

# The Effects of Feeding Reduced-Lignin Alfalfa on Growing Beef Cattle Performance: a Preliminary Study

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## Abstract

Gene suppression techniques down-regulating specific lignin biosynthesis pathway enzymes have permitted the development of an alfalfa variety with an altered lignin content and composition known as HarvXtra. This study was designed to compare the difference in forage nutrient quality between reduced-lignin alfalfa hay (variety HX-4114) and conventional alfalfa hay (variety WL336HQRR), and to evaluate Angus heifer performance when fed treatments of HX-4114 and WL336HQRR. Twenty-four Angus heifers (8 months of age; initial BW = 270 ± 21 kg) were selected to participate in an 84-day alfalfa hay feeding trial. Their performance was evaluated based on changes in body weight (BW), average daily gain (ADG), dry matter intake (DMI), and gain to feed ratio (G:F). Additionally, *in situ* dry matter disappearance was tested using four ruminally cannulated Hereford cows. Hay samples were collected and used to determine forage nutrient quality, leaf count, and leaf-to-stem ratio. Significance was set at  $P \leq 0.05$ . There were no differences ( $P \geq 0.05$ ) in forage nutrient quality between alfalfa treatments. Means did not differ by treatment for leaf count ( $P = 0.06$ ), but leaf-to-stem ratio was higher ( $P = 0.04$ ) for HX-4114 than WL336HQRR (1.67 vs. 1.17, respectively). There were no treatment or treatment by day interactions for BW, ADG, DMI, G:F, or *in situ* dry matter disappearance. Additionally, animal performance did not differ for growing Angus heifers consuming HX-4114 and WL336HQRR alfalfa hay. More research is warranted to determine the value of utilizing different genotypes of reduced-lignin alfalfa and their impact on the U.S beef industry.

**Keywords:** Alfalfa, Beef, Cattle, Heifer, HarvXtra, Performance, Reduced-lignin

## 1. Introduction

Gene suppression techniques used to decrease lignin content in plants focuses on down-regulating specific enzymes involved in lignin biosynthesis. The use of gene suppression technology to down-regulate the enzyme caffeoyl CoA 3-O-methyltransferase has helped foster the development of a more readily-digested variety of alfalfa known as HarvXtra (McCaslin et al., 2014). Compared to related null lines of alfalfa, HarvXtra is expected to exhibit a 150-200 g/kg reduction in lignin content and a 100-150 g/kg increase in neutral detergent fiber digestibility (NDFD) and relative forage quality (RFQ; McCaslin et al., 2014). Improvements in fiber digestibility and lignin concentration can have profound impacts on animal performance measurements such as daily gain, intake, and milk production. Casler and Vogel (1999) reported that when multiple forage species were averaged together, a 1 g/kg improvement in *in vitro* dry matter digestibility (IVDMD) improved average daily gain (ADG) in beef cattle by 3.2 g/kg. Similarly, Oba and Allen (1999) found that a one-unit increase in NDFD in forage fed to lactating dairy cattle increased dry matter (DM) consumption by 0.17 kg and increased production of 4 g/kg fat-corrected milk by 0.25 kg.

Previous studies indicate that reducing the lignin content in alfalfa increases NDFD and *in situ* digestibility when compared to related null lines (Guo et al. 2001; Reddy et al. 2005); however, published research describing the effects of feeding reduced-lignin alfalfa hay in ruminant feeding trials is lacking. One progress report created by the U.S Dairy Forage Research Center reported that feeding reduced-lignin alfalfa hay to lambs significantly improved fiber digestion by nearly 20 g/kg and increased ADG by 100 g/kg when compared to lambs consuming conventional alfalfa varieties (Mertens, 2009).

