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Effect of Blend-Pelleted Products Based on Carinata Meal or Canola Meal in Combination with Lignosulfonate on Ruminal Degradation and Fermentation Characteristics, Intestinal Digestion, and Feed Milk Value When Fed to Dairy Cows

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Abstract: The objectives of this study were to investigate the effect of newly developed blend-pelleted products based on carinata meal (BPPCR) or canola meal (BPPCN) in combination with peas and lignosulfonate on ruminal fermentation characteristics, degradation kinetics, intestinal digestion and feed milk values (FMV) when fed to high-producing dairy cows. Three dietary treatments were Control = control diet (common barley-based diet in western Canada); BPPCR = basal diet supplemented with 12.3%DM BPPCR (carinata meal 71.4% + pea 23.8% + lignosulfonate4.8%DM), and BPPCN = basal diet supplemented with 13.3%DM BPPCN (canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM). In the whole project, nine mid-lactating Holstein cows (body weight, 679 ± 124 kg; days in milk, 96 ± 22) were used in a triplicated 3 × 3 Latin square study for an animal production performance study. For this fermentation and degradation kinetics study, the experiment was a 3 × 3 Latin square design with three different dietary treatments in three different periods with three available multiparous fistulated Holstein cows. The results showed that the control diet was higher ($p < 0.05$) in total VFA rumen concentration (138 mmol/L) than BPPCN. There was no dietary effect ($p > 0.10$) on the concentration of rumen ammonia and ruminal degradation kinetics of dietary nutrients. There was no significant differences ($p > 0.10$) among diets on the intestinal digestion of nutrients and metabolizable protein. Similarly, the feed milk values (FMV) were not affected ($p > 0.10$) by diets. In conclusion, the blend-pelleted products based on carinata meal for a new co-product from the bio-fuel processing industry was equal to the pelleted products based on conventional canola meal for high producing dairy cattle.

Keywords: new co-product; bio-fuel processing; carinata meal; CNCPS fractions; Canola meal; rumen fermentation and degradation; intestinal digestion; feed milk value; high producing dairy cows

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1. Introduction

The use of biofuel industry co-products as feedstuffs for dairy cows is a realistic option to decrease feed costs and increase the production efficiency of high producing dairy cows [1]. A new co-product from bio-fuel processing is carinata meal. Carinata meal is a good source of crude protein (CP), at about 48% CP [2,3]. However, carinata meal is characterized by its higher level of rumen-degradable protein compared with canola meal [4,5].

In recent years, Canada has become the second country in terms of pea production [6]. In 2014, Saskatchewan grew about 64% of the dry pea crop and 90% of the chickpea crop of the total Canadian pea production [7]. Pea is relatively high in protein, at about 24% on a dry matter (DM) basis, and also contains a high level of starch: 46% DM [6]. The rumen-degradable protein (RDP) of peas is about 78% [8]. Decreasing the rumen degradability of protein supplements is an essential strategy used to improve the dietary amino acids (AAs)

supply to the small intestine. This concept assumes the enhancement of milk production from an increased amino acid supply to the lactating dairy cow.

Because of the high level of RDP in canola meal, carinata meal, and pea, it is essential to slow down the degradation (extent and rate) of ruminal degradation [4,5,9]. The most common methods used to maximize the utilization of protein and protect AAs are heat related treatments and the addition of special feed additives. Heat treatments include techniques such as pelleting, steam flaking, dry roasting, etc. [10,11]. Heat treatments are vital to improving the nutritional, chemical, physical, hygienic, and other animal feed characteristics. Feed additives such as formaldehyde [12], tannins [13], lignosulfonate (LSO_3), and xylose [14] could decrease the RDP of protein in different rations. The lignosulfonate can also improve blend pelleting product quality [15].

In a previous study, we reported the effects of feeding blend-pelleted co-products with carinate meal or canola meal on the nutrient intake, digestion, and production performance of high producing dairy cows [16]. The objectives of this study were to investigate the effect of blend-pelleted products based on carinata meal (BPPCR) or canola meal (BPPCN) in combination with peas and lignosulfonate on ruminal fermentation characteristics, ruminal degradation kinetics, intestinal digestion and feed milk values (FMV) when fed to high producing dairy cows.

2. Materials and Methods

All animal experimental procedures used in this study were approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 19910012) and were conducted in accordance with the Canadian Council of Animal Care guidelines [17].

2.1. Animals and Experimental Designs and Diets

In the previous study, nine mid-lactating Holstein cows (body weight, 679 ± 124 kg; days in milk, 96 ± 22) were used in a triplicated 3 × 3 Latin square study for an animal production performance study, which has been reported before [16]. In this study, on rumen fermentation and degradation kinetics, three multiparous fistulated lactating Holstein cows were used in a 3 × 3 Latin square design with three different dietary treatments and three different periods. Each experimental period lasted for 21 days, consisting of 14 days of diet adaptation and seven days of sample collection. The three multiparous fistulated lactating Holstein cows were housed in individual tie-stalls at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, Canada). The cows were randomly assigned to one of the following three treatment diets: Control = control diet: common barley-based diet used in western Canada (6.2% canola meal + 2.2% soybean meal + 3.9% peas), BPPCR diet: basal diet supplemented with 12.3%DM BPPCR (carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM), and BPPCN diet = basal diet supplemented with 13.3 %DM BPPCN (canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM). The diet formulation was undertaken by using NDS diet formulation software. The dietary ingredients of experimental diets fed to lactating dairy cows are presented in Table 1. The chemical composition of the diets was reported previously in a dairy production performance trial [16].

