed included group. Feed conversion ratio (FCR) was also better in 20% seed group. There was no significant difference in mortality and dressing percentage. Breast, leg, gizzard liver, intestine and abdominal fat weight were significantly higher in the 20% seed group. Heart weight was similar between all treatments. (Khan et al., 2009). It was concluded that these seeds could be used in broiler feed to get good FCR and potentially lead to decreases in broiler production cost.

Goldberg et al., (2013) studied the effect of hemp seed and hemp oil on sensory characteristics and fatty acid profile of eggs. White Bovan laying hens were offered 6 types of diets (control, 4% HO, 8% HO, 12% HO, 10% HS and 20%HS) for 12 weeks. Results showed that the total n-3 polyunsaturated fatty acid contents was significantly higher in all groups and was highest (15.3mg/g of yolk) in the 12% HO group as compared to controls (2.4 mg/g of yolk). There was no significant difference in aroma and flavor of the cooked eggs. The 20% HS group yielded the highest sweet flavor and the 4% HO group yielded the least sweet flavor. Hemp inclusion increased n-3 polyunsaturated fatty acids and color intensity in yolk without affecting the aroma and flavour of cooked eggs.

Mahmoudi et al., (2015) studied the effects of hemp seed and oligosaccharides on antibody titer response and growth performance of broiler chickens. A total of three hundred and eighty four male Ross 308 broilers were divided in 8 groups in a 4×2 factorial design. The broiler chickens were fed with 4 levels of hemp seed (0, 25, 50, 75 g/kg) and two levels of oligosaccharide (0 and 1g/kg). The experimental feeds were fed to broilers for 42 days. There were significant decreases in average daily feed intake and average daily gain in birds consuming 25 g/kg HS included group. Highest level of hemp seed (75g/kg) caused minimized levels of triglycerides, low and very low density lipoprotein and total serum cholesterol. High density lipoproteins (HDL) increased with increasing hemp seed level. Average daily gain and HDL was significantly increased and feed conversion ratio was also improved with the addition of dextran oligosaccharide (DOS). There were no significant differencesin antibody production, complete blood count (CBC), relative weight of spleen and bursa between the treatments. Highest levels of dietary hemp seed and dextran oligosaccharides (75g/kg of HS+ 1 g/kg of DOS) can be used to decrease lipids in blood serum without negative impact on broilers performance.

Kalmendal, 2008 used hemp seed cake in broiler diet. One day old Ross 308 broiler chickens (a total of 96) were used in this study. Hemp seed cake was fed to broiler chicken from 28 to 35 days post hatch. Four levels of hemp seed cake (0, 10, 20 and 30%) were included in finisher commercial broiler diet. There was no effect of hemp seed cake inclusion on feed consumption, weight gain and feed conversion ratio. Hemp seed cake inclusion up to 30% did not show any negative effect on broiler production performance and palatability of the feed

2.10 Cannabinoids

Cannabis is complex plant consists of more than 535 compounds. It has been of interest for hundreds of years because of its biologically active compounds (Hazekamp et al., 2010; Raharjo and Verpoorte, 2004). Cannabinoids are unique compound only present in cannabis plants. Glandular trichomes mostly present on aerial surface of cannabis plant are production house of cannabinoids (Turner et al., 1978). More than 70 cannabinoids have been discovered in cannabis plant.Cannabidiol (CBD), Cannabinol (CBN), Δ^9-tetrahydrocannabinol (THC), cannabigerol (CBG) and cannabichromne (CBC) are important cannabinoids (Turner et., 1980). THC is most important among cannabinoids because it has psychoactive effect (Elsohly and Slade, 2005).

Table 2.7 Identified compounds in *Cannabis sativa L*

Compound class	Compounds identified
Terpenoids	>120
Cannabinoids	>70
Hydrocarbons	50
Sugars and related compounds	34
Nitrogenous compounds	27
Noncannabinoid phenols	25
Flavonoids	23
Fatty acids	22
Simple acids	21
Amino acids	18
Simple ketones	13
Simple esters and lactones	13
Simple aldehydes	12
Proteins, glycoprotein, and enzymes	11
Steroids	11
Elements	9
Simple alcohols	7
Pigments	2
Vitamin	1 (vitamin K)

(Adapted from Hazekamp et al., 2010)

The current scientific cannabis classification is (Lehmann and Brenneisen, 1995):

Division	Angiosperm
Class	Dicotyledon
Subclass	Archichlamydeae
Order	Urticales
Family	Cannabinaceae
Genus	*Cannabis*
Species	*sativa* L, *Indica, ruderalis*

2.10.1 Classification of Cannabinoids

Cannabinoids are divided into three categories on the basis of their origin: phytocannabinoids originate from plants, endocannabinoids present inside human and animal tissues and synthetic cannabinoids. The common cannabinoids present in phytocannabinoids are Δ9-tetrahydrocannabinol, cannabinol, tetrahydrocannabivarin. Anandamide (AEA), 2-Arachidonyl glycerol (2-AG), 2-Arachidonyl glyceryl ether and N-Arachidonyl dopamine (NADA) are endogenous cannabinoids present inside human and animal tissue. Synthetic cannabinoids are Dronabinol (synthetic Δ9-THC), Nabilone (Δ9-THC analogue), CP-55940, WIN-55,212-2, HU-210, HU-211 and JWH-133(Elikottil et al., 2009).

2.11 Endocannabinoid system

The endocannabinoid (eCB) system is complex signaling system consists of receptors (CB1 and CB2 receptors), lignads and enzyme for lignad degeneration and biosynthesis. The endocannabinoids are not permanently present in cells but are synthesized in cell membrane of

mammalian cells (skeletal, neuron and adipocyte) from arachidonic acid-containing phospholipids on demand (Newman et al., 2007). The endocannabinoids synthesis could be started due to increase in intracellular calcium level (Cadas et al., 1996), depolarization of membrane and/or stimulation of receptors (Pagotto et al., 2006). Although endocannabinoid system is widely distributed in central nervous system (Katona and Freund, 2012; Marsicano and Lafenetre, 2009), But its activity is very specific. In recent years a large amount of data expressing the endocannabinoid (eCB) system role in the regulation of stresses coping, anxiety and acquired fever (McLaughlin et al., 2014;Gunduz-Cinar et al., 2013; Akirav, 2011; Ruehle et l., 2012; Riebe et al., 2012; Lafenetre et al., 2007).

2.11.1 CB1 and CB2 receptors

CB1 receptors are present in central and peripheral tissues, play important role in lipid and glucose metabolic pathways (Silvestri et al., 2011; Pagotto et al., 2006; Di Marzo et al., 2004). CB1 receptors are present in all parts of brain but most abundantly present in the brain structures related neurophysiological functions (Herkenham et al., 1991). CB_1 receptors are present in brainstem, midbrain and forebrain, in different locations of cells and varying densities. In locus coeruleus receptors were not only found presynaptically as expected, but also on postsynaptic somatodendritic compartments (Scavone et al., 2010). The lower density of CB_1 receptors are in brainstem region, ventral tegmental area and nucleus accumbens, with highest densities are basal ganglia, amygdale and cortex (Mackie, 2005). CB1 rectors are predominantly present on inhibitory neurons as compared to excitatory ones (Kano et al., 2009). CB2 receptors involved in immune and inflammatory processes (Howlett et al., 2002).

2.12 Pharmacokinetics of Tetrahydocannabinol

Following ingestion, tetrahydrocannabinol (THC) appears to be distributed in spleen, lungs, liver (Musshoff and Madea, 2006; Chiarotti and Costamagna, 2000; McBurney et al., 1986) and adipose tissue because it is highly lipophilic. Tetrahydocannabinol is metabolized in the liver. Δ^9-THC is first converted to 11-hydroxy Δ^9–Tetrahydrocannabinol (11-OH-THC) metabolite which is a psychoactive compound produced via hydroxylation and this metabolite is more potent than THC. 11-OH-THC further is converted into 11-nor-9-carboxy-Δ^9-tetrahydrocannbinol (THC-COOH) by oxidation. THC bioavailability is up to 50% when it enters the body via smoke inhalation. By contrast, a slower rate of absorption and 6-30% bioavailability are reported through oral ingestion (Ashton, 2001). After inhalation Δ^9-THC is absorbed through the lungs rapidly. About 90% of THC is circulated in blood plasma and the rest in red blood cells. After inhalation, Δ^9-THC is detectable in blood plasma just in few seconds while peak concentrations in plasma are attained in 3-10 minutes (Vandevenne et al., 2000; Widman et al., 1974). Approximately after 8 minute maximum Δ^9-THC concentration was observed and its metabolites like 11-OH-THC peak concentration was at 15 minutes and THC-COOH peak concentration could be observed at 81 minutes after inhaling (Huestis et., 1992). Peak Δ^9-THC concentration was observed 4.4 to 11ng/ml after 1 to 5 hours after oral ingestion of chocolate cookie containing 20 mg of Δ^9-THC (Huestis, 2007).

2.13 Summary

Feed cost contribute almost 70% of total broiler production cost. To maximize the net profit and minimize the feed cost, alternative feed ingredients are used in broiler production to achieve better profit. The increasing availability of hemp after lifting ban in 1998 create opportunities to use in poultry feed as alternative or additive feed ingredient. Whole hemp seed (HS) contain protein from 20% to 25% (Fortenbery and Bennet, 2004; Callaway, 2004), 34% carbohydrates and 31% fats (Leizer et al., 2000; Kelley and Rudolph, 2000; Callaway, 2004). Hemp seed contain mainly albumin (globular protein) and edestin (legumin protein), both are rich in essential amino acids (Callaway, 2004). Sulfur-containg amino acids like methionine and cysteine are also rich in hemp seed proteins (Odani and Odani, 1998). Hemp seed oil competes with all commonly used vegetable oils as it contains more than 80% polyunsaturated fatty acids (PUFA). Hemp seed oil is rich source of essential fatty acids, contains 56% linoleic acid (LA, 18:2 *n*-6) and 22% alpha linolenic acid (ALA, 18:3 *n*-3).Hemp seed oil also contains 4% gamma-linolenic acid (GLA) and 2% stearidonic acid (SDA) (Callaway, 2004). Major dietary sources of n-3 PUFA arefish and plants like flaxseed whereas n-6 PUFA sources are vegetable oils like soybean oil, safflower and corn (Schmitz and Ecker, 2008).N-3 PUFA are important for regulation of lipid and glucose metabolism, normal development and growth, and organ functioning. Particularly the n-3 long chain PUFA like eicosapentaenoic acid (EPA) is precursor of anti-inflammatory eicosanoids (Ajuyah et al., 2003) and docosahexaenoic acid (DHA) is important in early development of brain and eye (Lauritzen et al., 2001).N-3 PUFA also play important role in reducing cardiovascular diseases (CVD) incidents in humans (Simopoulous, 1997). Due to all thses charcteristics hemp could be used as alternative feed ingrideint to produce healthy chicken meat for human consumption. Industrial hemp commonly known as hemp contains less than 0.3% of tetrahydrocannabinol (THC) (Health Canada, 2016) and cannabis sativa variety containing 1-20%

of THC is called marijuana.Currently hemp use in animal feed is banned. We need more studies to prove its safety and efficacy in animals.