Although literature describing reduced-lignin alfalfa hay feeding trials are lacking, feeding trials utilizing other types of low lignin forages in the dairy industry indicate that reducing the lignin content improves dry matter intake (DMI), milk production, and ADG. Evidence of improved performance is demonstrated in dairy cows consuming brown midrib mutant forages (BMR) forages, which exhibit lower lignin content than their non-BMR counterparts. *In vitro* and *in situ* neutral detergent fiber (NDF) digestion trials using BMR mutant forages found that BMR forages had lower lignin content and higher NDFD than their null counterparts (Rook et al., 1977; Keith et al., 1979; Cherney et al., 1991). These studies also found that dairy cows consuming BMR varieties exhibited increased DMI, improved milk production, and better weight gain than dairy cows consuming non-BMR forages (Rook et al., 1977; Aydin et al., 1999).

The literature suggests that forages with lower lignin content, such as BMR mutant forages and reduced-lignin alfalfa, are valuable resources, and can be used to maximize animal production. On-going variety trials are looking to maximize reductions in lignin, evaluating impacts of environment on genotypes. Currently, because the development of reduced-lignin alfalfa is relatively new, there has been no research evaluating the potential of HarvXtra in beef cattle operations. Although small plot research provides evidence that reduced-lignin alfalfa is more digestible (Grev et al., 2017; Guo et al. 2001; Reddy et al. 2005), reduction in lignin content and improvement in digestibility varies between studies. This variation is caused by differences in gene selection for lignin down-regulation, environment, and harvest management. Therefore, research is necessary to determine differences in nutrient quality between reduced-lignin alfalfa varieties in small- and large-scale study settings, and subsequent animal feeding trials are necessary to validate improvements in nutrient quality associated with these reduced-lignin alfalfa varieties.

The first objective of this study was to evaluate differences in forage quality between HarvXtra alfalfa (HX-4114), a reduced-lignin alfalfa variety, and WL336HQRR, a conventional alfalfa variety. The second objective of this study was to evaluate Angus heifer performance in a feeding trial using HX-4114 and WL336HQRR alfalfa hay. Based on previously published literature describing the nutritive value of a cultivar similar to HX-4114 (Grev et al., 2017), we hypothesized that heifers consuming the HX-4114 alfalfa variety would weigh more on the final day of sampling and have higher ADG, DMI, and G:F compared to heifers consuming the WL336HQRR alfalfa variety.

## 2. Materials and Methods

All protocols for this study were reviewed and approved by the Montana State University Agricultural Animal Care and Use Committee (AACUC #2016-AA15).

### 2.1 Forage Establishment, Harvest, and Storage

The production site was located south of Townsend, MT. Two varieties of alfalfa were established on May 19, 2016, each on 8 ha; one variety of reduced-lignin alfalfa (HX-4114) and one variety of conventional alfalfa (WL336HQRR). Prior to planting, soil samples were collected to determine and apply appropriate fertilizer amounts. Alfalfa was drilled using a Great Plains 3S-3000HD double disc drill at a seeding rate of 3.6 kg pure live seed/ ha at a 1.27 cm depth, spaced 15 cm apart, into a firm, weed-free seedbed. The field was irrigated with a center-pivot that was already in place at the research site.

First and second harvests were taken on July 29, 2016 and October 10, 2016, respectively, at approximately 10% bloom. Both varieties of alfalfa were baled and twine wrapped at approximately 14 g/kg moisture into large square bales using a Krone 1290 HDP baler. Bales from the first harvest were not used in the feeding trial, so production and baling information for the first harvest will not be discussed further. The second harvest, which was used for the feeding trial in this research study, produced alfalfa yields of 2,741 and 2,682 kg/ ha, respectively, for the WL336HQRR and HX-4114 varieties. Average bale weight for both varieties was 609 kg (DM basis) and average bale dimension was 0.9 x 1.2 x 2.4 m for each bale. Bales in this study were allowed to reach a stable moisture and temperature prior to being stacked on a well-drained site. Bales were stored uncovered at this site for one month, until they were transported to the Bozeman Agricultural Research and Teaching (BART) Farm located west of the Montana State University campus in Bozeman, Montana. Bales were unloaded and stored under a covered hay shed that consisted of one wall on the west side to protect bales from wind and precipitation.

