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# 2007 South Dakota Beef Report

Department of Animal and Range Sciences, South Dakota State University

Agricultural Experiment Station, South Dakota State University

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South Dakota State University College of Agriculture and Biological Sciences Animal and Range Sciences Department



# SOUTH DAKOTA STATE UNIVERSITY

COLLEGE OF AGRICULTURE AND BIOLOGICAL SCIENCES

ANIMAL AND RANGE SCIENCES DEPARTMENT



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# **Department of Animal and Range Sciences**

## Mission

The overall mission of the Department of Animal and Range Sciences parallels South Dakota State University's land-grant mission of providing education, research, and professional outreach through the Cooperative Extension Service to the citizens of South Dakota. Two of the specific missions of the Department of Animal and Range Sciences are 1) to conduct research related to the animal and range sciences that will enhance the understanding and development of livestock and related industries and 2) to transfer to the citizens of South Dakota research technology and information on livestock production, range management, and related livestock industries, which will enhance the quality of life of all persons. The goal of this annual beef report is to disseminate new knowledge that is discovered at South Dakota State University to the producers and livestock industries of South Dakota.

# **Biological Variation and Treatment Differences**

Variability naturally exists among individual animals and plants. This variation can create problems when interpreting results from experiments. For example: when cattle in one treatment (X) have a numerically higher average daily gain compared to cattle in another treatment (Y), this difference in weight might be due to animal variation and not due to the treatments. Statistical analysis attempts to remove or reduce the natural variation that exists among animals and explains the difference due to the treatments.

In the following research papers, you will see notations similar to (P < 0.05). This means that there is less than a 5% chance that the difference between treatments is due to the natural variation that occurs. This indicates that that there is greater than a 95% probability that the differences between treatments are the result of the treatments. You will also notice notations similar to (P = 0.10). This means that there is a 10% chance that the difference between treatments is due to the natural variation that occurs. This indicates that the difference between treatments is due to the natural variation that occurs. This indicates that there is a 90% probability that the differences between treatments are the result of the treatments.

In most of the papers you will see an average, or mean, reported as  $25 \pm 2.3$ . The first number is the average value for the treatment. The second number is the standard error, or the variability that occurred, and explains how accurately the mean is estimated. There is a 68% probability that the true mean will fall within 1 standard error of the listed mean and a 94% probability that the true mean will fall within 2 standard errors. For this example we are 68% certain that the true mean is between the range of 27.3 and 22.7 and 94% certain that the true mean is between 29.6 and 20.4.

Ways we decrease variability and improve the chance of measuring differences due to treatments include having several animals in each treatment, replicating treatments several times, and using animals that are as similar as possible. The use of statistical analysis in research allows for unbiased interpretation of results. The use of statistical analysis in the research reported here increases the confidence in the results.

Editorial Committee: Dr. G. A. Perry, Editor Ms. Betty Knutsen, Word Processor and Formatter

# **Conversion Tables**

The metric system is frequently used for reporting scientific data. To aid in interpreting these data the following tables have conversions for common measurements from the metric system to the Standard English system.

Metric	English
0 C	32 Fahrenheit
1 milliliter	0.03 ounces
1 Liter	0.26 gallons
100 grams	0.22 pounds
1 kilogram	2.2 pounds
1 meter	3.28 feet

# **Commonly Used Abbreviations**

ADG	Average Daily Gain	mo	months
ADF	acid detergent fiber	MP	Metabolizable protein
Al	Artificial Insemination	NDF	neutral detergent fiber
BCS	Body Condition Score	NE	net energy
BW	Body weight	NEg	Net Energy gain
CIDR	Controlled Internal Drug Releasing device	NEm	Net Energy maintenance
сМ	Centimorgan	NEFA	Non-Esterified Fatty Acids
CP	Crude Protein	PCR	Polymerase Chain Reaction
d	days	PG	prostaglandin
DE	Digestible Energy	PSE	Pale, soft, and exudative
DFD	Dark, Firm, and Dry (meat)	ppb	parts/billion parts
DM	Dry matter	ppm	parts/million parts
DMI	Dry Matter Intake	<sup>32</sup> P	Phosphorus Radioactive Isotope
DNA	deoxyribonucleic acid	QTL	Quantitative Trait Locus (singular) or
EDTA	Ethylene Diamine Tetra Acetic Acid		Loci (plural)
F/G	feed to gain	RFLP	Restriction Fragment Length
g	gravity		Polymorphism
GH	Growth Hormone	RNA	Ribonucleic acid
GnRH	Gonadotropin Releasing Hormone	S	seconds
GLM	General Linear Model	SNP	Single Nucleotide Polymorphism
h	hours	TDN	total digestible nutrients
HCW	Hot Carcass Weight	VFA	Volatile Fatty Acid
Ins	Insulin	wk	weeks
KPH	Kidney, Pelvic, and Heart Fat	wt	weight
LMA	Longissimus Muscle Area	WW	Weaning Weight
MAS	Marker Assisted Selection	YG	Yield Grade
ME	Metabolizable energy	yr	years
min	minutes	YW	Yearling Weight

The faculty members of the Animal and Range Sciences Department are always ready to answer your questions. Our Brookings phone number is (605) 688-5165. Staff members in Rapid City (RC) may be reached at 605-394-2236. Please feel free to give any one of us a call, or check out our departmental website: http://ars.sdstate.edu. You can find this 2007 Beef Report and other information at http://ars.sdstate.edu/extbeef/Publications.htm

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# Effect of supplemental fat from dried distillers grains with solubles or corn oil on cow performance, IGF-1, GH, and NEFA concentrations<sup>1</sup>

Abigail Bartosh<sup>2</sup>, Cody Wright<sup>3</sup>, Aimee Wertz-Lutz<sup>4</sup>, and George Perry<sup>5</sup> Department of Animal and Range Sciences