Table 1. Ingredients of total mixed rations for the supplement diet treatments.

Items	Dietary Treatments		
	Control	BPPCR	BPPCN
Ingredient (%DM)			
Barley silage	38	38	38
Alfalfa hay	16	16	16
Barley grain	30	30	29
Canola meal	6.2	-	-
Soybean meal	2.2	-	-

Table 1. *Cont.*

Items	Dietary Treatments		
	Control	**BPPCR**	**BPPCN**
Peas	3.9	-	-
BPPCR	-	12.2	-
BPPCN	-	-	13.2
Minerals premix	2.0	2.0	2.0
Tallow	0.8	0.8	0.8
Palmitic acid	0.9	0.9	0.9

Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); Composition of the premix: Calcium = 16%; Phosphorus = 8.0%; Chloride = 10.4%; Sodium = 7.6%; Potassium = 1.8%; Sulfur = 1.0%; Magnesium = 4.5%; Copper = 535 ppm; Zinc = 2100 ppm; Manganese = 1500 ppm; Iron = max 1050 ppm; Selenium = 16 ppm; Iodine = 45 ppm; Cobalt = 16 ppm; Vitamin A (KIU) = 330; Vitamin D (KIU) = 60; Vitamin E (IU) = 2500.

2.2. Rumen Fluid Collection

On the last day of each experimental period (starting on day 21 at 08:00 h), the ruminal fluid was collected over 24 h every 3 h (0, 3, 6, 9, 12, 15, 18, 21, 24 h). About 250 mL of ruminal liquid was collected from four different locations of the rumen (ventral, anterior, posterior, and rumen mat). After that, the ruminal fluid went through two layers of cheesecloth, and the solids were discarded. Two 10 mL quantities of the filtrate samples were sub-sampled into 15 mL centrifuge tubes (Fisher Scientific, Waltham, MA, USA) [18]. For these parallel samples, one of the samples was added to a tube containing 2 mL of 25% metaphosphoric acid for VFA analysis and the other one of the samples was attached to a tube containing 2 mL of 1% sulphuric acid for ammonia analysis. All samples were stored at −20 °C.

The frozen ruminal volatile fatty acid (VFA) samples were melted overnight at 4 °C. Then, the samples were thoroughly mixed and centrifuged at 12,000× *g* for 10 min at 4 °C using a Beckman Centrifuge (Model Avanti J-E; Palo Alto, CA, USA). About 1.0 mL of this sample was placed into microcentrifuge tubes (VWR TM 1.5 mL Microcentrifuge tube with snap cap, Radnor, PA, USA). After that, samples were centrifuged at 16,000× *g* for 10 min at 4 °C using a Microcentrifuge (Beckman Coulter TM, Brea, CA, USA). An internal standard containing 300 µL isocaproic acid, 20 mL of 25% metaphosphoric acid, and double-distilled water (ddH_2O) were mixed with 1 mL of the supernatant sample in a gas chromatography (GC) vial (Agilent TechnologiesTM, Santa Clara, CA, USA) to determine the concentration of VFA by a comparison of peak areas using an Agilent 6890 series Gas chromatography system (Agilent Technologies TM, Santa Clara, CA, USA) with an Agilent 7683 series 5 µL injector, Zebron ZB-FFAP high performance GC capillary column (30 m × 320 µm × 0.25 µm, Phenomenex, Torrance, CA, USA) and an Agilent split focus liner (Agilent TechnologiesTM, Santa Clara, CA, USA). Samples were prepared daily at 4 °C to avoid volatilization until analysis. To build a calibration curve, acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, and isocaproic acids were used as a mixed standard.

For ammonia analysis [19], frozen samples were kept overnight at 4 °C, vortexed, and centrifuged at 12,000× *g* for 10 min at 4 °C using a Beckman Centrifuge (Model Avanti J-E; Palo Alto, CA, USA). After that, 1.0 mL of the sample was placed in microcentrifuge tubes (VWR TM 1.5-mL Microcentrifuge tube with snap cap, Radnor, PA, USA) and centrifuged at 16,000× *g* for 10 min at 4 °C using a Microcentrifuge (Beckman Coulter TM, Brea, CA, USA). The ammonia concentration of the ruminal fluid was analyzed using the phenol-hypochlorite method of Broderick and Kang [19].