### 2.2. Feeding Trial

Twenty-four Angus heifers (approximately 8 months of age; initial BW = 270 ± 21 kg) were utilized in a completely randomized design in an 84-day trial beginning on November 14, 2016 and ending on February 7, 2017. Heifers were stratified by BW to one of two alfalfa varieties (3 heifers/pen). There was a total of 8 pens in this study (4 pens per treatment). Each pen was equipped with a GrowSafe System (GrowSafe Systems Ltd., Alberta, Canada) to determine individual heifer intake. Heifers were fed diets consisting of 100% alfalfa at 3.40% BW, on a DM basis, once daily, and allowed *ad libitum* access to water and Easylix 12-12-12 mineral pressed blocks (Hubbard Feeds, Mankato, MN). Hay was not chopped or altered in any way for feeding. Heifers were fed at 3.40 g/kg BW, on a DM basis, because it was found that heifers would consume an unreasonable amount of hay if allowed *ad libitum* access. The number of bales available for use in this study were limited and it was necessary to limit to intake in order to have enough hay to meet the prerequisite 84-day recommendation for a valid feeding trial. Ort collection was implemented only on the days when the heifers left unwanted feed behind. Orts were collected into small buckets, examined, and weighed. Orts

were minimal and generally consisted of crushed leaves and dust, with the appearance of stems being negligible.

Heifers were acclimated to their respective treatments 10 days prior to the start of the trial. No data were recorded during this time. Heifers were weighed immediately prior to the start of the trial on day -1 and 0, and every 27 and 28 days thereafter. Unshrunk weights were taken for two consecutive days, and individual BW were averaged to obtain individual BW by period. Sampling occurred on days -1, 0, 27, 28, 55, 56, 83, and 84. Body weight and DMI were used to determine ADG.

### 2.3. Forage Analysis

Forage nutritive quality was analyzed using core samples taken with a Penn State electric drill mounted hay probe (Nasco Corporation, Modesto, CA). The protocol used for obtaining hay samples using an electric drill mounted hay probe is described by Filley (2005). Samples of both the HX-4114 alfalfa and the WL336HQRR alfalfa were taken during feeding one time each week (every Monday) to ensure an adequate number of samples were collected for analysis. Samples were combined bi-weekly for each treatment, totaling six composited samples per treatment ( $n = 6$ ). Samples were dried at 60°C to determine partial dry matter, which was used to calculate the amount of feed each heifer would receive on a DM basis. Samples were then ground in a Wiley mill to pass a 2-mm screen and sent to a commercial lab (Cumberland Valley Analytical Services, Hagerstown, MD) to be analyzed for DM (1<sup>st</sup> step included determining partial DM as described by Goering and Van Soest, 1970; 2<sup>nd</sup> step included placing samples into a force-air dried oven set at 105° C for 3 hours as recommended by Shreve et al., 2006), NDF (Van Soest et al., 1991), acid detergent fiber (ADF; AOAC, 2000), crude protein (CP; AOAC, 2000 and FP528 analyzer, LECO Corporation, St. Joseph, MI), acid detergent lignin (ADL; Goering and Van Soest, 1970), net energy for gain (NEg), relative feed value (RFV), relative forage quality (RFQ), total digestible nutrients (TDN), and neutral detergent fiber digestibility at 48 hours (NDFD48; Goering and Van Soest, 1970).

Separate core samples were taken (same method as above) to determine and analyze percent leaf and leaf-to-stem ratio. The leaves and stems of five core samples from each treatment ( $n = 5$ ) were hand-separated and weighed. Weights of the separated leaves and stems were used for analysis.

