

Replacing alfalfa hay with a novel alfalfa leaf pellet product (ProLEAF MAX) and/or alfalfa stems (ProFiber Plus) in the diet of developing dairy heifers alters dry matter intake, but does not negatively impact growth or development

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ABSTRACT

Alfalfa is a commonly grown forage in the Intermountain West region of the United States and is often included in the diet of dairy cattle. Alfalfa provides a variety of different nutrients, but the nutrient content of alfalfa varies depending on factors such as the soil, region, cutting, and climate. However, alfalfa leaves tend to have less variation in their nutrient content than alfalfa stems. Fractionating alfalfa may be one way to improve control of nutrients provided when developing a ration for developing dairy heifers. The purpose of this study was to determine whether including fractionated alfalfa in the diet impacts the growth or conception rates of developing dairy heifers. Heifers were allocated to one of three treatments: a control group fed a typical diet (CON: n = 8), a diet that replaced alfalfa with fractionated alfalfa leaf pellets and alfalfa stems (ProLEAF MAX + ProFiber Plus; PLM + PFP; n = 8), or a diet that replaced alfalfa with alfalfa stems (PFP; n = 8) for 85 d. Heifers were fed individually twice daily and weight, hip height (HH), and wither height (WH) were recorded every 14 d. Additionally, blood was collected every 28 d, and conception rates were recorded at the end of the trial. Heifers receiving the PFP diet consumed less dry matter (P = 0.001) than the CON treatment. Analyses were then conducted to determine nutrient intake and heifers receiving the PFP diet also consumed less neutral detergent fiber (P = 0.02), acid detergent fiber (P = 0.02), crude protein (P = 0.001), and net energy for maintenance (P = 0.001) than heifers consuming the CON diet; however, no differences (P > 0.10) were observed between heifers fed the CON and PLM + PFP diets. Analysis of body weight gain over the feeding period showed no difference (P = 0.52) among heifers consuming the different treatment diets. Additionally, treatment did not affect average daily gain (P = 0.49), gain:feed (P = 0.82), HH gain (P = 0.20), or WH gain (P = 0.44) among heifers receiving different diets. Treatment x time altered (P < 0.001) blood urea nitrogen when analyzed as a repeated measure. Total feed cost was lowest (P < 0.001) for the PFP diet and cost of gain tended (P = 0.09) to be increased for the PLM + PFP diet compared to the CON diet. Overall, these data indicate that including alfalfa stems in a developing heifer diet may decrease dry matter intake, lower input costs, and increase profitability, without negatively impacting growth.

LAY SUMMARY

In the Intermountain West of the United States, alfalfa is a common feedstuff in the diet of dairy cattle. Alfalfa is a relatively nutrient-dense forage, but nutrient content varies with soil, region, cutting, and climate. However, alfalfa leaves have a less variable nutrient content than alfalfa stems. Fractionating alfalfa into leaves and stems could improve control of nutrients when formulating diets. The purpose of this research was to determine whether including fractionated alfalfa in developing dairy heifer diets impacts growth or conception rate. To test this, 24 Holstein heifers of similar age and weight were fed a typical diet, a diet including alfalfa leaf pellets and alfalfa stems, or a diet including alfalfa stems had a lower dry matter intake than those receiving the typical ration. Total feed costs were lowest for heifers getting alfalfa stems, and the cost of gain tended to be highest for heifers getting both alfalfa leaves and stems. Overall, these data show that including alfalfa stems in a developing heifer diet may decrease dry matter intake, lower input costs, and increase profitability, without negatively impacting growth.

Key words: dairy, developing heifers, fractionated alfalfa, growth, reproduction

INTRODUCTION

Dairy heifers are responsible for replacing older and less productive cows in the milking herd. Of the total cost of raising dairy heifers, feed typically accounts for 50% of those costs (Zwald et al., 2007). The overall goal of dairy heifer development is to raise heifers that are efficient and profitable. In order to achieve the development of efficient and profitable dairy heifers, the impacts of nutritional management on heifer development must be understood (Akins, 2016). Heifers should be fed a diet that targets high feed efficiency, but also minimizes the risk of over conditioning (Akins, 2016), as excess adipose deposition in the

