Assessment of hemp meal as a protein supplement for non-lactating dairy cows

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ABSTRACT

Hemp meal (HM) may be a suitable protein supplement for dairy cows, but its use as a ruminant feed has not yet been approved in Canada. To obtain information regarding the potential to utilize hemp meal as a cattle feed, dry matter intake (DMI), rumen fermentation, blood metabolites, total tract digestibility, and concentrations of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in blood plasma, urine, muscle, and adipose tissues were compared between non-lactating dairy cows receiving either HM or canola meal (CM), or a combination of HM and CM. Six non-lactating, non-pregnant Holstein cows were used in a repeated 3 × 3 Latin Square design feed trial with three, 3-week experimental periods. The first two weeks served as an adaptation (washout) period, and sample collection occurred during the third week of each period. Cows were fed a basal, partially mixed ration (PMR) diet that was supplemented with either 10.2 % DM HM, 13.5 % DM CM or 6.25 % DM HM and 6.16 % DM CM (HC). All diets were isoenergetic and isonitrogenous. Total tract digestibility of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) were determined using acid insoluble ash contents of diets and feces as an internal marker. Supplement treatment did not affect DMI, pH, VFA, ammonia concentrations in the rumen, total tract DM digestibility, crude protein digestibility, urine nitrogen or blood serum concentration of glucose, urea, beta-hydroxybutyrate, and non-esterified fatty acids. However, the total tract NDF digestibility of the CM diet (43 %) was significantly higher ($p < 0.05$) than that of HM (38 %). No cannabinoids were detected in blood plasma, rumen fluid, kidney, liver, urine, muscle, and adipose tissues of cows. However, CBD and CBDA were detected in the feces of the cows (0.68 ug/g and 0.67 ug/g, respectively). Our data show that hemp meal may be a good and safe alternative to canola meal as a protein supplement for non-lactating dairy cows.

Keywords: hemp, protein supplementation, non-lactating dairy cow

DEDICATION

This thesis is dedicated to my parents late Mr. and Mrs. Addo who sacrificed their lives and happiness to help me achieve my academic goals. May their gentle souls rest in peace.

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FOREWORD

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LIST OF ABBREVIATIONS

AA	Amino Acids
ADC	Apparent Digestibility Coefficient
ADF	Acid Detergent Fibre
AIA	Acid-Insoluble Ash
BHBA	β-hydroxybutyric Acid
CBD	Cannabidiol
CBDA	Cannabidiolic Acid
CBN	Cannabinol
CBNA	Cannabinolic Acid
CM	Canola meal
CP	Crude Protein
DDGS	Dried Distillers' Grains with solubles
DG	Distillers' Grain
DM	Dry Matter
DMI	Dry Matter Intake
EAA	Essential Amino Acid
EFAs	Essential Fatty Acids
MCP	Microbial Crude Protein
ME	Metabolizable Energy
MP	Microbial Protein
MUN	Milk Urea Nitrogen
NDF	Neutral Detergent Fibre

NEB	Negative Energy Balance
NEFA	Non-Esterified Fatty Acid
NPN	Non-Protein Nitrogen
peNDF	Physical Effective Neutral Detergent Fibre
PMR	Partial Mixed Ration
PUFA	Polyunsaturated Fatty Acid
RDP	Rumen Degradable Protein
RUP	Rumen Undegradable Protein
SBM	Soybean Meal
THC	Delta-9-Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic Acid – A
TMR	Total Mixed Ration
VFA	Volatile Fatty Acid

1.0 GENERAL INTRODUCTION

The use of protein supplements such as canola meal and soybean meal to augment microbial crude protein in dairy cattle nutrition is vital since they help improve milk production, health, general growth, and reproduction. This is achieved through the increased supply of intestinal nitrogen and amino acids (Gidlund et al., 2015; Wanapat et al., 2011). There have been various reports on the use of various protein supplements in dairy cattle nutrition in different parts of the world. Protein supplements such as fish meal, soybean meal, cottonseed meal, canola meal, brewer's wet grain, blood meal, feather meal, distiller's grains, corn gluten meal, and many others have been reported (Broderick et al., 2015; Christen et al., 2010; Liu, 2011). High-yielding dairy cows (45 kg milk/day) generally require high dietary CP content (> 15 %) for body maintenance, milk protein synthesis, and the formation of body tissues (Mogensen et al., 2008). In contrast, dry cows do not require high dietary CP contents (10 % - 13 %) since they are not producing milk and therefore excess may protein may lead to problems such as excessive excretion of urea in urine which leads to loss of energy and essential AA, environmental impact, and reduction in fertility due to reduced survival of embryos in the uterus (Rodney et al., 2018).

Canola meal and soybean meal are the principal protein supplements used in the Canadian prairies and most parts of North America since they have been proven to help increase milk production and milk protein content (Broderick et al., 2015; Huhtanen et al., 2011). DDGS is an alternative protein supplement that has gained recent attention in the dairy cattle nutrition (Liu, 2011). This co-product which is obtained from the distillation process of ethanol has a CP content of 26 – 31.7% DM, 9.1 -14.1 % DM fat, and 33.1 – 43.9 % DM NDF (Liu, 2011). It was reported in a recent study on the comparison between corn DDGS and soybean meal as a protein supplement

for dairy cows that there was reduced urinary nitrogen excretion in cows fed a high-DDGS diet which led to a reduced manure ammonia concentration (Lee et al., 2020). Although research has demonstrated the benefits of the use of existing protein supplements like canola meal, soybean meal, and DDGS, there are some concerns surrounding their utilization. Some of these concerns include high prices of already existing and approved protein supplements, uncertainty in year-round availability, and the case of DDGS, possible contamination by mycotoxins such as aflatoxin during the production process because of the high moisture content of the product (Liu, 2011). Another challenge is the possible occurrence of milk fat depression when DDGS is fed as a protein supplement due to its high unsaturated fat content, especially when cows are fed low fiber and high DDGS diets (Ranathunga et al., 2018).

The search for a novel and low-cost alternatives to canola meal and soybean meal has been ongoing in recent years. One such alternative is hemp meal. Hemp (Cannabis sativa L.) is an annual herbaceous plant that is usually cultivated for its fiber or oils in Europe, the USA, and Canada (Mustafa et al., 1999; Stringer, 2018). In Canada, the production of industrial hemp (hemp varieties containing delta-9-tetrahydrocannabinol (THC) levels lower than 0.3 % in the flowering heads and leaves) especially in the Canadian prairies has increased significantly from 15,056 ha in 2011 to 52,000 ha in 2018 (Health Canada, 2018; Mooleki et al., 2017). Hemp meal is a by-product obtained from oil extraction from the hemp seed. It contains approximately 32-35 % CP DM, 78-85 % RUP, as well as 51 NDF % DM and 39 ADF DM % (Mustafa & Mustafa, 2002; NRC, 2001; Russo & Reggiani, 2015). Dietary proteins can also be classified into rumen degradable protein (RDP) and rumen bypass or rumen undegradable protein (RUP). A digestibility study conducted by Mustafa et al. (1999) on feeding hemp meal to rumen fistulated dairy cows and sheep showed that hemp meal is a good source of rumen undegradable protein (RUP) (77.4 % CP) and feeding

hemp meal instead of canola meal did not have harmful effects on dry matter intake (DMI) and nutrient utilization. The authors also demonstrated that the total tract NDF and CP were not negatively affected when hemp meal was fed instead of canola meal in sheep. Previous studies on the effect of using hemp meal as a total or partial replacement of soybean meal or canola in cows and small ruminants like sheep and goat showed that there are inconsistencies in DMI since in some cases an increase in DMI were reported and in others, no differences were found (Mustafa et al., 1999: Karlsson et al., 2010; Karlsson & Martinsson, 2011).

Despite the potential of hemp meal being used as an alternative protein supplement, there are some concerns about its use as a ruminant feed in Canada. The outstanding concern is the possible accumulation of cannabinoids (THC and CBD) in body tissues and biological fluids of animals which may also pose a threat to human consumers of animal products like milk and meat. To our knowledge, there is no information on the safety and nutritional quality of hemp meal as a potential alternative protein in the dairy and beef cattle industry. To obtain Canadian Food Inspection Agency (CFIA) approval on the use of hemp meal as ruminant feeds, the potential to utilize hemp meal as a potential supplement must be demonstrated. This study aims at feeding hemp meal and canola meal as protein supplements to non-lactating dairy cows and comparing their effect on the DMI, rumen fermentation, concentrations of blood metabolites, total tract digestibility, and concentration of cannabinoids in body tissues and biological fluids.

2.0 LITERATURE REVIEW

2.1 Role of dietary protein in dairy cow nutrition

In dairy cattle nutrition, the inclusion of CP (16 % - 18 % DM and 10 % - 12 % DM for lactating dairy cows and dry cows, respectively) is critical for milk production, health, general growth, and reproduction. During certain stages of the cow's life, such as reproduction, transition, and lactation periods, amino acids (AA) which is obtained from microbial crude protein (MCP) and RUP are critical. Their deficiency or excess may have detrimental effects on the cows and the environment. Some of these negative effects include reduced milk protein synthesis, excessive excretion of urea in urine and fecal nitrogen that may lead to loss of energy and essential AA, contamination of the environment, and reduction in fertility due to reduced survival of embryos in the uterus (Rodney et al., 2018).

The crude protein fraction of a feed also includes NPN such as urea and ammonium salts. In the rumen, these compounds are broken down into ammonia which can be used by rumen microbes for the synthesis of MCP. The AA produced from MCP synthesis as well as protein from feeds are absorbed in the small intestines (NRC, 2001). Generally, AA may be broadly classified into non-essential and EAA. Since AA are easily broken down in the rumen by microbes, it is important to ensure that EAA such as lysine and methionine which are the most critical in the determination of milk protein content of dairy cows reach the small intestines through the feeding of rumen-protected AA or high RUP content feed ingredients (Li et al., 2011). The form in which these EAA are incorporated into the diet of the cows is very important, since they may be degraded in the rumen if they are not protected. This was confirmed in a previous study which showed that feeding ruminal-protected lysine to lactating cows increased milk and milk lactose yields as well as milk protein content (Robinson et al., 2010).

Rumen microbes are the most important sources of protein in the intestines since they make available MP from the conversion of products from the breakdown of RDP (Zhu et al., 2013). RDP accounts for approximately 40 % to 60 % of total protein accessible for absorption in the small intestine for animal utilization due to the continuous movement (washout) from the abomasum to the small intestine (Zhu et al., 2013). According to NRC (2001), the protein source feed ingredients such as alfalfa and canola meal used for dairy cows' diets have different concentrations of RDP and RUP, and these influence the productivity of the cow (Table 2.1). Depending on the stage of the lifecycle, the concentrations of these fractions of protein in an ingredient are critical to ensure nutrient requirements are met. In the case of high producing lactating cows (40 – 50 kg milk/day), their diets are formulated so that they contain 40 % to 45 % of CP as RUP to augment microbial protein, enhance milk protein synthesis, and hence milk protein yield (NRC, 2001).