BEEF 2007-01

# Summary

Research has demonstrated that supplemental fat and(or) changes in growth hormone (GH) or insulinlike growth factor-1 (IGF-1) concentrations may affect reproductive performance in beef females. Dried distillers grains with solubles (DDGS) contain approximately 10% to 15% fat; however, minimal research to date has investigated DDGS specifically as a supplemental fat source. The objective of this experiment was to investigate whether supplemental fat from either DDGS or raw corn oil impacts cow growth performance and plasma GH, IGF-1, or non-esterified fatty acid (NEFA) concentrations. Sixty open beef cows [body weight (BW) =  $553.5 \pm 38.7$  kg; body condition score (BCS) =  $5.4 \pm 0.53$ ] were stratified by BW and BCS and allotted to 15 pens (n = 4 per pen; 14.6 x 37.2 m). Pens were randomly assigned to one of three dietary treatments: 1) DDGS, 2) a combination of high-protein dried distillers grain, corn bran, and corn oil (OIL), or 3) a combination of high-protein dried distillers grain and corn bran (HPBRAN). The DDGS, OIL, and HPBRAN treatments each comprised 35% of the diet dry matter (DM). Thirty-five percent was selected based upon the sulfur (S) content of dietary ingredients in the DDGS treatment and water, estimated water intake, and the maximum tolerable S concentration for cattle on forage-based diets (0.5%). In addition to dietary treatments, cattle were provided grass hay [7.7% crude protein (CP)] and a pelleted supplement containing vitamins and minerals as part of a totally mixed ration. Cows were fed once daily, in the morning, for 60 d. All diets were iso-nitrogenous (15.3% CP from d 0 to 47 and 15.1% from d 48 to 60) and total fat concentrations were 5.1% for DDGS and OIL and 3.5% for HPBRAN. Weights and blood samples were recorded prior to feeding on d -1, 0, 28, 59, and 60. Dry matter intake, average daily gain, final BW, and gain:feed were not affected by treatment. Treatment had no effect on plasma GH, IGF-1, or NEFA concentrations. These results suggest that providing low concentrations of supplemental fat as DDGS or raw corn oil to a forage-based diet does not influence growth performance, plasma GH, IGF-1, or NEFA concentrations in open beef cows.

## Introduction

Supplemental fat has been shown to increase serum growth hormone (GH) concentrations in beef cattle (Williams and Stanko, 2000). The increase in GH coincides with an increase in follicular fluid insulin-like growth factor-1 (IGF-1) concentrations, whereas peripheral IGF-1 levels are not affected. Accumulation of follicular fluid IGF-1 has been proposed as one of the mechanisms associated with follicle selection. Researchers have hypothesized that these changes in hormone concentrations may be associated with unsaturated fats (Williams and Stanko, 2000).

Dried distillers grains plus solubles (DDGS) are a co-product of the ethanol industry. Survey research suggests that distillers grains contain approximately 10.8% fat (Shurson, 2006). Furthermore, corn oil is high in unsaturated fatty acids (Griinari et al., 1998). Under normal circumstances, unsaturated fatty acids that are introduced into the rumen are biohydrogenated to more saturated fatty acids. However, recent research suggests that the fat contained in distillers grains may be somewhat protected from biohydrogenation in the rumen (Koger et al., 2004; Vander Pol et al., 2004). University of Nebraska

<sup>&</sup>lt;sup>1</sup> This project was funded by the South Dakota Corn Utilization Council and a portion of the co-products were provided by Dakota Gold Research Association.

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researchers reported decreased feedlot performance when 5% fat was included as corn oil, but no affect was observed when 5% fat was included as wet distillers grains (Vander Pol et al., 2004). These data suggest that the lipids contained in corn oil or distillers grains may be metabolized differently and elicit different production responses. Researchers at South Dakota State University examined the effect of 20% or 40% wet or dry distillers grains on the fatty acid composition of the longissimus muscle of steers (Koger et al., 2004). The researchers found that muscle tissue from cattle fed either wet or dry distillers grains of unsaturated fatty acids. Together, these data suggest that the unsaturated fats contained in distillers grains may escape rumen biohydrogenation and may be absorbed in an unsaturated form.

The current experiment was designed to compare the effect of supplemental fat as either DDGS or raw corn oil on cow performance and plasma GH, IGF-1, and non-esterified fatty acid (NEFA) concentrations.

#### **Materials and Methods**

Sixty open beef cows were purchased from public auction markets and transported to the Southeast Research Farm located near Beresford, SD. Initial body weights (BW; 1220.5  $\pm$  77.4 lb) were recorded and initial body condition score (BCS; 5.4  $\pm$  0.53) were determined as the average of two experienced evaluators. Cows were stratified by BW and BCS and allotted to 1 of 15 pens. The cows were provided with medium-quality grass hay *ad libitum* for a minimum of 14 d prior to trial initiation; after which the pens were randomly assigned to one of three dietary treatments.

The first dietary treatment (DDGS) was comprised solely of DDGS and contained approximately 15% dietary crude protein (CP) and 5% dietary ether extract (EE). The second dietary treatment (OIL) contained high-protein dried distillers grains (HP DDG), corn bran, and corn oil to provide the same amount of CP and EE as the DDGS diet. The final treatment (HPBRAN) was formulated provide the same amount of protein, but no additional fat; dietary ingredients included HP DDG and corn bran (Table 1).

