Final Report

Identification

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Project Title: Comparing the protein source and frequency of supplementation of forage intake, competitive feeding interactions, and nutrient utilization for beef cattle fed low-quality

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Supporting Organization: Saskatchewan Stock Growers Association
Saskatchewan Canola Development Comm.

Public Summary of Outcomes and Benefits to Canada

Forages in western Canada may not contain sufficient protein to meet nutrient requirements thereby requiring producers to utilize protein supplements to augment the dietary protein level. The major challenge addressed with this project was to determine if canola meal (CM; a major high protein byproduct produced in western Canada arising from canola oil production) could be a suitable protein source for beef cattle and whether feeding frequency influenced the response of cattle. We did this by comparing canola meal to dry distillers' grains (DDGS; a high protein byproduct from the ethanol industry) and comparing daily verses feeding every second day.

There were clear advantages associated with providing a protein supplement for beef heifers fed low quality forage such that those provided with either CM or DDGS had greater forage intake and growth than those without protein supplementation. Feeding the protein supplement on alternate days increased competitive feeding interactions suggesting that daily feeding is more desirable to ensure consistent nutrient delivery to each animal. Moreover, CM was more cost-effective than DDGS as a protein supplement. These results highlight that a locally produced byproduct which is unsuitable for human consumption can add significant value to diets for beef cattle fed low-quality forages.

The canola industry in Canada provides a major economic stimulus and important food sources including canola oil. Canola oil has also been investigated for use to produce biodiesel. These results highlight that cattle can up-cycle byproducts that are not suitable for humans and convert them into a high-quality meat source. Our study further showed that canola meal is a high-quality protein source and may be more economically favourable than distillers' grains.

Progress Towards Objectives/Milestones

To what extent were the objectives of the grant achieved? Rate your answer on a scale from 1 to 7.

Not at all			Somewhat		To a great extent	
○ 1	○ 2	○ 3	○ 4	○ 5	● 6	○ 7

REPORT ON PROGRESS

Brief Description of the Overall Objectives of the Grant as Awarded

The primary objectives were to compare use and digestion kinetics for **LO-DDGS** (low-oil dry distillers' grains) and canola meal (**CM**) as protein supplements for beef cattle consuming low-quality forage, and to determine whether the source of protein and the frequency of protein supplementation (daily vs. alternate days) affect nutrient utilization and feeding behaviour of cattle.

Description of the Progress Made Towards these Objectives as a Result of the Grant

The experimental activities were in alignment with the proposed methodology as two studies were conducted. All experimental procedures were reviewed and approved by the University of Saskatchewan Animal Research and Ethics Board (protocol 20100021) prior to initiation of the study and are described as follows.

Heifer Management

Five Hereford-cross yearling heifers from the University of Saskatchewan Goodale Research Farm (Floral, SK, Canada) were transported to the University of Saskatchewan Beef Cattle Research Unit (Saskatoon, SK, Canada) and fit with a 7.6-cm ruminal cannula (model 3C; Bar Diamond Inc., Parma, ID). Approximately 18 d after surgery, the cannulas were replaced with a 9-cm cannula (model 9C; Bar Diamond Inc.) and heifers were provided approximately 1 mo to recover. Prior to the start of the study, an additional 5 Hereford-cross heifers and 5 Black Angus-cross heifers were transported to the University of Saskatchewan Livestock Research Barn (Saskatoon, SK, Canada). Upon arrival, BW was recorded, and heifers were allocated 1 of 5 outdoor pens (8 m × 12 m) to minimize variation in BW within a pen. The final pen allocation consisted of one cannulated heifer and two non-cannulated heifers per pen. Heifers had access to both an indoor and outdoor area with water available ad libitum. Forage and the mineral and vitamin supplement were provided in a feeder outside at 0800 and 1500 h daily, and the supplemental protein source was provided at 0800 h using a wood-constructed box fit with removable artificial turf (EZ Lawn Pro, 15' x 6'7", EZ-grass, Saskatoon Landscape Store, SK, Canada) to facilitate collection of refusals when placed directly on the ground as described by Kelln et al. (2019). Heifers provided with supplement daily were provided a 1 m^2 feeding area and those provided the supplement on alternate days were provided with twice the supplement feeding area. The feeding box for the protein supplement was placed on the ground in the pen at the time of feeding and was maintained in the pen for the subsequent 2 h.