2.3. Cornell Net Carbohydrate and Protein System (CNCPS V.6.5)

In the CNCPS (Higgs et al., 2015), proteins are divided into PA1 ammonia (Kd = 200%/h), PA2 soluble true protein (Kd = 10–40%/h), PB1 (moderately degradable true protein, Kd = 3–20%/h), PB2 (slowly degradable true protein, Kd = 4–9%/h) and PC (unavailable protein) based on their rumen degradation features. The carbohydrate partition is described by Higgs et al. [20]. The eight subfractions include CA1, CA2, CA3, CA4, CB1, CB2, CB3, and CC, based on rumen fermentation and microbial activity on carbohydrate availability [21]). The CA1 fraction is VFA, consisting mainly of acetate, propionate, and butyrate, which are not degradable (0%/h). The CA2 fraction is lactic acid, with a degradation rate of 7%/h. The CA3 fraction degrades at 5%/h. The CA4 fraction has Kd 50%/h. The CB1 fraction with Kd rates equal to 30%/h. The CB2 fraction degrades at 30%/h. The CB3 fraction with Kd rates equal to 6%/h. The CC, mostly plant cell walls containing lignin, is considered undegradable. The Kp is 13.75%/h (mean retention time = 7.3 h) for CA4, PA1 and PA2, 7.60%/h for other CB1 and CB2 and PB1 fractions (mean retention time = 13.2 h), and 1.66%/h for PB2 and CB3 (mean retention time = 60.2 h).

2.4. Rumen Incubation Procedure and Sample Analysis

An in situ method was used to determine rumen degradation kinetics, as described by Yu et al. [22]. The in situ procedure included weighing 7 g of each diet in each number-coded nylon bag (10 × 20 cm), with multiple bags for each treatment and each incubation of 0, 3, 6, 9, 12, 24, and 48 h. The pore size of the nylon bag was ca. 41 µm. These bags were tied about 2 cm below the top, allowing a ratio of a sample size to bag surface area of 39 mg/cm^2. The rumen incubations were performed with three cannulated cows according to the "gradual addition/all-out" schedule (the bags were inserted sequentially and retrieved at the same time), and the samples were incubated in the rumens for 3, 6, 9, 12, 24, and 48 h). After incubation, the bags were collected from the rumen and washed with cool water by hand six times with 10 bags each round. The 0 h bags were washed under the same conditions four times. After washing the bags, the bags were dried at 55 °C for 48 h by placing all bags on stainless steel trays in a forced-air drying oven [23]. All dried bags were moved to lab room conditions (temperature room at 21 °C) for at least 24 h, then the bag + string + residue was weighed. The samples were ground through a 1 mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England) for chemical analysis. In situ samples were analyzed for ash (AOAC, 1990 [24]; method 942.05), CP (AOAC, 1990 [24]; method 990.03), neutral detergent fiber (NDF), and starch (ST [25]).

2.5. Measurement of Rumen Degradation Kinetics of Feed Nutrients Using the In Situ Technique

Degradation characteristics of DM, organic matter (OM), CP, NDF, and ST were determined using the first-order kinetics degradation model described by Ørskov and McDonald [26] and modified by Tamminga et al. [27]. The results were estimated using the nonlinear (NLIN) procedure of SAS 9.4 and iterative least-squares regression (Gauss–Newton method), as in the following equation:

$$R(t) = U + D \times e^{-Kd \times (t - T0)},$$

where R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (h^{-1}), and T0 = lag time (h).

The rumen undegradable (RU) or bypass (B) values of nutrients on a percentage basis were calculated according to NRC Dairy [28], as in the following equation:

$$\%BDM, BCP \text{ or } BNDF = U + D \times Kp/(Kp + Kd)$$

$$\%BST = 0.1 \times S + D \times Kp/(Kp + Kd),$$

where Kp stands for estimated passage rate from the rumen (4.5%/h); S stands for a soluble fraction (%). The factor 0.1 in the formula represents the approximate 100 g/kg of the soluble fraction (S) that escapes rumen fermentation [27].

The rumen undegradable or bypass DM, and starch (ST) in g/kg DM, were calculated as for the following equation:

$$\text{BDM or BST (g/kg DM)} = \text{DM or ST (g/kg DM)} \times \%\text{BDM or BST}$$

The rumen undegradable protein (RUP) and rumen bypass protein (BCP) were calculated differently in the Dutch model [27] and NRC Dairy 2001 model [28]:

$$\text{BCP}^{\text{DVE}}\text{ (g/kg DM)} = 1.11 \times \text{CP (g/kg DM)} \times \text{RUP (\%)},$$

$$\text{RUP}^{\text{NRC}}\text{ (g/kg DM)} = \text{CP (g/kg DM)} \times \text{RUP (\%)},$$

where DVE = truly digestible protein; 1.11 is the regression coefficient between in situ RUP and in vivo RUP [28].

