Quality and safety of hemp meal as a protein supplement for nonlactating dairy cows

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ABSTRACT

Hemp seed meal may be a suitable protein supplement for dairy cows, but its quality and safety as a dairy cow feed has not yet been fully investigated. As a result, dry matter intake (DMI), rumen fermentation, blood metabolites, total-tract digestibility, and concentrations of cannabinoids in blood plasma, urine, muscle, and adipose tissues were compared among nonlactating Holstein dairy cows receiving a basal partial mixed ration that was supplemented with either 10.2% dry matter (DM) hemp meal (HM treatment), 13.5% DM canola meal (CM treatment), or 6.25% DM hemp meal and 6.16% DM canola meal (HC treatment). Diets were formulated to be isoenergetic and isonitrogenous. Six nonlactating, nonpregnant Holstein cows were used in a repeated 3 × 3 Latin square design trial with three 3-wk experimental periods. The first 2 weeks of each served as adaptation. Sample and data collection occurred during the third week of each period. Neither the partial mixed ration nor canola meal contained cannabidiol (CBD), cannabidiolic acid (CBDA), d9-tetrahydrocannabinol (THC), or tetrahydrocannabinolic acid A (THCA). However, the hemp meal contained 3.0, 4.4, 0, and 0.1 μg/g DM of CBD, CBDA, THC, and THCA, respectively. Treatment did not affect DMI, pH, concentrations of volatile fatty acids or ammonia in the rumen, total-tract digestibilities of DM and crude protein, or blood plasma concentrations of glucose, urea, β-hydroxybutyrate, and nonesterified fatty acids. Hence, based on these metabolites, treatment did not affect the nutritional status of the cows. However, the total-tract neutral detergent fiber digestibility of the CM treatment (43%) was higher than that of the HM treatment (38%). No cannabinoids were detected in blood plasma, rumen fluid, and urine. Cannabinoids were also not detected in kidney, liver, urine, muscle, or adipose tissues at the end of the experiment when cows had undergone all treatments. Feces from all treatments did not contain detectable concentrations of THC or THCA, but feces of cows on the HC treatment contained 0.42 and 0.40 μg/g DM of CBD and CBDA, respectively. Feces of cows on the HM treatment contained 0.68 and 0.67 μg/g DM of CBD and CBDA, respectively. This indicated that most ingested CBD and CBDA were not absorbed but instead were excreted in the feces. Our data show dietary inclusion rates of up to 10.2% of DM. We find that hemp meal is a high-quality and safe protein supplement for nonlactating dairy cows. Key words: hemp meal, cannabinoids, nonlactating dairy cows

INTRODUCTION

Industrial hemp (Cannabis sativa L.) production in Canada has increased from 15,056 ha in 2011 to 54,941 ha in 2020 (Health Canada, 2022). As a result, the production of co-products from the processing and oil extraction of hemp seeds, including hemp meal, has also increased. Several studies have examined the potential of hemp meal as a feed for ruminants (Mustafa et al., 1999; Karlsson et al., 2010; Bailoni et al., 2021). Mustafa et al. (1999) reported that hemp meal has higher rumen-undegradable CP and intestinally available CP contents than canola meal. However, they observed that the total available CP did not differ among these meals. Mustafa et al. (1999) also concluded that replacing canola meal with hemp meal did not affect the DM intake and total-tract nutrient digestibilities of sheep. Mierlită et al. (2018) replaced a mixed concentrate with 160 g of hemp seed or 480 g hemp meal in the diets of milking sheep, and observed that, despite the diets being isoenergetic and isonitrogenous, the dietary inclusion of these hemp feeds increased milk yield and milk fat yield by 6.3% and 15.3%, respectively. Karlsson et al. (2010) fed diets containing up to 31.8% of DM of hemp meal to lactating dairy cows, and found that the highest milk production occurred at a 14.3% DM inclusion rate. Neither Mustafa et al. (1999) nor Karlsson et al. (2010) nor Mierlită et al. (2018) determined the effects of feeding hemp meal on rumen fermentation.
and blood metabolites. Hence, the effects of this meal on rumen function and nutritional status have not been fully characterized to date.

Despite the potential production benefits of using hemp meal, it has not been approved as a feed for ruminant livestock in Canada. One reason for this is the concern about the possible accumulation of psychoactive cannabinoids in animal products, such as body tissues and milk (Ware and Tawfik, 2005). As a result, much of the produced hemp meal is discarded in landfills, which poses an environmental issue and a loss of a potentially high-quality livestock feed.

Cannabinoids may be broken down by microorganisms in the rumen (McSweeney et al., 2002; Kleinhenz et al., 2020a,b), which enhances the safety of feeding hemp co-products to ruminants. Although the studies by Mustafa et al. (1999), Karlsson et al. (2010), and Mierlită et al. (2018) examined the potential of hemp meal as a feed for ruminants, they did not analyze cannabinoids in meat or what is necessary to confirm that feeding hemp meal to ruminants is safe for the animal and the consumer. The objectives of this study were, therefore, to compare DMI, rumen fermentation, concentrations of blood metabolites, total-tract digestibilities of nutrients, and concentrations of cannabinoids in body tissues and biological fluids between nonlactating dairy cows supplemented with hemp meal and those supplemented with canola meal. Canola meal was selected because it is commonly used as a dairy cow protein supplement.