3.0 Hypotheses and Objectives

3.1. Hypotheses

1) Production performance and mortality of broiler chickens will not be affected by dietary hemp seed products
2) Different levels of hemp seed, hemp meal and hemp oil will increase the polyunsaturated fatty acids in primary commercial parts of the chicken, including the breast and thigh muscles
3) Delta-9-tetrahydrcannabinol (THC) level in breast, thigh, liver and kidney tissues will not be higher than regulated levels.

3.2. Objectives

1) To provide evidence for the safety and efficacy of hemp products for use in broiler rations
2) To determine the optimum level of hemp products needed to get maximum level of essential fatty acids in edible parts of broiler chickens
3) To develop a method to determine the amount of delta-9-tetrahydrocannabinol (THC) in edible parts (breast and thigh muscles), liver and kidney after feeding different levels of hemp seed, hemp meal and hemp oil to broiler chickens.

CHAPTER 4: MATERIALS AND METHODS

4.1. Animal Care

The experimental research (animal and managemental procedures) were conducted under University of Manitoba Animal Care Protocol Management and Review Committee and guidelines of Canadian council on animal care (1993).

4.2. Feeding Experiment

In the first and second experiments, a total of 300 Ross 308 one day old broiler chicks were obtained from local hatchery. All birds were randomly assigned to 60 pens with 5 chicks in each pen. They were raised on wire floored battery in environmentally control room. The room temperature was maintained at 32° C for first three days and then gradually decrease 0.5° C each day until day 21. Chicks were exposed to 24h light. The bird had free access to water and feed. At day 35 birds were killed and muscles were immediately collected and frozen for analysis. Study design and conditions was same as in first experiment.

4.3. Experimental Diets

Two experiments were conducted with different levels of hemp seed, hemp meal and hemp oil. In first experiment total ten wheat and soybean based diets were formulated to meet all nutrient specifications for Ross 308 broiler. For better mixing hemp seed and wheat was mixed in 50:50 ratio. One of them was control (without hemp products) and other nine were three different levels of Hemp seed (10%, 20%, 30%), Hemp Meal (8%, 16%, 24%) and Hemp oil (3%, 6%, 9%) as shown in table 4.1 and 4.2. First 10 days chickens were fed starter diets and then after grower/ finisher diet. All the diets were isonitogenous and isocaloric. Experimental diets and water were offered *ad libitum* throughout the study. Feed intake and body weights were measured on weekly

basis. In second experiment diets were corn/soybean based. Total diets were ten, one of them was control and other nine were three different levels of Hemp seed (06%, 12%, 18%), Hemp Meal (5%, 10%, 15%) and Hemp oil (2%, 4%, 6%) as shown in table 4.3 and 4.4. Pen-wise live weight, feed intake and feed conversion ratio were determined on weekly basis. Feed samples from two experiments were analysed by same method used for the analysis of meat (breast and thigh) muscles. Fatty acid composition of feed is shown in table 4.5 and 4.6.

4.4. Sample and tissue collection

At the end of experiment 35 day, two birds from each pen total 12 birds 6 male and 6 females per treatment were randomly selected and slaughtered and breast and thigh muscles (skinless) were collected and then freezed in liquid nitrogen and then stored at -20°C until further analysis.

4.5. Laboratory Analyses

4.5.1. Feed Analysis

Feed samples (in duplicate) were analysed for dry matter (DM), crude protein (CP), crude fat (CF), calcium (Ca), phosphorous (P) and gross energy (GE). Dry matter was analysed using method 934.01 of AOAC (1990). For dry matter analysis of the feed, 1g samples of each diet were weighed in silica dishes and put into an oven set at 104 °C overnight. The next morning, feed samples were removed, cooled down in desiccator and re-weighed, with the moisture content/dry matter determined by difference. The crude protein (N× 6.25) content of the diets was determined through the use of a Leco nitrogen analyzer (Leco Corp., St. Joseph, model NS-2000). The calcium and phosphorus content of feed samples were analyzed according to method 990.08 of AOAC (1990). Feed samples were ashed in a 600°C muffle furnace for 12 h. Then, the ash was digested with 5N HCl and 1% HNO_3. An inductively coupled plasma optical emission spectrometer (AES Vista,

Varian Inc., Palo Alto, CA) was used to calculate the concentrations of Ca and P in diet samples. Crude fat (CF) was calculated using mehod 2003.06 of AOAC (2005). Gross energy (GE) content of the feed samples was determined throught the use of a bomb calorimeter (AOAC, 1995).

4.5.2. Determination of the fatty acid content

The concentration of fatty acids in feed, breast and thigh tissue were measured by capillary gas chromatography. Fatty acid analysis consist of two main parts extraction and methylation. Fatty acids were extracted by Folch method (Folch et al., 1957).

Diet or tissues (Breast and thifgh) lipids were extracted by the procedures similar to the Folch method. Chloroform/methanol (2:1, v/v) containing 0.025% CaCl2 was added (usually 4 ml solvent added to 50–100 µl sample) and homogenised vigorously for 2 min. One ml of 0.9% NaCl was added and mixed again. The chloroform phase containing fatty acids was collected. The remains (fatty acids) were extracted with another 2 ml chloroform. The dried samples were reconstituted in 1 ml of toluene. Fatty acid C17:1 (Nu-Chek Prep Inc., Elysian, MN) was added to the samples as an internal standard to monitor recovery rate.

Fatty acid methyl esters were prepared by using meyhanolic HCl. Lipid sample was mixed with1.5 ml of methanolic HCl in 8 ml glass tubes and then heated at 80°C in a oven for 2 hours, cooled to room temperature and methyl esters extracted in the hexane phase after addition of 1 ml petroleum ether. Then the upper hexane layer was removed and dried down under nitrogen

Table 4.1: Starter feed composition for broiler chickens – Experiment 1

	Control	Hemp Seed			Hemp Meal			Hemp Oil		
Ingredient	**0**	**10%**	**20%**	**30%**	**8%**	**16%**	**24%**	**3%**	**6%**	**9%**
Wheat	56.99	54.22	51.45	43.05	56.12	55.25	54.38	56.99	56.99	52.23
Soybean Meal	28.78	24.75	20.73	21.37	22.33	15.88	9.43	28.78	28.78	33.11
Hemp Oil	0	0	0	0	0	0	0	3	6	9
Hemp Seed	0	10	20	30	0	0	0	0	0	0
Hemp Meal	0	0	0	0	8	16	24	0	0	0
Limestone	2.24	2.26	2.28	2.29	2.27	2.29	2.31	2.24	2.24	2.23
Salt	0.14	0.15	0.15	0.16	0.15	0.15	0.15	0.14	0.14	0.15
Dicalcium Phosphate	1.24	1.20	1.16	1..09	1.21	1.19	1.16	1.24	1.24	1.21
Lysine	0.43	0.46	0.49	0.42	0.50	0.57	0.64	0.43	0.43	0.32
DL-Methionine	0.17	0.15	0.12	0.1	0.15	0.12	0.09	0.17	0.17	0.15
Threonine	0.19	0.17	0.14	0.10	0.16	0.14	0.11	0.19	0.19	0.17
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Vitamin mix supplied per kg of diet: 8260 IU of Vitamin A; 3000 IU of Vitamin D3; 30 IU of Vitamin E; 0.013mg of Vitamin B12; 2 mg of Vitamin K; 6 mg of Riboflavin; 11 mg of Pantothenic acid; 41.6mg of Niacin; 1300 mg of choline; 4mg of Folic acid; 0.25 mg of Biotin; 4mg of Pyridoxine; 4 mg of Thiamine.

[2]Mineral mix supplied per kg of diet: Manganese oxide, 66mg; zinc oxide, 150mg; Ferrous sulfate, 100mg; Copper sulfate, 10mg; Sodium selenite, 0.3mg; calcium iodate, 0.4mg.