The effective degradability (ED) of each nutrient was predicted according to NRC (2001), as in the following equation:

$$\%\text{EDDM (EDCP or EDST)} = S + D \times Kd/(Kp + Kd)$$

$$\text{EDDM (CP or ST, g/kg DM)} = \text{DM (CP or ST) (g/kg DM)} \times \%\text{EDDM (EDCP or EDST)}$$

2.6. Intestinal Digestibility of Feed Nutrients Using In Vitro Techniques

Intestinal digestion was evaluated using the three-step in vitro procedure described by Calsamiglia and Stern [29] and Gargallo et al. [30]. The In vitro digestion study included the following steps: (1) dried ground residues containing 15 mg of N after 12 h ruminal preincubation were placed into a 50 mL centrifuge tube; (2) 10 mL of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9) was added, vortexed, and incubated for 1 h at 38 °C in a water bath; (3) 0.5 mL of 1 N NaOH solution and 13.5 mL of pancreatin (Sigma P-7545) were added, vortexed and incubated at 38 °C for 24 h (vortexed every 8 h approximately); (4) 3 mL of TCA was added to stop enzymatic hydrolysis; (5) the tubes were vortexed and samples sat for 15 min at room temperature; and (6) all samples were centrifuged for 15 min at 10,000× *g* and supernatant (5 mL) was analyzed for soluble N using the Kjeldahl method. The intestinal digestion of protein was measured according to TCA-soluble N divided by the amount of N in the rumen residue sample [29,30].

2.7. Truly Digestible Protein in the Small Intestine

The metabolizable protein (MP) is composed of three major contributory protein sources using the NRC [28] model, as in the following equation:

$$\text{MP (g/kg DM)} = \text{AMCPNRC} + \text{ARUPNRC} + \text{AECP},$$

where AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP is the truly absorbable endogenous protein in the small intestine [28,31].

The DBP based on data from the NRC-2001 mode reflects the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on the energy available for microbial fermentation in the rumen. Thus, the DPBNRC was calculated as follows:

$$\text{DPBNRC (g/kg of DM)} = \text{RDPNRC} - 1.18 \times \text{MCPTDN}.$$

The FMV was estimated based on the characteristics of protein from NRC, 2001 model. The efficiency of the use of metabolizable protein for lactation was assumed to be 0.67 [28], and protein composition in milk was considered to be 33 g protein/1 kg of milk.

2.8. Statistical Analysis

The data were analyzed using Proc Mixed SAS 9.4 (SAS Institute, Cary, NC) to examine digestibility, ruminal fermentation, and ruminal pH profile by using the following model:

$$Y_{ijkl} = \mu + P_j + C_k + T_l + E_{ijkl},$$

where Y_{ijkl} was the dependent variable, μ was the overall mean, $P_{j(i)}$ was the fixed effect of j^{th} period, $C_{k(i)}$ was the random effect of k^{th} cow, T_l was the fixed effect of l^{th} dietary treatment, and E_{ijkl} was the residual error.

Multi-treatment comparisons were carried out using the Tukey method. The model assumptions were tested using Proc Univariate with Normal and Plot options. Normality was tested using the Shapiro–Wilk test. Differences were declared significant if $p < 0.05$, and values of $0.05 < p < 0.10$ were interpreted as tendencies towards significance.

3. Results

3.1. Ruminal Fermentation

The mean pH was not affected ($p > 0.10$) by different dietary treatments (Table 2). The total VFA was increased in the control diet ($p < 0.05$; 138 mmol, L) compared with the average of the BPPCR diet and BPPCN diet (averaging 119 mmol/L; Table 2). Acetate tended ($0.05 < p < 0.10$) to be higher in the control diet (78.8 mmol/L) compared with BPPCR and BPPCN diets (averaging 70.35 mmol/L). Our results showed that the control diet had higher proportions (37.2 mmol/L) compared with the BPPCR diet and BPPCN diet (averaging 29.05 mmol/L). In the same way, the control was higher for valerate and, on the other hand, lower for caproate. Iso-butyrate, butyrate, iso-valerate, and iso-caproate were not affected ($p > 0.10$) by different dietary treatments. For ammonia, there was no dietary effect ($p > 0.10$) on ammonia concentration; however, when comparing the control diet with the averaging of the BPPCR diet and BPPCN diet, the control tended ($0.05 < p < 0.10$) to be higher in ammonia relative to other different dietary treatments.

Table 2. Ruminal fermentation characteristics for high-producing dairy cows fed a total mixed ration with blend-pelleted products (BPP) *.

Items	Dietary Treatments			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Total VFA (mmol, L)	138.22 ^{a}	121.79 ^{ab}	117.01 ^{b}	3.531	0.02	0.04
VFA (mol/100 mol)						
Acetate	78.84	70.67	70.03	2.321	0.08	0.22
Propionate	37.19 ^{a}	30.40 ^{b}	27.70 ^{b}	0.983	<0.01	<0.01
Iso-butyrate	0.90	0.72	0.73	0.067	0.19	0.36
Butyrate	16.87	15.50	14.55	0.811	0.24	0.13
Iso-valerate	1.29	1.09	1.13	0.111	0.45	0.65
Valerate	2.60 ^{a}	2.11 ^{b}	2.08 ^{b}	0.087	0.02	0.15
Iso-caproate	0.71	0.65	0.77	0.088	0.66	0.43
Caproate	2.16 ^{b}	2.43 ^{a}	2.69 ^{a}	0.071	<0.01	<0.01
Ruminal pH	6.00	6.09	5.74	0.083	0.17	0.10
NH_3-N (mg/dL)	5.60	5.01	4.19	0.469	0.12	0.06

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; VFA: volatile fatty acids. SEM: standard error of mean; ^{a,b} Means with the different letters in the same row are significantly different ($p < 0.05$).