### 2.4. In situ Dry Matter Disappearance

Four ruminally cannulated Hereford cows (BW = 595 ± 35 kg) were fed grass hay *ad libitum* and alfalfa hay at 1% BW once daily. One Dacron bag (10 cm x 20 cm; pore size = 50 µm) per treatment containing 5 g of forage ( $n=12$ , 6 per treatment), one grass hay standard ( $n=6$ ), and one blank bag ( $n=6$ ) for each time point were placed into a 41 x 51 cm polyester mesh bag to allow for easier removal from the rumen. All 28 Dacron bags were placed into a single mesh bag, which was then placed into the rumen of each cow. The mesh bag was removed after 6, 12, 24, 30, 48, 96, and 240 hours. One dacron bag for each treatment (HX-4114, WL336HQRR, grass standard, and blank) was removed from the mesh bag at each of these

time points and rinsed in cold water until the water ran clear. Excess water was squeezed gently from each bag. Bags were then dried overnight in a forced-air oven at 60° C. Residue remaining in each bag was used for NDF analysis (Van Soest et al., 1991). This entire procedure was replicated two days following the removal of bags at 240 h. The procedure was replicated because space in the rumen is limited and it was not possible to replicate samples during the first *in situ* period. Only one Dacron bag for each treatment, at each time point, would fit in the rumen of each cow during the first *in situ* period. After replicating the procedure, *in situ* dry matter disappearance values were calculated and averaged from each *in situ* period, for each cow, at each time point, by taking the dry weight difference before and after the samples were placed in each cow.

### 2.5. Statistical Analysis

All data in this study were analyzed using SAS (version 9.4; SAS Inst. Inc., Cary, NC). Forage nutrient quality data was analyzed using the PROC GLM procedure of SAS. Individual core samples were treated as the experimental unit and treatment and day were set as fixed effects. Leaf-to-stem data and percent leaf data were analyzed using the PROC GLM procedure of SAS with core sample as the experimental unit. Feeding trial data were analyzed as a completely random design using the PROC MIXED procedure of SAS, with heifer as the experimental unit. Treatment and day were set as fixed effects, with heifer as a random effect. *In situ* data were analyzed using the PROC MIXED procedure of SAS with Dacron bag as the experimental unit. All means in this study were separated using the LSMEANS procedure and considered significant at  $P \leq 0.05$ , with a trend considered at  $P \leq 0.10$ .

## 3. Results

### 3.1. Forage Analysis

Forage nutrient quality parameters are reported in Table 1. There was a difference between treatments in DM ( $P = 0.01$ ), with HX-4114 alfalfa having 922.3 g/kg DM and WL336HQRR alfalfa having 928.7 g/kg DM. There were no significant differences ( $P \geq 0.11$ ) between treatments for CP, ADF, NDF, ADL, NEg, RFV, RFQ, TDN, or NDFD48. The HX-4114 alfalfa in this study did not meet the expected 150-200 g/kg reduction in lignin content and 100-150 g/kg increase in NDFD and RFQ. When compared to the WL336HQRR alfalfa treatment in this study, the lignin content for the HX-4114 alfalfa was only reduced by 55 g/kg and RFQ and NDFD48 only increased by 30 and 20 g/kg, respectively. Both varieties were extremely high quality, and it is possible that the high quality of the WL336HQRR control could have masked the increased nutrient quality of the HX-4114 variety.

Leaf count and leaf-to-stem ratios are reported in Table 2. There was a trend for an effect of treatment on leaf count ( $P = 0.06$ ) and a positive effect of treatment on leaf-to-stem ratio ( $P = 0.04$ ), with HX-4114 alfalfa having a higher leaf count and leaf-to-stem ratio than WL336HQRR alfalfa.



Table 1. Forage nutrient quality for reduced-lignin alfalfa hay (variety HX-4114) and conventional alfalfa hay (variety WL336HQRR) grown in Montana in 2016.