**MATERIALS AND METHODS**

**Animals, Diets, and Experimental Design**

The study was preapproved by the Fort Garry Campus Animal Care Committee of the University of Manitoba (protocol F20-015 AC11593). Cows were cared for in accordance with the Canadian Council on Animal Care Guidelines (CCAC, 1993). Hemp meal was obtained from Hemp Oil Canada Inc. (Ste Agathe, Manitoba, Canada). The hemp meal was produced from whole hemp seeds after mechanical extraction by cold pressing of hemp oil followed by sifting out hulls to increase the CP content.

Six nonlactating, nonpregnant Holstein cows with an average body weight of 704 kg and BCS of 3.25 (1–5 scale) were housed in individual tiestalls in the large animal metabolism unit of the Glenlea Research Station, University of Manitoba. Of these cows, 1, 3, and 2 cows had completed 1, 2, or 3 lactations, respectively. The stalls were lined with rubber mats, and chopped straw was used as bedding. No heifers were included in the study. The experimental design was a repeated 3 × 3 Latin square design with three 3-wk experimental periods. Six nonlactating cows were included in our study, as Li et al. (2012) were able to obtain a significant difference in DMI between 2 treatments with DMI of 15.4 and 16.7 kg/d, with a power of 0.80, a significance of 0.05, and a standard error of 0.02 using 6 dairy cows in a 3 × 3 Latin square arrangement of treatments. The first 2 weeks of the experimental periods served as adaptation, and sampling was conducted during the third week. Cows were blocked according to parity and milk production during the previous lactation, and were randomly assigned to 1 of 3 dietary treatments: (1) a basal partial mixed ration (PMR) with the addition of 10.2% DM hemp meal (HM), (2) a basal PMR diet with the addition of 13.5% DM canola meal (CM), or (3) a basal PMR diet with the addition of 6.16% DM canola meal and 6.25% hemp meal (HC). Diets were formulated to be isonitrogenous and isoenergetic. Cows were fed the PMR ad libitum once a day at 0900 h, allowing for 5 to 10% of refusal, with ad libitum access to fresh water. The hemp meal and canola meal were provided as a top-dress. The chemical composition of feeds and diets are given in Tables 1 and 2.

Rumination behavior of cows was monitored using the Lely Rumination Monitor (Qwes-HR, Lely, Maasvluis, The Netherlands). During the third week of each experimental period, fecal, urine, and blood samples were collected twice daily at 0900 and 1500 h, respectively. Body weight and BCS of cows were determined at the beginning and end of each experimental period.

**DMI and Feed Analyses**

Samples of PMR were collected once daily during each experimental period, pooled by treatment, and

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**Table 1. Ingredient composition (% DM) of experimental diets fed to nonlactating dairy cows**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CM</th>
<th>HC</th>
<th>HM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley silage</td>
<td>46.1</td>
<td>42.9</td>
<td>49.4</td>
</tr>
<tr>
<td>Dry cow premix</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Grass hay</td>
<td>12.8</td>
<td>13.5</td>
<td>9.40</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>26.2</td>
<td>29.7</td>
<td>29.5</td>
</tr>
<tr>
<td>Hemp meal</td>
<td>13.5</td>
<td>6.25</td>
<td>10.2</td>
</tr>
<tr>
<td>Canola meal</td>
<td>—</td>
<td>13.5</td>
<td>6.16</td>
</tr>
</tbody>
</table>

1 Treatment diets were as follows: CM = diet containing 13.5% DM canola meal; HC = diet containing 6.25% DM hemp meal and 6.16% DM canola meal; HM = diet containing 10.2% DM hemp meal.

2 Propulsion 1:1 Dry Cow Premix (Settlers Supplies; Sheffield, ON, Canada) contains: calcium (12%), phosphorous (12%), salt (0.002%), sodium (0.12%), potassium (0.06%), magnesium (12%) sulfur (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).

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stored at −20°C. Individual orts samples were collected every morning immediately before feed delivery and stored in a −20°C freezer during the last week of each experimental period. Pooled diet and orts samples were dried in a forced-air oven at 60°C for 48 h. The dried samples were ground using a Wiley mill through a 1-mm screen (Thomas-Wiley, Philadelphia, PA) but not homogenized, and stored at room temperature until further analyses.

The DMI was determined using the amounts and DM contents of PMR and protein supplements delivered, and the amounts and DM contents of orts remaining. Feed and orts samples were analyzed for analytical DM by heating to 105°C for 3 h, CP (AOAC 990.03; AOAC International, 2019). Neutral detergent fiber was analyzed according to Van Soest et al. (1991) using α-amylase (Sigma No. A3306; Sigma Chemical Co., St. Louis, MO) and sodium sulfite, and corrected for ash concentration, but not for protein, using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Acid detergent fiber was analyzed using AOAC method 973.18 (AOAC International, 1990) and the Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Starch was measured using a UV method (method 996.11; AOAC International, 2005). Acid insoluble ash (AIA) was analyzed using AOAC method 920.08 (AOAC International, 1990). Ether extract and ash analyses were conducted using AOAC methods AOAC 920.39 and 923.03, respectively (AOAC International, 2019). Inductively coupled plasma emission spectroscopy (AOAC method 968.08; AOAC International, 1990) using a plasma spectrometer (Thermo Jarrell Ash Corp., Grand Junction, CO), and acid digestion was used to analyze calcium, phosphorous, potassium, magnesium, and sodium.