Table 2.1. RDP and RUP content of protein source of feed ingredients

Ingredient	**RDP (% CP)**	**RUP (% CP)**
Soybean meal (mechanical extraction)	70	30
Soybean meal (solvent extraction)	70	30
Roasted whole soybean	45	55
Canola meal	70	30
Ground canola seeds	70	30
Corn gluten meal	35	65
Brewer's grain	35	65
Corn distillers' grain	35	65
Alfalfa hay	70	30
Whey	90	10

(NRC, 2001 & Singh Krishi Vigyan Kendra et al., 2019)

2.2 Importance of feeding protein supplements

In high-producing lactating cows, reliance solely on microbial protein cannot meet their needs of AA, which is critical for milk protein synthesis and the formation of body tissues (Mogensen et al., 2008). According to NRC (2001), during the dry period, cows are fed diets that contain low crude protein contents (12-13 % DM) because they are not producing milk. Furthermore, the diet usually contains high forage to concentrate ratio (80:20) which affects rumen papillae length and density, rumen absorptive capacity for volatile fatty acids (VFAs), and the composition of rumen microbes. However, during the close-up dry period, the diet is gradually changed to a lactating diet which contains higher CP content (14-15 % DM) and a lower ratio of forage in the diet (65:35). Immediately after calving, the diet of the cow is changed to include a lower forage to concentrate ratio (45:65) with CP content of between 18-19 % DM. Nevertheless, the DMI of cows during the close-up dry cow period is reduced compared to that of cows in mid to late lactation due to hormonal activities (NRC, 2001). Although during the fresh cow period DMI is slightly increased, changes in hormonal activities, as well as possible stress that the cow might have gone through during parturition inhibits optimum DMI. This reduction in DMI affects protein intake hence reducing the amount of AA available for milk protein synthesis and formation of body tissues. Moreover, the sudden and rapid rise in milk production after calving triggers the occurrence of negative energy balance (NEB); the mobilization of body reserves such as the labile protein and fat (adipose tissue) as energy for milk production (Zhu et al., 2013). Although the ability of the cow to utilize body protein reserves is limited in duration and quantity during early lactation, this leads to a substantial reduction in milk protein content (Souissi & Bouraoui, 2019).

Another reason for using supplements in dairy cows' diet is the benefits to human health. Using protein supplements such as canola meal, soybean meal, brewer's grain, corn gluten meal, and

hemp meal for ruminant diets could be beneficial for human health when dairy products such as milk and meat are consumed. For instance, hemp meal contains omega-3 and 6 fatty acids which have anti-inflammatory and anti-cancer properties, as well as a stimulatory effect on body metabolism (Russo & Reggiani, 2015).

The use of protein supplements such as canola meal and soybean meal in lactating cow diet provide EAA such as lysine, methionine, and histidine which are critical in milk production. Although EAA is critical most of them (70 %) are deaminated in the rumen which reduces availability for absorption (NRC, 2001). This causes limitation of important EAA like methionine, which is also low in MCP. Robinson et al. (2010) suggested that feeding rumen-protected lysine, isoleucine, valine, and histidine improves the milk and milk lactose yield of cows compared to feeding solely rumen-protected lysine since the combination of these EAAs create a balance, thereby, avoiding possible deficiencies. This confirms that microbial protein alone may not be able to satisfy or provide enough essential amino acids to maximize production. Therefore, to ensure that EAA is available for absorption in the small intestines, rumen-protected AA or feed ingredients that contain high RUP such as roasted whole soybeans and distiller's grain must be included in the diet to help satisfy the AA requirements and enhance health and productivity (NRC, 2001; Yoder et al., 2020).

Feed protein (true protein and non-protein nitrogen (NPN)) and MCP are digested, and its component AA is absorbed in the small intestine (NRC, 2001). It is important in the formulation of a dairy cattle diet to consider the type and quantity of feed ingredients (protein sources) being used since that determine the AA composition of metabolizable protein (true protein that is digested postruminally and its component AA absorbed by the small intestines). Protein supplements that have high contents of RUP such as roasted whole soybeans and corn gluten meal

have high contents of metabolizable protein. It is critical that the metabolizable protein (sum of all AA absorbed) content of the diet is not deficient or in excess based on the body weight of the cow since the excess AA will cause an increase in nitrogen excretion which is a major environmental concern, and the deficiency may cause problems such as reduction in milk production and fertility.

Ouellet & Chiquette (2016), conducted a study on the effect of different dietary levels of metabolizable protein and live yeasts on ruminal fermentation and nitrogen utilization in lactating dairy cows fed a high red clover silage diet. They concluded that when cows are fed moderately deficient in metabolizable protein diets compared to highly deficient diets, voluntary feed intake increases due to the decrease in the supply of AA to the small intestines associated with highly deficient diets.

2.3 Protein supplements in dairy cow nutrition

Various protein supplements are used to augment milk protein content during the lactating stage of dairy cows. The use of protein supplements such as fish meal, soybean meal, cottonseed meal, canola meal brewer's wet grain, blood meal, feather meal, distiller's grains, corn gluten meal, and many others have been reported (Santos et al., 1998). Distiller's grains and corn gluten meal have been used in recent years by many feed formulators due to their high RUP contents (65 % CP in each case). Distiller's grain (DG) is a co-product of cereals that are produced from the dry-grind stage of the distillation process in the production of ethanol. Corn gluten meal is a co-product of corn that is produced by using a wet milling process during distillation to produce ethanol (Liu, 2011). According to Liu, (2011), there are concerns with the use of DG as a livestock protein supplement since it may contain mycotoxins which may be harmful to the livestock. Similarly, there are also issues with the use of corn gluten meal as a livestock protein supplement

due to possible contamination with mycotoxins (Leeson & Summers, 2009; Rodrigues & Naehrer, 2012).

Another protein supplement gaining attention in recent years is dried distiller's grain with solubles (DDGS). This co-product of the distillation process of ethanol is obtained by recovering the non-volatile components following the distillation process, known as whole stillage (Liu, 2011). After the distillation process, two fractions; liquid (thin stillage) and solid (distillers' wet grains) are obtained, following the centrifugation of the whole stillage. The thin stillage at this point is evaporated into condensed distilled solubles that are mixed with the distiller's wet grains and dried (Liu, 2011; NRC, 2001). Research has shown that DDGS from cereals such as wheat contains arabinoxylans which have prebiotic potential (Monteagudo-Mera et al., 2018). A recent study on the feeding of corn DDGS compared to soybean meal in dairy cows showed that urinary nitrogen excretion is reduced and hence, there is low manure nitrogen concentration when corn DDGS is fed to dairy cows compared to feeding soybean meal (Lee et al., 2020). According to Ranathunga et al. (2018), the major concern with the feeding of DDGS to dairy cows is that there is a possibility of the occurrence of milk fat depression due to its high unsaturated fat content, especially when cows are fed low fiber diets and high amounts (> 18 % DM) of DDGS. The crude protein content of DDGS ranges between 26 and 31.7 %. Its fat, ADF and NDF contents range from 9.1 to 14.1%, 11.4 to 20.8%, and 33.1 to 43.9% respectively (Liu, 2011).

Amongst the existing protein supplements, soybean meal and canola meal are the most used in Canada, the USA, and many other countries in Europe (Huhtanen et al., 2011). According to Huhtanen et al. (2011), most dairy farmers preferred soybean meal to canola meal due to its higher CP content (approximately 49.9 % DM) compared to that of canola meal (37.8 % DM). However, according to Broderick et al. (2015), when soybean meal (15 % or 17 % DM CP) is replaced with

an equal amount of canola meal in diets formulated with corn silage, alfalfa silage, and high-moisture corn, there is increased milk production and milk protein, reduced urinary N-excretion and improved N-efficiency in lactating dairy cows due to increased DMI as well as better milk-N:N intake ratio. Similarly, Paula, Broderick, & Faciola, (2020) in their study on the replacement of soybean meal with canola meal concluded that by replacing soybean meal with canola, regardless of the ratio of alfalfa to corn silages, there is an increase in yields of milk, milk protein, and lactose, and decreased MUN and urinary N excretion of lactating dairy cows. These findings, coupled with a better price of canola meal compared with soybean meal, have led most farmers in Canada to prefer canola meal (Sánchez-Duarte et al., 2019). It is important to understand that the processing of soybean meal and canola meal using different methods such as solvent extraction, grinding, and roasting of whole seeds influences its digestibility in the rumen and absorption in the small intestines.

A study conducted on the use of solvent-extracted soybean meal, canola meal, and cottonseed meal as protein supplements in TMR diets containing alfalfa and corn silage fed to ruminally cannulated Holstein cows concluded that canola meal had the best results in terms of milk protein and milk yields due to increased nitrogen efficiency and increased DMI (Brito & Broderick, 2007). Christen et al. (2010) fed four different TMR diets containing 55 % forage and either high protein dried distiller's grain, canola meal, soybean meal, or dried distillers grain containing solubles to multiparous Holstein cows. The authors found similarities among the diets in terms of milk yield, dry matter intake, and feed efficiency. However, the high protein dried distiller's grain diet enhanced casein production in the milk compared to the other diets since its true protein content (total milk N minus NPN x 6.38) was the highest. In a similar study, a concentrated feed of SBM source containing 20 % DM CP was replaced with canola meal to

determine lactational responses (CM diet minus SBM diet). It was demonstrated that milk protein and apparent N efficiency (milk-N:N intake) of the CM diet was significantly greater (3.23 % and 277 g/kg, respectively) than that of SBM (3.19 % and 272 g/kg, respectively) (Martineau et al., 2013). However, the authors stated that the type of forage used in the diet played a vital role since their findings also proved that the increase in milk protein content and milk yield was due to the inclusion of grass or legume forage compared to corn or barley silage. The authors stated that grass or legume forages have higher peNDF content, which enhanced chewing and salivation, aided in rumen buffering, and hence increased the efficiency of rumen microbes.