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mammary gland during development can lead to reduced milk production in the future (Sejrsen et al., 1982). Alfalfa is a relatively nutrient-dense feedstuff that is commonly included in the diets of beef and dairy cattle. However, the alfalfa plant varies in nutrient content depending on the state of the soil, region, cutting, and climate. Alfalfa ranges from 12% to 20% crude protein (CP) and 20% to 28% crude fiber (Balliette and Torell, 2015), depending on the plant's stage of maturity. As the alfalfa plant matures, the alfalfa leaf protein content declines slightly, while the alfalfa stem protein content declines to a much greater extent (Sheaffer et al., 2000). The digestibility and neutral detergent fiber (NDF) concentration of alfalfa leaves decreases as the alfalfa plant matures, while the NDF and acid detergent fiber (ADF) concentrations of alfalfa stems increase as the plant matures (Fick and Onstad, 1988). The variability of the nutrient content that is seen in the alfalfa plant can make it difficult to develop a total mixed ration (TMR) for production livestock. Therefore, it is important to investigate the effects of including fractionated alfalfa in the diets of livestock animals. The objective of this study was to examine the effects of including a novel alfalfa leaf pellet product (ProLEAF MAX, Scoular, Omaha, NE; PLM; Pratt and Jackson, 2018) and/or alfalfa stems (ProFiber Plus, Scoular; PFP; Pratt and Jackson, 2018) in the diet on growth, conception rate, and economic cost of developing dairy heifers when compared to heifers fed a typical diet for the Intermountain West region of the United States. We hypothesized that heifers consuming diets that included fractionated alfalfa products would have altered growth and development when compared to heifers that consumed diets that included alfalfa hay.

MATERIALS AND METHODS

Heifers

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Utah State University (approval number IACUC-2821) and heifers were cared for in accordance with the Live Animal Use guidelines (FASS, 1999). Twenty-four Holstein heifers that were approximately 8 mo of age and similar in weight $(341 \pm 9.7 \text{ kg})$ were selected from two different herds, the Utah State University Caine Dairy Farm herd (n = 12) and the Ropelato Dairy Farm herd (n = 12). Heifers were housed in a covered barn in individual pens with free choice access to water. Pretrial, heifers were subjected to a 14 d adjustment period. Over the course of the adjustment period, all heifers were fed a typical diet that included the following ingredients (dry matter [DM] basis): alfalfa hay (28%), oat hay (18.3%), barley straw (0.1%), corn silage (37.2%), steam-flaked corn (14.6%), and a developing heifer mineral supplement (1.8%). The nutrient composition of all forages included in the adjustment diet and treatment diets can be seen in Table 1. Initially, heifers were stratified by body weight and randomly assigned to one of the three different treatment groups and fed their assigned experimental diet for 85 d. The three different treatment diets included oat hay, barley straw, corn silage, steam-flaked corn, a developing heifer supplement, and either alfalfa hay (control; CON; n = 8), alfalfa leaf pellets and alfalfa stems (PLM + PFP; n = 8) in place of alfalfa hay, or alfalfa stems (PFP; n = 8) in place of alfalfa hay. The nutrient composition of the different treatment diets can be found in Table 2. ProLEAF MAX was included in a treatment diet with PFP to ensure that there was an adequate amount of long-stem forage in the diet so rumen

 Table 1. Nutrient composition of forage sources used in the experimental diets fed to growing dairy heifers

ltem	Forage source'						
	Alfalfa hay	PLM	PFP	Corn silage	Oat hay	Barley straw	
DM, %	91.3	89.9	88.5	29.2	93.3	92.8	
Analysis, DM basis							
СР, %	13.4	24.1	12.1	8.0	9.9	3.6	
ADF, %	40.8	26.4	50.1	29.2	36.3	54.4	
aNDF, %	50.1	30.2	59.6	48.0	57.9	77.6	
NFC, %	27.5	31.0	22.7	36.1	20.2	8.2	
TDN, %	53.3	65.4	49.9	65.5	56.6	48.3	
NE _m , Mcal/kg	1.1	1.4	1.5	1.4	1.1	0.8	
NE _g , Mcal/kg	0.5	0.8	0.9	0.9	0.6	0.4	
Ash, %	9.1	13.2	6.4	6.6	12.0	10.6	
Calcium, %	1.1	2.2	0.7	0.2	0.3	0.2	
Phosphorus, %	0.3	0.3	0.2	0.2	0.3	0.1	
Magnesium, %	0.4	0.4	0.2	0.2	0.2	0.1	
Potassium, %	2.6	3.3	2.3	1.4	3.0	1.5	
Sodium, %	0.03	0.10	0.16	0.01	0.37	0.02	
Iron, mg/kg	166.0	627.5	94.0	104.0	208.0	116.0	
Manganese, mg/kg	25.0	57.0	17.5	68.0	82.0	39.0	
Zinc, mg/kg	16.0	24.0	16.17	27.0	26.0	25.0	
Copper, mg/kg	7.0	9.0	9.3	6.00	4.0	7.0	

¹Treatment diets consisted of alfalfa leaf pellets (PLM) and/or alfalfa stems.