3.2. Ruminal Degradation of Protein and Carbohydrate Subfractions

Ruminal degradable protein (RDP) subfractions such as rumen-degradable PA2 fraction (RDPA2), PB1 fraction (RDPB1), PB2 fraction (RDPB2), and total rumen-degradable protein (TRDP), and ruminal undegradable protein subfractions such as rumen-degradable PA2 fraction (RUPA2), PB1 fraction (RUPB1), and PB2 fraction (RUPB2), were not affected ($p > 0.10$) by different dietary treatments (Table 3). However, the BPPCN diet was higher ($p < 0.05$) in TRUP (4.50%DM) compared with the BPPCR diet and the BPPCN diet (averaging 4.16% DM).

Table 3. Ruminal degradable and undegradable subfractions of protein and carbohydrates for the total mixed ration with blend-pelleted products in lactating dairy cows using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5.

	Dietary Treatments *					**Contrast *p*-Value**
Items	**Control**	**BPPCR**	**BPPCN**	**SEM**	***p*-Value**	**Control vs. (BPPCR + BPPCN)**
Ruminal degradable protein fractions (%DM)						
RDPA2	4.92	4.96	4.69	0.051	0.24	0.17
RDPB1	5.70	5.53	6.35	0.070	0.11	0.08
RDPB2	0.07	0.06	0.03	0.038	0.82	0.61
TRDP	10.69	10.55	11.07	0.089	0.23	0.16
Ruminal undegradable protein fraction (%DM)						
RUPA2	0.66	0.67	0.63	0.006	0.24	0.16
RUPB1	2.28	2.21	2.54	0.026	0.10	0.07
RUPB2	0.20	0.19	0.10	0.115	0.84	0.64
TRUP	4.16 ^{b}	4.15 ^{b}	4.50 ^{a}	0.013	0.05	0.03
Ruminal degradable carbohydrate fraction (%DM)						
RDCA4	4.80	5.45	5.90	0.447	0.53	0.41
RDCB1	26.70	23.95	25.30	0.574	0.27	0.98
RDCB2	11.98	14.01	12.57	0.798	0.44	0.75
RDCB3	25.29	26.18	24.37	0.447	0.31	0.26
TRDC	46.19	46.25	44.36	0.756	0.21	0.09
Ruminal undegradable carbohydrate fraction (%DM)						
RUCA4	5.93	5.32	5.62	0.128	0.27	0.98
RUCB1	1.94	2.27	2.04	0.128	0.44	0.76
RUCB2	42.15	43.64	40.62	0.747	0.31	0.26
RUCB3	4.01	3.62	3.83	0.064	0.21	0.89
RUCC	56.17	57.27	54.73	0.485	0.25	0.20
TRUCC	2.13	2.43	2.62	0.198	0.53	0.41

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; ^{a,b} Means with the different letters in the same row are significantly different ($p < 0.05$); Multi-treatment comparison using Tukey method; RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RDPB2: ruminally degraded PB2; TRDP: total ruminally degraded CP; RUPA2: ruminally escaped PA2; RUPB1: ruminally escaped PB1; RUPB2: ruminally escaped PB2; RUPC: ruminally escaped PC; TRUP: total ruminally escaped CP; RDCA4: ruminally degraded CA4; RDCB1: ruminally degraded CB1; RDCB2: ruminally degraded CB2; RDCB3: ruminally degraded CB3; TRDC: total ruminally degraded CHO; RUCA4: ruminally escaped CA4; RUCB2: ruminally escaped CB2; RUCB3: ruminally escaped CB3; RUCC: ruminally escaped CC; TRUCC: ruminally escaped CHO.

Similarly, ruminally degradable carbohydrate subfractions such as rumen-degradable CA4 fraction (RDCA4), CB1 fraction (RDCB1), CB2 fraction (RDCB2), CB3 fraction (RDCB3), and total rumen-degradable carbohydrate (TRDC), and ruminal undegradable carbohydrate subfractions such as rumen undegradable CA4 fraction (RUCA4), CB1 fraction (RUCB1), CB2 fraction (RUCB2), CB3 fraction (RUCB3), and total rumen undegradable carbohydrate (TRUC), were not affected ($p > 0.10$) by different dietary treatments. However, when comparing the control diet with the BPPCR diet and BPPCN diet, TRDC tended to

be higher in the BPPCR diet (46.3% DM) compared with the control diet and BPPCN diet (averaging 45.3% DM).

3.3. In Situ Rumen Degradation Kinetics

The in situ ruminal degradation kinetics of dry matter are presented in Table 4. The rate of rumen degradation (Kd) was significantly ($p < 0.05$) higher in the control diet (9.1%/h) compared with the BPPCR diet and BPPCN diet (averaging 7.2%/h). However, the other ruminal degradation parameters of DM, including rumen-degradable fractions (D), rumen undegradable fractions (U), rumen bypass dry matter (BDM), and effectively degraded dry matter (EDDM), were unaffected ($p > 0.10$) by dietary treatments. Dietary treatments also did not affect ($p > 0.10$) the ruminal degradation kinetics of organic matter (OM), starch (ST), and crude protein (CP; Tables 5–7). The in situ ruminal degradation of OM, ST, and CP such as Kd, D, U, rumen bypass organic matter (BOM), effectively degraded organic matter (EDOM), rumen bypass starch (BST), effectively degraded starch (EDST), rumen bypass crude protein (BCP) in DVE/OEB system, and effective degraded crude protein (EDCP), were not affected ($p > 0.10$) by dietary treatments.