Table 4.1: (continued) Starter feed composition for broiler chickens – Experiment 1

	Control	**Hemp Seed**			**Hemp Meal**			**Hemp Oil**		
Nutrient	0	10%	20%	30%	8%	16%	24%	3%	6%	9%
Crude Fat (%)	10.0	10.0	10.0	11.09	10.0	10.0	10.0	10.0	10.0	10.57
Crude Protein	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.0	25.0
Total Lysine	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Methionine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Threonine	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Calcium	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Phosphorus	0.65	0.72	0.79	0.86	0.71	0.78	0.85	0.65	0.65	0.65
Available Phosphorus	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chloride	0.38	0.38	0.38	0.38	0.38	0.37	0.37	0.38	0.38	0.38
Linoleic Acid	5.05	5.32	5.58	6.47	5.05	5.05	5.05	4.98	4.90	5.16
LinolenicAcid	0.12	0.70	1.28	1.88	0.25	0.37	0.50	0.59	1.05	1.52
Anal. Nutrients[1]										
DM %	88.13	88.04	87.67	89.28	88.14	88.16	88.50	87.78	88.34	89.03
CP%	23.82	23.77	23.87	24.01	23.71	23.68	23.57	23.74	23.88	24.15
Crude Fat%	8.43	8.72	8.17	8.82	9.01	8.65	8.38	8.28	8.90	8.19
Calcium	1.19	1.21	1.22	1.18	1.14	1.20	1.17	1.18	1.16	1.12
Phosphorous	0.75	0.77	0.76	0.79	0.85	0.73	0.68	0.73	0.71	0.77

[1] Analyzed composition of nutrients

Table 4.2: Grower/finisher feed composition for broiler chickens – Experiment 1

	Control	Hemp Seed			Hemp Meal			Hemp Oil		
Ingredient	**0**	**10%**	**20%**	**30%**	**8%**	**16%**	**24%**	**3%**	**6%**	**9%**
Wheat	65.22	61.87	58.51	54.56	64.37	63.52	62.67	65.22	65.22	60.11
Soybean Meal	21.44	17.64	13.85	10.29	14.98	8.52	2.06	21.44	21.44	25.64
Hemp Oil	0	0	0	0	0	0	0	3	6	9
Hemp Seed	0	10	20	30	0	0	0	0	0	0
Hemp Meal	0	0	0	0	8	16	24	0	0	0
Corn Oil	8.3	5.49	2.67	0	7.55	6.8	6.05	5.3	2.30	0
Limestone	1.26	1.28	1.30	1.39	1.29	1.31	1.33	1.26	1.26	1.53
Salt	0	0	0	0.15	0	0	0	0	0	0.14
Dical.Phosp	1.54	1.49	1.45	1.41	1.51	1.48	1.45	1.54	1.54	1.51
Lysine	0.39	0.42	0.45	0.47	0.46	0.54	0.61	0.39	0.39	0.3
DL-Methionine	0.17	0.15	0.12	0.1	0.15	0.12	0.09	0.17	0.17	0.15
Threonine	0.18	0.16	0.14	0.13	0.19	0.21	0.23	0.18	0.18	0.12
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mix[2]	0.5	0.5	0.5	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Vitamin mix supplied per kg of diet: 8260 IU of Vitamin A; 3000 IU of Vitamin D3; 30 IU of Vitamin E; 0.013mg of Vitamin B12; 2 mg of Vitamin K; 6 mg of Riboflavin; 11 mg of Pantothenic acid; 41.6mg of Niacin; 1300 mg of choline; 4mg of Folic acid; 0.25 mg of Biotin; 4mg of Pyridoxine; 4 mg of Thiamine.

[2]Mineral mix supplied per kg of diet: Manganese oxide, 66mg; zinc oxide, 150mg; Ferrous sulfate, 100mg; Copper sulfate, 10mg; Sodium selenite, 0.3mg; calcium iodate, 0.4mg.

Table 4.2: (continued) Grower/finisher feed composition for broiler chickens – Experiment 1

Nutrient	Control	Hemp Seed			Hemp Meal			Hemp Oil		
	0	10%	20%	30%	8%	16%	24%	3%	6%	9%
Crude Fat (%)	10.1	10.46	10.81	11.29	10.1	10.09	10.08	10.1	10.1	10.71
Crude Protein	22	22	22	22	22	22	22	22	22	22.89
Total Lysine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionine	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Threonine	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Phosphorus	0.69	0.76	0.83	0.9	0.76	0.83	0.89	0.69	0.69	0.69
Available Phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.2	0.19	0.19	0.25	0.19	0.19	0.19	0.2	0.2	0.25
Chloride	0.29	0.29	0.29	0.37	0.29	0.29	0.28	0.29	0.29	0.38
Linoleic Acid	5.05	5.5	5.96	6.49	5.04	5.03	5.03	4.97	4.89	5.17
LinolenicAcid	0.12	0.71	1.29	1.88	0.25	0.38	0.5	0.59	1.05	1.53
Calc. Nutrients[1]										
DM %	90.20	90.60	90.40	90.50	90.90	89.90	91.24	91.56	90.95	90.03
CP%	22.25	21.77	21.92	21.72	21.32	21.64	21.78	21.46	21.56	22.89
Crude Fat%	8.33	8.98	8.47	8.74	9.15	8.55	8.54	8.82	8.70	8.08
Calcium	0.83	0.89	0.92	0.87	0.83	0.83	0.87	0.96	0.86	1.12
Phosphorous	0.79	0.77	0.76	0.79	0.85	0.73	0.68	0.73	0.71	0.84

[1] Calculated composition of nutrients

Table 4.3: Starter feed composition for broiler chickens – Experiment 2

	Control	Hemp Seed			Hemp Meal			Hemp Oil		
Ingredient	**0**	**6%**	**12%**	**18%**	**5%**	**10%**	**15%**	**2%**	**4%**	**6%**
Corn	49.63	50.58	51.50	52.42	50.04	50.44	50.83	49.63	49.63	49.31
Soybean Meal	39.32	34.21	29.10	23.99	34.30	29.29	24.27	39.32	39.32	39.60
Hemp Oil	0	0	0	0	0	0	0	2	4	6
Hemp Seed	0	6	12	18	0	0	0	0	0	0
Hemp Meal	0	0	0	0	5	10	15	0	0	0
Corn Oil	5.95	3.99	2.03	0.07	5.46	4.98	4.50	3.95	1.95	0
Limestone	1.49	1.50	1.50	1.51	1.50	1.50	1.51	1.49	1.49	1.49
Salt	0	0	0	0.0	0	0	0	0	0	0.0
Dical. Phosph	1.75	1.76	1.77	1.78	1.75	1.76	1.76	1.75	1.75	1.74
Lysine	0.08	0.18	0.28	0.38	0.16	0.25	0.33	0.08	0.08	0.07
DL-Methionine	0.11	0.11	0.11	0.11	0.10	0.09	0.08	0.11	0.11	0.11
Threonine	0	0	0.04	0.07	0	0.02	0.05	0	0	0
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Vitamin mix supplied per kg of diet: 8260 IU of Vitamin A; 3000 IU of Vitamin D3; 30 IU of Vitamin E; 0.013mg of Vitamin B12; 2 mg of Vitamin K; 6 mg of Riboflavin; 11 mg of Pantothenic acid; 41.6mg of Niacin; 1300 mg of choline; 4mg of Folic acid; 0.25 mg of Biotin; 4mg of Pyridoxine; 4 mg of Thiamine.

[2]Mineral mix supplied per kg of diet: Manganese oxide, 66mg; zinc oxide, 150mg; Ferrous sulfate, 100mg; Copper sulfate, 10mg; Sodium selenite, 0.3mg; calcium iodate, 0.4mg.

Table 4.3: (continued) Starter feed composition for broiler chickens – Experiment 2

	Control	Hemp Seed			Hemp Meal			Hemp Oil		
Nutrient	**0**	**6%**	**12%**	**18%**	**5%**	**10%**	**15%**	**2%**	**4%**	**6%**
AMEn(kcal/kg)	3150	3150	3150	3150	3150	3150	3150	3150	3150	3150
Crude Fat (%)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.04
Crude Protein	23.93	23.17	23.43	23.69	23.64	23.36	23.09	23.93	23.93	24.02
Total Lysine	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Threonine	0.93	0.90	0.90	0.90	0.90	0.90	0.90	0.93	0.93	0.93
Calcium	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Phosphorus	0.73	0.77	0.81	0.85	0.77	0.82	0.86	0.73	0.73	0.73
Available Phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chloride	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Linoleic Acid	4.35	4.49	4.64	4.78	4.34	4.33	4.33	4.30	4.24	4.22
LinolenicAcid	0.09	0.44	0.79	1.14	0.17	0.25	0.33	0.40	0.71	1.02
Calc. Nutrients[1]										
DM %	89.10	89.37	90.67	89.22	89.14	88.36	88.45	88.34	89.67	89.97
CP%	23.43	23.46	23.42	23.78	23.73	23.49	23.30	24.25	24.21	24.46
Crude Fat%	7.42	7.09	7.41	7.82	8.09	7.21	6.29	7.93	8.13	8.22
Calcium	1.00	1.04	0.93	0.91	1.05	1.06	1.05	0.95	0.98	0.99
Phosphorous	0.76	0.82	0.74	0.85	0.90	0.83	0.89	0.74	0.74	0.75

[1] Calculated composition of nutrients

Table 4.4: Grower/ finisher feed composition for broiler chickens – Experiment 2

	Control	Hemp Seed			Hemp Meal			Hemp Oil		
Ingredient	**0**	**6%**	**12%**	**18%**	**5%**	**10%**	**15%**	**2%**	**4%**	**6%**
Corn	55.39	56.3	57.2	55.02	55.7	56.1	56.5	55.3	55.3	52.2
Soybean Meal	33.39	28.2	23.1	21.3	28.3	23.3	18.3	33.3	33.3	36.4
Hemp Oil	0	0	0	0	0	0	0	2	4	6
Hemp Seed	0	6	12	18	0	0	0	0	0	0
Hemp Meal	0	0	0	0	5	10	15	0	0	0
Corn Oil	5.79	3.83	1.88	0.00	5.31	4.83	4.34	3.79	1.79	0
Limestone	1.48	1.49	1.49	1.50	1.49	1.50	1.50	1.48	1.48	1.49
Salt	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Dical.Phosphat	1.83	1.84	1.85	1.81	1.83	1.84	1.84	1.83	1.83	1.79
Lysine	0.24	0.34	0.45	0.46	0.33	0.41	0.5	0.24	0.24	0.16
Methionine	0.14	0.14	0.14	0.12	0.13	0.12	0.11	0.14	0.14	0.13
Threonine	0.06	0.09	0.12	0.11	0.09	0.11	0.13	0.06	0.06	0.02
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mix[2]	0.50	0.50	0.50	0.50	0.5	0.50	0.50	0.5	0.50	0.50

[1]Vitamin mix supplied per kg of diet: 8260 IU of Vitamin A; 3000 IU of Vitamin D3; 30 IU of Vitamin E; 0.013mg of Vitamin B12; 2 mg of Vitamin K; 6 mg of Riboflavin; 11 mg of Pantothenic acid; 41.6mg of Niacin; 1300 mg of choline; 4mg of Folic acid; 0.25 mg of Biotin; 4mg of Pyridoxine; 4 mg of Thiamine.

