EFFECT OF LEVEL AND FREQUENCY OF PROTEIN SUPPLEMENTATION ON UTILIZATION OF SOUTH TEXAS GRASS HAY

An Honors Fellows Thesis

by

GRETA RENEE MONSON

Submitted to the Honors Program Office
Texas A&M University
in partial fulfillment of the requirements for the designation as

HONORS UNDERGRADUATE RESEARCH FELLOW

April 2011

Major: Animal Science

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Approved by:

Research Advisor:	Tryon Wickersham
Associate Director for Honors Program Office:	Dave A. Louis

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ABSTRACT

Effect of Level and Frequency of Protein Supplementation on Utilization of Native South Texas Grass Hay. (April 2011)

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Reducing the frequency of supplementation is an effective means of reducing cost. However, little research has been conducted evaluating the level of supplemental protein required with infrequent supplementation. Therefore, our objective was to quantify forage utilization when grade levels of protein were delivered infrequently. Five ruminally cannulated Angus × Hereford steers (BW = 410 ± 43 kg) were used in a 5 × 4 incomplete Latin square. Steers were provided ad libitum access to native grass hay (2.3 % CP, 81.8% NDF). Treatments were control (0), 160/d, 160/3d, 320/3d, and 480/3d (mg of N/kg BW given daily (/d) versus every third day (/3d)). Supplemental protein was provided as a range cube (40.7% CP) fed at 0645h. Experimental periods were 18 d long. Forage intake was determined from d 10 through 15 to correspond with fecal grab samples collected from d 11 to d 16. Acid detergent insoluble ash was used as an internal marker to estimate fecal production. On d 16 to d 18 of each period ruminal fermentation profiles were performed. Hay OM intake was 4.41, 6.18, 5.46, 6.10, and 6.27 kg/d for 0/d, 160/d, 160/3d, 320/3d, and 480/3d. There was a linear increase ($P < 0.01$) with increasing level of protein provided every third d, but 160/d did not differ from 160/3d, 320/3d, and 480/3d ($P > 0.05$). Total OM intake responded in a similar fashion; however, 160/3d was less than 160/d ($P < 0.05$; 7.08 and 5.83 kg/d, respectively). Total digestible OM intake increased quadratically ($P = 0.06$) with increasing protein provision every third d (2.02, 2.82, 3.59, and 3.34 kg/d for 0/d, 160/3d, 320/3d, and 480/3d, respectively). When supplement was provided at 160/d total digestible OM intake was 3.30 kg/d, which did not differ from any treatment receiving supplement. There were no significant effects ($P > 0.05$) on digestibility. Reduced levels of protein supplemented infrequently may be an effective means of capturing additional cost savings.

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Above all, I want to acknowledge God because without Him, I am absolutely nothing, and I know that my whole purpose here is to bring glory to His name. He has covered all my failures, imperfections, and inadequacies through His Son, Jesus Christ, who took the punishment for all of us when He died on the cross, but then conquered death and sin by rising from the dead, allowing me to one day live with God.

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NOMENCLATURE

d	day
N	Nitrogen

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Cattle are often required to maintain themselves on low-quality forages, and do not receive the nutrients required for optimal performance. Significant quantities of research have been conducted evaluating the use of supplementation, specifically protein supplementation, to improve animal performance when the basal diet is low-quality forage. Cattle supplemented with protein have increased intake and digestion of low-quality forage (Köster et al., 1996, Wickersham et al., 2009) and this response translates into the improved ability to maintain BW and body condition (Mathis et al., 1999). [1]

Supplementation often elicits positive animal performance outcomes, however, the cost associated with this can be significant. In addition to the direct cost of the supplement, delivery of a supplement requires significant quantities of time and labor. For large cattle operations, supplementing cattle every day represents a significant expense.