Item	Treatment		SEM	P-value
	HX-4114	WL336HQRR		
DM (g/kg)	922.3	928.7	0.13	0.01
CP (g/kg)	205.1	207.0	0.32	0.69
ADF (g/kg)	241.7	256.1	0.54	0.11
NDF (g/kg)	306.1	311.7	0.72	0.60
ADL (g/kg)	56.9	60.2	0.19	0.25
NEg, mcal/kg	0.88	0.84	0.01	0.31
RFV	213.71	207.43	5.82	0.47
RFQ	218	212	6.31	0.53
TDN (g/kg)	646.7	639.6	0.33	0.18
NDFD48 <sup>3</sup> (g/kg)	474.4	464.9	0.72	0.39

DM: dry matter; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; ADL: acid detergent lignin; NEg: net energy for gain; RFV: relative feed value; RFQ: relative forage quality; TDN: total digestible nutrients; NDFD48: neutral detergent fiber digestibility at 48 hours

Table 2. Leaf count and leaf-to-stem ratio of reduced-lignin alfalfa hay (variety HX-4114) and conventional alfalfa hay (variety WL336HQRR) grown in Montana in

Item	Treatment		P-value
	HX-4114	WL336HQRR	
Leaf count (g/kg)	6.2	5.3	0.06
Leaf: stem	1.67 <sup>a</sup>	1.17 <sup>b</sup>	0.04

<sup>ab</sup>Within a row, means without a common superscript differ ( $P \leq 0.05$ )

### 3.2. Feeding Trial

Heifers in this study were fed at 3.4% BW (DM basis), permitting each heifer nearly unrestricted access to feed. This protocol was implemented to ensure adequate intake by all animals in each pen, to warrant a practical feeding application, and to maximize use of limited resources.

Main effects of alfalfa treatment on BW, ADG, DMI, and G:F during the 84-day study period are presented in Tables 3 and 4. No treatment by day interactions were observed for any of the animal performance parameters ( $P \geq 0.05$ ). Animal BW did not differ ( $P = 0.35$ ) between treatments at d 0, 28, 56, or 84. Average daily gain, DMI, and G:F did not differ ( $P > 0.32$ ) between treatments for d 0-28, 29-54, or 55-84.

Table 3. Least squared means and standard errors for body weight across dates and treatments for Angus heifers consuming two varieties of alfalfa hay: a reduced-lignin hay (variety HX-4114) and a conventional hay (variety WL336HQRR).

Body Weight (kg)				
Day	HX-4114	WL336HQRR	SEM	<i>P</i> -value
0	270	270	11.82	0.96
28	302	300	11.82	0.68
56	338	334	11.82	0.48
84	367	363	11.82	0.43
Avg	319	317	4.18	0.93

HX-4114: reduced-lignin alfalfa; WL336HQRR: conventional alfalfa

Table 4. Least squared means and standard errors for average daily gain, dry matter intake, and gain-to-feed ratio across dates and treatments for Angus heifers being fed two varieties of alfalfa hay: a reduced-lignin hay (variety HX-4114) and a conventional hay (variety WL336HQRR).

Trait												
Average Daily Gain (kg)					Dry Matter Intake (kg)				Gain-to-Feed Ratio			
Da	R	C	S	<i>P</i> -val	R	C	S	<i>P</i> -val	R	C	SE	<i>P</i> -val
0-2	1.	1.	0.	0.34	8.6	8.6	1.	0.97	0.	0.	0.0	0.24
28-	1.	1.	0.	0.54	10.	10.	1.	0.57	0.	0.	0.0	0.88
56-	1.	1.	0.	0.87	11.	11.	1.	0.92	0.	0.	0.0	0.73
Av	1.	1.	0.	0.85	10.	9.9	1.	0.69	0.	0.	0.0	0.71

R: reduced-lignin alfalfa hay, variety HX-4114; C: conventional alfalfa hay, variety WL336HQRR



### 3.3. *In situ* Dry Matter Disappearance

*In situ* dry matter disappearance for each treatment is reported in Table 5. There were no treatment or treatment by time interactions ( $P = 0.26$  and  $0.77$ , respectively) for *in situ* dry matter disappearance (Fig. 1). These results parallel the forage nutrient quality and feeding trial research results discussed in the previous section.