Table 4. In situ rumen degradation kinetics of dry matter (DM) with total mixed ration with blend-pelleted products (BPP) * in lactating dairy cows.

Items	Dietary Treatments			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen DM degradation						
Kd (%/h)	9.07 [a]	7.84 [ab]	6.49 [b]	0.444	0.04	0.02
T0 (h)	0.27	0.00	0.27	0.156	0.44	0.53
S (%)	27.68	28.26	27.99	0.775	0.88	0.99
D (%)	49.26	47.88	50.25	1.983	0.72	0.53
U (%)	23.05	23.86	21.76	1.919	0.75	0.51
BDM (=RUDM, g/kg DM)	394.21	416.60	421.93	13.964	0.30	0.29
EDDM (=RDDM, g/kg DM)	605.79	583.40	578.07	13.964	0.30	0.29
BDM (=%RUDM)	39.42	41.66	42.19	1.397	0.30	0.29
EDDM (=%RDDM)	60.58	58.34	57.81	1.397	0.30	0.29

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; [a,b] Means with the different letters in the same row are significantly different ($p < 0.05$); Multi-treatment comparison using Tukey method; DM: Dry Matter; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable degradable fractions; BDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

Table 5. In situ rumen degradation kinetics of organic matter (OM) of total mixed ration with blend-pelleted products (BPP) * in lactating dairy cows.

Items	Dietary Treatments *			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen OM degradation						
Kd (%/h)	10.30	6.45	8.42	0.764	0.13	0.97
T0 (h)	0.27	0.00	0.77	0.390	0.44	0.26
S (%)	27.68	28.26	27.99	0.775	0.88	0.99
D (%)	49.26	47.88	51.70	1.387	0.26	0.14
U (%)	23.05	23.86	20.31	1.404	0.28	0.14

Table 5. *Cont.*

Items	Dietary Treatments *			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
EDOM (g/kg DM)	569.52	548.14	566.91	15.265	0.60	0.69
BOM (g/kg DM)	371.31	387.02	370.55	16.560	0.75	0.69
%EDOM	60.54	58.61	60.49	1.711	0.69	0.69
%BOM	39.46	41.39	39.51	1.711	0.69	0.69

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; OM: Organic Matter; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable fractions; BOM: rumen bypass organic matter; EDOM: effective degradability of organic matter.

Table 6. In situ rumen degradation kinetics of starch (ST) of total mixed ration with blend-pelleted products (BPP) * in lactating dairy cows.

Items	Dietary Treatments *			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen starch degradation						
Kd (%/h)	26.27	31.09	21.88	8.057	0.74	0.53
T0 (h)	1.09	0.86	1.14	0.795	0.96	0.89
S (%)	10.86	4.34	8.58	2.861	0.36	0.94
D (%)	87.82	91.59	90.22	1.994	0.47	0.88
U (%)	0	3.96	2.81	2.026	0.53	0.81
BST (g/kg DM)	38.71	32.43	41.36	8.443	0.83	0.69
EDST (g/kg DM)	215.96	195.08	196.10	18.083	0.24	0.36
%BST	15.22	14.36	17.10	3.304	0.89	0.68
%EDST	86.61	82.33	84.38	2.107	0.51	0.98

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; St: Starch; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable fractions; BST: rumen bypass or undegraded feed starch; EDST: effective degraded starch.

Table 7. In situ rumen degradation kinetics of crude protein (CP) of total mixed ration with blend-pelleted products (BPP) * in lactating dairy cows.

Items	Dietary Treatments *			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen CP degradation						
Kd (%/h)	5.79	8.64	5.66	1.387	0.33	0.41
T0 (h)	0.86	2.43	3.34	1.409	0.51	0.38
S (%)	27.13	26.70	26.51	2.856	0.99	0.91
D (%)	60.17	57.75	62.15	2.718	0.57	0.39
U (%)	12.99	15.42	11.17	5.904	0.90	0.74
%BCP or %RUP	39.01	36.64	39.39	3.437	0.84	0.73

Table 7. *Cont.*

Items	Dietary Treatments *			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
RUP (g/kg DM, NRC)	56.61	49.20	47.92	3.477	0.39	0.39
BCP (g/kg DM, DVE)	62.83	54.62	53.19	3.862	0.39	0.39
%EDCP (=%RDP)	60.99	63.36	60.61	3.437	0.84	0.73
EDCP (=RDP, g/kg DM)	82.85	87.05	80.74	8.177	0.86	0.70

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; CP: crude protein; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable degradable fractions; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded crude protein.