²ADF, acid detergent fiber; aNDF, neutral detergent fiber alpha amylase; CP, crude protein; DM, dry matter; NE_m , net energy for maintenance; NE_g , net energy for gain; NFC, non-fiber carbohydrates; TDN, total digestible nutrients.

Table 2. Composition and nutrient density of treatment diets fed to growing heifers

Item	Treatment ¹				
	CON	PLM + PFP	PFP		
Composition of treatment diets					
Feed, % DM					
Alfalfa hay	27.98	_	_		
PLM	_	22.41	_		
PFP	_	12.07	24.70		
Oat hay	18.29	_	11.02		
Barley straw	0.12	13.08	_		
Corn silage	37.19	33.77	40.50		
Steam-flaked corn	14.60	16.88	21.37		
Heifer supplement ²	1.82	1.78	1.82		
Urea	—	—	0.61		
Nutrient density of treatment diets					
DM, %	58.55	58.50	56.87		
Analysis, DM basis ³					
CP, %	11.30	11.20	11.42		
ADF, %	27.35	28.00	29.37		
aNDF, %	39.45	40.22	42.07		
NFC, %	38.94	37.74	37.71		
TDN, %	64.48	63.88	64.48		
NE _m , Mcal/kg	1.46	1.43	1.46		
NE _g , Mcal/kg	0.88	0.84	0.88		
Ash, %	8.52	9.06	7.19		

¹Treatment diets consisted of the following ingredients as a percent of DM: oat hay, barley straw, corn silage, steam-flaked corn, a heifer supplement, and either alfalfa hay (CON; n = 8), alfalfa leaf pellets (PLM) and alfalfa stems (PFP; PLM + PFP; n = 8), or alfalfa stems (PFP; n = 8) and were fed to developing dairy heifers for 85 d.

²The guaranteed analysis for the heifer supplement is as follows: 4.6% crude protein, 1.0% crude fat, 2.6% crude fiber, 8.7% calcium, 0.3% phosphorus, 180 mg/kg copper, 11 mg/kg selenium, 425 mg/kg zinc, and 326.6 mg/kg Monensin.

³ADF, acid detergent fiber; aNDF, neutral detergent fiber alpha amylase; CP, crude protein; DM, dry matter; NE_m , net energy for maintenance; NE_g , net energy for gain; NFC, non-fiber carbohydrates; TDN, total digestible nutrients.

health was not compromised. All diets were balanced to include similar amounts of forage. Each of the three diets that were fed was formulated to be isocaloric and isonitrogenous using Agricultural Modeling and Training Systems Software (Groton, NY). Although treatment diets in the present study were all balanced to be isocaloric and isonitrogenous, posttrial nutrient composition analysis of the diets revealed that there were slight differences between the nutrient composition of the diets that were initially balanced and the actual nutrient compositions of the treatment diets that were delivered to heifers. Diets were mixed every 2 d and fed twice daily at 0800 and 1600 hours. All feed ingredients for the diets were weighed, loaded into a commercial mixer, and mixed for approximately 15 min to ensure a homogenous mixture of all ingredients.

Daily dry matter intake (DMI) was measured using the clean-bunk management system as previously described (Pritchard and Bruns, 2003). In brief, feed offered was weighed and the following day individual bunks were cleared out and feed refusals were weighed so that daily adjustments in feed offered could be made. To ensure that animals were receiving their feed *ad libitum*, feed bunks were managed to achieve 0.9 kg of refusals per day. The PFP diet required the addition of urea to ensure that all diets were isonitrogenous, thus, urea was top-dressed to all bunks receiving the PFP diet and manually mixed. Every 14 d, weight, hip height

(HH), and wither height (WH) were recorded at approximately 0700 hours. Every 28 d, blood was collected at approximately 0700 hours and serum was harvested for blood urea nitrogen (BUN) analysis. Feed efficiency calculated as gain to feed (G:F) was determined by assessing DMI and average daily gain (ADG). Intake of NDF, ADF, CP, and net energy for maintenance (NE_m) were calculated for each of the different treatment diets using the average DMI over the 85 d feeding period and average nutrient compositions from the feed analyses that were completed on diets.