[2]Mineral mix supplied per kg of diet: Manganese oxide, 66mg; zinc oxide, 150mg; Ferrous sulfate, 100mg; Copper sulfate, 10mg; Sodium selenite, 0.3mg; calcium iodate, 0.4mg.

Table 4.4. (continued) Grower/ finisher feed composition for broiler chickens – Experiment 2

	Control	Hemp Seed			Hemp Meal			Hemp Oil		
Nutrient	**0**	**6%**	**12%**	**18%**	**5%**	**10%**	**15%**	**2%**	**4%**	**6%**
AMEn(kcal/kg)	3200	3200	3200	3172	3200	3200	3200	3200	3200	3180
Crude Fat (%)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.12
Crude Protein	22.04	21.3	20.5	20.84	21.7	21.4	21.2	22.0	22.0	23
Total Lysine	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Threonine	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Calcium	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Phosphorus	0.72	0.76	0.8	0.85	0.76	0.81	0.85	0.72	0.72	0.72
Available Phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chloride	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Linoleic Acid	4.34	4.48	4.63	4.78	4.33	4.32	4.32	4.29	4.23	4.25
Linolenic Acid	0.09	0.44	0.79	1.14	0.17	0.25	0.33	0.4	0.71	1.02
Calc. Nutrients										
DM %	87.90	87.6	87.7	87.75	88.0	88.3	87.8	87.3	88.7	89.5
CP%	21.40	21.1	20.4	20.5	21.9	21.4	21.6	21.6	21.8	23.0
Crude Fat%	7.79	7.44	7.61	7.54	6.94	7.01	7.13	7.88	7.41	7.83
Calcium	1.09	1.01	0.94	0.98	0.97	1.03	1.05	1.02	0.93	0.99
Phosphorous	0.78	0.72	0.76	0.79	0.75	0.73	0.88	0.73	0.71	0.67

[1] Calculated composition of nutrients

Table 4.5. Fatty acid compostion (mg/g) of feed - Experiment 1

Fatty	**Control**	**Hemp Meal**			**Hemp Oil**		
acids	**0%**	**8%**	**16%**	**24%**	**3%**	**6%**	**9%**
C14:0	0.03	0.04	0.04	0.06	0.04	0.04	0.05
C16:0	9.01	9.77	8.43	9.06	9.18	7.79	7.45
C16:1	0.07	0.10	0.10	0.10	0.09	0.09	0.11
C18:0	1.59	1.53	1.56	1.52	1.74	1.96	2.44
C18:1	21.26	21.27	24.69	18.40	17.64	13.62	11.83
C18:2	41.81	45.01	40.03	42.56	48.28	48.45	54.49
C18:3 n-6	0.01	0.16	0.30	0.41	0.90	1.81	2.86
C18:3 n-3	1.43	2.12	3.95	3.27	5.33	9.32	14.00
C20:5	0.01	0.04	0.01	0.13	0.00	0.02	0.01
n-6	41.82	45.17	40.33	42.97	49.18	50.25	57.35
n-3	1.44	2.16	3.98	3.43	5.33	9.34	14.03
Ratio	29.03	20.88	10.14	12.51	9.22	5.38	4.09

Table 4.6. Fatty acid compostion (mg/g) of feed - Experiment 2

Fatty acids	**Control 0%**	**Hemp Seed**		
		6%	**12%**	**18%**
C14:0	0.03	0.03	0.03	0.04
C16:0	9.11	8.42	6.34	6.26
C16:1	0.07	0.08	0.07	0.08
C18:0	1.61	1.83	1.65	2.04
C18:1	21.68	19.19	13.9	13.12
C18:2	42.38	43.78	36.73	42.13
C18:3 n-6	0.01	0.65	1.05	1.85
C18:3 n-3	1.47	4.16	5.6	9.07
C20:5	0.01	0.01	0.01	0.01
n-6	42.38	44.44	37.78	43.97
n-3	1.48	4.17	5.61	9.08
Ratio	28.59	10.64	6.74	4.84

4.5.3. Determination of Tetrahydrocannabinols

4.5.3.1. Sample preparation

Standard solutions of Δ^9-THC (concentration 1mg/ml), 11-nor-9-carboxy- Δ^9-THC (THC-COOH) and 11-hydoxy-Δ^9-THC (THC-OH) at 100 µg/ml in methanol were obtained Cerilliant Sigma Aldrich (The Round rock TX, US). Δ^9-THC-d3, THC-COOH-d3 and THC-OH-d3 at 100 ug/ml in methanol were also obtained from the same company. All reagents used for extraction and preparation were GC or GC/MS grade.

A 500 mg muscle sample was mixed with 6 ml of deionized water and 9 ml of hexane/ethyl acetate (9:1) (Purschke et al., 2016; Fernandez et al., 2008) at 13500rpm for 2 minutes. A liquid-liquid extraction method was used to extract THC and its metabolites. First, samples were spiked with 100µl (concentration of 1µg/ml) of each internal standard,Δ^9-THC-d3,11-nor-9-carboxy- Δ^9-THC-d3 and 11-hydoxy-Δ^9-THC-d3 (Escrivá et al., 2017). Next, samples were centrifugede at 1000 g force for 20 min. Following centrifugation, the upper layer of the sample was removed to a new tube and dried under a stream of nitrogen gas. Once dry, the samples were derivatized by adding a 1:1 volume of pyridine: BSTFA mixture and heating the sample in single wall transit oven at 90ºC for 30 min. Samples were then cooled to room temperature for overnight in dark conditions. Blanks, standards and samples were analyzed within 24 h.

4.5.3.2. Generation of standard curves

For the generation of standard curves, seven different amounts (0.01, 0.05, 0.1, 0.5, 1, 2.5, 5 µg) of THC, THC-COOH and THC-OH were added to tubes along with 0.1µg THC-d3, THC-COOH-d3 and THC-OH-d3 respectively in methanol. The standards were taken to dryness under a stream of nitrogen gas. Once dry, a 1:1 volume of pyridine: BSTFA mixture (150µl of each) was added

to each tube and the tubes placed into an oven set at 90ºC for 30 min. The standards were then analyzed via GC/MS, using conditions described below.