Additionally, a study on organically raised Holstein cows showed that the addition of a protein concentrate supplement composed of 20 % DM each of sunflower and linseed cakes and 60 % soybean cake in a TMR diet compared to a diet without these protein concentrate supplements increased the milk fat, citric acid, and urea contents of the milk (Horký, 2014). However, it was explicitly stated that, although the protein concentrate used can yield positive results in terms of milk composition, the increase in the milk urea content poses a higher health risk to the cows by reducing the fertility of high producing cows, while accumulation of urea in the liver may lead to urea toxicity which can cause the death of the cow (Siatka et al., 2020). A recent study on the use of Fava bean as a replacement protein supplement for rapeseed (a parent crop of canola) meal in a grass silage-based TMR diet fed to lactating cows showed that the outcome was not desired, as there was a reduction in silage DMI, milk and protein yield, as well as an increase in urinary N excretion and a decrease in the blood concentrations of lysine, methionine, and cystine (Puhakka et al., 2016).

2.4 Alternative protein supplements

In recent decades, the use of locally available resources as alternative protein supplements has increased due to the high prices of commonly used protein supplements such as CM and SBM in Canada (Saavedra, 2019). Moreover, the need for cost-effective diet formulation strategies has triggered feed formulators to use alternative protein supplements (NRC, 2001). The production of hemp in Canada in the last decade has increased (from 15,056 ha in 2011 to 52,000 ha in 2018) which has led to the expansion of production acreages in provinces such as Manitoba and Saskatchewan (Chibisa et al., 2013; Health Canada, 2018; Kostuik et al., 2015; Mooleki et al., 2017; Saavedra, 2019; Wanapat et al., 2013). As a result, hemp processing companies produce large quantities of by-products such as hemp screenings, hulls, and hemp meal after the extraction of oil from the hemp seed, which has the potential to be used as a ruminant feed. The use of hemp meal as alternative protein supplements in dairy cow nutrition has been demonstrated in various trials based on *in-situ* and *in-vitro* methods and results show that they have the potential to substitute commonly used supplements such as canola meal and soybean meal (Mustafa et al., 1999; Mustafa & Mustafa, 2002; Pirzadeh Naeiny et al., 2019; Volpelli et al., 2012)

2.5 Nutritional benefits and chemical composition of hemp

The hemp (*Cannabis sativa* L.) plant is believed to have originated in Asia and was later spread to Europe and Africa (Eriksson, 2010). Hemp is an annual herbaceous plant that is commonly cultivated as a fiber crop in different parts of the world, however in Europe, the USA, and Canada, it is also grown for its oil, which is extracted from the seeds (Mustafa et al., 1999; Stringer, 2018). Two main types of hemp are being cultivated in various parts of the world: industrial hemp (fiber type) and drug-type hemp (Andre et al., 2016). Industrial hemp seed contains approximately 300 g of oil/kg and approximately 80 % of the oil is composed of polyunsaturated fatty acids (Karlsson,

Finell, & Martinsson, 2010; Eriksson, 2010). Phytochemicals, such as flavonoids and phenolics, as well as other secondary metabolites like lignans, terpenoids, and cannabinoids, are also present in the hemp leaves and seeds (Pihlanto et al., 2017). Research has shown that hemp hurds contains 80 % of cellulose and approximately 20 % of lignin. Eriksson (2010) and Kleinhenz et al. (2020) confirmed that the Ca and P contents of hemp seed are 0.17 % DM to 2.6 % DM and 0.6 % DM to 0.7 % DM, respectively. According to Andre et al. (2016), the hemp plant contains many cannabinoids. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are the psychoactive cannabinoids of most concern in the hemp plant (Andre et al., 2016; Semwogerere et al., 2020). However, amongst all the cannabinoids that are present in the hemp plant, tetrahydrocannabinolic acid – A (THCA-A), cannabidiolic acid (CBDA), and cannabinolic acid (CBNA) are the most predominant (Andre et al., 2016). However, according to Andre et al. (2016), THCA is the most predominant compound in the drug-type hemp, while CBDA is the most predominant in industrial hemp. These phytocannabinoid acids are the non-psychoactive forms of their neutral forms: THC and CBD. The THCA and CBDA are converted to their neutral forms through decarboxylation, which occurs within the plant, but is mostly triggered by heating during drying or curing (Andre et al., 2016). Various studies have reported differences in cannabinoid content among different parts of the hemp plant (Andre et al., 2016; Ross et al., 2000). According to Ross et al. (2000), the concentration of cannabinoids in the seed of industrial hemp varieties is very low (0 – 12 ug/g). As a result, the cannabinoid content of hemp seed or hemp seed oil should be very low. The concentration of cannabinoids in the hemp plant is affected by factors such as variety, age, humidity, stage of harvest, and storage conditions (Andre et al., 2016).

Research has shown that the hemp plant has various health benefits for humans such as the reduced risk of Type 2 diabetes and cardiovascular diseases due to properties such as the presence

of oil in the seed which contains essential fatty acids (EFAs); linoleic acid (LA) and α-linolenic acid (ALA) (Pihlanto et al., 2017). Studies have demonstrated that CBD possesses anti-inflammatory, immunomodulatory as well as therapeutic properties for controlling diseases, such as epilepsy and multiple sclerosis (Andre et al., 2016).

The essential oils extracted from hempseed have antimicrobial effects on pathogenic bacteria like *Staphylococcus aureus* and do not inhibit the growth of probiotic strains belonging to the genera *Lactobacillus* and *Bifidobacterium* (Semwogerere et al., 2020). Previous studies have demonstrated that hemp by-products exhibit potent antioxidant properties due to their phenol amides, lignanamides, and tocopherols contents (Semwogerere et al., 2020). Terpenes found in the hemp plant and its by-products have antimicrobial properties that inhibit the activity of undesirable microorganisms like *Staphylococcus aureus* in the rumen thereby reducing the fermentation in the rumen and nutritional disorders such as ruminal acidosis and bloat (Semwogerere et al., 2020). Moreover, condensed tannins found in the hemp plants and their by-products can reduce the population of gastrointestinal tract nematodes (Semwogerere et al., 2020).

For animal nutrition purposes, the seed of the plant is considered the part of most relevance because of its by-product; hemp meal. However, a recent study suggested the possibility of the use of the plant as a forage (Stringer, 2018). In the past decade, various animal trials were conducted on the utilization of hemp by-products as a protein supplement in monogastric and ruminants. This utilization appears to have little or no side effects on the accumulation of THC in milk, nutrient utilization, weight gain, rumen function, and milk production (FEEDAP, 2011; Hessle et al., 2008; Karlsson & Martinsson, 2011; Woods & Fearon, 2009).

2.5.1 Challenges in the utilization of hemp by-products in ruminant nutrition

Although hemp and its by-products have many beneficial properties to humans and animals, concerns exist surrounding compounds found in hemp that may be harmful to consumers. Cannabinoids such as THC, CBN and CBD are compounds of major concern (CONTAM, 2016; FEEDAP, 2011). According to FEEDAP (2016), the most concerning constituent is THC, since it is a psychoactive compound. The concentration of this compound in the hemp plant depends on the variety of hemp cultivated. Varieties of hemp cultivated purposely for oil extraction (industrial hemp) have relatively low levels (< 0.3 %) of THC compared to non-industrial strains which can contain up to 25 % of THC (Pojić et al., 2014). Most countries, however, have regulations governing hemp cultivation and these regulations are based on the level of THC. In USA and Canada for instance, the permitted levels of THC in industrial hemp seeds and its by products such as oil and powder are below 1 % and 0.3 %, respectively (Johnson, 2011). The use of hemp by-products in animal nutrition in Canada is currently not approved by the Canadian Food Inspection Agency (CFIA) due to insufficient research on the use of these by-products as animal feed. There are also concerns by the CFIA with the possible accumulation of CBD and THC in biological fluids such as milk, urine, and blood, and body tissues which may pose a risk to the animals and potentially to human consumers of animal products like milk and meat.

2.5.2 Hemp meal or cake

Hemp meal (HM) or Hemp cake (HC) refers to the by-product of hemp seed obtained after the mechanical or solvent extraction of oil from the seed. However, some researchers consider these terms to be different, based on the method of oil extraction. According to Karlsson, Finell, & Martinsson (2010), HC is obtained after the mechanical extraction process of hemp seed oil using a cold-pressing technique, and it contains high concentrations of fat (12.4 % DM to 13 % DM).

HM, on the other hand, is the by-product obtained after the mechanical (use of heat) or solvent extraction of the oil from the hemp seed and it contains a lower concentration of fats (5 % DM to 9.6 % DM) compared to HC (Mustafa, 2002; Eriksson, 2010). HC contains approximately 927 g/kg of DM, 36 % DM CP, 13 % DM fat, 41 % DM NDF, 36 % DM ADF and 7% DM NPN content (Karlsson et al., 2012) whereas HM contains 32-35 % CP DM, 78-85 % RUP, 51 % NDF and 39 % ADF (Mustafa & Mustafa, 2002; NRC, 2001; Russo & Reggiani, 2015).

HM is the by-product of the hemp plant that is used in ruminant nutrition. It contains polyunsaturated essential fatty acids, specifically linoleic and *alpha*-linolenic acids, and EAA such as arginine and lysine (Mustafa et al., 1999; Mustafa & Mustafa, 2002; Russo & Reggiani, 2015). According to Russo & Reggiani (2015), antinutritional compounds can be found in HM, and therefore, this should be taken into consideration during livestock diet formulation. These antinutritional compounds include condensed tannins, saponins, trypsin inhibitors, cyanogenic glycosides, and phytic acid. Phytic acid is the antinutritional compound of most concern since its presence in the gut reduces protein digestibility and increases the excretion of endogenous nitrogen and AA. However, this does not affect ruminants due to the presence of ruminal and intestinal microbes that produce the enzyme phytase, which breaks down the phytate (Russo & Reggiani, 2015).

It has been demonstrated that HM may be an alternative protein supplement to commonly used protein supplements, such as soybean meal and canola meal in ruminant diets (Eriksson, 2010). Karlsson, Finell, & Martinsson (2010) fed dairy cows a grass silage-based TMR diets containing four different concentrations of HC determined milk production and composition. The authors concluded that there was an increase in milk yield, milk fat content, milk protein, milk lactose, and energy corrected milk when dietary HC levels were below 25 % DM compared to when levels

were above 25 % DM. However, increasing the dietary inclusion of HC above this percentage led to a reduction in milk yield and milk fat, as well as a decrease in efficiency (yield/intake) of dietary CP conversion into milk protein. This was due to excess N in the blood which caused an increase in milk urea content. Serrapica et al. (2019) in an in-vitro digestibility study of HC reported that HC has higher RUP content (67 % CP) compared to sunflower cake, tobacco cake, and cardoon cake (31 %, 37 %, and 50 % CP, respectively), which may be useful in the nutrition of high producing lactating cows. The authors also reported that the intestinal digestibility of RUP of HC was higher (56 % CP) compared to that of sunflower cake, pomegranate cake, tobacco cake, and cardoon cake (12.1 %, 13.2 %, 14.3 %, and 48.9 % CP, respectively).