Harvest and Preparation of Fractionated Alfalfa Products

A leaf combine (Pratt and Jackson, 2018), which is carried on a self-propelled vehicle, was used to fractionate the alfalfa plant into alfalfa leaves (later pelleted to form PLM) and alfalfa stems (PFP). The leaf combine strips the alfalfa leaves from the standing alfalfa plant and the leaves were conveyed onto a trailer. The alfalfa leaf fraction was then transported by truck to a drying facility for curing and processing into pellets. The alfalfa stem fraction was cut, conditioned, and windrowed to be baled when dry. Of note, the hay utilized in the control diet did not come from the same field as the PLM and PFP products, as the fields utilized for PLM and PFP were designated for these products only.

Feed Sample Analysis

Samples of alfalfa hay, oat hay, barley straw, corn silage, steam-flaked corn, PLM, and PFP were collected pretrial and analyzed at a commercial lab (Cumberland Valley Analytical Services, Waynesboro, PA) for nutrient composition. Samples of the PLM and PFP were collected each time a new batch was delivered. A sample of the TMR was collected three times per week immediately following feed delivery to the bunks and urea top-dressing. Urea was top-dressed to ensure that accurate concentrations were provided and to achieve the desired isonitrogenous diets. The urea had to be top-dressed as we wanted to ensure it was mixed in consistently for all the heifers, which would have been difficult using a commercial mixer for the number of heifers used in this study. All samples were frozen at -20 °C and sent for nutrient composition analysis after completion of the trial. A composite TMR sample for each diet each week was sent for analysis at a commercial lab (Cumberland Valley Analytical Services).

Blood Urea Nitrogen

Blood was collected every 28 d into 10.0 mL, 16×100 mm BD Vacutainer serum blood collection tubes. After coagulation, blood was cooled at 4 °C, and serum was extracted the next day after a 15 min centrifugation at a speed of 100 xg. Serum was stored at -20 °C for future analyses. Blood urea nitrogen was determined using a commercially available BUN detection kit (Urea Nitrogen Colorimetric Detection Kit, Invitrogen, Carlsbad, CA) following the manufacturer's specifications. Results of the BUN detection assays were analyzed on a BioTek Synergy H1 plate reader (BioTek, Winooski, VT) using the program Gen5 version 2.09. Intraassay CV: 3.84%. Inter-assay CV: 1.69%.

Conception Rate

Once heifers reached an approximate average of 55% of their mature body weight (340 to 363 kg), they were synchronized using a 5 d CIDR synchronization protocol and bred to sexed semen from the Holstein bull, DIAMONDBACK, using single service artificial insemination. A licensed veterinarian checked heifers for pregnancy using ultrasound imaging 30 d after artificial insemination.

Economic Analysis

To make an economic comparison of the treatments, the total feed cost (TFC) and cost of gain (COG) were calculated and compared for each treatment. Total feed costs were calculated for each heifer as the summed product of total feed (kg as-fed) and the weighted cost (\$/kg) of each individual feed component where the weights were equal to the percentage of each feed component in the total diet. Five-year historical average prices (LMIC, 2020) were used for all feed components other than the PLM, PFP, urea, and mineral supplement for which actual prices were used. As corn silage, oat hay, and barley straw prices are seldom collected and reported, the following assumptions were relied upon to estimate the prices of those feedstuffs within the diets: corn silage price (\$/ ton) = $9 \times \text{corn price}$ (\$/bu), oat hay price = $2/3 \times \text{grass hay}$ price, and barley straw price = $1/3 \times \text{grass}$ hay price. Once the TFC for each treatment was calculated, comparisons were made with the intuitive understanding that greater relative TFC indicates additional expenses associated with feeding. Total feed costs were divided by total weight gain to calculate COG. The COG estimated in the present study

considers marginal changes to the cost of feed and represents the feed cost (\$) that could be expected to achieve one additional kilogram of weight gain.

Statistical Analysis

Individual animal (heifer) served as the experimental unit in all analyses. Initially, heifers were stratified by weight to ensure no differences in starting weights were present and then allocated to one of the three different treatment groups. All data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Treatment was the main effect and farm origin was included as a random effect in the model. The following variables were analyzed to provide averages across the entire 85 d feeding period: weight, total weight gain, ADG, G:F, DMI, aNDF intake, ADF intake, CP intake, NE_m intake, HH gain, WH gain, TFC, and COG. Blood urea nitrogen was analyzed as a repeated measure over time (day) using a REPEATED statement in the model. A Tukey-Kramer adjustment was used to determine treatment differences by separation of the least square means. A $P \le 0.05$ was considered significant and a P > 0.05 and $P \le 0.10$ were considered to be a tendency. Results are presented as the least squares mean ± standard error of the mean.