Table 5. Least squared means and standard errors for *in situ* dry matter disappearance of reduced-lignin (variety HX-4114) and conventional (variety WL336HQRR) alfalfa hay grown in Montana.

Time (hours)	Disappearance (g/kg)		SEM	P-value
	HX-4114	WL336HQRR		
6	295	234	4.32	0.16
12	383	414	4.17	0.45
24	493	461	4.17	0.44
30	534	505	4.17	0.49
48	526	542	4.17	0.70
96	582	556	4.17	0.53
240	649	623	4.32	0.54

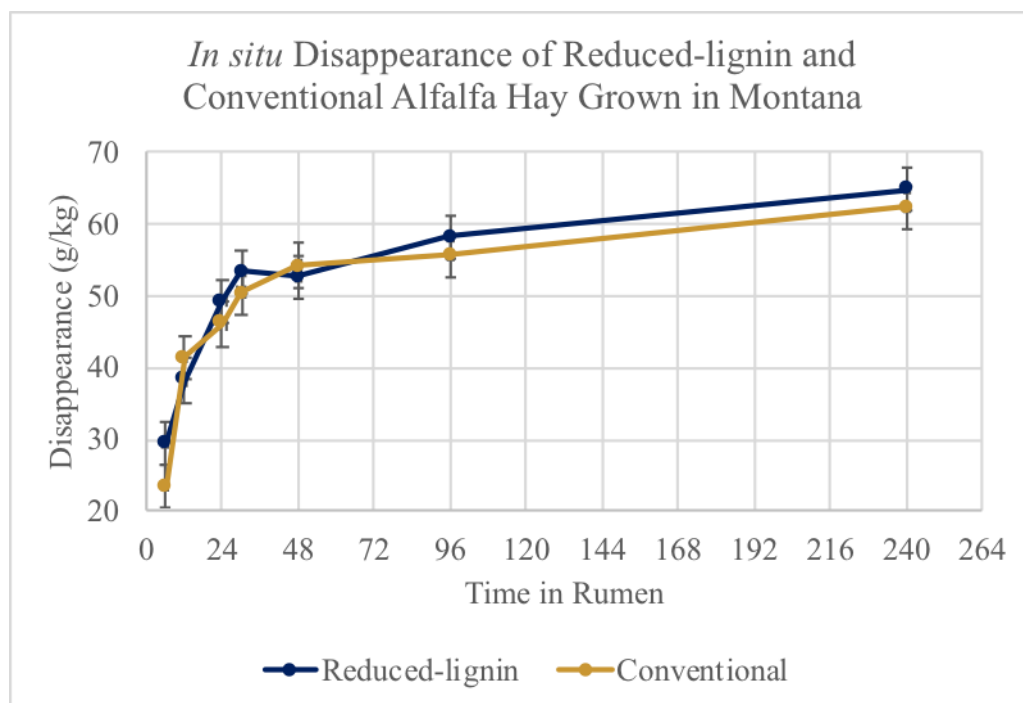


Figure 1. *In situ* dry matter disappearance at 6, 12, 24, 30, 48, 96, and 240 h for reduced-lignin (variety HX-4114) and conventional (variety WL336HQRR) alfalfa hay grown in Montana.

## 4. Discussion

### 4.1. Forage Analysis

Previous preliminary small plot research suggests that some of the available HarvXtra alfalfa varieties exhibit a reduction in lignin content and improvement in NDFD and RFQ in the environments they were tested in (McCaslin et al., 2014). It was unexpected that forage nutrient value between the HX-4114 and the WL336HQRR alfalfa varieties used in this study did not differ. Based on the results of leaf count and leaf-to-stem ratio, which were higher for the HX-4114 alfalfa variety, we would have expected the HX-4114 alfalfa treatment to have better forage nutrient quality than the WL336HQRR treatment. Leaves are lower in fiber content and more digestible than stems (Albrecht et al., 1987), so it is surprising that the HX-4114 alfalfa did not exhibit lower NDF, ADF, and ADL levels and higher RFQ, TDN, and NDFD48. This result is difficult to explain and follow-up research is needed to address this finding.