In the utilization of HC as a protein supplement, it is essential to consider the processing of this feed ingredient before its usage, because the processing method used may affect the availability, digestion, and absorption of AA (Karlsson et al., 2012). A recent study assessed the heat treatment of HC at different temperatures and suggested that heat-treating HC at 130 °C before feeding to cows led to the reduction in total ruminal degradability of AA (837 to 471 g/kg) and increased intestinal digestibility of AA (267 to 813 g/kg) compared to untreated HC. Therefore, RUP was increased while RDP was reduced after the heat treatment (Karlsson et al., 2012). Research carried out in the past decade on the feeding of cold-pressed hemp cake and soybean meal as protein supplements to fast-growing Holstein dairy bull calves and steers concluded that replacing soybean meal with cold-pressed HC at a rate of 0.1 kg/DM and 0.2 kg/DM, respectively yielded similar results in terms of production (live weight gain and carcass traits) (Hessle et al., 2008). The authors also emphasized that this was due to a higher fiber content (45 % DM and 18. 2 % DM for HC and soybean meal, respectively) and/or a lower starch content (30 % DM and 37 % DM, respectively), leading to selective retention of feed particles, enhanced rumination, extensive ruminal digestion

of carbohydrates, and a normal ruminal pH (Hessle et al., 2008). This result suggests that HC can be a good alternative protein supplement to replace soybean meal.

Turner (2010), compared feeding cold-pressed HC and soybean meal in a TMR diet containing grass silage to steers to determine their influence on fatty acid profile and tenderness of meat. He concluded that substituting soybean meal with HC resulted in an increase in PUFA content in the small intestines due to the increased lipid content in HC compared to soybean meal. Research has shown that increased levels of PUFA in the small intestine is beneficial to resulting in increased milk fat concentration of PUFA, improved reproductive efficiency of dairy cattle due to reduced prostaglandin-F2α secretion, and improved fertility (Lanier & Corl, 2015).

2.5.3 Effects of hemp by-products on ruminant nutrition

Various studies have examined the effects of hemp by-products on ruminant nutritional aspects such as DMI, total tract NDF, and CP digestibility (Karlsson et al., 2010; Karlsson & Martinsson, 2011; Krizsan & Huhtanen, 2013; Mustafa et al., 1999; Semwogerere et al., 2020). There have been differences in studies in terms of DMI of ruminants fed hemp by-products like HM. A review by Semwogerere et al. (2020) showed that feeding 1 kg to 1.4 kg/animal/day of HC to calves led to an increase in DMI due to the low degree of rumen fill of NDF from hemp meal compared to that of soybean meal. Other studies confirmed that feeding dairy cows and lambs HC at a proportion of up to 320 g/kg DM and 218 g/kg DM, respectively, led to an increase in DMI (Karlsson et al., 2010; Karlsson & Martinsson, 2011). Mustafa et al. (1999) also reported that no difference in DMI intake was recorded when lambs were fed diets containing up to 200 g of HC compared to CM. It has been reported that the NDF and CP digestibilities of ruminants (sheep) fed diets containing HM did not differ from sheep fed canola meal. Interestingly, in all these studies reviewed there was a difference in inclusion rates (32 % DM in dairy cows and between 20 - 21.8

% DM in lambs) and composition of basal diets, which may be the cause of the inconsistencies found in literature.

2.6 Summary

The protein supplements mostly used dairy cattle nutrition in Canada and the USA are CM and SBM. These supplements help increase milk production and improves milk quality. Despite these benefits there are constraints that dairy farmers may encounter such as high cost of the supplements. Therefore, the search for novel alternative protein supplements such as hemp meal is very important for the dairy cattle industry. The production of industrial hemp for its oils and fiber in the Canadian prairies has increased significantly from 15,056 ha in 2011 to 52,000 ha in 2018. This has caused processing companies like Hemp Oil Canada to increase its production of products such as hulled hemp seed and hemp oil, leading to the generation of large quantities of hemp by-products such as HM. Obtaining a viable market for these by-products would be beneficial to the Canadian hemp industry. Moreover, previous studies have reported the potential of hemp meal as a very good replacement for commonly used protein supplements such as CM and soybean meal. Although HM has the potential to be used as an alternative to CM and SBM, there concerns regarding its use in ruminant nutrition in Canada due to insufficient information its safety to animals. Moreover, the possible accumulation of cannabinoids (THC and CBD) in body tissues and biological fluids of ruminants is a major concern to the Canadian Food Inspection Agency (CFIA) since this accumulation may be harmful to animals and human consumers of animal products like milk and meat. If the potential and safety to utilize hemp meal as a ruminant feed can be demonstrated, it will help gain the CFIA approval.

3.0 HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis

1. Feeding hemp meal as a protein supplement at a rate of 10.2 % DM to non-lactating dairy cows will not negatively affect DMI, protein and nitrogen utilization as well as rumen fermentation compared to feeding canola meal.

2. Feeding hemp meal to cows will not lead to accumulation of cannabinoids (THC and CBD) in body tissues and biological fluids.

3.2 Objective

The main objectives of this study were to compare the dry matter intake, rumen fermentation (VFA, pH, ammonia nitrogen), concentration of blood metabolites, total tract digestibility as well as concentration of cannabinoids in body tissues and biological fluids between non-lactating dairy cows fed hemp meal and those fed canola meal.

4.0 MANUSCRIPT

4.1 Introduction

Dairy cows require protein in their diet since amino acids are critical for production, maintenance, growth, and development (NRC, 2001). Canola meal (CM) and soybean meal (SBM) are the principal protein supplements used in dairy cattle nutrition in North America (Budi et al., 2018; Chibisa et al., 2012). Feeding these supplements to dairy cows helps improve productivity and increase the supply of metabolizable protein compared to feeding other supplements such as dried distiller's grain with solubles and cottonseed meal (Brito & Broderick, 2007; Budi et al., 2018; Neal et al., 2014). Despite the many benefits that canola and soybean meals offer to dairy cows, their high cost may be a constraint for some farmers (Saavedra, 2019). Moreover, there are concerns about use of soybean meal in livestock nutrition because of concerns about the sustainability of its production, especially the excessive deforestation of the Amazon forest (Gasparri & le Polain de Waroux, 2015). The use of CM for starter mixtures for dairy calves is also a concern since it affects palatability and digestibility, there by reducing feed efficiency (Burakowska et al., 2021). As a result of those limitations of CM and SBM, the use of alternative protein supplements is on the rise in recent years. Dried distiller's grain with solubles (DDGS) for instance, is an alternative protein supplement to canola and soybean meals which has been used in dairy cattle nutrition (Budi et al., 2018). It helps increase milk fat composition due to the availability of more substrate available for ruminal biohydrogenation, which also increases omasal flow of critical poly unsaturated fatty acids such as C18:2n-6. Moreover, it enhances DMI, leading to increase in milk production, as well as energy and protein digestibility (Budi et al., 2018; Chibisa et al., 2012). However, according to Pecka-Kiełb et al. (2017), a major problem which may hinder the use of DDGS in dairy cattle nutrition is loss of nutritional value of total protein (about 40 %).

This occurs when high temperatures are rapidly used to dry the distiller grains, which leads to the occurrence of Maillard reaction and decreases digestibility of the total protein to about 40 % (Pecka-Kielb et al., 2017). Moreover, variations in the concentration of nutrients such as protein, fat, and phosphorous in DDGS is a concern, since it increases the feed cost of dairy farmers due to inaccuracy in diet formulation (Schingoethe et al., 2009).

An alternative to the aforementioned protein supplement sources in Canada could be hemp by-products. Industrial hemp (*Cannabis sativa* L.) production in the Canadian prairies has increased in recent years (from 15,056 ha in 2011 to 52,000 ha in 2018) due to increased demand for processed hemp seed products like hemp oil, hemp protein and hemp flour since the Industrial Hemp Regulation program came into effect in 1998 (Ashton & Galatsanou, 2015). This has triggered an increase in production by hemp processing companies such as Hemp Oil Canada. During the processing of hemp by-products such as hemp meal, hemp screenings, and hemp hulls are generated. Previous studies have shown that HM has the potential to replace CM and SBM as an alternative protein supplement to dairy cows due to its high crude protein content (over 36 %), favorable amino acid profile, high rumen undegradable protein content (66.7 % - 87.1 %), high intestinal availability of CP (53.5 % - 77.3 %) and its fast digestibility in the gut (Mustafa et al., 1999; Mustafa & Mustafa, 2002).

However, a few barriers have to be overcome before hemp by-products is allowed to be fed to dairy cows. According to Health Canada, hemp varieties that are permitted to be cultivated legally in Canada must contain less than 0.3 % of delta-9-tetrahydrocannabinol (THC). Feeding hemp meal as a protein supplement to cattle in Canada is not approved by the CFIA, due to concerns about the possible accumulation of cannabinoids, especially cannabidiol (CBD) and tetrahydrocannabinol (THC) in body tissues and biological fluids such as milk, blood, and urine

(Ware & Tawfik, 2005). These compounds may affect cow health due to cannabinoid intoxication and pose a possible risk to human consumers of food of cattle origin. According to Smith (1992) and McSweeney et al. (2002), rumen microbes can break down toxic plant compounds such as oxalate, neurolathrogen, nitropropanoic acid, and prussic acid. Therefore THC may also be broken down by these microorganisms (Kleinhenz, Magnin, Lin, et al., 2020; McSweeney et al., 2002).

Although earlier studies by Mustafa et al. (1999), Karlsson et al. (2010), and Karlsson & Martinsson (2011) have demonstrated the potential of hemp meal as a source of protein in cattle diets, they did not evaluate the possible accumulation of cannabinoids and safety of feeding it to cattle. The objective of this study was to compare the dry matter intake, rumen fermentation, concentrations of blood metabolites, total tract digestibility, and concentration of cannabinoids in body tissues and biological fluids between non-lactating dairy cows fed hemp meal and those fed canola meal. Canola meal was selected as the protein supplement to compare with hemp meal since it is frequently used in the Canadian prairies, and its nutrient profile is comparable to that of hemp meal.

4.2 Materials and methods

All procedures on the use of dry, non-pregnant cows in the experiment were pre-approved by the Fort Garry Campus Animal Care Committee (FG CCAC) of University of Manitoba, and the cows were cared for in accordance with the Canadian Council on Animal Care (CCAC) guidelines (CCAC, 1993).