Protein supplementation research has suggested that nitrogen (**N**) recycling by the ruminant may allow ruminally available N to remain at sufficient levels for long periods of time, posing an interesting question: can protein be supplemented in greater amounts, less frequently and yet provide for similar intake and digestion as an animal supplemented every day? While there has not currently been much research on this

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This thesis follows the format of the Journal of Animal Science.

subject, it would be beneficial to study the effects of these different frequencies on the intake and digestion of cattle consuming low-quality forage in order to determine the most cost effective way to supplement cattle.

Response to supplemental protein

Many cattle operations depend on low-quality forages (< 7% CP) for significant portions of the year. However, low-quality forage does not provide the proper balance of nutrients for optimum intake and digestion. Protein supplementation has been demonstrated to be effective in improving the maintenance of body weight and body condition (Mathis et al., 1999), and increasing the likelihood of reproductive success. Improvement in the ability of a cow to maintain herself in response to protein supplementation is likely driven by increased intake and digestion of low-quality forage. Protein, when supplemented to beef cattle consuming low-quality forage, has increased forage intake and total digestible OM intake (Köster et al., 1996) in a quadratic manner. Similarly, Wickersham et al. (2008) demonstrated quadratic increases in forage OM intake, total OM intake, and total digestible OM intake in response to supplemental protein. Mathis et al. (1999) also made similar observations when supplementing soybean meal to cattle consuming low-quality forage.

It has been suggested that cattle being supplemented protein less frequently and with greater amounts of supplement respond by decreasing forage intake on the day of supplementation (Wickersham et al., 2008). This is likely due to the large amounts of supplement being consumed, which may constrain forage intake; however, this has not been fully documented. Farmer et al. (2001) observed significant decreases in forage consumption on the days supplement was provided to cattle supplemented twice weekly. Cattle supplemented with protein have been reported to have increased weight gain and improved better body condition score, when compared to cattle receiving no supplement (Mathis et al., 1999; Bohnert et al., 2002b). According to research conducted by Farmer et al. (2001), all of the different supplementation frequencies resulted in a change in body condition score that was less than one during the winter months, suggesting that the frequency may not make much of a difference. Reproductive performance was also improved in supplemented cattle over un-supplemented cattle (Bohnert et al., 2002b). All of these results are highly desired by producers across the cattle industry and are important to consider when feeding a herd. Research is consistent in showing that supplementing protein has positive effects on cattle consuming low-quality forage.

Source of supplemental protein

Crude protein can be fractionated into two broad classifications, degradable intake protein (**DIP**) and undegradable intake protein (**UIP**). Ruminal microbes can degrade and utilize DIP, whereas UIP bypasses ruminal degradation (Wickersham, 2006). The response to supplemental protein is, in part, dependent on the ration of DIP:UIP (Wickersham, 2004). While DIP provides N directly to the ruminal microbes, UIP must first be absorbed, metabolized, and then recycled in order to be utilized by ruminal microbes. Cattle receiving protein supplemented in the form of DIP have a greater increase in ruminal ammonia concentration than cattle receiving UIP (Bandyk, et al., 2000; Wickersham., 2004). However, Wickersham, et al. (2004) observed a linear relationship between DIP and plasma urea N, and observed that additional N provided as UIP increases the plasma urea N a little more. Similarly, Bandyk et al, (2000) observed increases in plasma urea N with both DIP and UIP. When supplementation of DIP is increased, there is an increase in ruminally available nitrogen causing an increase in microbial N flowing to the duodenum (Köster et al., 1996; Wickersham et al., 2009). Ruminally available N is necessary for the synthesis of microbial crude protein (**MCP**). In cattle consuming low-quality forages, there is a shortage or ruminally available nitrogen, limiting MCP production. Supplementation of protein, specifically DIP, increases microbial efficiency through increasing ruminally available N (Wickersham et al., 2009). However, the increase in forage intake and utilization due to the supplementation of DIP is only visible when the forage source is deficient in DIP (Wickersham et al., 2009). When UIP is supplemented, there is a smaller increase in forage intake than with DIP (Bandyk et al., 2000; Wickersham et al., 2004). Wickersham et al. (2008) demonstrated that while UIP does not provide N immediately in the rumen, it can be used as a N source for urea which can then be recycled to the rumen and increase forage utilization.