RESULTS

Heifer Performance

No differences between heifers receiving different treatment diets were found in body weight gain (P = 0.52), ADG (P = 0.49), or G:F (P = 0.82; Table 3). However, the average daily DMI was different (P = 0.001) between heifers receiving different treatments such that heifers consuming the PFP diet consumed less (P = 0.001) than those receiving the CON diet. No differences (P > 0.10) were noted between heifers consuming the PLM + PFP diet and the other treatments (Table 3). Additionally, nutrient intake was analyzed for NDF, ADF, CP, and NE_m to discern how nutrient intake varied between heifers receiving different diets. Similar to DMI results, heifers receiving the PFP diet consumed less ($P \le 0.03$) total ADF, NDF, CP, and NE_m than the CON heifers, but no differences (P > 0.10) were found between PLM + PFP and the other two treatments (Table 3). Further, no differences were observed in HH gain (P = 0.20) or WH gain (P = 0.44) between heifers receiving the different treatment diets (Table 4).

Blood Urea Nitrogen

Blood urea nitrogen was analyzed as a repeated measure over time and demonstrated there was a treatment × time effect (P < 0.001; Fig. 1). The BUN of heifers consuming PFP declined throughout the trial, BUN of heifers consuming PLM + PFP was variable, and BUN of heifers consuming the CON diet stayed relatively consistent (Fig. 1). On d 85, heifers consuming the PLM + PFP diet had increased (P = 0.004) BUN compared to heifers consuming the PFP diet (Fig. 1). Additionally, on d 56, heifers consuming the CON diet had an increased (P < 0.05) BUN compared to heifers consuming the PLM + PFP diets.

Conception Rates

Heifers that received the PLM + PFP diet had the highest conception rates, numerically, out of the three different treatment

Item ²	Treamtent ¹			SEM	P-value ³
	CON	PLM + PFP	PFP		
BW gain, kg	92.9	87.8	82.1	6.6	0.52
ADG, kg	1.24	1.19	1.08	0.09	0.49
G:F	0.11	0.12	0.12	0.01	0.82
DMI, kg	9.88 ^a	9.07 ^{ab}	7.97 ^b	0.32	< 0.01
NDF intake ³ , kg	3.90ª	3.65 ^{ab}	3.35 ^b	0.13	0.02
ADF intake ³ , kg	2.70ª	2.54 ^{ab}	2.34 ^b	0.09	0.03
CP intake ³ , kg	1.12ª	1.02 ^{ab}	0.91 ^b	0.04	< 0.01
NE _m intake ³ , kg	2.96ª	2.72 ^{ab}	2.39 ^b	0.09	< 0.01

Table 3. Effects of feeding fractionated alfalfa on heifer body weight gain, feed efficiency, DMI, and nutrient intake over the 85 d feeding trial

¹Treatment diets consisted of the following ingredients: oat hay, barley straw, corn silage, steam-flaked corn, a heifer supplement, and either alfalfa hay (CON; n = 8), alfalfa leaf pellets (PLM) and alfalfa stems (PFP; PLM + PFP; n = 8), or alfalfa stems (PFP; n = 8) and were fed to developing dairy heifers for 85 d. Values represent the least squares means.

²Intake of NDF (neutral detergent fiber), ADF (acid detergent fiber), CP (crude protein), and NE_m (net energy for maintenance) were calculated for each of the different treatment diets using the average DMI over the 85 d feeding period and average nutrient compositions from the feeding analyses. ^{3a,b}Means within a row without a common superscript represent differences (P < 0.05) between treatments over the 85 d feeding period.

Table 4. Effects of feeding fractionated alfalfa on HH and WH gain over the 85 d feeding trial

Item	Treamtent ¹	Treamtent ¹			P-value
	CON	PLM	PLM + PFP		
HH, cm	5.63	2.63	2.94	1.25	0.20
WH, cm	4.69	4.13	4.89	0.42	0.44

¹Treatment diets consisted of the following ingredients: oat hay, barley straw, corn silage, steam-flaked corn, a heifer supplement, and either alfalfa hay (CON; n = 8), alfalfa leaf pellets (PLM) and alfalfa stems (PFP; PLM + PFP; n = 8), or alfalfa stems (PFP; n = 8) and were fed to developing dairy heifers for 85 d. Values within columns represent least squares means.