Experimental Design and Dietary Treatment

The study was conducted as a 5 × 5 Latin square design balanced for carry-over effects. The overall study design included a 2 × 2 + 1 factorial treatment arrangement. The main factors in the factorial arrangement consisted of the supplement type (CM versus LO-DDGS) and the frequency of supplementation (daily versus every second day; **D** vs. **A**). A negative control (no CP supplementation) was also included.

Each period was 21 d in duration and consisted of 15 d for dietary adaptation followed by 6 d for data sample collection. Within the data and sample collection

portions, the first 4 d were used for behavioral assessment and the last 2 d were used for ruminal fluid collection. Samples of forage, vitamin and mineral supplement, and the protein supplements were also collected throughout the 6-d sampling period. Body weights were measured on d 20 and 21 of each period and body condition score (BCS) was evaluated by three experienced individuals blinded to the experimental treatments. The average of the 3 observer scores were used as the final BCS for each heifer in each period. All heifers were fed a basal (control) diet of mature grass hay and the vitamin and mineral supplement (Table 1). Diets were formulated using the CNCPS model using the Nutritional Dynamic System (RUM&N Sas, Reggio Emilia, Italy). The vitamin and mineral supplement was provided daily and formulated to provide 0.4 mg of melengestrol acetate (Zoetis, Parsippany, New Jersey) for each heifer daily. Dietary treatments included a forage-based control diet (CON) in which heifers did not receive any CP supplementation. The other treatments included the same mature grass hay-based diet with the provision of canola meal provided once daily (CM-D), canola meal provided once every second day (CM-A), LO-DDGS provided once daily (LO-DDGS-D), or LO-DDGS provided once every second day (LO-DDGS-A). Both supplements were fed in pellet form and were manufactured using a 11-mm Muyang pellet mill die (Crawfordsville, Indiana). The production rate of the canola meal pellets were 1.8 tonnes/h and LO-DDGS pellets were manufactured at 1.5 tonnes/h with resulting pellet durability indices at 97.5 and 86.8%, respectively. The control diet, by design, did not meet predicted net energy or metabolizable protein requirements for yearling heifers, but treatments provided with CM or LO-DDGS were formulated to meet metabolizable energy and metabolizable protein requirements to achieve 0.5 kg/d of BW gain (Table 1). Given the differences in CP among LO-DDGS and CM (Table 2), differing quantities of LO-DDGS and CM were provided to ensure total dietary CP was similar.

The quantity of forage provided for each pen was adjusted to target refusals equating to 10% of the weight offered to ensure ad libitum intake. The supplement heifers receiving the alternate-day treatments were provided with twice the quantity of supplement every second day relative to those fed on a daily basis. This approach ensured that the same amount of supplement was provided to each treatment group when considering a 2-d cycle, with the exception of the control.

Feed Sampling

During the 6-d sampling period, representative samples of hay were collected daily and samples of refused hay were collected every second day to determine average forage DMI. Representative samples of the CM and LO-DDGS pellets were also collected daily. Following supplementation for the CM and LO-DDGS treatments, the uneaten pellets were collected to determine pellet DMI as described by Kelln et al. (2019). All feed ingredient samples were pooled by type (forage, vitamin and mineral supplement, CM pellet and LO-DDGS pellet) and particle size was analyzed using the Pennsylvania State Particle Size Separator (PSPS) using sieves with aperture openings of 19, 8, and 4 mm, and a bottom pan. Refusals of forage and pellet were pooled by pen and period and analyzed using the PSPS to determine the sorting index (Leonardi and Armentano, 2003). Briefly, the sorting index was calculated by expressing the proportion of each particle size refused relative to the theoretical propotion that would be refused should no sorting occur. Pooled feed samples were dried in a forced-air oven at 55°C until a constant

weight was achieved for DM determination. Dried grass hay samples were ground to pass through a 1-mm sieve using a hammer mill (Christy and Norris Ltd, Chelmsford, UK). Dried LO-DDGS, CM, and vitamin and mineral supplements were ground to pass through a 1-mm sieve using a Retch ZM 200 cyclone mill (Haan, Germany). The ground samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD) and were analyzed for DM, OM, CP, aNDFom, ADF, starch, Ca and P as described by Rosser et al. (2013) and ether extract determined by acid hydrolysis (AOAC 2005; Methods 922.06 and 954.02).