4.2.1 Animals, diets, and experimental design

Hemp meal was obtained from Hemp Oil Canada Inc, Ste Agathe, Manitoba. The hemp meal was produced from whole hemp seeds after the mechanical extraction (cold pressing) of hemp oil. Six non-lactating, non-pregnant Holstein cows with an average body weight of 704 kg and body

condition score of 3.25, were housed in a metabolism unit, where they were individually kept in tie stalls. The stalls were lined with rubber mats and chopped straw was used as bedding.

The experimental design used was a repeated 3 × 3 Latin Square Design with three, 3 experimental periods. The cows were blocked according to their parity and milk production of the previous lactation and were randomly assigned to one of three dietary treatments: (1) a basal partial mixed ration (PMR) diet and the addition of 10.2 % hemp meal (HM) protein supplement, (2) a basal PMR diet and the addition of 13.5 % canola meal (CM), or (3) a basal PMR diet and the addition of 6.16 % CM plus 6.25 % hemp meal (HC). All the diets were formulated such that they were isonitrogenous and isoenergetic. Cows were fed once daily, and protein supplements were supplied to the cows as a top-dressing during feed delivery. The chemical composition of protein supplements and the ingredient composition of experimental diets fed to cows are displayed in Tables 4.2 and 4.3, respectively.

Table 4.2. Chemical composition of hemp meal relative to canola meal and soybean meal (% DM or as indicated)

	Supplement		
Diet Property	HM	CM	SBM[1]
DM	91.8 - 95.1	90.3 - 92.1	90.0 - 90.7
CP	36.0 - 40.7	36.5 - 41.8	45.6 - 53.8
Fat	9.12 - 12.5	5.40 - 6.00	1.10 - 2.00
NDF	20.0 - 42.8	27.5 - 33.0	8.10 - 8.80
ADF	21.5 - 35.3	18.6 - 20.5	4.80 - 6.20
Ash	6.70 - 7.33	7.00 - 7.50	5.50 - 7.00

Values of HM, CM and SBM are adapted from Abu-Ghazaleh, Schingoethe, & Hippen, (2001); Canadian Hemp Trade Alliance (CHTA), Hristov et al. (2011); Lopes et al. (2017); Mejicanos et al., (2016); Mustafa, (2002); NRC, (2001); Paula et al. (2018); Paula, Broderick, & Faciola, (2020); Xin & Yu, (2013); Yehudi Coura de Assis et al. (2019): Bailoni et al. (2021) and House et al. (2010).
[1]SBM= soybean meal

The first two weeks of each experimental period served as an adaptation period and sample collection occurred during the third week, after which treatments were changed amongst cows. Cows were fed once a day at 0900 h, accounting for 5 % to 10 % of refusals and had *ad libitum* access to fresh water all day. The chemical composition of PMR diet and supplements are displayed in Table 4.4. Rumination behaviour of cows was monitored using the Lely Rumination Monitor (Qwes-HR, Lely). During the third week of each experimental period, fecal, free catch urine and blood samples were collected twice daily at 0900 h and 1500 h, respectively. Rumen fluid was collected two days (3^{rd} and 7^{th} days) once daily at 1500 h through rumenocentesis. Body weight and condition score of cows were taken at the beginning and end of each experimental period.

4.2.2 Dry matter intake and feed analyses

PMR diet samples were collected and stored at –20 °C once daily during each experimental period and were pooled by treatment, by period at the end of the experimental period. Pooled basal diet samples were later oven dried in a forced-air oven at 60 °C for 48 hrs. The dried basal diet samples were ground using a Wiley mill through a 1-mm screen (Thomas-Wiley, Philadelphia, PA) and were bagged and stored at room temperature until further analysis. Individual orts samples were collected every morning before feeding and stored in a –20 °C freezer during the last week of each experimental period. The pooled orts samples were processed using the same procedure used for PMR diet samples. DMI was estimated using the dry matter contents of PMR diets offered, orts (refusals), and the dry matter content of the protein supplements fed. Chemical analyses of diet and feed samples were analysed by Central Testing Laboratory Ltd (Winnipeg, MB). Samples were analysed for DM, CP (Modification of AOAC 990.03; Horwitz & Latimer, 2005), NDF (Ankom Technology, Fairport, NY), ADF (Ankom Technology, Fairport, NY), AIA, starch, fat,

ash (AOAC 942.05; Horwitz & Latimer, 2005) and minerals (Ca, P, Mg, K, and Na) (Modification of AOAC 985.01, 968.08, 935.13a; Horwitz & Latimer, 2005) using wet chemistry.

The DMI, %CP, %NDF and AIA concentrations of experimental diets as well as the AIA contents of feces of cows during the last week of each period were determined and used in the estimation of the apparent digestibility coefficient (ADC) of nutrients. The AIA content of diets was used as internal digestion marker (Wilson & Winter, 1984) and ADC of DM, CP and NDF were estimated using the following equations:

$$\text{DMD} = 1 - \left(\frac{\text{AIA \% DM in diet}}{\text{AIA \% DM in feces}}\right)$$

$$\text{ADC CP} = 1 - \left[\left(\frac{\text{CP \% DM in feces}}{\text{CP \% DM in diet}}\right) \text{x} \left(\frac{\text{AIA \% DM in diet}}{\text{AIA \% DM in feces}}\right)\right]$$

$$\text{ADC NDF} = 1 - \left[\left(\frac{\text{NDF \% DM in feces}}{\text{NDF \% DM in diet}}\right) \text{x} \left(\frac{\text{AIA \% DM in diet}}{\text{AIA \% DM in feces}}\right)\right]$$

4.3 Sample collection and analyses

4.3.1 Rumen fluid

Approximately 10 mL of rumen fluid (RF) were collected via rumenocentesis (Garrett & Nordlund, 1994), and pH was immediately recorded using an Accumet Basic 15 pH meter (Fisher Scientific, Fairlawn, NJ), equipped with a Sensorex 450C Flat Surface Combination pH/Reference Electrode (Sensorex, Stanton, CA). The cows were given 10 units of xylazine IV before rumen fluid collection. Subsequently, two sub samples (1 mL each) of RF was pipetted into two, 2 mL tubes and immediately flash frozen in liquid nitrogen before storing at a temperature of -80 °C for subsequent cannabinoid analysis. Also, 2 mL of RF was transferred into tubes prepared with 0.4 mL 25% metaphosphoric acid and 0.4 mL sulphuric acid (H_2SO_4) and stored at -20 °C for rumen VFA and ammonia nitrogen (NH_3N) analyses, respectively.

For rumen VFA analysis, frozen samples were thawed and centrifuged at 3000 RPM for 20 min at 4 °C and 1 mL of the supernatant was gently decanted using a syringe with filter into clean GC vails. The vials were then placed into an autosampler (8410 Varian, Walnut Creek, CA) and analyzed for VFA using the gas chromatography method (model 3900 GC, Varian, Walnut Creek, CA) as described by Bhandari et al. (2007). The injector and detector of the GC were set at a temperature of 170 °C and 195 °C, respectively, and the initial and final column temperatures at 120 °C and 165 °C, respectively. The samples were run for a period of 4 minutes followed by a 2-minute thermal stabilization period. The ammonia nitrogen concentration of stored rumen fluid samples was determined using the Indole-Phenol Blue Method as described by Novamsky et al. (1974).

4.3.2 Blood

Blood samples were taken from the tail vein and collected into 10 mL vacutainer tubes for serum and 10 mL heparinized vacutainer tubes for plasma analyses. For blood serum extraction, collected blood in vacutainer tubes was kept at room temperature for 30 min to ensure clotting before being centrifuged at 3000 RPM at 4°C for 10 minutes. Serum samples were later transferred into plastic tubes and stored immediately in a -20 °C freezer until final analyses. The analysis of serum samples was conducted by Veterinary Diagnostic Services (Manitoba Agriculture, Food and Rural Initiatives, Winnipeg, MB). Samples were analysed for NEFA and BHBA using the RX Monza analyser and Randox kits (Randox Laboratories Ltd, Crumlin, BT29 4QY, UK). Serum glucose and urea were analysed using the cobas c 502 analyser (Roche Diagnostics GmbH, Mannheim, Germany).

Blood samples collected for plasma analysis were centrifuged at 4 °C for 15 min immediately after collection. Plasma samples were then transferred into prelabelled tubes and immediately flash

frozen in liquid nitrogen and later kept at a temperature of -80 °C until final analysis for cannabinoids and plasma urea concentrations.

4.3.3 Urine

Approximately 80 mL of mid-stream urine samples were collected by stimulating the perirenal area to initiate urination. After collection, a total of 30 mL of urine sample was transferred into tubes pre-filled with approximately 2 mL 3N hydrochloric acid (HCl), to ensure that the pH of the urine samples was below 2.0 and to minimize N volatilization and samples were later stored in a -20 °C freezer until final analysis for urea nitrogen. Approximately 4 mL of urine from the initial sample was transferred in 5 mL screw-top vails, immediately flash frozen in liquid nitrogen, and later stored at -80 °C until analyzed for cannabinoids.

Total urine protein analysis was conducted by the Veterinary Diagnostic Services (Manitoba Agriculture, Food and Rural Initiatives, Winnipeg, MB) using the procedure developed by Watanabe et al. (1986) and Fujita et al. (1983). Urea nitrogen concentration of samples were determined using procedure described in AOAC 990.03 (Modified: CTL-PDSOP).

4.3.4 Feces

Fecal samples were collected twice a day at 0900 h and 1500 h over a 5-day period during the last week of each experimental period. Fecal sample collection was conducted as described by S. Li et al., 2012. Approximately 250 g of feces was collected via grab sample from the rectum of each cow and stored at -20 °C. Samples were later thawed and pooled by animal by period and oven dried at 60 °C for seven days. Dried fecal samples were later ground using a Cyclotec 1093 Sample Mill through a 1-mm screen (Cyclotec, 1093 Sample mill, Foss Tecator), bagged in whirl-pack bags and stored at room temperature until further analysis. Fecal samples were analyzed for DM, CP (Modification of AOAC 990.03; Horwitz & Latimer, 2005), NDF, ADF, AIA (AOAC

942.05; Horwitz & Latimer, 2005), and Starch (AOAC 942.05; Horwitz & Latimer, 2005) using wet chemistry.

4.3.5 Tissue

On the last day of the trial, all the cows were euthanized with a pentobarbital solution, and liver biopsy, adipose tissue, muscle, and kidney samples were collected from each cow. Immediately after the euthanasia cows were slaughtered and a paracostal incision was made to gain access to the liver and kidney. A 20 g liver sample was collected as described by Coetzee et al. (2015). The sample was rinsed with deionized water, placed immediately in whirl-pack bags, flash frozen in liquid nitrogen, and stored at -80 °C until analysis was carried out. A transverse-section of the kidney lobe was made, and a sample was extracted ensuring that it included both the medulla and cortex. The kidney sample was then prepared and stored using the same procedure as described for liver samples.