Frequency of protein supplementation

A unique feature of the ruminant is the ability of recycled N to be incorporated into microbial crude protein (**MCP**) and utilized by the animal as a source of MP. Nitrogen from protein is not completely excreted when fed in excess, but is recycled as urea-N and brought back to the rumen at a later time, allowing this urea-N to play an important role in supplying N to the rumen and ultimately to the animal (Wickersham et al., 2009). Similar results were observed when wethers were supplemented infrequently as compared to daily. Ammonia was determined to be important as the infrequent supplementation of large amounts of protein at one time caused an excess in ruminal ammonia concentration (Bohnert et al., 2002b). The excess ammonia was likely absorbed from the rumen, metabolized into urea-N, and a portion was likely recycled back to the rumen (Bohnert et al., 2002a). Farmer et al. (2004a) observed that the amount of N retained per amount of N supplemented was comparable between steers supplemented infrequently and those supplemented daily.

Research in sheep has shown that the recycling of urea is effective enough to allow the animal to be supplemented as little as once every 6 d while not significantly affecting N retention or performance of sheep (Bohnert et al., 2002b). Research has shown that N recycling can act as a buffer, minimizing the effects of less frequent supplementation (Wickersham et al., 2009; Farmer et al., 2004a; Lapierre and Lobley, 2001). Ammonia levels within the rumen remained elevated even on the days that the infrequently supplemented cattle were not given supplement (Farmer et al., 2001). Ruminal ammonia concentrations were slightly different in the cattle supplemented daily than those supplemented infrequently, peaking at different times after supplementation, but quickly returning to normal levels (Farmer et al., 2001).

Research on supplementation frequency has yielded varying results. Some studies have shown that there is not a significant difference in supplementing daily, every other day, every three days, or even just once a week, on intake and digestion in cattle consuming low-quality forages (Schauer et al., 2005). Together, a large majority of research indicates that intake, is similar both in the infrequently supplemented cattle and the cattle being supplemented daily (Schauer et al., 2005). However, other research has shown that supplementing less frequently actually decreases intake. Some studies have show an increase in the forage intake and digestibility when cattle are supplemented more frequently (Farmer et al., 2004b; Farmer et al., 2001). This is supported by other research by Bohnert et al. (2002b) in sheep showing a similar trend in wethers supplemented protein. Wethers were observed to have a decreased forage and total intake as supplementation frequency decreased (Bohnert et al., 2002b). However, daily forage intake increased in the five days immediately following supplementation, followed by a large decrease in intake on the sixth day (Bohnert et al., 2002b).

Many contrasting results have also been reported regarding the relationship between digestibility and supplementation frequency. Differences have been observed depending on the type of supplement, percentage of protein, and type of forage being fed. Those who observed an increase in digestibility, often credit it to the improved N availability to ruminal microbes, allowing them to better utilize the forage. However, in many studies, no effect was observed between DM digestibility and supplementation frequency (Bohnert et al., 2002a; Schauer et al., 2005). According to Farmer et al. (2001), there is a slight improvement in forage intake and digestibility when supplementation frequency is increased (when all treatments provided the equal amounts of supplement weekly); however, it was not a large enough difference to significantly impact performance. For this reason, they concluded that because the results are so similar, it would be worth supplementing less frequently in order to alleviate the excess labor and equipment costs required to supplement every day. Research provided by Beaty et al. (1994) provides similar results and agrees that although supplementing daily provides the optimal digestion characteristics, it is worth giving up the small differences in performance if it would reduce the costs from labor by supplementing less frequently, such as three times weekly in this case (Beaty et al., 1994). Beaty et al. (1994) observed that it is evident that there was a large change in forage intake for the steer being supplemented daily, while the steers being supplemented less frequently did not consume nearly as much forage. However, the relationship between body condition scores of the steers with the different supplementation frequencies, which do not change much. Farmer et al. (2001) concurs with these ideas, agreeing that the differences in performance between the beef cattle are very slight between the different frequencies. Supplementing at as low a frequency as two times per week did not lead to large declines in performance of cattle (Farmer et al., 2001). These conclusions seem to match up with most of the findings found in most of the research previously done; however, there are still some results that disagree. Due to some contrasting results, the actual relationship between supplementation frequency, intake and digestion is still largely unknown. When observing data, there are many areas of contradiction between sources and results obtained, and therefore the data is difficult to consider reliable to make a decision by.