The DMI, CP, NDF, and AIA contents of experimental diets and pooled feces of individual cows during the last week of each period were used to estimate apparent total-tract digestibility coefficients (ADC) of nutrients.

The AIA content of diets was used as internal digestion marker (Van Keulen and Young, 1977; McGeough et al., 2010), and ADC of DM, CP, and NDF were estimated using the following equations:

\[
DMD = 100 \times \left[ 1 - \frac{\text{AIA % DM in diet}}{\text{AIA % DM in feces}} \right]; \\
ADC CP = 100 \times \left[ 1 - \frac{\text{CP % DM in feces}}{\text{CP % DM in diet}} \times \frac{\text{AIA % DM in diet}}{\text{AIA % DM in feces}} \right]; \\
ADC NDF = 100 \times \left[ 1 - \frac{\text{NDF % DM in feces}}{\text{NDF % DM in diet}} \times \frac{\text{AIA % DM in diet}}{\text{AIA % DM in feces}} \right].
\]

The proportions of ingested cannabinoids that were excreted in the feces were also estimated using AIA as a marker.

**Sample Collection and Analyses**

**Rumen Fluid.** Approximately 10 mL of rumen fluid (RF) was collected once daily at 1500 h on d 3 and 7.
of the sampling periods via rumenocentesis (Duffield et al., 2004). Cows were given between 0.05 and 0.15 mg/kg of xylazine (Rompun, Bayer, Leverkusen, Germany) intravenously depending on the cow’s response before the RF collection. Immediately following collection, pH was determined with an Accumet Basic 15 pH meter (Fisher Scientific, Fairlawn, NJ), equipped with a Sensorox 450C Flat Surface Combination pH/Reference Electrode (Sensorox, Stanton, CA). Subsequently, 2 subsamples (1 mL each) of RF were pipetted into two 2-mL tubes and immediately flash frozen in liquid nitrogen before storing at a temperature of −80°C until subsequent cannabinoid analysis. In addition, 2 mL of RF was transferred into tubes prepared with either 0.4 mL of 25% metaphosphoric acid or 0.4 mL of sulfuric acid (H2SO4) and stored at −20°C for rumen VFA and ammonia nitrogen (NH3-N) analyses, respectively.

For rumen VFA analysis, frozen samples were thawed and centrifuged at 1,900 × g for 20 min at 4°C, and 1 mL of the supernatant was gently decanted using a syringe with filter to clean GC vials. The vials were then placed into an autosampler (8410 Varian, Walnut Creek, CA) and analyzed for VFA using GC on a model 3900 gas chromatograph (Varian, Walnut Creek, CA) as described by Bhandari et al. (2007). The injector and detector of the GC were set at temperatures 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 min, followed by a 2-min thermal stabilization period. The ammonia nitrogen concentration in RF was determined using the indophenol blue method, as described by Novamsky et al. (1974).

**Blood.** Blood samples were taken from the tail vein and collected into 10-mL vacutainer tubes and 10-mL heparinized evacuated tubes to obtain serum and plasma, respectively. Blood samples collected for serum extraction were maintained at room temperature for 30 min to ensure clotting before being centrifugated at 1,900 × g at 4°C for 10 min. Serum samples were then transferred into plastic tubes and stored immediately in a −20°C freezer until subsequent analyses. Serum samples were analyzed for nonesterified fatty acids (NEFA) and BHB using the RX Monza analyzer and Randox kits (Randox Laboratories Ltd., Crumlin, UK), respectively. Serum glucose and urea were analyzed using the CobasCc 502 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) using COBAS GLUC2 (ref. 0767131 322, Roche Diagnostics GmbH, Mannheim, Germany) and COBAS UREAL (ref 04460715 190 Roche Diagnostics GmbH, Mannheim, Germany), respectively.

Blood samples collected for plasma analyses were centrifuged at 4°C for 15 min at 1,900 × g immediately after collection. Plasma samples were immediately flash frozen in liquid nitrogen and stored at −80°C until further analyses for cannabinoids and plasma urea concentrations.

**Urine.** Approximately 80 mL of midstream urine samples were collected by stimulating the perirenal area to initiate urination. After collection, 30 mL of each sample was transferred into tubes prefilled with approximately 2 mL of 3 N hydrochloric acid (HCl), to ensure that the pH of the urine samples was below 2.0 to minimize N volatilization. Samples were later stored in a −20°C freezer until final analysis for nitrogen, using a Leco NS 2000 analyzer (Leco Corporation, St. Joseph, MI). Approximately 4 mL of urine from the initial sample was transferred into 5-mL screw-top vials, immediately flash frozen in liquid nitrogen, and stored at −80°C until subsequently analyzed for cannabinoids.

**Feces.** Fecal samples were collected twice a day at 0900 h and 1500 h over a 5-d period during the last week of each experimental period. Fecal sample collection was conducted as described by 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 and period, and oven-dried at 60°C for 7 d. Dried fecal samples were later ground using a Cyclotec 1093 Sample Mill (Foss Tecator, Hillerød, Denmark) through a 1-mm screen, bagged in Whirl-Pak bags (Whirl-Pak; Fort Atkinson, WI), and stored at room temperature until further analysis. Feces were analyzed for CP, NDF, ADF, and AIA as described in the Feed Analysis section, and for cannabinoids.