Behavioral Assessment

Video cameras were used to record feeding behavior while consuming the protein supplement. Recording started at 0800 h on d 16 and 18 of each period. Behavior was recorded only on days when all CM and LO-DDGS treatments received their supplement. The meal duration for each heifer and the total eating time for each pen were determined. The eating rate for each pen was determined by dividing the amount of pellet consumed in each pen by the total time that heifers in a pen spent eating the pellet. The amount of time when one, two or three heifers consuming supplement at a time were also determined. Competitive interactions while eating the pellet were assessed. Competitive interactions were defined as the occurrence of one heifer displacing another during supplement consumption. For competitive interactions, the heifer acting as the aggressor and the reactor were recorded. These data were used to determine the proportion of times a single heifer acted as the aggressor and reactor.

Ruminal Fermentation

On d 20 and 21 of each period, ruminal digesta samples were collected at 0800, 1400, 2000, and 0200 h from the five cannulated heifers in each pen. These sampling days represented 1 d where CM and LO-DDGS treatment groups received their pellet and 1 d where the alternate day supplemented treatments did not receive their pellet. Three 250-mL samples were taken from the ruminal fluid-ruminal mat interface in the cranial, central, and caudal regions. The three samples were then pooled, and the resulting 750-mL ruminal digesta sample was strained through two layers of cheesecloth. Two 10-mL aliquots of ruminal fluid were transferred with one added to 2 mL of 25% (wt/v) metaphosphoric acid while the other was added to 2 mL of 1% (wt/v) sulfuric acid for determination of SCFA concentration and ruminal ammonia-N concentration, respectively. Samples were immediately placed on ice and ruminal fluid samples were stored at -20ºC after collection until being analyzed. Ruminal SCFA concentration were separated and quantified using gas chromatography (Agilent 6890, Mississauga, ON, Canada) as described by Khorasani et al. (1996). The phenol-hypochlorite method was used to determine ruminal ammonia-N concentration as described by Broderick and Kang (1980).

From d 16 to d 21, ruminal pH was measured using an indwelling pH measurement system to obtain 96 h of data as described by Penner et al. (2006). The pH system was standardized using pH buffers 7 and 4 at 39ºC, and then inserted into the ventral sac of the rumen. On d 1 of the following period, the pH systems were removed, washed, standardized as described above, and data were downloaded. The data were then converted from mV to pH using linear regression and assuming linear drift between the

starting and ending regression (Penner et al., 2006). The minimum, mean, and maximum pH was determined, and the data were sub-divided to represent days when the supplement was provided and days for when the LO-DDGS and CM treatments in the alternate-day supplementation frequency did not receive supplementation.

Statistical Analysis

Data were summarized into 2 data sets. The first data set included all 5 treatments and data were analyzed as a 5 × 5 Latin square design using the MIXED PROC of SAS (SAS version 9.4, SAS Institute, Inc, Cary, NC, USA). Pen was always considered as the experimental unit, treatment was considered as a fixed effect, and pen and period were included as random effects. This model was used to compare the CON to all other treatments using a single contrast statement and this model was used for normality testing using visual appraisal of residual plots and the Shapiro-Wilk test. No outliers were detected, and data and residuals were normally distributed. A second data set was generated that did not include the control treatment. Data within this data set were analyzed as a 2 × 2 +1 factorial treatment design with the main effect of protein source, frequency of supplementation, and the 2-way interaction. The random effect of pen and period were included in the model.

For ruminal pH, ruminal SCFA concentration, and ruminal NH_3-N concentration, data were divided into days where all CM and LO-DDGS treatments received their supplement (day of supplement) and days where only the daily supplement frequency received their supplement (day without supplement). The data were analyzed using the same approaches as described above with the exception that data for SCFA and NH_3-N concentration also included the fixed effect of time and the 2- and 3-way interactions. For this analysis, time was considered a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian information criterion for each variable was used. For all analysis, effects were considered significant when $P < 0.05$ and means were separated using the Tukey's test.