Figure 4.1. Standard curve for Δ9-THC

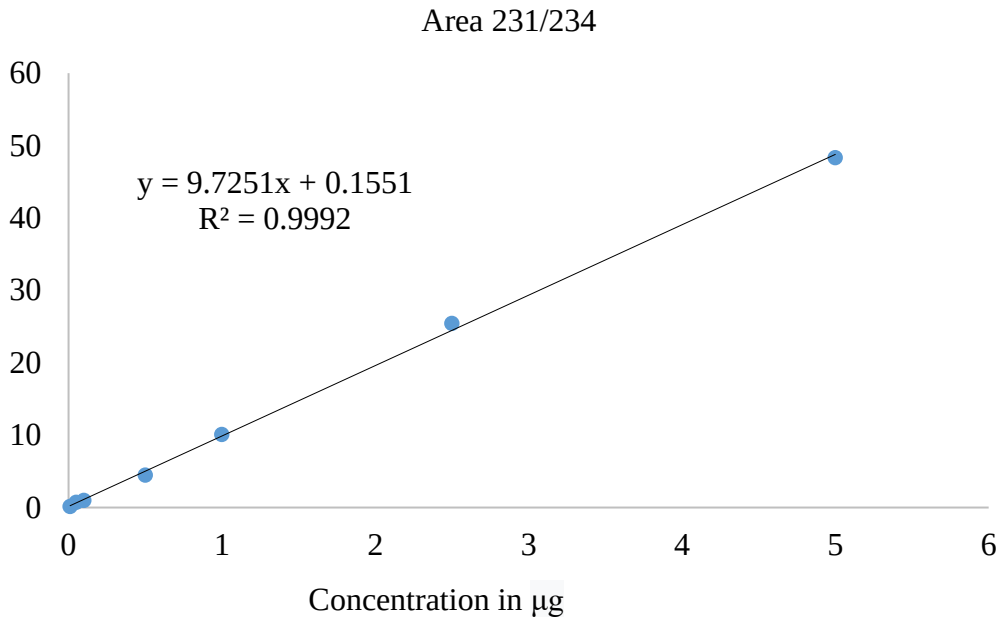


Figure 4.2. Standard curve for 11-nor-9-carboxy- Δ^9-THC

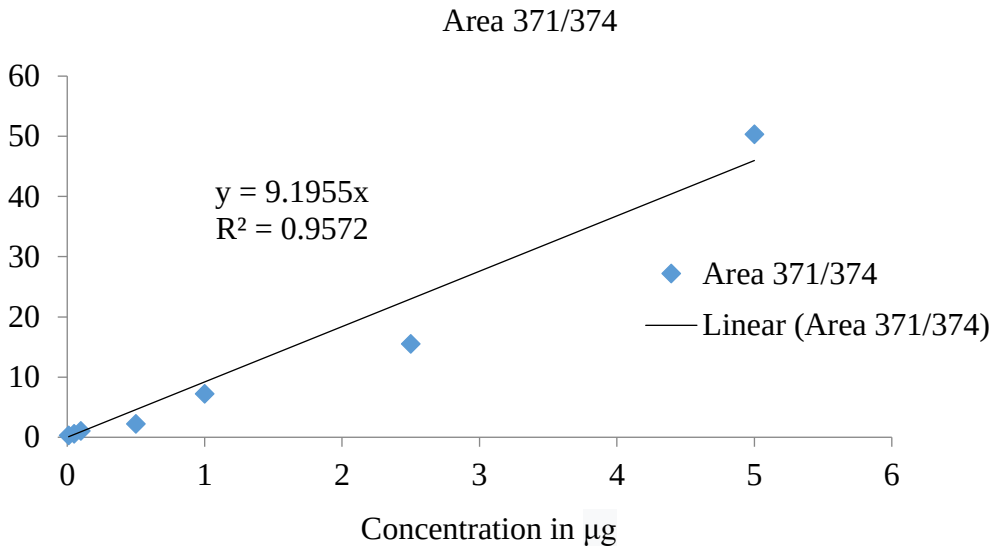
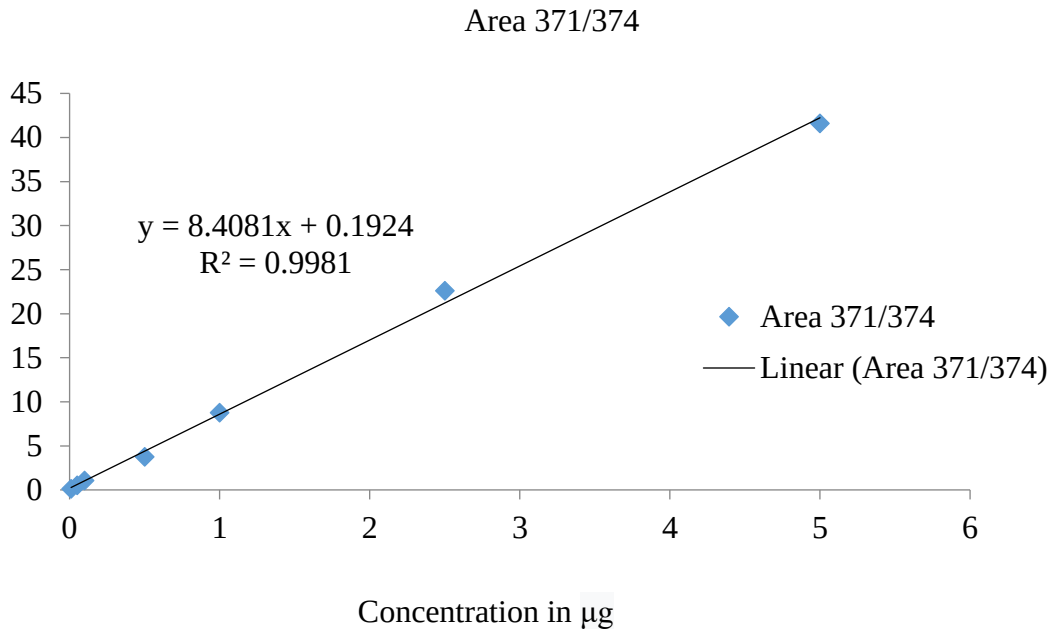


Figure 4.3. Standard curve for 11-hydoxy-Δ^9-THC



4.5.3.3. Analytical conditions for tetrahydrocannabinols

The following GC/MS instrument specifications were used to detect the -Δ^9-THC -d3

Conditions:

Instrument Type:	Varian 450 GC
Column Type:	DB-5ms, 30mm ×0.25mm ID, 0.25 µm film thickness
Temperature Program :	50°C to 300°C @ 10°C/min, 05min hold
Injector column:	cool on column
Carrier gas:	Helium
Flow Rate:	0.80ml/min
Transfer Line Temp:	280 °C

The following GC/MS instrument specifications were used to detect the 11-hydoxy-Δ^9-THC -d3

Conditions

Instrument Type:	Varian 450 GC
Column Type:	DB-5ms, 30mm ×0.25mm ID, 0.25 µm film thickness
Temperature Program:	50°C to 200°C @ 40°C/min, 200°C to 300°C @ 10°C/min, 16 min hold
Injector column:	cool on column
Carrier gas:	Helium
Flow Rate:	0.80ml/minTransfer Line Temp: 280 °C

The following GC/MS instrument was used to detect the 11-nor-9-carboxy- Δ^9-THC

Conditions:

Instrument Type:	Varian 450 GC
Column Type:	DB-5ms, 30mm×0.25mm ID, 0.25 µm film thickness
Temp. Program:	50°C to 200°C @ 20°C/min, 200°C to 250°C @ 5°C/min, 250°C to 300°C @ 2°C/min.
Injector column:	cool on column
Carrier gas:	Helium
Flow Rate:	0.80ml/min
Transfer Line Temp:	280 °C

4.6. Statistical Analyses

The current study was designed as a completely randomized design and all analyses were conducted using the Statistical Analysis System (SAS) institute version 9.3. Growth performance was analyzed via the Proc General linear model (GLM) procedure. Probability difference (PDIFF) was used to determine if the differences were significant ($P<0.05$). For growth performance, each pen was used as the experimental unit. Fatty acids were analyzed using the Proc Mixed procedure of SAS. Differences in selected means were calculated by PDIFF when significant ($P<0.05$). The data are presented in least square means±SEM. Differences were considered significant when $P<0.05$.

5. Results

5.1. Growth Performance

Growth performance (average daily feed intake, average daily gain and feed conversion ratio) data of Experiment 1 is shown in tables 5.1, 5.2 and 5.3. Average daily feed intake (ADFI) was significantly highest in 3% hemp oil group and was lowest in the 16% hemp oil fed broiler. 30% hemp seed fed animals showed ADFI very similar to control diets. Average daily gain (ADG; table 5.2) was lowest in 16 % hemp meal-fed animals. ADG was highest in 9% hemp oil group. However hemp seed (10% and 20%) fed animals ADG was the same as the control group. Feed conversion ratio (FCR; table 5.3) was highest in the 30% hemp seed group and significantly lowest in 9% hemp oil fed chickens. The data provided evidence that diets containing 9% hemp oil facilitated good feed conversion ratios. Growth performance (ADFI, ADG and FCR) data from Experiment 2 are shown in tables 5.4, 5.5 and 5.6. There was significant difference in ADFI in first fourteen days but after that there was no significant difference in average daily feed intake in all groups. Average daily gain presented in 5.5 showed no significant difference in all groups at any stage. Feed conversion ratio (FCR) presented in table 5.6 showed no significant difference in all treatments

Table 5.1: Average daily feed intake (g/d) of broiler chickens consuming diets containing hempseed-derived products – Experiment 1

ADFI	Control	Hemp seed			Hemp Meal			Hemp Oil			SEM	P-value
	(g)	10%	20%	30%	8%	16%	24%	3%	6%	9%		
1-14d	33.98^{ab}	35.26^{ab}	32.30^{b}	32.26^{b}	35.25^{ab}	27.81^{c}	36.82^{a}	34.62^{ab}	34.35^{ab}	35.53^{a}	1.100	<.0001
15-35	103.96^{b}	106.49^{ab}	109.96^{ab}	104.54^{ab}	106.93^{ab}	90.14^{c}	108.71^{a}	113.32^{a}	111.78^{ab}	109.67^{ab}	3.140	0.0004
1-35d	75.97^{b}	78.00^{ab}	78.89^{ab}	75.62^{b}	78.25^{ab}	65.21^{c}	79.95^{ab}	81.84^{a}	80.81^{ab}	80.01^{ab}	2.040	<.0001

Data within a row with different superscripts are significantly different (P<0.05).

Table 5.2: Average daily gain (g/d) of broiler chickens consuming diets containing hempseed-derived products – Experiment 1

ADG	Control (g)	Hemp seed			Hemp Meal			Hemp Oil			SEM	P-value
days		10%	20%	30%	8%	16%	24%	3%	6%	9%		
1-14	30.61^{a}	31.23^{a}	27.46^{b}	27.65^{b}	30.92^{a}	24.36^{c}	31.28^{a}	31.09^{a}	30.77^{bc}	32.31^{a}	1.020	<.0001
15-35	67.78^{c}	65.48^{cde}	68.23^{bcd}	60.29^{ef}	68.58^{abcd}	54.83^{f}	65.01^{de}	73.98^{ab}	70.87^{abc}	74.08^{a}	2.045	<.0001
1-35	52.91^{c}	51.78^{c}	51.92^{c}	47.24^{d}	53.52^{bc}	42.64^{e}	51.52^{c}	56.82^{ab}	54.83^{abc}	57.37^{a}	1.240	<.0001

Data within a row with different superscripts are significantly different (P<0.05).

Table 5.3: Feed conversion ratio of broiler chickens consuming diets containing hempseed-derived products – Experiment 1

FCR	Control	Hemp seed			Hemp Meal			Hemp Oil			SEM	P-
	(g)	10%	20%	30%	8%	16%	24%	3%	6%	9%		value
1-14d	1.00	1.03	1.06	1.05	1.04	1.01	1.07	1.01	1.01	1.01	0.018	0.1052
15-35	1.54^{cd}	1.64^{abc}	1.61^{bcd}	1.73^{a}	1.55^{cde}	1.64^{abc}	1.67^{ab}	1.52^{de}	1.57^{bc}	1.48^{e}	0.039	0.0009
1-35d	1.40^{de}	1.47^{bcd}	1.48^{bc}	1.56^{a}	1.42^{cde}	1.48^{abc}	1.51^{ab}	1.41^{de}	1.43^{cde}	1.36^{e}	0.026	<.0001

Data within a row with different superscripts are significantly different (P<0.05).

Table 5.4: Average daily feed Intake (g/d) of broiler chickens consuming diets containing hempseed-derived products – Experiment 2

ADFI	Control (g)	Hemp seed			Hemp Meal			Hemp Oil			SEM	P-
Days		6%	12%	18%	5%	10%	15%	2%	4%	6%		value
1-14	36.7^{ab}	37.07^{ab}	37.01^{ab}	36.6^{ab}	36.83^{ab}	37.35^{a}	37.75^{a}	33.76^{c}	36.16^{ab}	34.94^{bc}	0.79	0.0286
15-35	106.6	107.50	108.58	106.38	107.04	104.56	109.05	104.86	107.75	104.91	2.26	0.8915
1-35	78.68	79.33	79.98	78.48	78.96	77.67	80.53	76.42	79.12	76.92	1.51	0.698

Data within a row with different superscripts are significantly different (P<0.05).

Table 5.5: Average daily gain (g/d) of broiler chickens consuming diets containing hempseed-derived products – Experiment 2

ADG	Control (g)	Hemp seed			Hemp Meal			Hemp Oil			SEM	P-
Days		6%	12%	18%	5%	10%	15%	2%	4%	6%		value
1-14	31.23	31.30	29.88	29.97	30.74	31.24	30.70	28.59	30.88	29.76	0.90	0.5001
15-35	67.05	65.63	68.03	62.64	65.32	63.91	66.85	66.19	71.05	69.77	2.48	0.4127
1-35	52.72	51.90	52.77	49.57	51.49	50.84	52.39	51.15	54.98	53.77	1.64	0.5442

Data within a row with different superscripts are significantly different ($P<0.05$).