**Tissues.** Collection of animal tissues required euthanization of the cows. Hence, these tissues could only be collected at the last day of the trial, when all cows had undergone all treatments. On that day, all the cows were euthanized with a pentobarbital solution, 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). Once obtained, the samples were rinsed with deionized water, placed immediately in Whirl-Pak 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 20-g sample was extracted, ensuring that it included both the medulla and cortex. The sample was stored using the same procedure as described for liver samples.

Adipose tissue samples of 20 g were collected from the perirenal, mesenteric, and omental tissues, as well as the subcutaneous fat pad around the pin bone, and pooled by cow by mixing equal amounts of these samples. Also, muscle tissues were extracted from the semitendinosus and semimembranosus muscle of each
cow and pooled by cow by mixing equal amounts of these samples. Approximately 20 g of tissue were extracted using scalpels cleaned with alcohol between collections to prevent any possible cross-contamination. The samples were washed with deionized water, placed in Whirl-Pak bags, flash frozen in liquid nitrogen, and stored at −80°C before analyses for cannabinoids.

**Cannabinoid Analysis**

**Sample Preparation.** Sample preparation and quantification of cannabinoids were conducted by Innotech Alberta in Vegreville, AB, Canada, which holds a license for analytical testing of cannabinoids under the Canadian Cannabis Act (Government of Canada, 2018).

**Feed Samples.** A total of 1 g of dried samples that had been ground using a Wiley mill through a 1-mm screen (Thomas-Wiley, Philadelphia, PA) but not homogenized, and stored at room temperature, were mixed with 5 mL of HPLC-grade methanol (Thermo Fisher, Waltham, MA). Samples were subsequently loaded in a Spex Geno/Grinder (Spex SamplePrep, ThermoFisher, Metuchen, NJ) and shaken for 5 min. at rate of 1,500 stroke/min. After this, the samples were centrifuged at room temperature for 12 min at 250 × g and transferred to Eppendorf vials (Eppendorf, Hamburg, Germany). After centrifugation for another 10 min at 1,600 × g, aliquots were transferred to screw-cap vials with flat bottom inserts.

**Feces Samples.** This procedure was the same as for feed samples, with the exception that after the addition of HPLC-grade methanol (Thermo Fisher, Waltham, MA), samples were sonicated at room temperature for 20 min.

**Blood Plasma Samples.** A total of 100 μL of blood plasma was mixed with 200 μL of cold HPLC-grade methanol stored at −20°C (Thermo Fisher, Waltham, MA). The tubes were vortexed at 1,600 × g for 10 min and transferred to autosampler vials with inserts for liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis.

**Urine Samples.** A total of 200 μL of urine was added to 1.5-mL centrifuge tubes (VWR, Radnor, PA), mixed with 400 μL of water, and vortexed. After this, 160 μL of 10% acetic acid was added, and the mixture was vortexed. This was followed by adding 1.6 mL of a 9:1 hexane:ethylacetate solution and sonication for 30 min. Subsequently, the mixture was centrifuged at 280 × g for 15 min to achieve separation. The organic upper layer was transferred, and the fluid was evaporated. The samples were reconstituted in 200 μL of a 50:50 acetonitrile:water solution. After centrifugation for 10 min at 1,600 × g, the reconstituted samples were transferred to autosampler vials with inserts for LC-MS/MS analysis.

**Rumen Fluid Samples.** The processing of RF was similar to that used for blood plasma samples, with the exception that 100 μL of cold HPLC-grade methanol (Thermo Fisher, Waltham, MA) was added.

**Tissue Samples.** A total of 1 g of tissue was homogenized with 5 mL of HPLC-grade methanol (Thermo Fisher, Waltham, MA) and vortexed. The mixture was sonicated at room temperature for 20 min, after which it was centrifuged for 10 min at 250 × g. A total of 1 mL of extracted solvent was transferred to 1.5-mL centrifuge tubes (VWR, Radnor, PA), and centrifuged for 15 min at 1,600 × g. Subsequently, the mixture was transferred to 2-mL GC screw vials (VWR, Radnor, PA).

**Quantification of Cannabinoids.** The cannabinoid analysis was performed using LC/MS and analyzed using a Waters Quattro Micro API (Waters Corporation, Milford, MA), following the procedure described by Meng et al. (2018). The chromatographic separation was performed with a Cosmosil 2.5C18-MS-II 30-mm × 100-mm column (Nacalai Tesque, Kyoto, Japan) at a column temperature of 25°C. The flow rate was set at 0.5 mL/min, and the mobile phase consisted of water containing 0.1% acetic acid (A), 0.1% acetic acid in LC/MS grade methanol (B), and isotopanil (C) as follows: 0 min, 25% A, 75% B, 0% C; 10 min, 15% A, 85% B, 0% C; 14 min, 5% A, 95% B, 0% C; 16 min, 0% A, 100% B, 0% C; 21 min, 0% A, 0% B, 100% C; 23 min, 0% A, 100% B, 0% C; 24 min, 25% A, 75% B, 0% C. After every 20 samples, 1 solvent blank sample, 1 duplicate sample, and 1 sample that was spiked with 0.01 μg/mL of cannabinoid standards were run.