Justification for any Deviations from the Original Objectives

In the original proposal we planned for an *in situ* study to evaluate disappearance rates of disappearance for the CM and LO-DDGS prior to and after pelleting. This study was attempted, and the methodology is described below. However, approximately 30% of the bags failed to hold their seal and residue was lost. Hence, we did not have sufficient replication to trust the data. We were willing to re-run the incubations; however, our storage room and the samples within were infested with beetles. As a consequence, we were unable to complete this portion of the study. That said, the in vivo study completed did provide information on ruminal fermentation and provided insight into ruminal digestion, albeit rates could not be determined.

The LO-DDGS and canola meal was sourced to evaluate in situ digestibility of DM, OM, CP, and NDF and to produce LO-DDGS and CM pellets for the *in situ* study. The original lot (prior to pelleting and after pelleting) was sub-sampled (n=3). Prior to in situ incubation, pelleted samples were crushed using a mortar and pestle while, samples of the original LO-DDGS and CM were not altered. Samples were weighed into nylon bags (7 g/bag) and incubated in the rumen of beef heifers fed a low-CP forage diet (Table 1) to evaluate digestion kinetics as described by the NRC (2001). Incubation times were

0, 2, 4, 8, 12, 24, 30, 48, 72, 96, and 120 h using the sequential-in all-out process. Bags were incubated with a maximum of 40 nylon bags/heifer using 5 ruminally cannulated heifers. Placement of bags in each heifer was randomized. Following incubation, bags were rinsed 5 times in cold water with 1 min/wash and dried at 55°C until achieving a constant weight. The same washing procedure was applied for bags not incubated in the rumen (0 h bags). Unfortunately, 60 of the 198 bags had seals that failed and hence, samples and data from this study were not utilized.

Description of the Scientific Significance of the Results Achieved

Effect of Supplementation

Results of this study confirm that providing supplemental protein when fed a protein deficient diet stimulates forage intake and average daily gain. While this finding was expected, it demonstrates that the model imposed to evaluate protein source and frequency of protein supplementation was suitable. Previous research has clearly reported that protein-deficient diets limit forage intake, cattle ADG, and ruminal fermentation (Caton et al., 1988, Delcurto et al., 1994, Bodine et al., 2001). This is in part due to high NDF concentrations found in many forage species (Baron et al., 2004) and their effect to stimulate rumen fill (Buxton, 1996). In the present study, we provided a low-quality grass hay containing 66.34% NDF. Buxton (1996) also explains that high ADF concentrations also present in low-quality forages (Glover et al., 2004) limits forage digestibility which was also replicated in this study with grass hay containing 43.26% ADF concentration.

Protein supplementation of cattle grazing low-quality forages has been reported to correct for deficiencies and stimulate forage intake, ADG, and DM digestibility (Bodine et al., 2001, Wickersham et al., 2004, Bohnert et al., 2011). Furthermore, Li et al. (2013) reported that total SCFA and ammonia-N concentration increased with CM or DDGS supplementation to cattle fed low-quality forage. Providing a protein supplement in the current study showed increased heifer performance, forage DMI, and ruminal fermentation relative to the control diet which was deficient in CP and high in NDF and ADF. Providing a diet deficient in CP resulted in low ADG (0.45 kg/d), forage DMI, and low fermentation activity confirming the need for protein supplementation demonstrated in other studies previously mentioned.

Comparing CM and LO-DDGS

There has been little research conducted to directly compare the effects of canola meal and DDGS as protein supplements for grazing beef cattle fed low-quality forage (Li et al., 2013). This study aimed to determine the differences in using the two sources as protein supplements and their effects on cattle performance, DMI, feeding behavior and ruminal fermentation. In dairy cattle, protein source had an effect on forage DMI when comparing canola meal with soybean meal (Huhtanen et al., 2011); however, Li et al. (2013) found no differences in dietary DMI between canola meal supplementation and corn or wheat DDGS supplementation. Results from this study also found no differences in forage DMI between protein supplements but did show a difference in pellet DMI. However, as previously mentioned, protein supplements were fed at different levels in the diet and thus the differences in pellet intake are an artifact of the experimental model.

Despite no change for DMI, a protein source × frequency interaction was detected for ADG as LO-DDGS when fed daily had the greatest ADG relative to the control

compared to LO-DDGS and CM when fed every second day. The difference in ADG between protein sources is affected in part by the difference in the ether extract concentration of the CM (2.96%) and LO-DDGS (8.50%). Greater ether extract concentration supplies more energy to those supplemented with LO-DDGS than those supplemented with CM. Heifers supplemented with LO-DDGS daily had greater ADG than heifers supplemented with LO-DDGS in an alternate-day pattern because they received a more stable nutrient supply by being supplemented on a daily basis rather than being protein deficient every second day.