Table 5.6: Feed conversion ratio of broiler chickens consuming diets containing hempseed-derived products – Experiment 2

FCR	Control (g)	**Hemp seed**			**Hemp Meal**			**Hemp Oil**			SEM	P-value
Days		**6%**	**12%**	**18%**	**5%**	**10%**	**15%**	**2%**	**4%**	**6%**		
1-14	1.07	1.08	1.13	1.11	1.09	1.08	1.11	1.07	1.07	1.07	0.02	0.1456
15-35	1.61	1.64	1.60	1.71	1.65	1.64	1.62	1.60	1.52	1.51	0.05	0.1985
1-35	1.47	1.49	1.48	1.55	1.50	1.49	1.49	1.47	1.41	1.40	0.03	0.1457

Data within a row with different superscripts are significantly different ($P<0.05$).

5.2 Fatty acid Analysis

Fatty acids concentrations (percentage of total FA) of breast and thigh muscles from Experiment 1 are given in Tables 5.7 and 5.8. Dietary treatments affected the breast and thigh muscles (Table 5.7 and 5.8). Fatty acid analysis of breast and thigh muscles of hemp meal and hemp oil treatments was done from 1st experiment. Fatty acid analysis of hemp seed treatment was done from 2nd experiment. In breast muscles, with increasing levels of hemp meal from 8% to 16% in the diet, there was an increase in the n-3 fatty acids and n-6 fatty acids and n-6 to n-3 ratio is decreased. In the hemp oil group, the inclusion of 9% hemp oil in broiler diets led to significantly more n-6 and n-3 fatty acids. A similar trend was seen in the hemp oil group. With the increase of hemp oil concentration there was an increase in the n-6 and n-3 fatty acid deposition and decrease in n-6 to n-3 ratio as shown in table 5.7. In thigh muscles there was no significant difference in total n-6 fatty acids among the treatments however, total n-3 fatty acids increased with the increase of hemp meal and hemp oil concentration in feed. The ratio (n-6 to n-3) was significantly lowest in 6% hemp oil treatment. Fatty acid analyses of breast and thigh muscles of hemp seed fed broiler chickens are shown in table 5.9 and 5.10 respectively. There were no significant differences in total n-6 fatty acids in breast muscles however, total n-3 fatty acids in breast muscle increased with increases in dietary hemp seed inclusion (Table 5.9). The ratio (n6/n-3) was lowest in 24% hemp meal treatment. Increases in hemp seed concentrations decreases the n-6 to n-3 ratio of fatty acids. In thigh muscles the same trend was seen as in breast muscles. Total n-3 deposition was almost three times more in the 18% hemp seed group as compared to control. Increases in dietary hemp seed concentrations also led to increases in the n-3 fatty acids in thigh muscles and decreases in the total n-6 to total n-3 ratio.

Table 5.7. Fatty acid concentrations (percentage of total FA) in breast muscles of broiler chickens consuming diets containing hempseed-derived products – Experiment 1

Fatty Acid	Control	Hemp Meal			Hemp Oil			SEM	P-value
		8%	16%	24%	3%	6%	9%		
C14:0	0.24	0.24	0.20	0.24	0.26	0.25	0.23	0.014	0.0852
C16:0	15.49	16.19	14.93	15.66	16.34	15.20	14.75	0.424	0.0821
C16:1	0.91^{bc}	1.10^{ab}	0.83^{bc}	1.33^{a}	1.05^{b}	1.08^{ab}	0.76^{c}	0.092	0.0028
C18:0	8.20^{a}	6.73^{bc}	7.92^{ab}	6.38^{c}	8.24^{a}	8.27^{a}	9.04^{a}	0.425	0.0011
C18:1	18.81^{ab}	20.49^{a}	21.02^{a}	21.08^{a}	17.85^{b}	17.68^{b}	12.27^{c}	0.878	<.0001
C18:2	24.94^{c}	30.17^{ab}	26.99^{bc}	32.91^{a}	31.09^{ab}	31.45^{ab}	32.94^{a}	1.562	0.0055
C18:3 n-6	0.41^{cd}	0.31^{d}	0.31^{d}	0.39^{cd}	0.60^{bc}	0.82^{b}	1.17^{a}	0.076	<.0001
C18:3 n-3	2.10^{cd}	1.44^{d}	1.93^{cd}	2.04^{cd}	2.997^{c}	4.26^{b}	6.19^{a}	0.425	<.0001
C20:4	5.73	4.84	6.01	3.93	4.99	4.81	5.17	0.504	0.1266
C20:5	0.24^{cde}	0.14^{e}	0.27^{bcd}	$0.14d^{e}$	0.29^{bc}	0.38^{b}	0.54^{a}	0.043	<.0001
C22:5	1.14^{a}	0.70^{ab}	1.28^{b}	0.70^{ab}	1.36^{a}	1.66^{a}	2.14^{ab}	0.173	<.0001
C22:6	0.87^{a}	0.40^{b}	0.96^{a}	0.43^{b}	0.94^{a}	0.99^{a}	1.20^{a}	0.127	0.0004
n-6	31.10^{c}	35.33^{ab}	33.32^{bc}	37.23^{ab}	36.70^{ab}	37.09^{ab}	39.25^{a}	1.370	0.0039
n-3	4.37^{cd}	2.70^{e}	4.45^{cd}	3.33^{de}	5.58^{c}	7.31^{b}	10.08^{a}	0.459	<.0001
Ratio	8.30^{c}	13.26^{a}	7.47^{c}	11.21^{b}	6.74^{cd}	5.23^{de}	3.89^{e}	0.630	<.0001

Data within a row with different superscripts are significantly different.
NS $P \geq 0.05$. P Total n-6 = Total omega-6 fatty acids; Total n-3 = Total n-3 fatty acids.

Table 5.8: Fatty acid concentrations (percentage of total FA) in thigh muscles of broiler chickens consuming diets containing hempseed-derived products – Experiment 1

Fatty Acid	Control	Hemp Meal			Hemp Oil			SEM	P-value
		8%	16%	24%	3%	6%	9%		
C14:0	0.28	0.27	0.26	0.29	0.28	0.29	0.26	0.017	0.7300
C16:0	15.62	14.02	14.22	15.82	15.79	15.1	14.79	1.217	0.9000
C16:1	1.29^{ab}	1.35^{ab}	1.27^{ab}	1.62^{a}	1.35^{ab}	1.20^{ab}	1.03^{b}	0.164	0.3256
C18:0	6.79	7.13	6.47	6.10	7.39	7.75	8.40	0.530	0.0686
C18:1	23.77^{ab}	20.17^{bcd}	24.55^{a}	21.62^{abc}	19.47^{cd}	16.41^{de}	14.89^{e}	1.411	0.0001
C18:2	30.38	32.12	32.98	35.88	34.24	31.30	33.58	1.416	0.1458
C18:3 n-6	0.24^{c}	0.31^{bc}	0.35^{bc}	0.44^{bc}	0.51^{b}	0.76^{a}	0.77^{a}	0.083	0.0001
C18:3 n-3	1.44^{bc}	1.34^{bc}	2.45^{b}	2.39^{b}	2.67^{b}	4.10^{a}	4.30^{a}	0.474	0.0002
C20:4	4.22	4.63	3.78	3.19	4.66	4.36	4.81	0.627	0.5376
C20:5	0.09^{c}	0.10^{bc}	0.13^{bc}	0.10^{bc}	0.18^{b}	0.29^{a}	0.30^{a}	0.027	<.0001
C22:5	0.57^{d}	0.69^{cd}	0.87^{cd}	0.64^{d}	1.06^{bc}	1.45^{ab}	1.53^{a}	0.138	<.0001
C22:6	0.39^{b}	0.36^{b}	0.58^{ab}	0.38^{b}	0.71^{a}	0.79^{a}	0.84^{a}	0.111	0.0090
n-6	34.85	37.42	37.12	39.52	39.43	36.43	39.18	1.181	0.0641
n-3	2.50^{c}	2.51^{c}	4.04^{bc}	3.53^{bc}	4.64^{b}	6.64^{a}	6.98^{a}	0.570	<.0001
Ratio	14.14^{a}	15.55^{a}	9.21^{bc}	11.20^{b}	9.05^{bc}	5.73^{d}	6.64^{cd}	0.899	<.0001

Data within a row with different superscripts are significantly different.

NS $P \geq 0.05$. Total n-6 = Total omega-6 fatty acids; Total n-3 = Total n-3 fatty acids**.**

Table 5.9: Fatty acid concentrations (percentage of total FA) in breast muscles of broiler chickens consuming diets containing hempseed-derived products – Experiment 2

Fatty Acid	Control	Hemp Seed			SEM	P-value
		6%	12%	18%		
C14:0	0.24	0.24	0.25	0.23	0.014	0.8563
C16:0	16.34	17.22	16.42	16.34	0.625	0.7051
C16:1	0.87	1.00	1.09	0.80	0.108	0.2565
C18:0	8.36	8.90	8.21	10.05	0.523	0.0871
C18:1	17.16^{b}	19.40^{a}	16.62^{b}	15.06^{b}	0.725	0.0039
C18:2	27.67	27.86	25.58	27.73	1.288	0.5526
C18:3 n-6	0.47	0.42	0.55	0.69	0.089	0.1914
C18:3 n-3	2.09	1.87	2.61	3.49	0.516	0.1561
C20:4	6.04	6.64	5.50	6.19	0.444	0.3598
C20:5	0.22^{c}	0.35^{bc}	0.44^{ab}	0.52^{a}	0.049	0.0026
C22:5	1.05^{c}	1.38^{bc}	1.87^{ab}	2.13^{a}	0.202	0.0054
C22:6	0.91	1.24	1.31	1.60	0.179	0.088
n-6	34.20	34.93	31.61	34.62	1.142	0.1924
n-3	4.29^{b}	4.85^{b}	6.25^{ab}	7.75^{a}	0.745	0.0169
Ratio	7.97^{a}	7.30^{ab}	5.10^{b}	4.50^{b}	1.616	0.0077

Data within a row with different superscripts are significantly different.
NS $P \geq 0.05$. Total n-6 = Total omega-6 fatty acids; Total n-3 = Total n-3 fatty acids.