Standards of cannabidiol (CBD, C-045), cannabidiolic acid (CBDA, C-144), d9-tetrahydrocannabinol (THC, T-005), and tetrahydrocannabinolic acid (THCA, T-093) were obtained from Sigma-Aldrich (St. Louis, MO). Cannabinoid standards were stored at −20°C.

A calibration stock solution was then prepared by combining all cannabinoid compounds with methanol to provide a stock solution of 50 μg/mL. This stock solution was serially diluted to give standards of 1, 2, 5, 25, 100, 200, and 1,000 ng/mL. Calibration curves were linear in these ranges for all cannabinoids, with coefficient of correlation greater than 0.99. The limit of detection was 2 ng/g for feed and feces samples, 1 ng/mL for blood plasma, urine, and RF samples, and 10 ng/g for tissue samples. Average intra-day and inter-day accuracies were 80 to 120% and 70 to 130%, respectively. No internal standards were used. For spiking and recovery analysis, cannabinoid-free matrices were spiked with 0.05 μg/g of each cannabinoid (Sigma-Aldrich, St.
Louis, MO) for feed and feces samples, whereas RF, blood plasma, tissue, and urine samples were spiked with 0.01 μg/g of these cannabinoids (Sigma-Aldrich, St. Louis, MO).

**Statistical Analysis**

Data were analyzed using a repeated Latin square model with the MIXED procedure of SAS (Ver. 9.4, SAS Institute Inc., Cary, NC). The effect of treatment (CM, HM, and HC) was considered fixed. The effects of square, cow within square, and period were considered random. For dependent variables that had repeated measurements, the repeated measurement option within the SAS MIXED procedure was used. The UNIVARIATE procedure and Levene’s test were used to test for normal distribution of the error and homogeneity of variances, respectively. No transformation of the data or adjustment of the degrees of freedom in case of unequal variances was needed to meet the assumption of the statistical analysis. The PDIF statement in SAS was used to determine differences between treatments; significant effects of treatments as well as interactions were discussed at $P < 0.05$, and tendencies were reported at $0.05 < P < 0.10$.

**RESULTS**

We detected no differences in DMI, rumination time, or blood concentrations of urea, glucose, BHB, and NEFA among treatments (Table 3). Similarly, the pH, VFA, and ammonia nitrogen concentrations of RF and the N content of urine did not differ between treatments (Table 4), nor did total-tract digestibility coefficients of DM, CP, and NDF (Table 5). Treatment did not affect the total-tract digestibilities of DM and CP. However, the total-tract NDF digestibility of the CM diet was higher than that of the HC and CM diets (43.0 vs. 39.0 and 38.0%, $P = 0.03$).

The results from the spiking and recovery analysis are given in Table 6. Averaged across matrices, the recoveries of CBD, CBDA, THC, and THCA were 103.9, 95.4, 62.6, and 67.2%, respectively.

The concentrations of CBD, CBDA, THC, and THCA in RF, blood plasma, and urine of cows were all below the detection limit of 1 ng/mL. The concentrations of these cannabinoids in kidney, adipose, liver, and muscle tissues at the end of the experiment, when cows had undergone all treatments, were also below the detection limit of 10 ng/g. The feces of cows offered the HC treatment contained 0.42 and 0.40 μg/g DM.

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**Table 3.** Dry matter intake, rumination time, and blood metabolite concentrations of nonlactating dairy cows fed experimental diets containing canola meal (CM), a mixture of hemp meal and canola meal (HC), or hemp meal (HM)

<table>
<thead>
<tr>
<th>Item</th>
<th>CM</th>
<th>HC</th>
<th>HM</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>14.9</td>
<td>14.5</td>
<td>14.8</td>
<td>0.97</td>
<td>0.56</td>
</tr>
<tr>
<td>Rumination time, min/d</td>
<td>487</td>
<td>474</td>
<td>477</td>
<td>48.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>4.71</td>
<td>4.90</td>
<td>4.86</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.38</td>
<td>4.47</td>
<td>4.43</td>
<td>0.16</td>
<td>0.74</td>
</tr>
<tr>
<td>BHB, mmol/L</td>
<td>0.39</td>
<td>0.42</td>
<td>0.38</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>NEFA,1 mmol/L</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.02</td>
<td>0.97</td>
</tr>
</tbody>
</table>

1NEFA = nonesterified fatty acids.

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**Table 4.** Ruminal pH, ammonia nitrogen, and VFA and urine nitrogen concentrations of nonlactating dairy cows fed experimental diets containing canola meal (CM), a mixture of hemp meal and canola meal (HC), or hemp meal (HM)

<table>
<thead>
<tr>
<th>Item</th>
<th>CM</th>
<th>HC</th>
<th>HM</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.42</td>
<td>6.34</td>
<td>6.50</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Acetate, mmol/L</td>
<td>71.0</td>
<td>73.3</td>
<td>69.5</td>
<td>1.93</td>
<td>0.41</td>
</tr>
<tr>
<td>Propionate, mmol/L</td>
<td>21.4</td>
<td>22.9</td>
<td>21.3</td>
<td>1.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Butyrate, mmol/L</td>
<td>13.6</td>
<td>13.7</td>
<td>12.7</td>
<td>0.54</td>
<td>0.27</td>
</tr>
<tr>
<td>Other VFA,1 mmol/L</td>
<td>4.95</td>
<td>5.36</td>
<td>4.93</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Total VFA, mmol/L</td>
<td>111</td>
<td>115</td>
<td>108</td>
<td>3.14</td>
<td>0.32</td>
</tr>
<tr>
<td>Ac/Pr2</td>
<td>3.33</td>
<td>3.22</td>
<td>3.27</td>
<td>0.13</td>
<td>0.55</td>
</tr>
<tr>
<td>NH₃-N, mg/dL</td>
<td>8.48</td>
<td>10.4</td>
<td>9.24</td>
<td>0.99</td>
<td>0.15</td>
</tr>
<tr>
<td>Urinary N</td>
<td>1.36</td>
<td>1.31</td>
<td>1.29</td>
<td>0.13</td>
<td>0.83</td>
</tr>
</tbody>
</table>

1Other VFA = isobutyric acid + valeric acid + isovaleric acid.