Part of the aim of this study was to address the differences in supplement feeding behaviour between canola meal and LO-DDGS. Although past studies have observed the effects of supplementation on grazing behavior (Sarker and Holmes, 1974, Krysl et al., 1993), little research has been conducted to observe supplement intake behavior and competitive interactions. Longer meal times for LO-DDGS-supplemented groups can be explained by the differences in levels of supplementation between CM treatments and LO-DDGS treatments. This also explains why the time that one and three heifers spent eating pellets were longer for LO-DDGS treatments than for CM treatments. Eating rates were not different between heifers consuming CM and LO-DDGS suggesting that both protein supplements were readily consumed by heifers.

Canola meal supplementation in dairy (Huhtanen et al., 2011, Martineau et al., 2013, Mustafa et al., 2015) and feedlot diets (Nair et al., 2015) has been reported to have positive effects on lactational and growth responses, respectively. However, little research has been conducted to investigate its effects on ruminal fermentation in grazing beef cattle consuming low-quality forage, especially in comparison to DDGS supplementation. Li et al. (2013) reported no differences in total SCFA concentration and the proportion of acetate in backgrounded heifers supplemented with either canola meal or corn DDGS. Results from this study also found that protein source did not affect total SCFA concentration on the day of supplementation, but CM treatments resulted in higher proportions of acetate and isobutyrate compared to LO-DDGS treatments. Additionally, on the day of no supplementation, total SCFA concentration was greater for CM when fed daily than all other treatments suggesting that when LO-DDGS is fed daily it results in similar ruminal fermentation activity levels in comparison to both CM and LO-DDGS fed in an alternate day pattern on the day when supplement was not received. Consistent with Li et al. (2013), the current study showed greater ruminal ammonia-N concentrations with CM treatments compared to LO-DDGS treatments which suggests that canola meal is more readily degraded in the rumen, driving rumen fermentation and acetate production. The lower proportions of butyrate for CM treatments compared to LO-DDGS treatments is likely a result of driving fermentation in the rumen towards acetate production.

Effect of the Frequency of Supplementation

Greater forage DMI has previously been observed in ruminants fed CP supplementation daily compared to ruminants fed CP supplementation three times per week (Beaty et al., 1994) or fed a high CP diet for two days followed by a low CP diet for two days (Doranalli et al., 2011). However, Klein et al. (2014) reported no differences in forage intake in cattle fed DDGS daily compared to cattle fed DDGS three times per week. Results from this study showed no differences in average forage DMI between heifers

supplemented daily and heifers supplemented every second day. These results suggest that there may be a difference in supplementing grazing cattle three times per week compared to supplementation every second day, and that more frequent supplementation results in greater forage intake.

The increase in meal time for alternate-day supplementation treatments compared to daily treatments is a direct result of feeding protein supplement at a higher level in an alternate-day pattern to supply the same amount of protein over two days as the daily supplementation treatments. The difference in eating rate for the alternate-day treatments and the daily treatments suggests that heifers on the day of supplementation in alternate-day treatments are exhibiting gorging behavior and extremely motivated to eat. The most surprising result from this study was the difference in heifer displacements between daily and alternate-day treatments. Competitive interactions increased when heifers were offered twice the amount of supplement (and head space) every second day compared to heifers offered the same amount of supplement every day. One possibility to explain this result is that, again, gorging behavior was exhibited resulting in increased competitiveness in heifers after protein being deficient for one day. Negative implications can be drawn from this result as alternate-day supplementation could reduce labor and machinery costs but would result in increased competitive behavior between cattle which could lead to less-aggressive, or subordinate, cattle receiving less supplement than more aggressive cattle. Differences in supplement intake within a herd could result in greater variation in performance, forage intake and ruminal fermentation between individual animals.