Table 5.10: Fatty acid concentrations **(**percentage of total FA) in thigh muscles of of broiler chickens consuming diets containing hempseed-derived products – Experiment 2

Fatty Acid	Control	Hemp Seed			SEM	P-value
		6%	12%	18%		
C14:0	0.28	0.32	0.30	0.28	0.019	0.4054
C16:0	16.15^{a}	16.94^{a}	16.43^{a}	14.53^{b}	0.496	0.0160
C16:1	1.72	1.64	1.49	1.35	0.162	0.4102
C18:0	7.15	7.26	7.50	7.57	0.400	0.8668
C18:1	22.70^{a}	21.55^{a}	18.92^{b}	17.84^{b}	0.867	0.0025
C18:2	33.28	32.18	31.35	28.64	3.078	0.7458
C18:3 n-6	0.33	0.48	0.74	7.23	3.090	0.3420
C18:3 n-3	1.29^{c}	2.32^{c}	3.71^{b}	5.62^{a}	0.411	<.0001
C20:4	4.53	4.46	4.40	3.71	0.395	0.4475
C20:5	0.06^{c}	0.17^{b}	0.28^{a}	0.27^{a}	0.024	<.0001
C22:5	0.64	0.87	1.33	1.21	0.168	0.0346
C22:6	0.31^{c}	0.57^{b}	0.84^{a}	0.81^{a}	0.078	0.0003
n-6	38.15	37.13	36.50	39.59	0.935	0.1351
n-3	2.32^{d}	3.95^{c}	6.18^{b}	7.93^{a}	0.413	<.0001
Ratio	19.27^{a}	9.56^{b}	5.97^{bc}	5.05^{c}	1.517	<.0001

Data within a row with different superscripts are significantly different.

NS $P \geq 0.05$. Total n-6 = Total omega-6 fatty acids; Total n-3 = Total n-3 fatty acids.

5.3 Tetrahydrocannabinol Analysis

Quantification of THC and THC metabolites (11-nor-9-carboxy- Δ^9-THC and 11-hydoxy-Δ^9-THC) in broiler meat is necessary to prove its safety for human consumption after feeding chickens different concentrations of hemp seed, hemp meal and hemp oil. In breast, thigh, liver and kidney samples THC and THC metabolites (11-nor-9-carboxy- Δ^9-THC and 11-hydoxy-Δ^9-THC) were not found as shown in table 5.11. The contents were below the limits of detection. Internal standard peaks are shown in figure 5.1 and figure 5.2. Our limit of detection was 10ng which is way less than allowed amount (10ppm) of THC in food in Canada. In all samples, the internal standards were detected

Figure. 5.1. Chromatogram of breast muscle (THC-COOHd3)

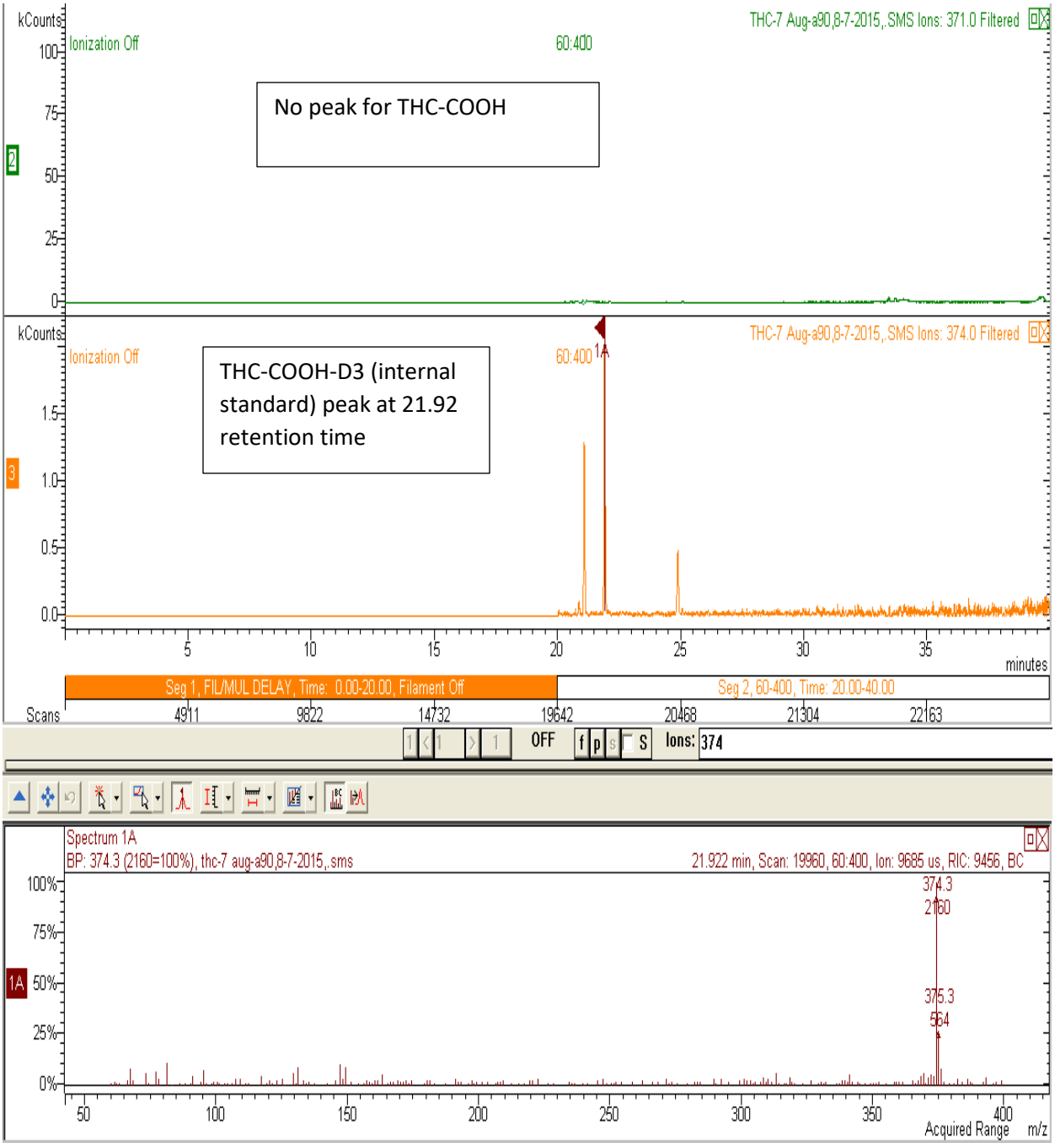


Figure 5.2. Chromatogram of breast muscle (THC-OHd3)

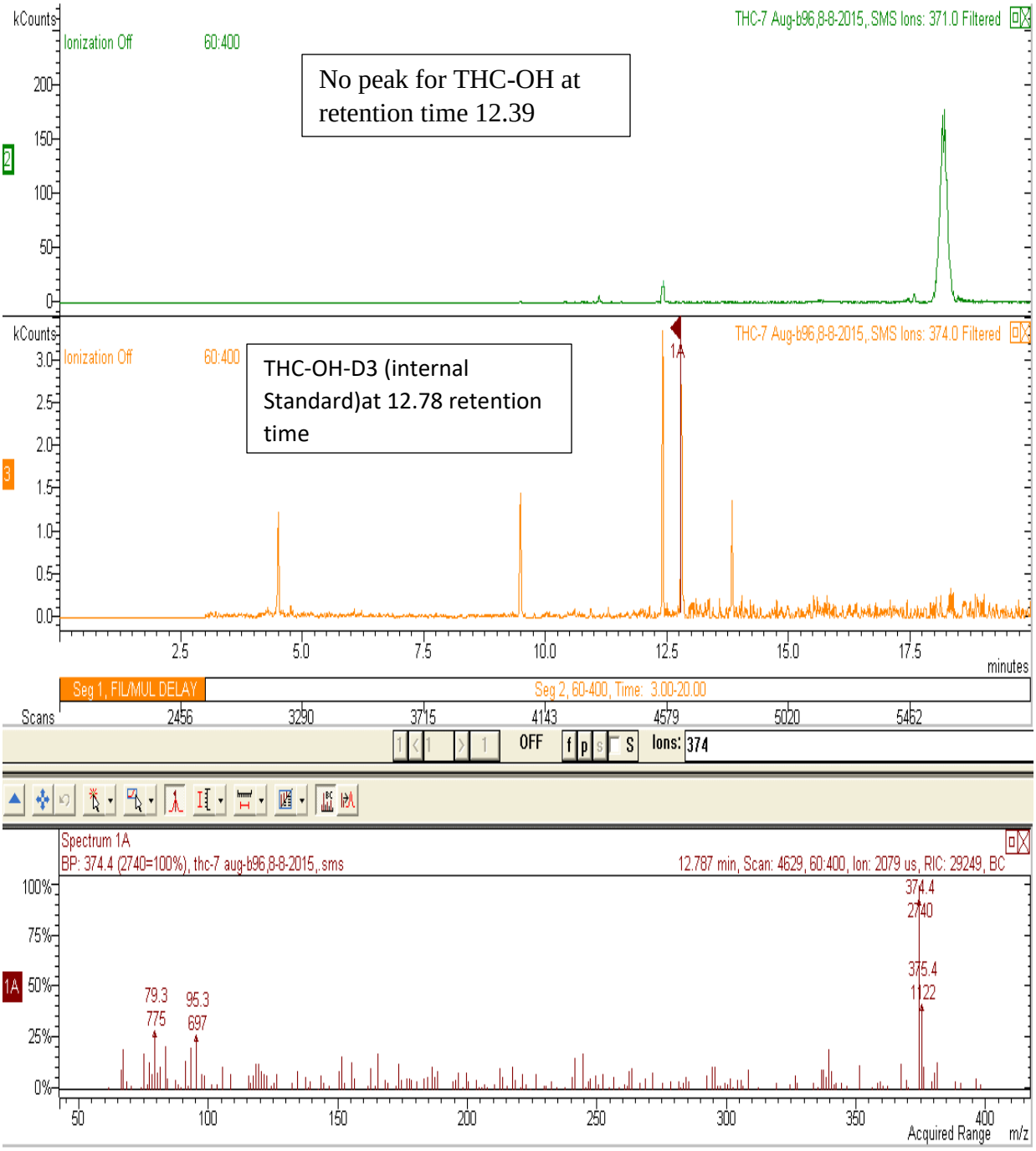


Table 5.11: Estimation of the THC and its metabolites content in breast, thigh, liver and kidney tissues derived from broiler chickens consuming diets containing hempseed-derived products.