2Ac/Pr = acetate-to-propionate ratio.
of CBD and CBDA, respectively, whereas the feces of cows offered the HM treatment contained 0.68 and 0.67 μg/g DM of CBD and CBDA, respectively (Table 7). The CBD and CBDA contents of the feces of cows offered the HM treatment were 102.2 and 68.7% of the ingested amounts of these cannabinoids, respectively. No cannabinoids were detected in the feces of cows on the CM treatment. The THC and THCA contents of feces were below the detection limit of 2 ng/g for all treatments.

DISCUSSION

Feed Quality and Diet Composition

The chemical composition of oil seed meal depends on the chemical composition of the seed and the method of fat extraction (Bell, 1993; Bailoni et al., 2021). The former is affected by the variety or cultivar, preliminary treatments, soil and climatic conditions, and agronomic practices (Bell, 1993; Bailoni et al., 2021). Hence, variations in the chemical composition of hemp meal and canola meal between processing plants and between batches from the same plant are expected (Jacobson et al., 2021). The Canadian Hemp Trade Alliance (2020, personal communication) reported average DM, CP, crude fat, and NDF contents of Canadian hemp meal of 92.0%, 34.3% DM, 12.7% DM, and 43.1% DM, respectively. The hemp meal in our study contained similar proportions of DM and crude fat but higher proportions of CP and lower proportions of NDF than these industry values. These differences were caused by the sifting out of hulls. Bell and Keith (1991) reported averages on the CP, crude fat, and NDF contents of canola meal from several commercial plants of 41.9, 3.9, and 23.5% of DM, respectively. The composition of the canola meal used in our study is comparable to these averages.

DMI

The absence of a treatment effect on DMI in our study confirms the findings of Mustafa et al. (1999), who showed that replacing 20% canola meal with 20% hemp meal in the diet of growing lambs did not affect their DMI. Also, Abrahamsen et al. (2021) found that including up to 33% DM hemp seed meal in the diet of growing meat goats did not affect DMI. Hessle et al. (2008) replaced 1 kg of hemp meal with 0.5 kg of barley grain and 0.5 kg of soybean meal in the diet of calves and replaced 0.2 kg of hemp meal with 0.1 kg of barley grain and 0.5 kg of soybean meal in the diet of finishing steers. This increased feed intake in calves but not in steers. Further, as live weight gain of the calves and steers did not differ, including hemp meal in the diet of calves reduced the feed efficiency. Gibb et al. (2005) included up to 14% of full-fat hemp seed in the diet of feedlot cattle, with no effect on DMI. Karlsson et al. (2010) replaced up to 32% DM of a basal diet with hemp seed meal, with no effect on DMI of lactating dairy cows. A comparison of these studies shows that differences in inclusion levels and composition of basal diets affect the influence of the addition of hemp meal on DMI. However, it is apparent that, when included up to 15% DM in the diet of ruminants, DMI of hemp meal–fed cattle is comparable to those of canola meal–fed cattle.

Table 5. Apparent total-tract digestibility coefficients (ADC) of DM, CP, and NDF of cows fed experimental diets containing canola meal (CM), a mixture of hemp meal and canola meal (HC), or hemp meal (HM)

<table>
<thead>
<tr>
<th>ADC</th>
<th>CM</th>
<th>HC</th>
<th>HM</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>55.0</td>
<td>54.0</td>
<td>54.0</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>CP, %</td>
<td>63.0</td>
<td>64.0</td>
<td>65.0</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>NDF, %</td>
<td>43.0</td>
<td>39.0</td>
<td>38.0</td>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a,bMeans with different letters within rows differ \((P < 0.05)\).

Table 6. Recoveries of spiking matrices with cannabinoids (%)

<table>
<thead>
<tr>
<th>Cannabinoid1</th>
<th>Matrix</th>
<th>Feed2</th>
<th>Feces2</th>
<th>Blood plasma3</th>
<th>Rumen fluid1</th>
<th>Urine3</th>
<th>Liver3</th>
<th>Kidney3</th>
<th>Adipose3</th>
<th>Muscle3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBD</td>
<td></td>
<td>89</td>
<td>87</td>
<td>117</td>
<td>110</td>
<td>101</td>
<td>111</td>
<td>101</td>
<td>124</td>
<td>95</td>
</tr>
<tr>
<td>CBDA</td>
<td></td>
<td>86</td>
<td>90</td>
<td>87</td>
<td>97</td>
<td>101</td>
<td>100</td>
<td>95</td>
<td>105</td>
<td>98</td>
</tr>
<tr>
<td>Δ9-THC</td>
<td></td>
<td>87</td>
<td>55</td>
<td>69</td>
<td>80</td>
<td>72</td>
<td>41</td>
<td>66</td>
<td>60</td>
<td>33</td>
</tr>
<tr>
<td>THCA</td>
<td></td>
<td>88</td>
<td>64</td>
<td>71</td>
<td>80</td>
<td>62</td>
<td>53</td>
<td>71</td>
<td>61</td>
<td>55</td>
</tr>
</tbody>
</table>