	Treatments			
Item	DDGS	OIL	HPBRAN	
		% of diet DM		
Hay	62.5	62.5	62.5	
Supplement <sup>a</sup>	2.5	2.5	2.5	
DDGS <sup>b</sup>	35			
HP- DDG $^{\circ}$		20.7	20.3	
Corn bran		12.6	14.7	
Corn oil		1.7		
Analyzed nutrient comp	oosition			
Dry matter	84.5	84.1	83.7	
Crude protein	15.1	15.1	15.1	
Ether extract	5.1	5.1	3.5	
Sulfur	0.5	0.5	0.5	

Table 1. Composition of treatment diets

<sup>a</sup> Provides vitamins and minerals to meet or exceed nutrient requirements (Nutrient Requirements of Beef Cattle, National Research Council, 2000).

<sup>b</sup> Dried distillers grains with solubles.

<sup>c</sup> High-protein dried distillers grains.

Dietary CP fed in this experiment (15%) is substantially more than these cows require; diets were formulated to provide the maximum amount of fat from distillers as possible without causing health problems due to sulfur (S) intake. Mineral Tolerances of Animals states the maximum tolerable S concentration for cattle on forage-based diets as 0.5%. Daily water intake was estimated to be 7 gal/d

and available water and all feed ingredients were analyzed to determine S content prior to trial initiation. Cows were fed a totally mixed ration which consisted of the dietary treatment, medium-quality grass hay and a vitamin/mineral supplement to meet or exceed the cows' mineral requirements as suggested by the NRC (2000).

Weights were recorded on 2 consecutive d prior to trial initiation and at trial termination and one day near the midpoint of the trial. Blood samples were collected on d 0, 28, and 60. Growth hormone and IGF-1 concentrations were analyzed using radioimmunoassay. Non-esterified fatty acid concentrations were analyzed using a colorimetric assay. Cow performance data were analyzed using the PROC GLM method of SAS with pen as the experimental unit. Plasma GH, IGF-1, and NEFA data were analyzed as repeated measures using the mixed procedure of SAS with pen as the experimental unit.

#### **Results and Discussion**

Cow performance data are presented in Table 2. Dietary treatments had no effect on animal performance. Cows consumed 2.4 to 2.6% of their body weight and gained 1.2 to 1.3 kg per head per day.

Table 2. Cow growth performance.							
Item	DDGS	OIL	HPBRAN	SEM	<i>P</i> -value		
DMI, Ib	34.4	36.2	34.2	2.9	0.87		
ADG, Ib	2.9	2.6	2.9	0.4	0.78		
G:F	0.08	0.07	0.08	0.01	0.78		

Table 3. Plasma GH, IGF-1, and NEFA concentrations.

	Treatment				
Item	DDGS	OIL	HPBRAN	SEM	P-value
GH, ng/mL	32	20	28	4.0	0.09
IGF-1, ng/mL	78	57	60	9.3	0.23
NEFA, µEq/L	247	218	195	19.8	0.18

Plasma GH, IGF-1, and NEFA concentrations were not affected by treatment by time interactions. Table 3 shows the main effect of treatment on the hormone and NEFA concentrations.

Figures 1, 2, and 3 illustrate the main effect of time on plasma GH, IGF-1, and NEFA, respectively. Plasma IGF-1 concentration was greater (P < 0.001) on d 60 than on d 0 or 28 (Figure 1). From d 0 to d 28, plasma GH concentration decreased (P < 0.013); concentrations on d 28 and d 60 were similar (Figure 2). Plasma NEFA concentration decreased (P < 0.001) from d 0 to d 28; concentrations on d 28 and d 60 were not different. This decrease in plasma NEFA concentrations is likely the result of the grass hay diet the cows were fed prior to the initiation of the experiment. It is possible that the grass hay was not meeting the energy demands of the cows, and consequently, they were mobilizing body fat stores to meet their energy needs. Then, once the experiment began and the plane of nutrition was increased, the circulating NEFA concentrations decreased in a similar pattern to that of GH.







FIGURES 1, 2, 3

#### Implications

This experiment suggests that, when total dietary fat concentrations are equal to or below 5.1%, supplemental fat from either DDGS or corn oil does not impact growth performance or plasma GH, IGF-1, or NEFA concentrations in open beef cows.

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# Prolonged, moderate nutrient restriction in beef cattle results in persistently-elevated plasma ghrelin concentrations<sup>1</sup>

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# BEEF 2007-02

## Summary

Four steers (BW 1281±28.2 kg) were used in a crossover design to determine the effects of prolonged. moderate energy and protein restriction on plasma ghrelin and GH concentrations. A common highenergy diet was offered at 240% of the intake necessary for BW maintenance (2.4xM) or 80% of the intake necessary for BW maintenance (0.8xM). As a common starting point, all steers were adjusted to 2.4xM during a 23-d pre-trial adaptation period. At initiation of period 1, 2 steers remained at 2.4xM, whereas intake for the remaining 2 steers was restricted to 0.8xM. Feed allotments were offered twice daily in equal aliquots at 0800 and at 2000 h. On 7, 14, and 21 d following initiation of restriction, serial blood samples were collected via indwelling jugular catheter at 15-min intervals throughout a 12-h feeding interval. Following period 1, steers were weighed and intake amounts were recalculated. Dietary treatments were switched between steer groups, 2.4xM intake was established, and sampling period II was initiated as described for period I. Plasma samples were assayed for ghrelin, GH, insulin (INS), and NEFA concentrations. Subsequent to analyses, hormone data were pooled by hour for statistical analyses. The energy and protein restriction resulted in decreased BW for 0.8xM (-108.9 lb) steers compared with 2.4xM (127.9 lb) steers. Body weight loss along with decreased plasma INS concentrations and elevated plasma NEFA and GH concentrations indicate that these steers were in a catabolic state and mobilizing body tissue stores to meet nutrient requirements not met by dietary intake. Plasma ghrelin concentrations also were elevated for the 0.8xM steers compared with those of 2.4xM steers throughout the 21-d treatment period. These data are consistent with the hypothesis that plasma ghrelin concentrations are elevated in cattle throughout a prolonged, moderate energy and protein restriction that result in a catabolic state.