Although total SCFA concentration was not affected by frequency on day of supplementation, it was greater for daily treatments than for alternate-day treatments on the day where no supplementation was provided. Providing adequate CP on a daily basis increased nutrient stability of the diet and contributes to greater ruminal fermentation for heifers provided a CP supplement on the day where the alternate-day supplementation groups did not receive a CP supplement. However, the proportion of acetate was higher for alternate-day treatments than daily treatments on both day of supplementation and on day of no supplementation suggesting that increased urea recycling with alternate-day supplementation patterns drove rumen fermentation toward acetate production to a greater capacity than daily treatments. That being said, results from this study did not show a difference in ruminal ammonia-N concentration to support this theory, but based on previous work (Cole, 1996, Doranalli et al., 2011) could still be a valid explanation for these results.

In conclusion, there is a clear need for CP supplementation of heifers grazing low-quality forages as it increases forage DMI, heifer ADG, total ruminal SCFA concentration and ruminal ammonia concentration. Protein source may appear to have an effect on supplement meal-time, but differences could result from varying levels of supplement inclusion. Furthermore, protein source does not have an effect on competitive interactions among heifers during supplement intake. Canola meal protein is degraded to a greater extent in the rumen than LO-DDGS protein resulting in greater levels of ruminal ammonia-N. Frequency of supplementation does not affect forage or pellet DMI, but alternate-day supplementation leads to increased competitive behavior between heifers and faster eating rate of the supplement provided due to increased motivation to feed when feed is offered less frequently.

Dissemination of Research Results

Refereed Journal Articles Submitted :	0
Refereed Journal Articles Accepted or Published:	0
Conference Presentations/ Posters:	2
Other (Technical Reports, Non-Refereed Articles, etc.):	1
How many of the publications, conference presentations, etc. identified above were co-authored with a non-academic partner?	1

DISSEMINATION OF RESEARCH RESULTS

Refereed Journal Articles, Submitted

None

Refereed Journal Articles, Accepted or Published

None

Conferences Presentations and Posters

G.B. Penner, K. Ginther, K. Williamson, C. MacPherson, and E. Willinberg. 2019. Using canola meal pellets as a protein source for beef cattle. Saskatchewan StockGrowers Annual General Meeting. Moose Jaw, SK, June 19-21.

K. Ginther. 2019. Effect of protein source and frequency of feeding on performance of heifers fed low-quality forage. Department of Animal and Poultry Science Mini-Conference. March 15, Saskatoon, SK.

Other (Technical Reports, Non-Refereed Articles, etc.)

G.B. Penner. 2019. Using canola meal pellets as a protein supplement for beef cattle. Beef Business. May edition. Pp 22-23.

Table 1. Ingredient and chemical composition for non-supplemented heifers (Control) and heifers provided pelleted low-oil dry distillers grain (LO-DDGS) or canola meal.

	Dietary Treatment		
Variable	Control	LO-DDGS	Canola Meal
Ingredient, % DM			
Grass Hay	95.9	86.9	89.0
Mineral and vitamin mash	4.1	3.7	3.7
Protein Supplement			
Canola Meal	0.0	0.0	7.3
LO-DDGS	0.0	9.4	0.0
Chemical composition (DM basis)			
DM, %	95.22 ± 1.26	95.21 ± 1.03	95.21 ± 1.20
OM, %DM	90.72 ± 0.40	91.10 ± 0.36	90.97 ± 0.37
CP, %DM	6.98 ± 0.70	9.41 ± 0.58	9.41 ± 0.65
ADF, %DM	41.79 ± 1.25	39.19 ± 1.13	40.15 ± 1.17
NDF, %DM	64.17 ± 1.11	60.63 ± 0.98	61.45 ± 1.08
Starch, %DM	1.22 ± 0.10	1.56 ± 0.07	1.47 ± 0.05
Ether extract, %DM	1.89 ± 0.34	2.51 ± 0.32	1.97 ± 0.32
Calcium, %DM	0.90 ± 0.12	0.83 ± 0.11	0.88 ± 0.11
Phosphorus, %DM	0.33 ± 0.05	0.38 ± 0.04	0.37 ± 0.04

Table 2. Chemical composition of treatment ingredients.