Protein supplementation is effective in many ways to improve the intake, digestion, and productivity of cattle consuming low quality forages. When supplemented, microbes within the rumen are provided with the N they need to function correctly and properly digest the forages being consumed, causing greater intake and digestion. Together, this leads to greater weight gain of cattle and also improved body condition scores (BCS), which should consequently provide a greater profit to the cattle producer.

However, the costs of supplementation are also important to consider. Supplementing cattle requires large labor and operational costs including the transportation of supplements, etc. Therefore, when supplemented every day, the overall profit for the producer is greatly reduced. This is not advantageous and therefore has led to supplementation research to try to determine possible solutions.

Based on our understanding of how the rumen works, we expect that supplementing protein less frequently (while still providing the same amount of protein overall, or possibly even less), could provide similar intake and digestion responses. This is likely attributable to urea recycling allowing excess N to be recycled to the rumen on subsequent days; therefore, allowing the forages being fed to continue to benefit from the protein supplementation even on days when supplement is not provided to the animal. While there are some results that show this to be true, other research has contrasting results making it difficult to make a recommendation.

Objective of project and hypothesis

The objective of this research project is to determine the effects of both level and frequency of supplemental protein on the utilization of low-quality forage by cattle.

CHAPTER II

MATERIALS AND METHODS

Study description

This study evaluated the effects of frequency and amount of supplemental protein on the intake and utilization of low quality hay by beef cattle. Five ruminally fistulated Angus × Hereford steers (400 ± 52 kg of initial BW) were used in a 5 × 4 incomplete Latin square (Cochran and Cox, 1957) involving 5 treatments and 4 periods. Treatments consisted of a negative control, which consisted of no supplementation (0/d), 160/d, 160/3d, 320/3d, and 480/3d , the numbers representing the amount of N (mg N per kg BW) given every day (/d) verses every third day (/3d). Supplement was provided as range cubes (40% CP; Table 1). Prior to feeding, hay was chopped through a 75mm × 75mm screen and fed at 130% of the previous 4-d average intake to ensure that access to forage did not restrict intake. Steers were housed in a continuously lit, enclosed and climate-controlled (21ºC) barn in individual stalls (2 m × 3 m) with ad libitum access to water and a trace mineral salt block. Supplements were weighed and fed once daily at 0645h. Hay was fed immediately afterward at 0700h.

The experimental protocol was approved by the Institutional Animal Care & Use Committee at Texas A&M University.

Sampling periods

Experimental periods were 18 d long with 9 d for adaptation and 9 d for sample collection. Intake and digestion were determined from d 10 to 15. Hay and supplement samples were collected from d 10 to 15, orts were collected from d 10 to d 15, and fecal grab samples were collected every 12 h between d 11 and 16. Fecal sampling was advanced 2 h every day to gather representative samples from each even hour of the day. Partial DM of hay, orts, and fecal samples was determined by drying samples at 55ºC for 96 h in a forced-air oven. Samples were ground (Wiley mill) to pass a 1 mm screen. Samples of hay and supplement were composited across days within period and ort and fecal samples were composited across days within steer for each period. Digestion coefficients were calculated using acid detergent insoluble ash (**ADIA**) as an internal marker as described by Cochran and Galyean (1994).