## Introduction

Inadequate nutrient intake relative to demand for maintenance and (or) production can result in economic loss from poor production efficiency and metabolic disorders. Therefore, understanding feed intake regulation and energy expenditure in cattle is important. Ghrelin is a peptide hormone synthesized by abomasal and ruminal tissues of cattle (Hayashida et al., 2001; Gentry et al., 2003). In rodents, ghrelin stimulates feed intake through neuropeptides in the hypothalamus (Nakazato et al., 2001) and is reported to influence energy metabolism and body composition (Tschöp et al., 2000). Most research that has been conducted with livestock to evaluate the relationship of plasma ghrelin with feed intake restriction has been done with short-term periods of complete feed deprivation without sufficient length to result in differences in body composition. Wertz-Lutz et al. (2006) observed elevated plasma ghrelin concentrations that persisted for 48 h in mature beef cattle completely deprived of feed. Length and severity of the nutrient restriction may influence plasma ghrelin concentrations. More often than complete feed deprivation, ruminant livestock encounter periods of prolonged, moderate nutrient restriction, whereby nutrient resources are limiting relative to expected production. Currently, research that evaluates the effects of prolonged, moderate nutrient restriction sufficient to result in BW loss on plasma ghrelin concentrations does not exist. The objective of this experiment was to evaluate effects of prolonged,

<sup>&</sup>lt;sup>1</sup> Project funded by NRICR Grant No. 2004-34206-14372.

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<sup>&</sup>lt;sup>4</sup> Professor

moderate energy and protein restriction on plasma ghrelin concentrations and its relationship to other hormones indicative of nutritional status.

#### **Materials and Methods**

**Dietary treatments.** Dietary treatments for this experiment were two different amounts of a common high-energy diet. Feed amounts were 80% of that necessary to meet the NEm requirement **(0.8xM)** or 240% of the feed intake necessary to meet the NEm requirement **(2.4xM)** of a given steer and were calculated as described below. To determine the amount of feed intake necessary to meet the NEm requirement (Mcal/d), the equation 0.077 x empty BW(kg<sup>0.75</sup>) was used (NRC, 2000). This NEm requirement (Mcal/d) then was divided by the energy density of the diet (Mcal/lb) to determine the amount of feed (lb/d) necessary to meet the maintenance requirement of each particular steer on the basis of its own BW. The amount of feed intake for the steers in positive nutrient balance (2.4xM) or multiplied by 0.80 to determine the amount of intake assigned to negative nutrient balance treatment (0.8xM). Once each steer's given amount of feed was determined, the MP content of the feed was estimated based on the degradability of the dietary protein (43.4%; Beef NRC, 2000). The amount of dietary MP consumed then was compared with the MP required for BW maintenance as calculated using the equation 3.8 g MP x BW(kg<sup>0.75</sup>) (NRC, 2000).

**Animals and procedures.** Four ruminally-cannulated (3-yr-old) Angus crossbred steers (BW 1281  $\pm$  28.2 lb; 581.4  $\pm$  12.8 kg) steers fitted with an indwelling jugular catheter were used in a crossover design to evaluate the effects of prolonged, moderate protein and energy restriction on plasma hormone and metabolite concentrations. This experiment was conducted in a climate-controlled metabolism facility at South Dakota State University, and all animal procedures were approved by the Institutional Animal Care and Use Committee.

During a 23-d pre-experiment adaptation period, steers were acclimated to the climate-controlled facility. Equal aliquots of feed were offered twice daily at 0800 and 2000 h, and this 12-h feeding interval was maintained throughout the trial. To establish a common starting point, feed intake of the common high-grain finishing diet (Table 1) was increased during this acclimation period until feed intake was 240% greater than the amount required to meet the NEm requirement of each steers.

Table 1. Ingredient composition of experimental diet			
Ingredient	%, DM Basis		
Beet pulp	20.00		
Corn	65.00		
Soybean meal	5.67		
DDGS <sup>a</sup>	8.00		
Limestone	1.00		
TM salt <sup>b</sup>	0.30		
Vitamin A D E <sup>c</sup>	0.0055		
Zinc sulfate <sup>a</sup>	0.0056		
Rumensin <sup>e</sup>	0.02		
Calculated Nutrient Composition	DM Basis		
CP, %	12.5		
Degradable intake protein, %	43.4		
NEm, Mcal/lb	0.95		
<sup>a</sup> Dried distiller's grains with solubles			

<sup>b</sup> NaCl 94.0-98.5%, Zn 0.35%, Fe 0.20%, Co 0.005%, Mn 0.20%, Cu

0.30%, 10.007%.

 $^{c}$  30,000 IU/g Vitamin A, 500 IU/g Vitamin E, Vitamin D\_3 500,000 IU/g  $^{d}$  35.54% Zn.

<sup>e</sup> Formulated to contain 32 g/T.

Once all steers had reached 240% of feed intake required to meet NEm, the experiment was initiated. The experiment was conducted as a crossover design with two, 21-d treatment periods. During treatment period I, 2 steers were maintained at 240% of the intake required to the NEm requirement (2.4xM) established during the acclimation period and the remaining 2 steers were limited to 80% of the intake required to meet NEm requirement. Serial blood and rumen fluid samples were collected on d 7, 14, and 21 following initiation of the restriction. Following period I, dietary treatments were switched between steer groups, steers were weighed, and feed amounts were re-calculated as described for period I on the basis of the BW recorded at the end of sampling period I. Feed intake gradually was increased until steers that had been fed 0.8xM reached 2.4xM intake. A second 21-d treatment and sampling period then was conducted as described for period I.