Figure 1. Average BUN of heifers fed treatment diets consisting of oat hay, barley straw, corn silage, steam-flaked corn, a heifer supplement, and either alfalfa hay (control; CON; n = 8), alfalfa leaf pellets (ProLEAF MAX, Scoular, Omaha, NE) and alfalfa stems (ProFiber Plus, Scoular; PLM + PFP; n = 8), or alfalfa stems (PFP; n = 8) for 85 d. *P*-values represent the effects of treatment × time, time, and treatment when analyzed with time as a repeated measure, and values represent the least square mean ± SEM. Different superscripts represent differences (P < 0.05) between treatments.

groups (average conception rates: CON: 37.5%, PLM + PFP: 62.5%, PFP: 57.1%). Of note, although conception rates were measured and presented, statistical analysis was not completed on the conception rate due to the relatively small number of animals to measure this metric.

Feed Cost Comparison

Least square means for TFC were calculated for each treatment equal to \$123.48, \$136.52, and \$97.27 for heifers receiving the CON, PLM + PFP, and PFP treatments, respectively (Table 5). Over the 85 d trial, heifers receiving the PFP diet had a lower TFC than heifers receiving the PLM + PFP (P < 0.001) and CON diets (P = 0.001; Table 5). Heifers receiving the CON diet had a lower (P = 0.05) TFC than the PLM + PFP diet (Table 5). The least square means for COG were \$1.20, \$1.51, and \$1.11 for the heifers receiving the CON, PLM + PFP, and PFP treatments, respectively (Table 5). Over the 85 d feeding period, COG tended (P = 0.09) to be increased for heifers receiving the PLM + PFP diet when compared to heifers receiving the PFP diet. However, no differences (P > 0.10) in COG were observed between heifers receiving the CON diet and heifers receiving the PLM + PFP or PFP diets.

DISCUSSION

The present study aimed to determine whether the inclusion of fractionated alfalfa in the diet of developing heifers impacts heifer development. The nutrient composition of the alfalfa plant is variable, and by fractionating the plant into the leaf and stem portions, producers and/or nutritionists could help control for some of the variability that is present in this plant. The inclusion of alfalfa leaf products, such as alfalfa leaf meal (ALM), in the diet of livestock animals has been studied previously and found to be a beneficial feed. ALM has an energy content that is similar to a high-quality hay or small grain

Item ²	Treatment ¹	Treatment ¹			P-value
	CON	PLM + PFP	PFP		
TFC	\$123.26ª	\$136.52ª	\$97.27 ^b	6.68	<0.001
COG	\$1.20	\$1.51	\$1.11	0.13	0.09

Table 5. Effects of feeding fractionated alfalfa on TFCs and COG of developing dairy heifers

a-bMeans within a row without a common superscript represent differences (P < 0.05) between treatments throughout the 85 d feeding period.

¹Treatment diets consisted of the following ingredients: oat hay, barley straw, corn silage, steam-flaked corn, a heifer supplement, and either alfalfa hay

(CON; n = 8), alfalfa leaf pellets (PLM) and alfalfa stems (PFP; PLM + PFP; n = 8), or alfalfa stems (PFP; n = 8) and were fed to developing dairy heifers for 85 d. Values represent the least square mean \pm SEM.

²TFC (\$) is the total cost of all feeds in each treatment fed for the 85 d feeding period; COG is equal to the TFC/total weight gain.

silage (DiCostanzo et al., 1999) and a CP content of 22% to 28% (Jorgensen et al., 1997; DiCostanzo et al., 1999), which is two to three times the CP content of alfalfa stems (Mowat et al., 1965; Mowat and Wilton, 1984; Albrecht et al., 1987). However, alfalfa stems can also be utilized as a feed source in livestock diets. In contrast to alfalfa leaves, alfalfa stems have a high fiber content, similar to that of straw, but have twice the CP content of straw (Su et al., 2017). As such, alfalfa stems could be used to replace straw in livestock diets while providing more protein, which is especially important for growing animals and lactating dairy cattle.

In the present study, heifers were fed one of three different treatment diets that were initially balanced to be isocaloric and isonitrogenous. After completion of the feeding trial, samples of treatment diets collected throughout the trial were analyzed for nutritional composition. These post-trial analyses showed differences in nutrient content between the three treatment diets. The differences observed in the nutrient compositions between the treatment rations are most likely due to variability in nutrient content between the different forage sources and variability in mixing the ration. The CON and PLM + PFP diets had less ADF and NDF when compared to the PFP diet. This is important to note because the variations that were present between the different treatment diets could have influenced the results in the present study.