A rumen fermentation profile was conducted on d 16. Samples of rumen fluid were collected just prior to feeding (0h) and at 0, 3, 6, 9, 12, 18, 27, 36, 51, and 60 h post feeding with a suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh). A combination electrode was used to measure rumen pH immediately after samples were collected. Eight mL of rumen fluid was mixed with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen (-20ºC) for VFA and ammonia analysis.

A blood sample was obtained from each steer at 1900, 12 hours after feeding, on day 16 through 18 from the jugular vein into Vacutainer tubes. Tubes containing blood samples were immediately stored upright in ice and were shortly after centrifuged at 5,000× *g* for 20 minutes. Plasma was retained and frozen (-20ºC) for determination of urea-N concentration.

Laboratory analysis

Hay, supplement, ort, and fecal samples were dried for 24h at 105ºC in a forced-air oven to calculate DM and then combusted for 8 h at 450ºC in a muffle furnace to determine OM. Crude protein was calculated as 6.25 × %N, which was determined by total combustion (Rapid N cube, Elementar Americas, Inc., Mt. Laurel, NJ) on hay & supplement samples. The ANKOM-Fiber Analyzer (Ankom-Technology, Fairport, NY) was used to determine NDF and ADF of hay, supplement, ort, fecal, samples, with sodium sulfite and amylase omitted and without correction for residual ash. To determine ADIA of hay, supplement, ort, fecal, and samples, the Ankom bags from the ADF analysis were combusted for *h at 450ºC in a muffle furnace. Ruminal VFA were determined by OLC as described by Vanzant & Cochran (1994). Colorimetric determination of ruminal ammonia (Broderick and Kang, 1980) and plasma urea (Marsh et al., 1956) were made using a UV/UIS IDU 730 UV/UIS Spectrometer, (Bechman Coulter, Inc., Fullerton, CA).

Statistical analysis

Intake and digestion data were analyzed with PROC MIXED (SAS). Period and treatment were included in the model with steer included as the random term. Daily intake and PUN data were analyzed using PROC MIXED. Terms in the model were treatment, period, day and day × treatment with steer and treatment × period × steer included as random terms. The repeated term was day with treatment × steer serving as the subject. Compound symmetry was used for covariance structure. Fermentation profile variables were analyzed using PROC MIXED and the model was the same as for PUN data except day was replaced with hour Treatment means were calculated using the LSMEANS option and were separated using linear and quadratic contrast for amount of supplemental N provided per 3 d for 0/d, 160/3d, 320/3d, and 480/3d and contrast comparing 160/d to each treatment was made.

CHAPTER III

RESULTS

Hay OM intake (Table 2) was greater ($P < 0.05$) when cattle were supplemented, regardless of amount or frequency. When supplements were provided every 3d there was a linear ($P = 0.01$) increase in HOMI with increasing level of supplemental protein. Differences in HOMI between 160/d and 160/3d, 320/3d, and 480/3d were not significant ($P > 0.05$). There was no significant treatment × day interaction ($P = 0.85$) for HOMI. There was no significant effect of day ($P = 0.35$) on HOMI (Table 3). Total OMI was greater ($P < 0.05$) in supplemented cattle than 0/d. A linear increase ($P = 0.01$) in TOMI was observed as level of supplemental protein provided every third d increased. Supplementation of 160 mg N/kg BW every 3d resulted in less TOMI ($P < 0.05$) than daily provision of the same amount of protein. There was a significant treatment × day interaction ($P < 0.01$). This interaction was largely driven by greater intakes ($P < 0.05$) of OM on days when steers supplemented every third d, were supplemented then on the days when they weren't supplemented. In contrast, TOMI of steers supplemented daily were not different across days ($P > 0.05$). There was no significant effect ($P > 0.05$) of treatment on OMD or NDFD, which averaged 48.6 and 49.1%, respectively. In contrast to digestion, forage utilization, reported as TDOMI, was greater for supplemented steers ($P < 0.05$) than 0/d. Increasing provision of supplemental protein from 0/d to 480/3d, within steers supplemented every third d, resulted in a quadratic ($P = 0.06$) increase in TDOMI, with the largest increase occurring between 0/d and 160/3d, a small increase between 160/3d and 320/3d, followed by a slight decrease from 320/3d to 480/3d. There was no significant ($P > 0.05$) difference between 160/d and the infrequently supplemented treatments for TDOMI.