**Blood collection.** During each 21-d treatment period, blood and rumen fluid samples were collected at 7, 14, and 21 d after the intake restriction was invoked. On each sampling day, blood samples were collected at 15-min intervals from 0700 and 1145, 1300 to 1345, 1600 to1645, and 1800 to 1845 h. Aliquots of plasma (1.0 mL each) were processed and stored at –20°C for the subsequent analyses of GH, INS, NEFA and ghrelin according to procedures outlined by Wertz-Lutz et al. (2006).

**Statistical analyses.** To verify that differences in NEm and MP intake resulted from the planned differences in DMI, these characteristics and BW change were analyzed statistically as a crossover design with a model that accounted for variation from sampling period, steer, and amount of feed intake. Differences in the characterization parameters that resulted from amount of feed intake were separated using a Fisher's t-test. Plasma ghrelin, GH, INS, and NEFA concentrations were analyzed statistically as repeated measures in time by using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with independent errors that accounted for error correlation of sampling times. The model included length of treatment (7, 14, and 21 d), amount of feed intake (0.8xM vs. 2.4xM), steer, sampling period (I or II), and the interactions of length of treatment and amount of feed intake as independent variables. Differences in least squares means for plasma ghrelin, GH, INS, and NEFA were separated by using a Fisher's t-test.

#### **Results and Discussion**

*Nutritional state of steers*. Imposed dietary treatments resulted in less ( $P \le 0.002$ ) DMI of the common compositional diet for steers assigned to the 0.8xM compared with the 2.4xM steers (8.6 and 22.9 ± 0.40 lb/d, respectively). This feed intake restriction resulted a net energy intake that was below the NEm requirement for 0.8xM (-1.0 Mcal/d) and a net energy intake that was lower ( $P \le 0.001$ ) than that of the steers assigned to the 2.4xM treatment (9.0 Mcal/d). The feed intake restriction also resulted in MP intake below that required to meet the maintenance requirement for the 0.8xM steers (-107 g/d relative to maintenance requirement). Metabolizable protein intake also was lower ( $P \le 0.001$ ) for 0.8xM steers compared with 2.4xM steers (500 g/d in excess of maintenance requirement). The energy and protein restriction resulted in decreased ( $P \le 0.001$ ) BW for 0.8xM (-108.9 lb) steers compared with 2.4xM (127.9 lb) steers.

Length of restriction on hormone and metabolite profiles. Plasma hormone and metabolite profiles are reported in Figure 1. Plasma GH and NEFA concentrations were elevated ( $P \le 0.001$ ) for 0.8xM steers throughout the 21-d treatment sampling period (Figures 1B and 1D, respectively). In contrast, plasma INS concentrations were lower ( $P \le 0.001$ ) for 0.8xM steers throughout the 21-d treatment period (Figure 1C). A dietary treatment by length of treatment interaction resulted for plasma INS concentrations were similar across the 21-d sampling period for 0.8xM steers, whereas plasma INS concentrations were higher at d 7 and 14 compared with d 21 for 2.4xM steers. Plasma GH, NEFA, and INS concentrations indicate that 0.8xM steers were in catabolic state throughout the 21-d treatment period. Body weight loss along with decreased plasma INS concentrations and elevated plasma NEFA and GH concentrations indicate that these steers were mobilizing body tissue stores to meet nutrient requirements not met by dietary intake. Plasma ghrelin concentrations were elevated ( $P \le 0.001$ ) for the 0.8xM steers compared with 2.4xM steers throughout the 21-d treatment period (Figure 1A). There was an interaction of dietary treatment by length of treatment ( $P \le 0.01$ ) for plasma ghrelin concentrations. Plasma ghrelin concentrations for 2.4xM steers were similar regardless of length of treatment, whereas plasma ghrelin concentrations for 0.8xM steers were higher at d 14

compared with d 7 and 21. These data are consistent with the hypothesis that plasma ghrelin concentrations remain elevated through a prolonged, moderate energy and protein restriction that result in a catabolic state in cattle.



**Figure 1**. Effects of prolonged energy and protein restriction sufficient to result in body weight loss on plasma hormone and metabolite concentrations in beef cattle. TRT = 0.8xM - 80% of the feed intake needed to meet the energy requirement for maintenance; 2.4xM - 240% of the feed intake needed to meet the requirement for maintenance. DAY = sampling day relative to the invoked DMI restriction. TRTxDAY = interaction of the main effects.

Rumen distention must be considered as a plausible explanation of the interaction of treatment with length of treatment for plasma ghrelin concentrations. Sugino et al. (2003) reported that cholinergic activity of the vagus nerve suppressed ghrelin secretion, suggesting that distension of the rumen may regulate ghrelin secretion. However, Arnold et al. (2006) acknowledged that phasic increases in ghrelin influence activity of some load-sensing vagal afferents, but concluded that the acute eating-stimulatory effect of ghrelin did not require vagal afferent signaling. By design, steers in the current experiment were adapted to an intake amount that would supply 240% of the NEm requirement as a means of standardizing the group before the restriction was invoked. Intake did differ for the two treatment groups throughout the treatment period such that the amount of feed consumed was greater for 2.4xM steers (22.9 lb) compared with 0.8xM steers (8.6 lb), and therefore distention and suppression of ghrelin secretion would be expected to be greater for the 2.4xM steers. In the current experiment, no objective measure of rumen distention was used and this observations warrants further investigation before a definitive conclusion can be drawn. However, adaptation of the rumen to the quantity of feed provided

may explain partially the fluctuation in plasma ghrelin concentrations that was observed during the 21-d sampling period for 0.8xM steers but not 2.4xM steers. The relationship of ghrelin and the vagal nerve only partially explains the relationship of ghrelin and feed intake and has not need demonstrated as a link between ghrelin and body composition.