To the authors' knowledge, this is the first study that has investigated the effects of feeding fractionated alfalfa to developing heifers. Previously, our group published a paper that examined the effects of feeding fractionated alfalfa on the growth and performance of finishing beef steers (Motsinger et al., 2021). Several other studies have looked at the effects of feeding ALM or alfalfa stem haylage and, as such, the results of the present study will be compared to those studies.

In the present study, no differences in body weight gain, ADG, HH gain, or WH gain were observed between heifers consuming the three different treatment diets. However, Su et al. (2017) performed a similar study investigating the effects of feeding alfalfa stem haylage on the performance of Holstein dairy heifers. In their study, Su et al. (2017) fed three different treatments (corn silage and alfalfa haylage; corn silage, alfalfa haylage, and alfalfa stem haylage; or corn silage, alfalfa haylage, and wheat straw) to Holstein heifers and measured weight gain and growth (as measured by HH, WH, heart girth, and body condition score). Similar to the results of the present study, Su et al. (2017) observed decreased weight gain and decreased growth when alfalfa stem haylage was included in the diet. In contrast to the results of the present study, in a similar study conducted by our research group with finishing beef steers, increased body weight gain and ADG were observed in steers receiving the PFP diet when compared to the steers receiving the PLM + PFP diet (Motsinger et al., 2021). However, the treatment diets in the present study utilized higher concentrations of PLM and PFP in the treatment diets than those used previously by Motsinger et al. (2021). In the present study, the PLM + PFP diet consisted of 22.4% PLM and 12.1% PFP and the PFP diet consisted of 24.7% PFP on a DM basis, while in the study completed by Motsinger et al. (2021), the PLM + PFP diet consisted of 13.8% PLM and 5.8% PFP and the PFP diet consisted of 14.0% PFP on a DM basis. The results of the present study also contrasted those of Gossett and Riggs (1956) whom observed improved weight gain and daily weight gains in steers that consumed a finishing beef steer diet consisting of low-quality prairie hay, cottonseed meal, and ground milo grain that was supplemented with varying amounts (7%, 14%, or 21% of the diet DM) of ALM when compared to the diet without ALM supplementation. Of note, all diets fed in the study completed by Gossett and Riggs (1956) were, overall, formulated to be isocaloric and isonitrogenous. However, unlike the present study, Gossett and Riggs (1956) did not include alfalfa in their control diet. In the present study, including PLM in the diet did not improve heifer growth; however, findings from previous studies indicate that supplementation of alfalfa leaf products has the potential to improve weight gain and growth in cattle. As such, additional research needs to be completed to determine the effects of different alfalfa leaf products and inclusion rates relative to heifer growth.

While no differences were seen in heifer growth, decreased DMI was observed in heifers consuming the PFP diet when compared to CON heifers. These differences could be explained by the greater fiber content in the PFP diet when compared to the other two treatment diets. However, because DMI was lower, the calculation of total nutrients consumed showed these heifers received less total NDF, ADF, CP, and NE than heifers receiving the CON diet. The relatively high ADF and NDF in the PFP diet could have limited DMI, which resulted in lower total nutrient intake. In contrast to the present study, a previous study conducted by our research group in finishing beef steers found that steers receiving the PFP diet had the highest DMI after d 42 and the highest body weight gain throughout the feeding trial when compared to the other two treatments (CON and PLM + PFP), but there were no differences in G:F between the different treatments (Motsinger et al., 2021). However, Gossett and Riggs (1956) observed improved feed efficiency in steers that received a diet that was supplemented with ALM when compared to the steers that received no supplemental ALM. In the present study, PLM + PFP did not result in improved DMI when compared to CON, however, DiCostanzo et al. (1999) demonstrated that ALM has the potential to improve DMI of finishing beef steers when constituting 12% (DM) of the diet. An additional study analyzed the effects of including alfalfa stem havlage in the ration and found no differences in DMI of Holstein dairy heifers when alfalfa stem haylage was used to dilute a basal diet that consisted of corn silage and alfalfa haylage, but no differences between alfalfa stem haylage and straw diet treatments were observed (Su et al., 2017). Additionally, in beef heifers fed a corn-based diet that replaced soybean meal with ALM, no differences in DMI were observed (Zehnder et al., 2010). Previous studies indicate that including alfalfa leaf products (ALM or PLM) in the diet of finishing beef steers results in improved DMI (DiCostanzo et al., 1999; Motsinger et al., 2021) and subsequent weight gain (Motsinger et al., 2021). However, in the present study, when PLM + PFP was included in the diet for developing dairy heifers, improvements in DMI or growth were not observed. This finding warrants future research on feeding alfalfa leaf products to developing dairy heifers. Additionally, feeding PFP in the diets of developing dairy heifers results in decreased DMI. However, it is important to note that no previous studies have looked at including alfalfa stems in the diet of cattle at any stage of production, just alfalfa stemlage. As such, further research is needed to determine the ideal concentrations of PLM and PFP in the diet of developing dairy heifers.