1CBD = cannabidiol; CBDA = cannabidiolic acid; Δ9-THC = d9-tetrahydrocannabinol; THCA = tetrahydrocannabinolic acid A.
2Spiked with 0.05 μg/g of cannabinoids.
3Spiked with 0.01 μg/g of cannabinoids.
The effects of including hemp meal in ruminant diets on rumen fermentation depend on the composition of the feed that it replaces. In our study, hemp meal replaced canola meal. The latter had lower CP, NDF, and crude fat contents compared with hemp meal, but the diets were formulated to be isoenergetic and isonitrogenous. Mustafa et al. (1999) and Vastolo et al. (2021) reported that the DM and CP degradability of hemp meal in the rumen are lower than those of canola meal. These differences could have affected the ammonia and VFA contents of rumen digesta, but this was not observed in our study. This was likely due to the isoenergetic and isonitrogenous contents of the experimental diets and to the fact that most of the fermentable DM and CP in the diets were not provided by hemp meal and canola meal. This explains why, in contrast to our findings, Abrahamsen et al. (2021) observed that including up to 33% DM hemp seed meal on a DM basis in the diet of growing meat goats increased the dietary NDF, ADF, and lignin contents, and decreased the dietary NEL contents, resulting in lower rumen VFA concentrations. The isoenergetic and isonitrogenous diets of our study may also explain why, in contrast to our findings, inclusion of 20% DM hemp seed meal in the diet of steers, which increased the dietary CP and gross energy contents, also increased the rumen concentrations of ammonia and VFA of rumen digesta (Winders et al., 2023).

**Rumen Fermentation**

In our study, treatment did not affect the total-tract digestibilities of DM and CP. This agrees with the findings of Mustafa et al. (1999) and Semwogerere et al. (2020), who also reported that hemp seed meal had similar DM and CP total-tract digestibilities compared with canola meal. Semwogerere et al. (2020) also suggested that, although the rumen degradation rate of hemp seed meal may be lower than that of canola meal, this may be compensated by an increase in rumen retention time. Winders et al. (2023) replaced 20% DM of dry-rolled corn in the diet of steers with either DDGS or hemp seed meal. In contrast with our findings, their hemp meal-fed steers had the highest true rumen and apparent total-tract N digestibilities and N retention. However, because their diets differed in energy, crude protein, fiber, and ether extract contents, their findings may not be representative for our study.

In contrast to the findings of Mustafa et al. (1999), our study showed that cows offered hemp meal had lower total-tract digestibilities of NDF than cows offered canola meal. This may be due to the higher indigestible NDF content and lower in situ NDF degradability of hemp seed meal compared with canola meal (Karlsson et al., 2010; Krizsan and Huhtanen 2013).

**Blood Metabolites**

Treatment did not affect the blood serum concentrations of glucose, NEFA, and BHBA, which were in the normal range for nonlactating dairy cows (LeBlanc, 2010; Piccione et al., 2012; Soulat et al., 2020). This suggests that the supply and status of energy were similar among treatments. That was to be expected, as diets were formulated to be isoenergetic. The calculated NEL content of hemp seed meal was higher than that of canola meal, but to make diets isoenergetic, the NEL content of the PMR of the diet fed to hemp meal-supplemented cows was lower than that of the PMR given to canola meal–fed cows. This, combined with a treatment effect on DMI, resulted in a similar NEL content in all treatments.

Experimental diets were also formulated to be isonitrogenous. However, as Mustafa et al. (1999) and Semwogerere et al. (2020) reported that the rumen undegradable protein content of hemp meal was higher than that of canola meal, treatment differences in the concentrations of rumen ammonia and blood urea could have occurred. This, nevertheless, was not observed. A reason for that may be that the crude protein content of the hemp meal in our study was comparatively high due to the sifting out of hulls.
Cannabinoids

A concern associated with feeding hemp co-products to livestock is the possible accumulation of psychoactive cannabinoids, including CBD and THC, in edible animal products such as meat and milk. Validation of the LC/MS technique used for the analysis of cannabinoids used in our study showed that the average recoveries of CBD, CBDA, THC, and THCA across matrices were 103.9, 95.4, 62.6, and 67.2%, respectively. These values are comparable to the ranges of recoveries of these cannabinoids in various matrices of 70.0 to 115%, reported by Aizpurua-Olaizola et al. (2017), Escrivá et al. (2017), and Christodoulou et al. (2023). Limits of detection of cannabinoids depend on the matrix, dilution of the samples, and technique used for detection (Escrivá et al., 2017; Pourseyed Lazarjani et al., 2020). The most common techniques for analysis of cannabinoids are GC and HPLC, combined with MS (Pourseyed Lazarjani et al., 2020). Using these techniques, detection limits of cannabinoid detection have been reported to range from 0.2 to 2.0 ng/g in feeds and feces, 0.5 to 2.2 ng/mL in blood plasma and urine, and 1.4 to 30 ng/g in animal tissues (Escrivá et al., 2017; Rasool, 2018; Kleinhenz et al., 2020a,b; Krebs et al., 2021; Stevens et al., 2022; Wagner et al., 2022). Hence, our detection limits were in the same range as those in earlier studies.