The observation that plasma ghrelin concentrations differ between cattle in a positive nutritional state compared with a negative nutritional also warrants further investigation. A link between ghrelin and adipositiy has been established. Patel et al. (2006) reported that ghrelin stimulated deoxyglucose uptake by rodent epididymal adipocytes in the presences but not in the absence of insulin. Patel et al. (2006) also reported differential expression of the receptor to which ghrelin binds (GHS-R) in various adipose depots. The GHS-R was expressed in adipocytes that responded to ghrelin stimulation and not expressed in adipocytes that did not respond to ghrelin stimulation. Although completed with rodents, these data suggest a complex interaction of nutritional state, form of ghrelin, and adipose depot, whereby investigating the role of ghrelin in altering composition of gain in cattle is warranted.

#### Implications

Data from the current experiment and other experiments in our laboratory indicate that plasma ghrelin concentrations fluctuate both with acute feed deprivation and prolonged moderate nutrient intake restriction. The fluctuation of ghrelin with other hormones and metabolites indicative of nutritional status may influence the ability of ghrelin to communicate nutritional status, as evidence that ghrelin alters glucose uptake by adipocytes and altered use of metabolic fuels suggest that ghrelin also has the potential to influence nutrient expenditure. As a result, the potential role of ghrelin in regulating composition of gain and (or) feed efficiency warrants further exploration.

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# Relationship of plasma ghrelin concentrations with end-products of carbohydrate fermentation for beef cattle during a feeding interval <sup>1</sup>

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# Summary

Four steers (BW 1281±28.2 lb) were used to determine the relationship of plasma ghrelin concentrations with end-products of carbohydrate fermentation and hormones and metabolites indicative of nutrition status during a 12-h feeding interval. A common high-energy diet was offered at 240% of the intake necessary for BW maintenance (2.4xM) or 80% of the intake necessary for BW maintenance (0.8xM). At initiation of period I, 2 steers were allowed 2.4xM intake, whereas intake for the remaining 2 steers was restricted to 0.8xM. Equal aliguots of feed were offered at 0800 and at 2000 h. On 7. 14. and 21 d following initiation of intake restriction, serial blood samples were collected via indwelling jugular catheter at 15-min intervals through the 12-h feeding interval. Plasma samples were assayed for ghrelin, GH, insulin (INS), and NEFA concentrations. Rumen fluid samples were collected throughout the feeding interval and processed for subsequent analyses of VFA concentrations. Following period I, steers were weighed, dietary treatments were switched between steer groups, intake amounts were recalculated, and sampling period II then was initiated as described for period I. Regardless of amount of DMI, plasma ghrelin and GH concentrations fluctuated as a result of sampling time relative to feeding. Plasma ghrelin concentrations were elevated prior to feeding at 0800 and 2000 h and reached a nadir from 1 and 3 h post-feeding. Although GH was elevated prior to the 0800 h feeding, it was not elevated at 1800 h despite increasing ghrelin concentrations. A tendency for an interaction of dietary treatment by sampling time relative to feeding which indicated an inverse relationship of plasma INS and ghrelin concentrations for cattle in a positive nutrient balance but no relationship between the two hormones when cattle were in negative energy balance. An interaction of dietary treatment by sampling time relative to feeding also resulted for plasma NEFA concentrations. A positive relationship of NEFA and ghrelin concentrations resulted for cattle when energy and protein intake were below the requirement for maintenance of BW. Ruminal VFA concentrations were weakly correlated to plasma ghrelin concentrations. These data are consistent with the hypothesis that, whereas plasma ghrelin concentrations fluctuate with nutritional status of the ruminant animal, the fluctuation is not completely explained by fluctuations in GH, NEFA, INS or ruminal VFA concentrations.

## Introduction

Inadequate nutrient intake relative to demand for maintenance and (or) production can result in economic loss from poor production efficiency and metabolic disorders. Therefore, understanding feed intake regulation and energy expenditure in cattle is important. Ghrelin is a peptide hormone synthesized by abomasal and ruminal tissues of cattle (Hayashida et al., 2001; Gentry et al., 2003). In rodents, ghrelin stimulates feed intake through neuropeptides in the hypothalamus (Nakazato et al., 2001) and is reported to influence energy metabolism and body composition (Tschöp et al., 2000). Plasma ghrelin concentrations increased with acute (48 h) complete feed deprivation in mature cattle (Wertz-Lutz et al., 2006. Wertz-Lutz et al. (2006) also demonstrated an increase in time spent feeding and a tendency for increased DMI with pulse doses of ghrelin in cattle. Additionally, in rodents, circulating ghrelin concentrations decreased with re-feeding or infusion of glucose but not water (Tschöp et al., 2000). For ruminants, plasma glucose concentrations are one-half that of monogastric animals, and, because ruminants generate the majority of their glucose from the metabolism of propionate in the liver, plasma

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glucose fluctuates less relative to meal consumption. Given the differences in gastrointestinal intestinal tract anatomy and glucose metabolism in ruminants, this experiment was designed to evaluate whether a relationship exists between plasma ghrelin concentrations and end-products of carbohydrate fermentation in the rumen during a 12-h feeding interval when DMI of a high-grain diet is restricted.

#### Materials and Methods

**Dietary treatments.** Dietary treatments for this experiment were two different amounts of a common high-energy diet. Feed amounts were 80% of that necessary to meet the NEm requirement (0.8xM) or 240% of the DMI necessary to meet the NEm requirement (2.4xM) of a given steer and were calculated by using the equations from the Beef NRC (2000) as described below. To determine the amount of DMI necessary to meet the NEm requirement (Mcal/d) the equation 0.077 x empty BW(kg<sup>0.75</sup>) was used (NRC, 2000). This NEm requirement (Mcal/d) then was divided by the energy density of the diet (Mcal/lb) to determine the amount of feed (lb/d) necessary to meet the maintenance requirement of each particular steer as based on its own BW. The amount of feed required to meet the NEm requirement then was multiplied by 2.4 to determine the target amount of DMI for the steers in positive nutrient balance (2.4xM) or multiplied by 0.80 to determine the amount of intake assigned to negative nutrient balance treatment (0.8xM). Once a given amount of feed was determined for each steer, the MP content of the feed was estimated on the basis of degradability of the dietary protein (43.4%) by using the Beef NRC (2000) equation. The amount of dietary MP consumed then was compared with the MP required for BW maintenance as calculated using the equation 3.8 g MP x BW(kg<sup>0.75</sup>) (NRC, 2000).