Measurement of BUN levels is used to assess the protein status of an animal (Hammond, 1997) and lean tissue anabolism (Smith and Johnson, 2020). Generally, if cattle are consuming diets that are isonitrogenous at similar rates, decreased serum urea nitrogen is an indication that protein is being incorporated into lean tissue (Smith and Johnson, 2020). In the present study, analysis of BUN concentration as a repeated measure demonstrated that BUN decreased over time in heifers that received the PFP diet, was variable in heifers that received the PLM diet, and stayed level in heifers consuming the CON diet. However, no differences were present in CP content between the different diets when analyzed at a commercial laboratory and as such, the decreased BUN over time in heifers that received the PFP diet was not the result of lower CP in the diet. These heifers did eat a lower amount of CP though, once calculations accounting for CP were completed. Additionally, these heifers exhibited the lowest weight gain (numerically, not statistically significant) throughout the feeding trial when compared to heifers receiving the CON and PLM + PFP diets which could indicate that heifers metabolized the protein differently, resulting in alterations in circulating metabolites, which could have impacted protein turnover. It is also important to note that the heifers receiving the PFP diet also received supplemental urea, which could have impacted nitrogen cycling. Blood urea nitrogen concentrations can also affect reproductive performance, such that plasma urea nitrogen over 19 mg/dL in cows and heifers can decrease conception (Butler et al., 1996). However, in the present study, no BUN concentrations that neared 19 mg/dL were observed and the sample size in the present study was not sufficient for assessing conception rate.

The economic results demonstrate that, as was expected after analyzing the DMI, potential cost saving can be expected through feeding PFP in the diet when compared to a diet with traditional alfalfa (CON). However, the cost per kilogram of weight gain for heifers receiving the PFP diet was not different from heifers receiving the CON diet and tended to be decreased when compared to heifers receiving the PLM + PFP diet. These results demonstrate that the

inclusion of PFP, rather than whole alfalfa, in a developing dairy heifer diet decreased DMI, while having no effect on growth measurements. As such, if PFP can be procured at a price significantly less than traditional alfalfa, cost savings can be expected in feed costs per head. Additionally, COG will not be decreased when feeding PFP to developing dairy heifers when compared to feeding a traditional alfalfa-based diet. However, the \$25.99 difference in expected feed costs $(TFC_{CON}-TFC_{PFP})$ has the potential to have a large impact on the profit of dairy producers. Given a 1,000-cow herd with a 33% turnover rate, approximately 330 replacements would be required per year. Thus, based on the data from this study, a dairy of this size may expect total cost savings of \$8,576.70 per year when feeding the PFP diet as compared to the CON when considering raising replacement heifers. Overall, additional research needs to be completed to determine the optimal concentrations of fractionated alfalfa to include in a developing dairy heifer diet that could result in decreased TFC and decreased COG.

CONCLUSION

Feed costs account for the majority of input costs required for raising dairy heifers (Zwald et al., 2007) and, therefore, it is necessary to maximize the efficiency of dairy heifer production. Through an improved understanding of nutritional management practices and their impacts, we can enhance the efficiency, productivity, and profitability of developing heifers (Akins, 2016). Overall, the findings of the present study demonstrate that the inclusion of PFP, which can be purchased at a lower price than alfalfa hay or PLM, in a developing dairy heifer diet decreases DMI, but does not negatively impact growth measurements and has the potential to decrease TFC, but does not affect COG. Through the inclusion of PFP in a developing dairy heifer diet, dairy heifer producers may be able to lower their input costs and increase the profitability of their operation. However, more research on a larger number of animals is needed to determine the optimal concentrations of fractionated alfalfa to include in diets for developing dairy heifers. Additionally, research on lactating dairy cows is needed to further investigate the effects of feeding fractionated alfalfa on dairy production.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Conflict of interest statement

The authors each declare there are no conflicts of interest associated with the data presented in this manuscript.

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