One possible explanation for the results of our forage nutrient analysis is the late timing of harvest. Alfalfa harvested later in the year is associated with lower yields and lower quality (Ottman et al., 2014). Alfalfa produced in the fall typically has smaller leaves, lower leaf percentage, and carbohydrates that are being rapidly metabolized due to higher temperatures (Ottman et al., 2014). These factors explain why fiber (NDF and ADF) values are higher and CP is lower in alfalfa during the fall. Both varieties of alfalfa in this study were rated as “premium quality” and the lignin content was considered low; however, we cannot be certain that late harvest date did not confound the results in this study. It is quite possible that late harvest date is the reason we did not see a reduction in lignin content and an increase in RFQ and NDFD in our reduced-lignin alfalfa.

Another explanation describing the forage nutrient analysis results in this study might be associated with testing the HX-4114 alfalfa variety during the establishment year. Reduced-lignin alfalfa is a relatively new technology and we are unsure as to whether reduced-lignin alfalfa will consistently exhibit reductions in lignin content and increases in RFQ and NDFD during the establishment year as in subsequent years. Comparing the results of our study to the results of other studies (Grev et al., 2017; Guo et al. 2001; Reddy et al. 2005), we find a lack of consistency in the nutrient values of reduced-lignin alfalfa varieties, which may be due to a genotype x environment effect. More research is warranted to determine differences in forage quality for reduced-lignin alfalfa during the establishment year, and during subsequent years, as well as impact of growing environment on performance.

### 4.2. Feeding Trial

One noticeable observation during this study was the lower ADG and higher DMI during d 57-84 compared to the ADG and DMI during days 0-28 and days 29-56. Average daily gain and DMI values during this time may have been the result of less efficient use of feed associated with larger animals as they mature or cold stress associated with extreme temperatures. There were extended periods of time during this study (d 57-84) where heifers

were exposed to extremely low temperatures which are outside of their thermoneutral zone (-5° and 23.9° C; Collier et al., 2012). Exposure to cold temperatures outside of their thermoneutral range causes cattle to expend extra energy in order to maintain normal body temperatures. The low and high temperatures for the month of January were -33° and 7.22° C, respectively, with an average of -12° C (Underground, 2016). Houseal and Olson (1995) concluded that animals exposed to lower critical temperatures (-13° to -47° C depending on size, sex, age plane of nutrition, and previous acclimatization) for prolonged periods of time must increase their metabolic heat production in order to maintain homeostasis. Birkelo et al. (1991) found that cold temperatures increased requirements for both body weight maintenance as well as muscle gain, and when exposed to acute cold stress their feedlot performance was decreased. Therefore, we find it likely that the heifers in our study were cold-stressed during January and February due to the prolonged low temperatures in these months which may have resulted in lower ADG.

Temperature could have been a confounding factor masking any difference in animal performance for heifers being fed the HX-4114 and WL336HQRR alfalfa varieties, although, this is doubtful based on the forage nutrient values of the two varieties. It is possible that we would have seen a difference in overall heifer performance had a different reduced-lignin alfalfa cultivar been used that was better-suited to the environment this study was conducted in.

## **5. Conclusion**

Based on the results of this study, additional research is necessary to further explore the quality and value of feeding reduced-lignin alfalfa on growing beef cattle performance. Research exploring the effects of feeding reduced-lignin alfalfa to growing Angus heifers post-seeding year and in a restricted feed setting is necessary to determine the potential impact of reduced-lignin alfalfa on the U.S beef industry.

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