As a result, the hemp meal used in our study contained 3.0 μg/g of the psychoactive cannabinoid CBD, whereas the psychoactive THC was not detected. This is below the Canadian legal limit of THC in commercial hemp products, which has been set at 10 μg/g (Meng et al., 2018; Chicoine et al., 2020). The hemp meal also contained 4.4 and 0.10 μg/g of the nonpsychoactive cannabinoids CBDA and THCA, respectively. The highest dietary inclusion of hemp meal was 10.2% DM. Hence, compared with the legal THC limit of commercial hemp products, the cannabinoid contents of the experimental diets of our study were low.

Fecal concentrations of CBD and CBDA were 102.2 and 68.7% of the ingested amounts of these cannabinoids, respectively. This suggest that the majority of ingested CBD and CBDA were excreted in the feces. This supports the finding of Chayasirisobhon (2020), who reported that the bioavailability of orally ingested CBD is only 12%. Despite this, Semwogerere et al. (2020) and Huestis (2007) reported that absorbed CBD and THC are easily released from the feed matrix and absorbed. This was also shown by Kleinhenz et al. (2020b), who demonstrated that a one-time oral administration of industrial hemp flower material to cattle designed to achieve a CBDA dose of 5.4 mg/kg body weight resulted in a maximum blood plasma concentration of CBDA of 72.9 ng/mL at 11.8 h after dosing, and a maximum THCA blood plasma concentration of 12.1 ng/mL at 25.2 h after dosing. In contrast, no cannabinoids were detected in blood plasma in our study. However, due to the low intake of cannabinoids in our study, the concentrations of digested cannabinoids in blood plasma would have been below the detection limits of the assay. The absence of cannabinoids in RF in our study also suggests that, even if cannabinoids were released from the feed in the rumen, this did not result in detectable concentrations of these cannabinoids in RF. As rumen digesta was collected by rumenocentesis, no solid rumen digesta that probably contained cannabinoids was sampled. Chayasirisobhon (2020) concluded that most absorbed CBD and THC are eliminated or metabolized in the liver, which may also have contributed to the absence of cannabinoids in blood plasma in our study.

As cannabinoids are highly lipophilic, absorbed cannabinoids that are not excreted or metabolized are deposited in adipose tissues. This was demonstrated by Brum et al. (2006), who showed that following an intravenous injection of 200 μg/kg of THC in pigs, this cannabinoid was no longer detectible in liver, kidney, muscle tissues, and blood plasma at 24 h after this injection, but that THC was still detectible in adipose tissues at this time. Also, Rawitch et al. (1979) observed that an intraperitoneal injection of THC increased the THC content of adipose tissues, especially in gonadal fat. This implies that a continuous supply of cannabinoids through the feeding of hemp meal could result in accumulation of cannabinoids in animal tissues. Despite this, feeding diets containing up to 18% DM hemp meal and up to 9% DM hemp oil for 35 d did not result in detectible levels on THC in edible broiler tissues (Rasool, 2018). This supports our finding that, at the end of our trial, no detectible levels of cannabinoids were detected in muscle and adipose tissues, and that this was likely due to the low intake of cannabinoids and low bioavailability of cannabinoids in our study.

Limitations of the Study

Heat treatment can cause decarboxylation of acidic cannabinoids; however, this has been shown to occur at temperatures above 80°C. Hence, the drying of the feed and feces samples in our study is expected to have caused little or no decarboxylation of cannabinoids (Wang et al., 2016). The grinding of the feed and feces samples in our study could also have affected the contents and profiles of cannabinoids in these samples (Paulik et al., 2019). However, as these samples were ground through a 1-mm screen and no heat development was observed during this grinding, it is believed
that this effect of grinding must have been absent or limited.

As the cows were not rumen cannulated, rumen digesta was collected by rumenocentesis. This technique only allows for the collection of liquid rumen digesta. As the cannabinoids are likely found in the solid rumen digesta, this also explains why no cannabinoids were detected in RF digesta in our study.

Collection of animal tissues required euthanization of the cows in our study. Hence, these tissues could only be collected at the end of the study, when all cows had undergone all treatments. Hence, we can only conclude that the combination of the treatments, and not any individual treatment, did not result in detectable levels of cannabinoids in liver, kidney, muscle, and adipose tissues.

CONCLUSIONS

The replacement of canola meal with hemp meal as a protein supplement in diets formulated from barley silage, wheat straw, and 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, or blood BHB. A higher total-tract NDF digestibility in the CM diet was apparent compared with the HM diet. This may be due to the relatively low digestible NDF and high insoluble dietary fiber (29% DM to 32% DM) contents of the hemp seed meal protein used in our study. Cannabinoid contents in RF, blood plasma, urine, and kidney, liver, adipose, and muscle tissues of the cows were below detection limits. This shows that feeding hemp meal to nonlactating dairy cows does not lead to accumulation of cannabinoids in body tissues and biological fluids. Our data indicate that, up to a dietary inclusion rate of 10.2% of DM, hemp seed meal is a good and safe alternative for canola meal as a protein supplement for nonlactating dairy cows.

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