Animals and procedures. Four ruminally-cannulated (3-yr-old) Angus crossbred steers (BW 1281±28.2 lb; 581.4 ± 12.8 kg) steers fitted with an indwelling jugular catheter. This experiment was conducted in a climate-controlled metabolism facility at South Dakota State University, and all animal procedures were approved by the Institutional Animal Care and Use Committee.

During a 23-d pre-experiment adaptation period, steers were acclimated to the climate-controlled facility. Equal aliguots of feed were offered twice daily at 0800 and 2000 h, and this 12-h feeding interval was maintained throughout the trial. To establish a common starting point, DMI of the common high-grain finishing diet (Table 1) was increased during this acclimation period until DMI was 240% greater than the amount required to meet the NEm requirement of each steers. Once all steers had reached 240% of DMI required to meet NEm, the experiment was initiated.

Table 1. Ingredient composition of experimental diet			
Ingredient %, DM Basis			
Beet pulp	20.00		
Corn	65.00		
Soybean meal	5.67		
DDGS <sup>a</sup>	8.00		
Limestone	1.00		
TM salt <sup>b</sup>	0.30		
Vitamin A D E <sup>c</sup>	0.0055		
Zinc sulfate <sup>a</sup>	0.0056		
Rumensin <sup>e</sup>	0.02		
Calculated Nutrient Composition	DM Basis		
CP, %	12.5		
Degradable intake protein, %	43.4		
NEm, Mcal/lb	0.95		
<sup>a</sup> Dried distiller's grains with solubles			
<sup>o</sup> NaCl 94.0-98.5%, Zn 0.35%, Fe 0.20	0%, Co 0.005%,		
Mn 0.20%, Cu 0.30%, I 0.007%.			
$^{\circ}$ 30,000 IU/g Vitamin A, 500 IU/g Vitamin E, Vitamin D 500,000			

IU/g <sup>d</sup> 35.54% Zn.

<sup>e</sup> Formulated to contain 32 g/T.

The experiment was conducted as a crossover design with two 21-day treatment periods. During treatment period I, 2 steers were maintained at 240% of intake required to meet the NEm requirement (2.4xM) established during the acclimation period and the remaining 2 steers were limited to 80% of the intake to meet NEm requirement. Serial blood and rumen fluid samples were collected on d 7, 14, and 21 following invocation of the restriction. Following period I, dietary treatments were switched between steer groups, steers were weighed, and feed amounts were re-calculated as described for period I based-on the BW recorded at the end of sampling period I. A DMI amount of 2.4xM again was established, and a second 21-d treatment and sampling period II was conducted as described for period I.

**Blood collection.** During each 21-d treatment period, blood and rumen fluid samples were collected at 7, 14, and 21 d after the intake restriction was invoked. On each sampling day, blood samples were collected at 15-min intervals from 0700 and 1145, 1300 to 1345, 1600 to1645, and 1800 to 1845. One 10-mL aliquot of blood was collected into a glass tube containing  $K_3$  EDTA for plasma separation. Aliquots of plasma (1.0 mL each) were processed and stored at -20°C for the subsequent analyses of GH, NEFA, INS, and ghrelin according to procedures outlined by Wertz-Lutz et al. (2006).

*Rumen fluid collection.* Rumen fluid (50 mL) was collected the beginning of each hour in which blood samples were collected and pH was recorded immediately. A 5-mL aliquot of rumen fluid was acidified, centrifuged at 20,000 x *g*, and frozen at -20  $^{\circ}$ C for subsequent quantification of molar proportion of VFA via gas chromatography.

**Statistical analyses.** To verify that differences in NEm and MP intake resulted from the invoked differences in DMI, these characteristics and BW change were analyzed statistically as a crossover design with a model that accounted for variation from sampling period, steer, and amount of DMI. Differences in the characterization parameters that resulted from amount of DMI were separated by using a Fisher's t-test. Plasma ghrelin, GH, INS, and NEFA concentrations and ruminal VFA concentrations and pH were analyzed statistically as repeated measures in time by using the MIXED procedure of SAS with independent errors that accounted for error correlation during the sampling times. The model included length of treatment, sampling time relative to feeding, amount of DMI (0.8xM vs. 2.4xM), steer, period, and the interactions of length of treatment, sampling time, and amount of DMI as independent variables. Differences in least squares means for plasma ghrelin, GH, INS, NEFA, and ruminal VFA concentrations and pH were separated by using a Fisher's t-test. The data set then was divided by dietary treatment and Pearson correlation and stepwise regression was performed to characterize the relationship between plasma hormones and metabolites and end-products of ruminal fermentation for steers in different nutritional states.

#### **Results and Discussion**

*Nutritional state of steers*. Imposed dietary treatments resulted in less ( $P \le 0.002$ ) DMI of the common compositional diet for steers assigned to the 0.8xM compared with the 2.4xM steers (8.6 and 22.9 ± 0.4 lb/d, respectively). This DMI restriction resulted in lower ( $P \le 0.001$ ) energy and MP intake for 0.8xM steers compared with 2.4xM steers. The energy and protein restriction that resulted from restricted DMI decreased ( $P \le 0.001$ ) BW for 0.8xM (-108.9 lb) steers compared with that for 2.4xM (127.9 lb) steers.