	Ingredient		
Variable	Grass Hay	Canola Meal	LO-DDGS
Chemical Composition			
DM, %	95.28 ± 1.28	95.16 ± 1.68	95.16 ± 1.36
OM, %DM	93.06 ± 0.10	93.63 ± 0.13	94.65 ± 0.21
CP, %DM	6.98 ± 0.74	40.28 ± 0.77	32.86 ± 0.68
ADF, %DM	43.26 ± 1.32	18.82 ± 0.37	14.14 ± 0.59
NDF, %DM	66.34 ± 1.12	26.20 ± 0.88	26.44 ± 0.54
Starch, %DM	0.66 ± 0.15	4.80 ± 0.80	4.82 ± 0.22
Ether Extract, %DM	1.90 ± 0.36	2.96 ± 0.29	8.50 ± 0.24
Calcium, %DM	0.41 ± 0.07	0.81 ± 0.01	0.17 ± 0.02
Phosphorus, %DM	0.13 ± 0.01	0.95 ± 0.02	0.84 ± 0.02
Pellet Quality			
Fines, %[z]	-	4.15 ± 2.39	9.48 ± 5.89

[z]Determined as the particles that fall through a 4-mm sieve.

Table 3. Effect of dietary treatment on average daily gain, body condition score, dry matter intake, and supplemental pellet refusal.

	Treatment								
		DDGS		CM			*P* value		
Variable	Control	Daily	Alternate	Daily	Alternate	SEM	Con vs. all	Protein	Frequency	Protein × frequency
BW, kg	425	430	427	425	425	13	0.52	0.23	0.54	0.57
ADG, kg/d	0.20	0.78[a]	0.46[b]	0.47[b]	0.62[ab]	0.11	< 0.001	0.32	0.27	0.007
BCS	4.35	4.4	4.5	4.25	4.35	0.13	0.81	0.055	0.17	1.0
DMI, kg/hd/d										
Forage	6.54	6.74	6.77	6.92	6.85	0.54	0.018	0.19	0.75	0.59
Pellet	-	0.77	0.93	0.60	0.59	0.08	-	0.012	0.31	0.42
Pellet refusal, g/d	-	105	50	83	66	22	-	0.90	0.19	0.47

[abc]For the interaction of protein source and frequency of supplementation, means within a row with uncommon superscripts indicate means that differ ($P < 0.05$).

Table 4. Effect of protein source and frequency of supplementation on meal time, eating rate and competitive interactions at the time of supplementation.

	Treatment					P value		
	DDGS		CM					
Pellet eating characteristics	Daily	Alternate	Daily	Alternate	SEM	Protein	Frequency	Protein × frequency
Cumulative meal time, min/pen/d	51.68	87.33	40.41	58.48	9.68	0.004	0.001	0.12
Meal time, min/heifer/d	17.23	29.11	13.47	19.49	3.23	0.004	0.001	0.12
Eating rate, g/min	55	71	66	79	11	0.053	0.006	0.63
Time with all heifers eating, min/d	5.68	9.77	4.49	7.58	0.78	0.039	< 0.001	0.51
Time with two heifers eating, min/d	2.73	8.21	2.76	4.4	1.9	0.28	0.061	0.28
Time with one heifer eating, min/d	28.18	39.45	19.89	26.46	6.28	0.009	0.021	0.48
Heifer displacements, no./d	8	12	4	12	3	0.36	0.022	0.41
Aggressor, % of displacements[z]	57	66.8	64.3	74.6	10.7	0.33	0.20	0.97
Subordinate, % of displacements[y]	54.2	62.3	66.1	64.2	9.4	0.45	0.73	0.59

[z]The % of displacements caused by the heifer with the most displacements.

[y]The % of displacements of the heifer being displaced the most.

Table 5. Effect of dietary treatment and supplementation day on rumen fermentation parameters.