Adipose tissue samples were collected from the perirenal tissue, mesenteric tissue, omental tissue, and subcutaneous fat pad around the pin bone. Approximately 20 g of both tissues were carefully extracted ensuring that scalpels were cleaned with alcohol between sample collections to prevent any possible cross-contamination. The collected samples were washed with deionized water, placed in whirl-pack bags, flash frozen in liquid nitrogen, and stored at -80 °C before analyses for cannabinoids.

Approximately 20 g of muscle tissues were extracted from the semitendinosus and semimembranosus muscle of each cow. Collected samples were processed and stored following the aforementioned procedure.

4.3.6 Cannabinoid Analysis

Cannabinoids in diet samples were determined using a liquid chromatography mass spectrometry (LC/MS) and analyzed using a Waters Quattro micro API, following the procedure described by Meng et al. (2018). Fecal samples and frozen tissue (liver, kidney, adipose tissue, and muscle tissue) and were homogenized by grinding and 1 g was mixed with 5 mL of methanol and vortexed. The vortexed mixture was then sonicated for 20 min and centrifuged for 10 min at 4750 rpm. One mL of extracted solvent was further centrifuged for 15 min at 12000 rpm. The supernatant was then transferred into a vial and analysed for cannabinoids using an Agilent 6430. Rumen fluid (100 µL) and thawed blood plasma (100 µL) samples were mixed with 100 µL of cold methanol and vortexed. The mixture was then centrifuged for 10 minutes at 12000 rpm and transferred into autosampler vials for cannabinoid quantification. A calibration stock solution of 50 µg/mL was prepared by combining the individual cannabinoids in methanol and was used to prepare working calibration standards at a minimum of 5 levels ranging between 0.1 - 2 µg/mL A detection limit of 0.048 ng/mL and 0.024 ng/mL was used for CBD and CBDA, and THC and THCA-A, respectively.

Table 4.3. Ingredient composition of experimental diets

	Diets (% DM)		
Ingredient	CM	HC	HM
Barley silage	46.1	42.9	49.4
Dry Cow Premix[1]	1.50	1.50	1.50
Grass hay	12.8	13.5	9.40
Wheat straw	26.2	29.7	29.5
Hemp meal	--	6.25	10.2
Canola meal	13.5	6.16	--

[1]Propulsion 1:1 Dry Cow Premix contains: calcium (12 %), phosphorous (12 %), salt (0.002 %), sodium (0.12 %), potassium (0.06 %), magnesium (12 %) sulphur (1 %), iron (6,880 mg/kg), zinc (2,250 mg/kg), manganese (1,875 mg/kg), copper (892 mg/kg), iodine (27 mg/kg), cobalt (10 mg/kg), fluorine (0.0009 mg/kg), vitamin A (500,000 IU/kg DM), vitamin D (147,055 IU/kg DM), vitamin E (7,066 IU/kg DM).

Table 4.4. Chemical composition of partial mixed ration (PMR) diets and HM and CM protein supplements

	PMR CM	PMR HC	PMR HM	CM	HM
DM, %	94.2	94.1	94.1	88.1	90.1
CP, % DM	11.3	11.4	11.4	42.1	55.2
NDF, % DM	52.2	50.9	51.5	25.5	15.8
ADF, % DM	35.1	34.7	34.8	20.6	13.1
Crude fat, % DM	2.80	2.75	2.54	3.47	6.55
Ash, % DM	11.5	11.3	11.3	7.56	11.9
NFC, % DM	25.7	26.9	26.3	21.6	18.2
Starch, % DM	12.5	10.0	9.99	1.09	0.42
Ca, % DM	0.57	0.55	0.53	0.76	0.20
P, % DM	0.31	0.33	0.31	1.11	2.31
Mg, % DM	0.31	0.30	0.30	0.55	0.97
Na, % DM	0.05	0.05	0.05	0.15	0.002
K, % DM	1.78	1.78	1.76	1.26	1.86
NE_L, Mcal/kg	1.37	1.39	1.39	1.64	1.73

4.3.7 Statistical Analysis

Data were analysed using the MIXED procedure of SAS (Ver. 9.4 SAS Institute Inc., Cary, NC) using the following models:

$$Y_{ij(k)} = \mu + C_i + P_j + T_{(k)} + e_{ij(k)}$$

Where $Yij_{(k)}$ = observation of dependent variables, μ = overall mean, C_i = random effect of cows, P_j = random effect of period, $T_{(k)}$ = fixed effect of treatments (CM, HM, and HC) and $e_{ij(k)}$ = residuals. Treatment effects on apparent total tract digestibility coefficients of CP, NDF and dry matter were determined using this model.

$$Y_{ij(k)} = \mu + C_i + P_j + T_{(k)} + E_{ij(k)} + e_{ij(k)l}$$

Where $Yij_{(k)}$ = observation of dependent variables, μ = overall mean, C_i = random effect of cows, P_j = random effect of period, $T_{(k)}$ = fixed effect of treatments, $E_{ij(k)}$ = interaction of cow x period x treatment, and $e_{ij(k)l}$ = residual error due to the l'th sample from the I'th cow in the j'th period and

k'th treatment. The effects of treatments on rumen pH, VFA and NH_3N were determined using this model.

$$Y_{ij(k)} = \mu + C_i + P_j + T_{(k)} + E_{ij(k)} + W_l + TW_{kl} + e_{ij(k)l}$$

Where $Yij_{(k)}$ = observation of dependent variables, μ = overall mean, C_i = random effect of cows, P_j = random effect of period, $T_{(k)}$ = fixed effect of treatments, $E_{ij(k)}$ = interaction of cow x period x treatment, W_l = effect of week, TW_{kl} = the interaction of treatment x week and $e_{ij(k)l}$ = residual error due to the l'th sample from the I'th cow in the j'th period and k'th treatment. Week in the dry matter intake analysis and time of sample collection in the blood metabolites analysis were considered as repeated measures for the subject of interaction between treatment, period, and cow. In case of unequal variances, the (UN), (CSH) and (CS) covariance structures for DMI; blood metabolites and urine nitrogen concentrations, respectively were included in the REPEATED statement to allow for separate estimation of variances. If the assumption of equal variances was not met, the DDFM=KR option of the PROC MIXED procedure was added to the model to adjust the degrees of freedom of the unequal variances. The PDIFF statement in SAS was used to determine differences between treatments and the significant effects of treatments as well as interactions were discussed at $P < 0.05$, and tendencies were reported at $0.05 < P < 0.10$.

4.4 Results

4.4.1 Dry matter intake and blood metabolites

The DMI and blood metabolites variables recorded are shown in Table 4.5. There was no significant difference in DMI among the treatments. No effects of supplement treatment and the interaction between treatment and week were observed. There was no significant effect of treatment on rumination behaviour of the cows.

Serum urea, glucose, BHBA and NEFA levels did not differ among treatments. There were no effect of treatment and the interaction between treatment and time for any of the blood metabolite concentrations.

Table 4.5. Blood metabolites concentration, average daily dry matter intake and rumination time of non-lactating dairy cows fed canola meal (CM), hemp meal + canola meal (HC) or hemp meal (HM)

	Treatment				**Significance, *p value*[4]**		
Item	**CM**	**HC**	**HM**	**SEM[3]**	**T**	**Week**	**T x Week**
DMI, kg/day	14.9	14.5	14.8	0.97	0.56	0.03	0.13
Rumination time (min)	487	474	477	48.3	0.62	0.05	0.13
	CM	**HC**	**HM**	**SEM**	**T**	**Time**	**T x Time**
	(N=6; n = 36)						
Urea, mmol/L	4.71	4.90	4.86	0.24	0.84	<.0001	0.64
Glucose, mmol/L	4.38	4.47	4.43	0.16	0.74	0.07	0.46
BHBA[1], mmol/L	0.39	0.42	0.38	0.02	0.25	<.0001	0.58
NEFA[2], mmol/L	0.07	0.07	0.07	0.02	0.97	0.0004	0.69

[1] BHBA = beta-hydroxybutyrate.
[2] NEFA = non-esterified fatty acids.
[3] SEM = standard error of mean.
[4] T = Treatment.
N = number of cows.
n = number of samples.

4.4.2 Rumen pH, VFA, and cannabinoid concentration of body tissues and fluids

The pH, concentrations of VFA and ammonia nitrogen of collected rumen fluid are provided in Table 4.6. No significant differences in rumen pH were observed among treatments. The concentrations of acetate, propionate, butyrate, total VFA, other VFA and acetate to propionate ratio, as well as rumen ammonia nitrogen did not differ among treatments.

Table 4.6. Ruminal pH, ammonia nitrogen and volatile fatty acids concentrations of non-lactating dairy cows fed canola meal (CM), hemp meal + canola meal (HC) or hemp meal (HM)

Item	**CM**	**HC**	**HM**	**SEM[1]**	**Significance, *P-values***
	(N=6; n=36)				
pH	6.42	6.34	6.50	0.06	0.20
Acetate, mmol/L	71.0	73.3	69.5	1.93[y]	0.41
Propionate, mmol/L	21.4	22.9	21.3	1.09[y]	0.29
Butyrate, mmol/L	13.6	13.7	12.7	0.54[y]	0.27
Other[2] VFA, mmol/L	4.95	5.36	4.93	0.27[y]	0.28
Total VFA, mmol/L	111	115	108	3.14[y]	0.32
Ac/Pr[3]	3.33	3.22	3.27	0.13[y]	0.55
NH_3-N, mg/dL	8.48	10.4	9.24	0.99	0.15

[y]Simple average of the standard errors due to one missed observation.
[1]SEM = standard error of mean.
[2]Other = isobutyric acid + valeric acid + isovaleric acid
[3]Ac/Pr = acetate to propionate ratio
N = number of cows.
n = number of samples.

The total tract digestibility coefficients of DM, CP, and NDF are shown in Table 4.7. Total tract NDF digestibility was the only variable that differed significantly among treatments ($p < 0.03$). We observed that the average NDF digestibility coefficient of the CM was the highest (43 %) compared to the hemp meal + canola meal and hemp meal diets which had 39 % and 38 % NDF, respectively.

Table 4.7. Total tract digestibility coefficients of dry matter, crude protein and neutral detergent fibre of cows fed experimental diets

Digestibility coefficient	**CM**	**HC**	**HM**	**SEM[1]**	**Significance, *P-values***
DMD[2]	55.0	54.0	54.0	0.01	0.28
CP digestibility, %	63.0	64.0	65.0	0.02	0.22
NDF digestibility, %	43.0 *a*	39.0 *b*	38.0 *b*	0.01[3]	0.03

a, b LSM with different letters within rows differ (p <0.05)
[1]SEM = standard error of mean.
[2]DMD = dry matter digestibility.
[3]SED = standard error of difference.