		THC and its metabolites quantity			
		Breast	**Thigh**	**Liver**	**Kidney**
Control	0%	<LOD	<LOD	<LOD	<LOD
Hemp seed	6%	<LOD	<LOD	<LOD	<LOD
	12%	<LOD	<LOD	<LOD	<LOD
	18%	<LOD	<LOD	<LOD	<LOD
Hemp meal	8%	<LOD	<LOD	<LOD	<LOD
	16%	<LOD	<LOD	<LOD	<LOD
	24%	<LOD	<LOD	<LOD	<LOD
Hemp oil	3%	<LOD	<LOD	<LOD	<LOD
	6%	<LOD	<LOD	<LOD	<LOD
	9%	<LOD	<LOD	<LOD	<LOD

6. Discussion

6.1. Growth performance

These results indicate that hemp seed, hemp meal and hemp oil do not have any detrimental effect on broiler performance. Eriksson and Wall, (2012) found no significant difference in feed intake, average daily gain and feed conversion ratio when fed hemp seed cake in organic broiler diet. Lopez-Ferrer et al., (1999) findings were similar for chickens fed diet supplemented with fish oil and rapeseed oil, combination of various vegetable oils including soyabean oil and linseed oil (Kavouridou et al., 2008), coconut oil (Marco et al., 2013) and Mandal et al, (2014) also reported same results. El-Katcha et al., 2014 used linseed oil and sunflower oil to get different ratios of n-6 and n-3 fatty acids in diets. The results of this study are also in agreement with our study. These different treatments did not show any affect on body weight gain and feed conversion ratio. Results were consistent with study Mirghelenj et al., (2016) fed four (tallow fat, olive oil, soy oil and canola oil) different types of experimental broiler finisher diets. Mirghelenj et al., 2016 results showed no significant difference in average daily weight gain and feed conversion ratio among all four treatments. Elkin et al., (2015) used conventional soybean oil and stearidonic acid (18:4n-3) enriched soybean oil in broiler chickens diets. There was no significant difference in weight gains and feed conversion ratio. Our results are consistent with Gallardo et al., (2012) fed different percentages (0, 5, 10, 15%) of canola oil. Gallardo results showed non-significant difference in water consumption, feed intake, feed consumption and final body weight. Some researches (Shen et al., 2005; Rodriguez., 2001) showed reduction in production performance on the inclusion of flax seed or linseed meal. Coetzee and Hoffman (2002) demonstrated the effect of various dietary n-6 to n-3 fatty acid ratios on the performance of broiler. Six different combinations of canola acid oil and famarol acid oil were included in diet. There was no difference in weight gain and feed

conversion ratio. Kamran Azad et al., (2009) used flax seed and canola seed to enrich the chicken meat with the n-3 fatty acids. Feed consumption was not significantly different among the treatments however inclusion of flaxseed and canola seed had negative effect on production.

6.2. Fatty acid analysis

The broiler white meat consist of 33.5% saturated fatty acids, 30.5% unsaturated fatty acids and almost 32% polyunsaturated fatty acids (Ratnayake et al., 1989). Broiler meat is more favourable for human consumption as compared to red meat, which contains high level of saturated fats and low levels of polyunsaturated fatty acids (Yau et al., 1991). The fatty acid composition of poultry meat can be modified relatively easy. In 1963, Marion and Woodroof noted that fatty acid composition of breast, thigh and skin tissues were similar to feed composition. As early as 1960s, Neudoerffer and Lea study reported, increased EPA and DHA in muscle lipids of turkey after feeding fish oil. Fatty acids results are in agreement with Zuidhof et al., (2009) who found that dietary inclusion of flax seed (10% and 17%) resulted into increase n-3 fatty acids in breast and thigh muscle (double in 17% flax seed as compared to 10% flaxseed in diet). The results is consistent with the findings of Maroufyan et al., 2012. Maroufyan et al., (2012) data showed dietary treatments influence the fatty acid composition of breast muscle. Breast muscles n-3 PUFA significantly increase with the inclusion of fish oil in diet. Morales-Barrera et al., (2013) study also showed same results. Three levels (0.75%, 1% and 1.25%) of tuna oil was included in feed to observe the fatty acids in breast and thigh muscles. There was significant increase in n-3 PUFA in breast and thigh muscles with the inclusion of tuna oil in diet. El-Katcha et al., (2014) results were also consistent who found that total n-3 fatty acid deposition increase with the decrease in n-3 to n-6 ratio. Highest total n-3 fatty acids deposition was recorded in the group 5 fed 1:9 ratio of n-3 to n-6 fatty acids. Mandal et al., (2014) study results are also in agreement with our results. In this

study they fed three levels of n-6 to n-3 ratio diets. With the decrease of n-6 to n-3 ratio (medium and low) in diets increased approximately 2 to 3 folds of n-3 fatty acid deposition in breast and thigh muscles. Meat-type chickens can convert ALA or SDA into DHA. DPA is most abundant long-chain n-3 PUFA in breast and thigh muscles. Elkin et al., (2015) used conventional soybean oil and stearidonic acid (18:4n-3) enriched soybean oil in broiler chickens diets to see its effect on deposition of n-3 fatty acids. Results of this study was similar to our study. There was significant difference (3 times) higher in long-chain n-3 PUFA in stearidonic acid (18:4n-3) enriched soybean oil group as compared to conventional soybean oil fed chickens. It concluded that broiler fed ALA and SDA together as compared to ALA alone has more capability to deposit long-chain n-3 fatty acids. Gallardo et al., (2012) reported increase in canola oil percentage (0 to 15%) in broiler diet increase omega 3 and omega-9 fatty acids and decrease in omega 6 fatty acids in plasma, fat and meat. Our results are in agreement with Ozpinar et al., (2003) study. Ozpinar et al., (2003) fed four types of diets. N-3 Polyunsaturated fatty acids were increased, n-6 fatty acids were decreased with increase in fish oil in diet. Kamran Azad et al., (2009) studies showed similar type of results. They used full-fat flaxseed and canola seed as n-3 fatty acid resources in feed. Inclusion of full-fat flaxseed and canola seed significantly increased n-3 fatty acids and decrease the arachidonic acid and also decrease n-6:n:3 ratio. Salamatdoustnobar et al., (2008) used canola oil (4% and 6%) to see deposition of n-3 fatty acids in broiler. Results of this study showed that inclusion of canola oil modify the fatty acid profile and improve quality of meat. Increase in canola oil in diet from 0 to 4% increase omega 3 almost double in breast and thigh muscles as compared to control diet. So it is concluded that we can easily modify the fatty acid contents of breast and thigh muscles by changing the fatty acid composition of feed. Konieczka et al., (2017) used fish oil flax seed and

rapeseed to enrich the broiler meat with omega3 fatty acids. The levels of EPA and DHA increased in breast and thigh muscle in groups fed lard, flaxseed and rapeseed.

6.3. THC content

THC and THC metabolites (11-nor-9-carboxy- Δ^9-THC and 11-hydoxy-Δ^9-THC) were not found as shown in table 1. Even if present may be lower than limit of detection. Our limit of detection was 10ng which is way less than allowed amount (10ppm) of THC in food in Canada. In all samples internal standards were detected. Escrivá et al., (2017) study showed similar type of results are seen. They used liquid chromatography-mass spectrometry to analyse cannabinoids in milk, liver and hemp seed. They did not detect any metabolites in milk and liver. Purschke et al (2016) used same liquid-liquid extraction (LLE) to extract the THC and THC metabolites in blood serum. They used same method GC-MS as used in our study method to analyse the samples. Limit of detection (LOD) and limit of quantification (LOQ) for THC were 0.3 and 0.6 µg/l, for 11-nor-9-carboxy- Δ^9-THC were 0.3 and 1.1µg/l and for 11-hydoxy-Δ^9-THC were 0.1 and 0.8 µg/l. However in our study only LOD that was 10 ng/g was used. The procedure for quantification of THC and its metabolites used in our study was different as compared to use by Teixeira et al., (2007).Teixeira et al., (2007) used LC-MS procedure to analyse THC and THC-COOH in oral blood and urine samples of human. They used solid phase extraction method to extract THC and THC-COOH. As compared to GC-MS method used in our study, LC-MS method does not require any silylation and derivatization. LC-MS is less time consuming. This quantification method has high sensitivity, specificity and selectivity. So it could be good alternative to GC-MS.

7. Summary and Future Directions

In conclusion, hemp seed, hemp meal and hemp oil had no detrimental effect on the production performance (average daily feed intake, average daily weight gain and feed conversion ratio). However, these hemp products have positive effect on fatty acid deposition in breast and thigh muscle. Lowering the n-6 to n-3 fatty acids increase EPA and DHA by 2 to 3 folds. Concentration of long chain n-3 PUFA also increase in breast and thigh muscle with the decrease in n-6 to n-3 ratio that lead to healthy meat for human consumption. Hemp products could be alternative to increase n-3 PUFA without deteriorating production performance of broiler. All samples (breast, thigh, kidney and liver) do not contain THC and THC metabolites (LOD is 10ng/g). If THC or its metabolites are present, they may be lower than the limit of detection. Our limit of detection was far less than allowed THC in food (10 ppm). It is concluded that meat produced by chicken fed different hemp products is safe for human consumption. A method was also developed to analyse the THC and its metabolites in different edible parts of chicken. The other cannabinoids present in hemp plant also have purported therpeutical benefits, yet these remain to be studied. Methods to improve the cost efficiency of hemp used in chicken diets should also be developed, following a comprehensive economic review of the potential utilization of this crop by the livestock feed industry. Additionally, other hemp products, including hemp protein, could be evaluated for their potential utilization in high value livestock and poultry feeds. The current data provide strong evidence for the safety and efficacy of hemp product utilization within feeds designed for broiler chickens.

8. References

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