		Treatment					P value			
		DDGS		CM						
Variable	Control	Daily	Alternate	Daily	Alternate	SEM	Con vs all	Protein	Frequency	P × F[z]
Day of Supp.										
Total SCFA, m*M*	86.08	92.27	90.41	91.98	89.84	3.59	0.010	0.82	0.27	0.94
Acetate %	70.38	68.94[b]	68.97[b]	69.14[b]	70.36[a]	0.57	< 0.001	< 0.001	0.006	0.008
Propionate %	19.58	20.34	19.48	20.17	19.38	0.60	0.30	0.44	< 0.001	0.84
Isobutyrate %	0.49	0.42	0.45	0.50	0.51	0.05	0.69	0.077	0.62	0.75
Butyrate %	8.63	9.28[b]	9.96[a]	8.97[bc]	8.69[c]	0.18	< 0.001	< 0.001	0.11	< 0.001
Valerate %	0.42	0.48	0.49	0.52	0.47	0.05	0.009	0.62	0.50	0.22
Caproate %	0.07	0.10[b]	0.15[a]	0.12[a]	0.07[b]	0.04	0.019	0.39	0.026	< 0.001
Ammonia-N, mg/dL	0.41	0.59	0.66	0.94	0.88	0.17	0.002	0.012	0.99	0.57
Mean pH	6.38	6.45	6.53	6.43	6.42	0.09	0.68	0.45	0.69	0.52
Min pH	5.86	5.37	6.18	5.89	6.20	0.30	0.90	0.48	0.16	0.51
Max pH	6.78	6.80	6.87	6.73	6.66	0.08	0.58	0.028	0.97	0.20
Day of No Supp.										
Total SCFA, m*M*	85.74	89.95[b]	89.10[b]	95.95[a]	85.09[b]	3.47	0.005	0.47	< 0.001	< 0.001
Acetate %	69.33	68.5[c]	71.28[a]	69.56[b]	70.93[a]	0.50	0.036	0.072	< 0.001	< 0.001
Propionate %	19.85	20.41	18.37	20.33	18.66	0.61	0.12	0.58	< 0.001	0.31
Isobutyrate %	0.47	0.48	0.37	0.38	0.46	0.07	0.15	0.90	0.71	0.006
Butyrate %	9.36	9.53[a]	9.04[b]	8.69[b]	9.03[b]	0.18	0.10	0.001	0.56	0.002
Valerate %	0.43	0.49	0.37	0.50	0.38	0.07	0.80	0.75	< 0.001	0.89
Caproate %	0.10	0.09	0.09	0.10	0.08	0.43	0.32	0.97	0.51	0.32
Ammonia-N, mg/dL	0.42	0.65	0.70	0.79	0.77	0.14	< 0.001	0.17	0.83	0.61
Mean pH	6.33	6.42	6.58	6.40	6.49	0.08	0.44	0.51	0.16	0.62
Min pH	5.83	5.39	6.30	5.61	6.23	0.30	0.89	0.83	0.065	0.71
Max pH	6.77	6.82	6.81	6.72	6.64	0.08	0.57	0.042	0.40	0.53

Table 6. Effect of time and supplementation day on rumen fermentation parameters.

	Time (h)					P Value		
Variable	800	1400	2000	200	SEM	Time	Protein × Time	Frequency × Time
Day of Supp.								
Total SCFA, m*M*	85.08	96.32	88.84	94.64	3.59	< 0.001	0.72	0.13
Acetate %	71.65	66.59	68.73	70.43	0.57	< 0.001	0.011	0.039
Propionate %	18.45	21.10	20.24	19.59	0.60	< 0.001	0.73	0.17
Isobutyrate %	0.63	0.51	0.41	0.34	0.05	< 0.001	0.32	0.84
Butyrate %	8.04	10.77	9.75	8.35	0.18	< 0.001	< 0.001	0.24
Valerate %	0.37	0.65	0.49	0.46	0.05	< 0.001	0.95	0.16
Caproate %	0.10	0.11	0.08	0.11	0.04	0.15	0.95	0.100
Ammonia-N, mg/dL	1.04	0.93	0.55	0.55	0.17	0.002	0.009	0.89
Day of No Supp.								
Total SCFA, m*M*	87.51	92.14	87.89	92.55	3.47	< 0.001	1.00	0.14
Acetate %	71.28	67.97	69.73	71.29	0.50	< 0.001	0.64	0.028
Propionate %	18.95	20.45	19.55	18.81	0.61	< 0.001	0.67	0.20
Isobutyrate %	0.60	0.33	0.31	0.44	0.07	< 0.001	0.15	0.78
Butyrate %	7.97	10.33	9.59	8.40	0.18	< 0.001	0.13	0.68
Valerate %	0.43	0.49	0.41	0.40	0.07	0.060	0.91	0.066
Caproate %	0.08	0.08	0.09	0.10	0.43	0.75	0.86	0.58
Ammonia-N, mg/dL	1.19	0.67	0.51	0.54	0.14	< 0.001	0.39	1.00