CHAPTER IV

DISCUSSION: SUMMARY AND CONCLUSIONS

Observed increases in HOMI with provision of supplement protein were expected based on the low CP content (2.3%) and were in accordance with previous observations (Köster et al., 1996; Wickersham et al., 2004, Wickersham et al., 2008). When supplement was delivered daily at 160 mg N/kg BW HOMI was approximately 140% greater than unsupplemented. When the same total amount of supplemental N, 480 mg N/kg BW, was provided every 3 d (480/3d) HOMI intake was 142% greater than unsupplemented and not different from daily supplementation. In accordance with our observations, Wickersham et al. (2008) reported that when 549 mg N/kg BW was provided every 3 d as either 183 mg N/kg BW daily or 549mg N/kg BW every third d HOMI was identical. In the same paper, provision of 183 mg N/kg BW every third d, the equivalent of 61 mg N/kg BW daily, resulted in HOMI that was similar (7.4 versus 7.6 kg/d) to 183 mg N/kg BW every d. In contrast, Farmer et al., (2004b) observed supplementation twice weekly resulted in HOMI that was 85% of steers supplemented daily. In an earlier study, Farmer et al. (2001) reported that in steers supplemented twice weekly HOMI was 75% of steers supplemented daily. In our study, reduced (linear, $P < 0.01$) consumption of hay was observed when the total amount of N provided every 3 d was 33 (160/3d) and 66% (320/3d) of 480/3d; however, the efficiency of HOMI stimulation (g of additional HOMI/g of supplemental N) was greater when reduced amounts of supplement were provided (11, 20, 16, and 12 for 160/d, 160/3d, 320/3d, and

480/3d, respectively). When equal amounts of supplemental protein were provided daily or every third day the effectiveness of supplementation at stimulating hay intake was not diminished. Farmer et al. (2001) observed decreased hay intake when supplements were delivered twice weekly, which is in contrast to our observation of no effect of day on HOMI. This difference can likely be attributed to the provision of less supplement at each supplementation event in our study versus Farmer et al. (2001), 0.8 versus 1.3% of BW, respectively.

Previous research (Farmer et al., 2001 and 2004b) has shown that with a decrease in protein supplementation frequency, there is a decrease in TOMI. In contrast to these results we found that when the same amount of protein was supplemented per 3-d period (480 mg N/kg BW) TOMI was largely unaffected. Farmer et al. (2001) attributed a portion of the decreased TOMI to the aforementioned reduction in HOMI on days when large amounts of supplement was provided. We did not observe reduced HOMI when supplement was provided. However, TOMI was greater for infrequently supplemented steers on the day supplement was provided, with this increase being accounted for by the consumption of the supplement. These data suggest that adequate amounts of N remain in the rumen in order to provide the positive effects of supplementation, even a 3d after supplementation occurred. Urea recycling has been shown to play an important role in maintaining ruminal N levels, especially when large amounts of supplemental protein are provided infrequently (Wickersham et al., 2008). Also, TOMI was the same, even when supplemented every 3d at only 2/3 the total amount (320/3d). However, there was a significant decrease in TOMI when supplemented at only 1/3 the amount (160/3d). Similar results were seen from Wickersham et al. (2008). Wickersham et al. (2008) suggests that the reasoning is based on the fact that there is simply not enough N provided to the rumen when only 160 mg N per kg BW was delivered only every 3 d.