3.4. Intestinal Digestion of Dietary Nutrients

For intestinal digestion (Table 8), the BPPCN diet tended ($0.05 < p < 0.10$) to be higher for the intestinal digestibility of rumen-bypassed DM (dBDM; 47.80%BDM) compared with control diet and BPPCR diet (averaging 41.05%BDM). The intestinal digestible rumen-bypassed DM (IDBDM) tended to be affected ($0.05 < p < 0.10$) by diet. The total digestible DM (TDDM) was not affected ($p > 0.10$) by diet. There was no treatment effect ($p > 0.10$) on the intestinal digestion of CP, such as digestible intestinal protein (IDP) or total digestible protein (TDP), by diet. Furthermore, there was no dietary effect ($p > 0.10$) on the intestinal digestion of ST such as intestinal digestible starch (IDBST) or total digestible starch (TDST) by diet.

Table 8. Intestinal digestion and availability of total mixed ration with blend-pelleted products (BPP) * in lactating dairy cows.

Items	Dietary Treatments			SEM	*p*-Value	Contrast *p*-Value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
DM intestinal digestion						
dBDM (% BDM)	39.57	42.53	47.80	1.956	0.09	0.05
IDBDM (% BDM)	15.60	17.57	20.27	1.002	0.07	0.04
IDBDM (g/kg DM)	61.70	72.97	86.03	6.458	0.13	0.08
TDDM (%DM)	75.93	76.26	77.94	1.130	0.21	0.12
CP intestinal digestion						
dIDP (%)	76.33	77.03	70.27	3.264	0.53	0.32
IDP (% CP)	29.83	28.00	27.70	2.520	0.82	0.71
IDP (g/kg DM)	40.17	37.10	36.83	2.019	0.50	0.51
TDP (%CP)	89.35	91.48	89.71	1.341	0.62	0.73
TDP (g/kg DM)	123.03	124.13	117.57	5.352	0.33	0.18
Starch intestinal digestion						
dBST (%BST)	94.03	94.13	94.13	2.856	1.00	0.99
IDBST (%BST)	14.47	13.50	16.13	3.419	0.91	0.71
IDBST (g/kg DM)	6.17	4.40	7.57	2.419	0.78	0.59
TDST (%DM)	99.27	99.13	99.03	0.292	0.86	0.67
TDST (g/kg DM)	219.96	201.12	204.23	17.619	0.19	0.41

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN:

canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; dBDM: intestinal digestibility of rumen bypassed DM, calculated as: (BDM-DM residual after 48 h rumen incubation)/BDM × 100; IDBDM: intestinal digestible rumen bypassed DM, calculated as: BDM × dBDM; TDDM: total digestible DM, calculated as: EDDM + IDBDM; IDP: intestinal digestible protein, calculated as: BCP × dIDP; dIDP: intestinal digestibility of rumen undegraded protein; TDP: total digestible protein, calculated as: EDCP +IDP; dBST: intestinal digestibility of rumen bypassed ST, calculated as: (BST-ST residual after 48 h rumen incubation)/BST × 100; IDBST: intestinal digestible rumen bypassed ST, calculated as: BST × dBST; TDST: total digestible ST, calculated as: EDST + IDBST.

3.5. Truly Absorbed Metabolizable Protein

Table 9 shows the predicted truly absorbed metabolizable protein, in which there was no significant difference ($p > 0.10$) among diets in the truly absorbable rumen synthesized microbial protein, truly absorbable rumen-undegradable protein, and endogenous rumen protein in the small intestine for the different dietary treatments. There was no dietary effect ($p > 0.10$) on the total metabolizable protein (MP) in the small intestine. Moreover, the degraded protein balance (DPB) and feed milk value (FMV) were not affected ($p > 0.10$) by different diets.

Table 9. Predicted truly absorbed metabolizable protein in dairy cows and feed milk value of the total mixed ration with blend-pelleted products (BPP) * in lactating dairy cows *.

	Dietary Treatments					Contrast *p*-Value
Items	**Control**	**BPPCR**	**BPPCN**	**SEM**	***p*-Value**	**Control vs. (BPPCR + BPPCN)**
Rumen-synthesized microbial protein truly absorbable in the small intestine (g/kg DM)						
MCP_{RDP}	82.25	84.91	83.47	4.611	0.92	0.99
MCP_{TDN}	94.25	95.72	95.14	0.522	0.25	0.82
AMCP	52.64	53.76	53.42	2.659	0.96	0.95
Rumen-undegradable feed protein truly absorbable in the small intestine (g/kg DM)						
RUP	39.01	36.64	39.39	3.437	0.84	0.73
ARUP	5.98	5.81	6.34	1.052	0.78	0.55
Rumen endogenous protein truly digested in small intestine (g/kg DM)						
ECP	10.93	11.01	11.07	0.048	0.24	0.15
AECP	4.37	4.40	4.43	0.019	0.29	0.19
Total truly absorbed (metabolizable) protein in the small intestine (g/kg DM)						
MP	62.99	63.96	64.19	2.238	0.92	0.81
Degraded protein balance (g/kg DM)						
DPB	−14.45	−13.06	−14.06	5.328	0.98	0.96
Feed milk value (kg milk/kg feed)						
FMV	1.28	1.30	1.31	0.044	0.91	0.77

* Control diet: common barley-based diet used in western Canada; BPPCR: basal diet supplemented with 12.2%DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); BPPCN: basal diet supplemented with 13.2% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4% + pea 23.8% + lignosulfonate 4.8%DM); blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea and lignosulfonate; SEM: standard error of mean; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; MCP_{TDN}: microbial protein synthesized in the rumen based on available energy; AMCP: truly absorbable rumen-synthesized microbial protein in the small intestine; RUP: rumen-undegradable protein in the small intestine; ARUP: truly absorbable rumen-undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: total metabolizable protein; DPB: degraded protein balance; FMV: feed milk value, kg milk/kg feed.