The cannabinoid concentrations of rumen fluid, blood plasma, urine, feces, kidney, liver, adipose and muscle tissues of cows are shown in Table 4.8. No accumulations of cannabinoids were found in any of the body tissues and biological fluids studied except in feces of cows that were fed the HC and HM diets which had 0.42 ug/g and 0.68 ug/g of CBD and 0.40 ug/g and 0.67 ug/g of CBDA, respectively (Table 4.9).

Table 4.8. Cannabinoid concentration of blood plasma, rumen fluid, urine, liver, kidney, adipose and muscle tissue of non-lactating dairy cows fed experimental diets

Body tissues and fluids	**Cannabinoid**	**Content (µg/g)**
Blood plasma	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND
Rumen fluid	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND
Urine	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND

Body tissues and fluids	Cannabinoid	Content (µg/g)
Liver	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND
Kidney	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND
Adipose tissue[1]	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND
Muscle tissue[2]	Δ^9-THC	ND
	THCA-A	ND
	CBD	ND
	CBDA	ND

[1] Adipose tissue = pooled omental, mesenteric, subcutaneous fat pad near pin bones and perirenal adipose tissues
[2] Muscle tissue = pooled semimembranosus and semitendinosus tissues
Δ^9-THC= delta 9-Tetrahydrocannabinol
THCA-A= Tetrahydrocannabinolic acid A
CBD= Cannabidiol
CBDA= Cannabidiolic acid
ND= not detected
n = number of samples

Table 4.9. Cannabinoid concentration of feces of non-lactating dairy cows fed experimental diets

Item	CM	HC	HM	SEM[1]	Significance, *P-values*
CBD, ug/g	ND[2]	0.42	0.68	0.04	0.008
CBDA, ug/g	ND	0.40	0.67	0.14	<0.0001
Δ^9-THC, ug/g	ND	ND	ND	--	--
THCA-A, ug/g	ND	ND	ND	--	--

A detection limit of 0.048 ng/mL for CBD and CBDA and 0.024 ng/mL for THC and THCA-A was used
[1] SEM = standard error of mean
[2] ND = not detected
Δ^9-THC= delta 9-Tetrahydrocannabinol
THCA-A= Tetrahydrocannabinolic acid A
CBD= Cannabidiol
CBDA= Cannabidiolic acid

4.5 Discussion

4.5.1 Feed quality and diet composition

The CP content of CM used in the present study was 42.1 % DM, which is comparable to published values (Brito & Broderick, 2007; Broderick et al., 2015; Maxin et al., 2013; Mustafa & Mustafa, 2002; Mutsvangwa, 2017). The NDF content of canola meal used in this study (25.5 % DM) was lower than values published by the NRC (2001), Mutsvangwa (2017), Mustafa (2002), and Broderick et al (2015), which were all above 30 % DM. This variability is possibly due to factors such as differences in variety, growing conditions of the crop, and processing conditions of the seed and meal (Mutsvangwa, 2017). The ADF content in our study was similar to the values published by these authors. The crude fat content of 3.47 % DM obtained in our study was similar to those published by other authors (Broderick et al., 2015; Mejicanos et al., 2016; Mutsvangwa, 2017).

The HM protein supplement had a CP concentration of 55.2 % DM. This value was higher than concentrations published by Bailoni et al. (2021), Wang et al. (2017), Mierlita (2016), Gibb et al. (2005) and Mustafa (2002). The NDF and ADF contents of HM (15.8 and 13.1 % DM, respectively) obtained in this study were low compared to reported values by other researchers: 21.3 % DM to 39.3 % DM and 23.3 % DM to 33.4 % DM (Gibb et al., 2005; Karlsson et al., 2010; Mierliţă, 2018). The crude fat content of the HM used in this study was 6.55 % DM which was lower than recent published values which ranges between 9.6 % - 12.4 % DM (Bailoni et al., 2021) but it was higher than the value (5 % DM) published by Mustafa (2002). Research has shown that the seed composition of hemp meal may be affected by factors such as geography, climatic conditions, local agronomic factors and method of extraction oil from hemp seed which may lead

to inconsistencies in chemical composition of hemp by-products like hemp meal (House et al., 2010).

4.5.2 Effects of protein supplements in the diet of non-lactating dairy cows

The DMI intake of cows may be affected by factors such as particle size of feed ingredients, health status of cows, stage of lactation, frequency of feed delivery, palatability of diet, and facility and management practices (Mertens, 1997; Weiss, 2015). There was no effect of treatment on DMI. This was expected since the nutrient content of dietary treatments were similar as diets were isoenergetic and isonitrogenous and were formulated to meet the nutrient requirements of the cows. Similarly, Mustafa et al. (1999) demonstrated that replacing canola meal completely with hemp meal (20 % DM HM) had no effect on DMI of growing ram lambs. The results obtained in the present study indicate that feeding non-lactating dairy cows up to 10.2 % DM HM does not affect dry matter intake.

Mustafa et al. (1999) also reported that substituting CM with HM did not affect the total tract digestibility coefficient of dry matter, CP and NDF. In the present study, the total tract digestibility coefficients of DM and CP were not affected by feeding HM instead of CM to the cows. HM, according to Semwogerere et al. (2020) and Krizsan & Huhtanen (2013) has low *in situ* NDF degradability due to its high indigestible NDF content (40.1 % DM) compared to that of CM (10 % DM). This was confirmed in the present study since the results shows that the NDF digestibility coefficient of the cows fed the CM diet was higher than that of the HM diet. The HM protein supplement which was used in the present study was obtained from the mechanical extraction of whole hemp seed which might have caused a low digestible NDF composition due to its high insoluble dietary fibre content (29.4 % to 32.4 % DM), which reflected in the low total tract NDF digestibility of the HM diet (Mattila et al., 2018).

Blood serum NEFA and BHBA of the cows were not affected by feeding HM; the average concentrations found in our trial (0.07 mmol/L and 0.42 mmol/L, respectively) were within the normal range (< 0.2 mmol/L) for positive energy balance cows (Adewuyi et al., 2005; Leblanc, 2010; Piccione et al., 2012; Soulat et al., 2020). Moreover, serum NEFA and BHBA concentration of non-pregnant and dry cows are normally low compared to lactating cows since body reserves are not being mobilized to support milk production and low feed intake, especially during the first two weeks of lactation (Leblanc, 2010; Maurer et al., 2017). Concentrations of blood serum urea and glucose in our study were also normal for positive energy balance cows and the obtained values were within the range reported by others (Hussein et al., 2020; Mokhber Dezfouli et al., 2013; Piccione et al., 2012). According to Hussein et al. (2020), the time of feeding and sampling may have an effect on the blood serum glucose concentration since the cows' body tissues absorbs and utilize the glucose, as well as BHBA when the frequency of feed intake is reduced, especially in the night. It has also been reported in previous studies that blood serum urea concentration may be affected by the time of feeding and diet composition since these two factors play a major role in the concentration of this metabolite (Gustafsson & Palmquist, 1993; Hussein et al., 2020). Soon after feeding (1 to 4 hrs) blood metabolites (BHB, glucose, and urea) concentration increase, reaching a peak and, subsequently reduce with time (Gustafsson & Palmquist, 1993; Hwand et al., 2001; Niu et al., 2014). Moreover, when dietary CP is supplied in excess, this leads to the excess availability of ruminally available N for MCP synthesis. Rumen microbes might not be able to utilize all the N especially when there is not enough energy available in the rumen, and ammonia will be absorbed and converted into urea in the liver, and later released into the blood (Aguilar et al., 2012). According to the study conducted by Hussein et al. (2020), dry cows were fed at 0930 h and the highest concentration of serum urea was recorded in the afternoon (1400 h). Similarly,

in our study the highest serum urea concentration was recorded at 1500 h which confirms the role of time of feeding and sampling on serum urea.

Feeding the experimental diets to the cows did not negatively affect the rumen pH. The ruminal pH was always above 6.0 during sample collection for all diets. The mean pH that were recorded for diets were comparable to values reported by Broderick et al. (2015) and Brito & Broderick (2007). The VFA concentrations recorded were normal for healthy dry cows, and were similar to reported values by other researchers (Bettero et al., 2017; Jolicoeur et al., 2014). Unlike lactating dairy cows, the low feed intake of dry cows coupled with the low grain and high forage diets causes a low production of rumen VFA (Andersen et al., 1999).

One of the major concerns of feeding hemp by-products to ruminants in Canada is the possible the accumulation of cannabinoids in body tissues and biological fluids. The Canadian legal limit of THC in commercial hemp products is 10 µg/g (Chicoine et al., 2020; Meng et al., 2018). The detection limit of cannabinoid used in the present study was 0.04.8 ng/mL for CBD and CBDA and 0.024 ng/mL for THC and THCA-A, respectively. The data obtained in this study showed that no accumulation of cannabinoids was detected in rumen fluid, blood plasma, urine, kidney, liver, adipose tissue nor muscle tissues. The low dietary cannabinoid (0.00 µg/g THC and 1.10 µg/g THCA-A, and 3.0 µg/g CBD and 4.4 µg/g CBDA) content of the hemp meal which was used in the trial possibly resulted in a low availability of cannabinoids in the digestive tract of the cows. Both CBD and CBDA were detected in feces of cows that were fed hemp meal while THC and THCA-A were not. The average fecal CBD and CBDA (0.68 µg/g and 0.67 µg/g, respectively) contents show that approximately 10.4 % of CBD intake (44.4 mg/d) and 7.1 % of CBDA intake (65.1 mg/d) were excreted in the feces of cows fed HM. The digestibility, bioavailability in the rumen, and pharmacokinetics of these cannabinoids in the cows, however, is unclear. However, it

appears that cannabinoids may be metabolized in the digestive tract. Semwogerere et al. (2020) and Huestis (2007) reported, that CBD and CBDA are volatile compounds and as such, they are easily released from the feed matrix and excreted unchanged. Brunet et al. (2006) reported that intravenous injection (IV) of 200 µg/kg of THC in pigs, resulted in the elimination of cannabinoids (based on WinNonlin software's linear trapezoidal method of calculating kinetics parameters) from the liver, kidney, muscle tissue and blood within 24 hours. However, the initial concentration of THC in fat tissues was reduced to 34.7 % after 24 hours of injection. In the present study, no THC or THCA-A were detected in biological fluids and body tissues, as well as feces, possibly due to the low dietary THC and THCA-A contents (1.1 µg/g).