In contrast to digestion, forage utilization, reported as TDOMI, was greater for supplemented steers than 0/d. Increasing provision of supplemental protein from 0/d to 480/3d, within steers supplemented every third d, resulted in a quadratic increase in TDOMI, with the largest increase (40%) occurring between 0/d and 160/3d, a small increase (27%) between 160/3d and 320/3d, followed by a slight decrease (-7%) from 320/3d to 480/3d. There was no significant difference between 160/d and the infrequently supplemented treatments for TDOMI, indicating that among supplemented steers, the frequency of supplementation (every 1d compared to every 3d) did not greatly affect digestible OMI .

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APPENDIX

Table 1. Chemical composition of forage and supplement

Item	Hay	Supplement
	---------- % DM ----------	
OM	88.9	88.4
CP	2.3	40.7
NDF	81.9	29.3
ADF	58.3	11.5
Acid Detergent Insoluble ash	8.9	1.6

Table 2. Effect of level and frequency of protein supplementation provided on intake and digestion of South Texas grass hay

Item	Treatment[1]					SEM	Contrast *P*-value[2]	
	0/d	160/d	160/3d	320/3d	480/3d		Linear	Quadratic
Hay Intake, kg/d								
OM	4.41[a]	6.18	5.46	6.10	6.27	0.4	< 0.01	0.14
NDF	4.03[a]	5.65	4.96	5.49	5.75	0.4	< 0.01	0.22
Total Intake, kg/d								
OM	4.42[a]	7.08	5.83[a]	6.72	7.20	0.4	< 0.01	0.15
NDF	4.03[a]	5.94	5.09[a]	5.69	6.06	0.4	< 0.01	0.23
Digestible OM	2.02[a]	3.30	2.82	3.59	3.34	0.3	< 0.01	0.06
Digestible NDF	1.97[a]	2.68	2.53	3.16	2.71	0.3	0.02	0.05
Digestibility, %								
OM	47.9	47.5	48.8	52.2	46.1	3.3	0.87	0.24
NDF	51.0	45.9	50.4	53.7	44.5	3.5	0.25	0.19

[1]0/d = 0 mg of N/kg BW daily; 160/d = 160 mg of N/kg BW daily; 160/3d = 160 mg of N/kg BW every 3/d; 320/3d = 320 mg of N/kg BW every 3/d; 480/d = 480 mg of N/kg BW every 3/d; all supplemental N was provided as range cubes. Means with a superscript differ from 160/d ($P < 0.05$).

[2]Linear = linear effect of increasing supplemental N provided every 3/d including unsupplemented controls. Quadratic = quadratic effect of increasing supplemental N provided every 3/d including unsupplemented controls.

Table 3. Effect of supplement delivery on hay and total organic matter

Item	Treatment[1]					SEM	Contrast *P*-value		
	0/d	160/d	160/3d	320/3d	480/3d		Trt	Day	Trt × Day
Hay OM Intake, kg									
Day 1	4.53	6.15	5.42	6.29	6.26	0.41	< 0.01	0.35	0.85
Day 2	4.46	6.13	5.26	6.06	6.40				
Day 3	4.27	6.30	5.21	5.90	6.18				
Total OM Intake, kg									
Day 1	4.55	7.04	6.32[a]	8.14[a]	9.06[a]	0.43	< 0.01	< 0.01	< 0.01
Day 2	4.48	7.03	5.27[b]	6.05[b]	6.38[b]				
Day 3	4.29	7.20	5.22[b]	5.88[b]	6.16[b]				

[1]0/d = 0 mg of N/kg BW daily; 160/d = 160 mg of N/kg BW daily; 160/3d = 160 mg of N/kg BW every 3/d; 320/3d = 320 mg of N/kg BW every 3/d; 480/d = 480 mg of N/kg BW every 3/d; all supplemental N was provided as range cubes. Means within treatment (column) with different superscripts differ ($P < 0.05$). Steers receiving supplement every 3/d were supplemented on day 1.

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