4. Discussion

Canola meal is widely used as a protein source for lactating dairy cows' rations in North America. In recent years, carinata meal (from bi-energy/bio-fuel processing), a new protein feed, has been introduced in feedlot diets and dairy heifers (Guidotti 2018 [32,33]. However, there has been no study on the effect of feeding this new feed to lactating cows in

terms of ruminal fermentation characteristics, degradation kinetics and intestinal digestion, or in feed milk value.

Carinata and canola meal have been shown to a have high ruminal digestion of protein [2–5,34], thus, it is essential to slow down their ruminal digestion in the rumen by applying a heat treatment (pelleting or extrusion) or using feeding additives (i.e., lignosulfonate and tannins). A previous study by Guevara-Oquendo et al. [2] reported that blend-pelleted products based on carinata meal would exhibit a higher nutritive value relative than blend-pelleted products based on canola meal [15].

Thus, in the current study, blend-pelleted products based on a new co-product—carinata meal (lignosulfonate 4.8% + carinata meal 71.4% + pea 23.8%)—were used in a comparison with blend-pelleted products based on the conventional co-product—canola meal (lignosulfonate 4.8% + canola meal 71.4% + pea 23.8%). It was found that a heat treatment and the addition of lignosulfonate could reduce the proportion of ruminal degradable protein, thereby increasing the available essential amino acids to the mammary gland for milk synthesis [25,35].

The CNCPS model was updated to estimate the ruminal digestion of CHO and protein. The updated system used different degradation rates and passage rates. The PA1 and PA2 had higher passage and degradation rates, while the PB2 had a slow degradation rate and passage rate similar to CB3 in carbohydrates fractions [20]. The RUP tended to be higher in BPPCN than in the other diets, while the TRDC tended to be lower in the BPPCN compared with different diets. The lower TRDC would be due to the higher lignin content in BPPCN, as reported in the previous chapter. The higher TRDC would, in turn, enhance the ruminal bacteria growth and increase the MCP in dairy cows.

There were no effects of different diets on the in situ ruminal digestion of DM, CP, starch, and NDF. Adding lignosulfonate to BPPCN or BPPCR did not increase the bypass protein as expected. These findings were not in agreement with an earlier study by Wright et al. [34], who reported an improvement in the N bioavailability in dairy cows and milk production. The higher N utilization was due to improving the bypass protein and a reduction in urinary N excretion in dairy cows. These findings were not in line with Wright et al. [34] or Neves et al. [36], who found that the RDP and total tract digestibility decreased after adding lignosulfonate to canola meal. The TMR based on BPPCR exhibited similar BCP to other diets (averaging 38%CP). In contrast, Guevara-Oquendo [15] reported a lower BCP in BPPCR relative to BPPCN (50 vs. 63%CP). Carinata meal was also reported to contain lower BCP than canola meal in previous studies (25 vs. 40% CP [4,5,37]). Using the omasal sampling technique to evaluate the ruminal digestion and omasal nutrient in beef cows, Guidotti [32] reported similar ruminal DM, OM, NDF, and CP for feedlot fed diets based on canola meal or carinata meal. The same author also reported a similar RDP (averaging 65% of N intake). In the literature, there was no report on ruminal digestion and intestinal digestion in lactating cows fed TMR based on carinata meal versus canola meal. The obtained results in this study indicate that BPPCR has the same digestion behavior as BPPCN in dairy cows.

Using the in vitro technique to evaluate the intestinal digestion of dietary treatments, the result in the current study showed that the predicted intestinal digestion of protein was the same for all diets. The total ruminal and intestinal digestions of DM, CP, and starch were similar for all diets. These results were in agreement with the total tract digestibility results for the same dietary treatment in the lactating ration shown in the previous study. The MP content of a feed is the total protein content that contributes to milk production. The total MP in the NRC model is the summation of AECP, ARUP, and AMCP [28]. The results of the current study showed that the MP content was the same for all dietary treatments (averaging 74 g/kg DM). These results were not in line with Guevara-Oquendo et al. [15], who reported high MP values for BPPCR relative to BPPCN (231 vs. 163 g/kg DM). The findings in the current study would explain the non-significant results found in production performance in the previous study.

5. Conclusions

Blend-pelleted products based on carinata meal as a new co-product from the bio-fuel processing industry are equal to the pelleted products based on conventional canola meal as a protein source for dairy cattle. Blend-pelleted products based on carinata meal are similar to the pelleted products based on canola meal in rumen fermentation, rumen degradation kinetics, intestinal digestion, and feed milk values in lactating dairy cows. It is safe to use the new bio-fuel processing co-product—carinata meal—as an alternative source of protein for dairy cows.

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