4.6 Conclusion

The replacement of canola meal with hemp meal as protein supplements in diets formulated from barley silage, wheat straw, grass hay and a CP content averaging between 10.2 % and 13.5 % did not alter rumination, rumen pH, rumen VFA, rumen ammonia nitrogen, total tract digestibility of DM and CP, blood urea, blood glucose, blood NEFA, and blood BHBA. We detected a higher total tract NDF digestibility in the CM diet compared to the HM diet. This may be due to the low digestible NDF, and high insoluble dietary fiber (29 % DM to 32 % DM) content of the hemp meal protein supplement used in the study. Cannabinoid contents in rumen fluid, blood plasma, urine, kidney, liver, adipose, and muscle tissues of the cows were below detection limits of 0.048 ng/mL and 0.024 ng/mL for CBD and THC, respectively, which are below the permitted limit in Canada. This shows that feeding hemp meal to non-lactating dairy cows does not lead to accumulation of cannabinoids in body tissues and biological fluids. Our data indicates that hemp meal is a good and safe alternative for canola meal as a protein supplement for non-lactating dairy cows.

5.0 GENERAL DISCUSSION, CONCLUSION AND FUTURE DIRECTIONS

5.1 General discussion

This study sought to assess the effect of hemp meal as a protein supplement for non-lactating dairy cows. CB and SBM have traditionally been used as a protein supplement source. However, challenges such as the high cost of these supplements in the Canadian prairies and other parts of North America has led to the search for novel alternative protein supplements to CM and SBM (Chibisa et al., 2012).The use of alternative protein supplements such as DDGS and brewers' grains as replacements or partial replacements for CM and SBM has been demonstrated in Canada (Budi et al., 2018; Chibisa et al., 2012). Mustafa et al. (1999) demonstrated the potential to utilize hemp meal as an alternative protein supplement to CM and SBM. Due to insufficient information on the possible accumulation cannabinoids in body tissues and biological fluids and safety of hemp by-products, the use of hemp meal as ruminant feeds has not been approved by the CFIA. A safety concern of the use of hemp meal as ruminant feed in Canada is the possible accumulation of cannabinoids such as THC and CBD in body reserves and biological fluids (Ware & Tawfik, 2005).

Studies have demonstrated the effect of the use of hemp meal as protein supplements to small ruminants, such as sheep, as well as steers and dairy cows (Hessle et al., 2008; Karlsson et al., 2010; Karlsson & Martinsson, 2011; Mustafa et al., 1999). The effect of feeding hemp meal instead of protein supplements such as canola meal on DMI has been shown to be variable, although in most cases there was no significant effect. Hessle et al. (2008) reported that feeding hemp meal instead of soybean meal to dairy calves increased DMI, but no differences in were observed in steers. The authors attributed the increase in DMI in the dairy calves to improved rumen function, specifically in terms of selective retention of feed particles, and adequate rumination and/or rumen

motility. Karlsson et al. (2010) and Karlsson & Martinsson (2011) fed hempseed cake instead of rapeseed cake and compound pellets to dairy cows and lambs, respectively, and recorded an increase in DMI. Mustafa et al. (1999) however, reported no difference in DMI when lambs were fed hemp meal instead of canola meal. In our study we also found no differences in DMI between hemp meal-fed and canola-meal fed cows. These inconsistencies in DMI may be due to the differences in animal species (lambs and cows), ingredient composition, and inclusion rates (20 % kg DM and 32 % kg DM for lambs and dairy cows, respectively) that were used in these studies (Semwogerere et al., 2020).

Various studies have demonstrated that the normal rumen pH for healthy cows is ≥ 5.6 and any depression in this pH for extended periods of time in a day may lead to metabolic diseases such as SARA (Li et al., 2012; Plaizier et al., 2018). The pH of the cows used in the current study was always above 6.0 during sampling. Hence, no SARA was induced.

The blood serum and plasma metabolite concentrations of dairy cows is an indicator of their nutritional status. The normal concentration of blood serum NEFA and BHBA for positive energy balance dairy cows have been reported to be within the range of < 1.0 mmol/L and < 1.4 mmol/L for NEFA and BHBA, respectively (Adewuyi et al., 2005; Fiore et al., 2014; García et al., 2011; Leblanc, 2010; Piccione et al., 2012). The average concentrations of NEFA and BHBA found in our study were 0.07 mmol/L and 0.42 mmol/L, respectively. This is an indication that the energy status of the cows was not affected by feeding hemp meal compared to canola meal.

Blood serum glucose and urea concentrations in dairy cows are affected by factors such as diet composition, time of feeding, and time sample collection. Recent studies have demonstrated the effect of these factors on serum urea and glucose concentrations. Hussein et al. (2020) and Gustafsson & Palmquist (1993) reported that the time of feeding and sampling have a significant

effect on serum glucose and urea concentrations since the concentration of the metabolites reduces when feeding frequency is reduced especially during the night. The authors also confirmed that diet composition influences the concentration of these blood metabolites. Diets that contain high inclusion rates of grain will enhance the production of propionate, which is essential for gluconeogenesis (Miyaji et al., 2014). Furthermore, excess dietary rumen degradable protein will result in high serum urea content, which may be detrimental to the cows and contaminate the environment when excreted (Rodney et al., 2018). In the present study, we found no effect of feeding hemp meal instead of canola meal on blood serum glucose and urea concentrations.

Rumen VFA concentrations are also important indicators of the nutritional status of cows. Diet composition is one of the main factors that determines these concentrations. The transition from a dry cow diet to a lactating cow diet that contains a higher inclusion rate of grains, enhances the production of butyrate and propionate, which are critical for the proliferation of rumen epithelium and gluconeogenesis, respectively (Andersen et al., 1999; Rodney et al., 2018). Reports from previous studies shows that the concentration of rumen acetate, propionate, butyrate, and total VFA concentrations of dry cows are 69 – 71 mmol/L, 18 – 21 mmol/L, 11 – 13 mmol/L and 97 – 104 mmol/L, respectively (Jolicoeur et al., 2014; Peyrat et al., 2016). Similar concentrations of these VFA were observed in the present study.

The possible accumulation of cannabinoids in body tissues and biological fluids of livestock as well as insufficient research on the nutritional quality and safety in ruminants are some of the concerns regarding the use of hemp seeds and its by-products like hemp meal as ruminant feed in Canada. The major cannabinoids of concern are THC and CBD due to their psychoactive nature (Ware & Tawfik, 2005). As part of Health Canada's regulations, industrial hemp varieties that are permitted to be cultivated in Canada must have a THC concentration of 0.3 % DM or less in any

part of the plant (Health Canada, 2018). Although this maximum allowable concentration is low compared to that of the USA (1 % or less), proving that these cannabinoids do not transfer into body tissues and biological fluids such as milk of dairy cows is indispensable for obtaining CFIA approval of hemp plant by-products such as hemp meal as ruminant feed in Canada.

According to Meng et al. (2018), the current legal concentration of THC in commercial hemp products as well hemp plant materials is < 10.0 µg/g. However, there are no defined detection limits for CBD in industrial hemp varieties due to the lack of required tests and general information on the safety of this cannabinoid (Meng et al., 2018). Previous studies on the possible accumulation of cannabinoids in various body tissues of pigs and ewes injected (IV) with approximately 1 mg/kg and 200 µg/g of THC, respectively showed that accumulations may occur initially but with time they are excreted without any harm to the animals (Brunet et al., 2006; Huestis, 2007; Semwogerere et al., 2020). In the present study, no accumulations of cannabinoids were detected in urine, kidney, liver, blood plasma, rumen fluid, adipose tissue nor muscle tissue of cows, which might be due to the low levels of cannabinoids in the hemp meal used in the trial. However, since CBD and CBDA are easily released from the feed matrix and excreted unchanged, we detected these cannabinoids in the feces of the cows (Huestis, 2007; Semwogerere et al., 2020).

5.2 General Conclusion

The use of novel alternative protein supplements such as hemp meal in cattle nutrition is beneficial for the cattle industry since there are challenges that dairy farmers face such as high cost of existing and commonly used protein supplements like canola meal and soybean meal. Hemp meal has been proposed as an alternative protein source. However, one major concern of feeding hemp meal as ruminant feed in Canada is the possible accumulation of cannabinoids in body tissues and biological fluids, which may also be a potential health hazard to human consumers of

animal by-products like milk and meat. To ensure the safe use of hemp meal as protein supplement for dairy cows, and hence attain CFIA approval, it is important that its nutritional quality and safety be demonstrated.

In our study, the comparison between hemp meal and canola meal as protein supplements in a PMR diet composed of barley silage, wheat straw, and grass hay, fed to non-lactating dairy cows showed that replacing canola meal with hemp meal at a rate of 10.2 % DM did not affect palatability of the cows, as DMI was not affected. Rumen fermentation and blood serum glucose, urea, BHBA, and NEFA concentrations of the cows were not depressed by feeding hemp meal. The rumen concentration of VFA and ammonia nitrogen, and blood serum concentration of urea, glucose, BHBA, and NEFA obtained were within the range for healthy non-lactating dairy cows. There were no differences in the total tract DM and CP digestibility between cows fed hemp meal and those that fed canola meal. However, the total tract NDF digestibility of the canola meal diet was higher than that of the hemp meal diet. This difference might be due to the low NDF degradability and high indigestible NDF contents of the hemp meal that was used in the trial since it was produced through the mechanical extraction of whole hemp seeds. No cannabinoids were detected in rumen fluid, blood plasma, kidney, liver, urine, muscle nor adipose tissues of the cows but some cannabinoids (CBD and CBDA) were detected in their feces. Data obtained in this study shows that hemp meal is a good and safe alternative protein supplement to canola meal for non-lactating dairy cows. An important step to take from here will be to conduct further studies of the use of hemp meal as protein supplements for lactating dairy cows to evaluate its effects on milk production and milk composition. Assessing the variability of cannabinoid concentration in hemp meal produced by different methods and/or facilities would also be important to characterize the risk of varying cannabinoid contents.

5.3 Future directions

To ensure CFIA approval of the use of hemp meal as protein supplement in the dairy cattle production industry, follow-up studies may include the following:

1. Feeding hemp meal instead of canola meal to lactating cows to determine its effects on the milk yield, composition, and milk production efficiency.
2. Determine the cost effectiveness of the use of hemp meal instead of canola meal as a protein supplement.
3. Conduct a toxicological assessment on hemp meal to determine its safety as a protein supplement for dairy cows.
4. Assess the variability in hemp meal nutritional characteristics and cannabinoids content.

6.0 References

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