**Relationship of plasma hormones and metabolites with end-products of fermentation.** For monogastric animals, carbohydrate but not water ingestion decreased plasma ghrelin concentrations elevated by fasting (Tschöp et al., 2000). Monogastric animals traditionally exhibit a transient elevation in plasma glucose subsequent to a carbohydrate-containing meal. In contrast, ruminants generate the majority of their glucose from the metabolism of propionate in the liver, and plasma glucose fluctuates less relative to meal consumption (Fahey and Berger, 1988). Wertz-Lutz et al. (2006) demonstrated no difference in plasma glucose concentrations. Because the majority of carbohydrate in a ruminant animal diet is fermented by rumen microbes to produce VFA and then converted to glucose, we investigated the relationship of ruminal VFA concentrations and hormones and metabolites indicative of

nutritional status with the fluctuation plasma ghrelin concentrations during a 12-h feeding interval for cattle consuming a high-grain diet.

There was no three-way interaction of dietary treatment by length of treatment by sampling time relative to feeding for any of the measured variables. For this reason, data were pooled for d 7, 14, and 21 and are reported as the interaction of dietary treatment by sampling time relative to feeding in Figure 1. Whereas differences in plasma ghrelin and GH concentrations resulted from the main effects of dietary treatment and sampling time relative to feeding, there was not a significant interaction of dietary treatment by sampling time for these parameters. Plasma ghrelin concentrations were elevated (P < 0.001) for 0.8xM compared with those for 2.4xM steers (181.8 and 86.0 ± 4.8 pg/mL, respectively) throughout the 12-h feeding interval. Plasma GH concentrations also were elevated (P < 0.001) for 0.8xM compared with those for 2.4xM steers (15.0 and  $9.4 \pm 0.45$  ng/mL, respectively) throughout the 12-h feeding interval. Regardless of dietary treatment, plasma ghrelin and GH concentrations fluctuated as a result of sampling time relative to feeding (P < 0.003). Plasma ghrelin concentrations were elevated ( $P \le 0.05$ ) prior to feeding at 0800 and 2000 h, reached a nadir from 1 and 3 h post-feeding, and then began to increase as time progressed toward the next feeding (Figure 1A). Average plasma GH concentration the hour before the 0800 h feeding was higher than plasma GH concentrations throughout the remainder of the sampling period (Figure 1B). Although GH was elevated at the 0800 h feeding along with ghrelin, plasma GH was not elevated at 1800 h prior to the evening feeding despite increasing ghrelin concentrations.



**Figure 1**. Relationship of plasma ghrelin with hormones and metabolites during a 12-h feeding interval for cattle consuming energy and protein above or below that necessary for maintenance of BW. TRT =  $0.8 \times M - 80\%$  of the DMI needed to meet the energy requirement for maintenance;  $2.4 \times M - 240\%$  of the DMI needed to meet the requirement for maintenance. TIME = sampling time relative to feeding times of 0800 and 2000h. TRTxTIME= interaction of the main effects.

Plasma INS concentrations were lower (P < 0.001) for 0.8xM steers compared with 2.4xM steers (0.9 and 1.9 ± 0.07 ng/mL, respectively). There was an interaction of dietary treatment by sampling time relative to feeding ( $P \le 0.05$ ) where plasma INS concentrations did not vary relative to feeding time for 0.8xM cattle but increased through 5 h post-feeding for 2.4xM steers and then began to decline (Figure 1C). Plasma NEFA concentrations were elevated for 0.8xM steers compared with those of 2.4xM. An interaction of dietary treatment by sampling time relative to feeding also resulted for plasma NEFA concentrations (P < 0.001). Plasma NEFA concentrations were elevated 0.8xM steers prior to both the 0800 and the 2000 h feeding and reached a nadir between the two feedings (Figure 1D), whereas a strong relationship between plasma NEFA and ghrelin concentrations did not exist for 2.4xM steers.

Fluctuation of ruminal fermentation characteristics relative to feeding. There was not an interaction of dietary treatment by sampling time relative to feeding for ruminal pH or VFAs. These data therefore, have been reported on the basis of main effects of dietary treatment and sampling time relative to feeding (Figure 2A-H). Ruminal pH was lower (P < 0.001) for 2.4xM steers compared with 0.8xM steers. Ruminal acetate (61.7 and  $50.8 \pm 0.39$  molar%) and butyrate (14.3 and 7.7  $\pm 0.28$  molar%) concentrations were higher (P < 0.001) for 0.8xM steers compared with 2.4xM steers. However, both acetate and butyrate concentrations were similar regardless of sampling time relative to feeding. In contrast, ruminal valerate (1.2 and 2.1 ± 0.07 molar%) and propionate (19.4 and 37.9 ± 0.42 molar%) concentrations were lower (P < 0.001) for 0.8xM steers compared with 2.4xM steers. Ruminal valerate concentrations were similar regardless of sampling time relative to feeding; however, ruminal propionate concentrations were higher (P < 0.05) at 3 and 5 h post-feeding compared with pre-feeding propionate concentrations. Ruminal isovalerate (2.3 and  $0.9 \pm 0.07$  molar%) and isobutyrate (1.2 and  $0.6 \pm 0.02$ molar%) concentrations were higher (P < 0.001) for 0.8xM compared with 2.4xM steers. For both isovalerate and isobutyrate, concentrations in the rumen were elevated (P < 0.05) at sampling times prior to both the 0800 and 2000 h feedings and reached a nadir from 1 to 5 h post-feeding (Figure 2G and 2H). Acetate : propionate was higher (P < 0.001) for 0.8xM steers compared with 2.4xM steers, (3.3 and 1.4 ± 0.05, respectively). Acetate : propionate was higher ( $P \le 0.05$ ) before the 0800 h feeding compared with the remainder of the sampling times relative to feeding. Differences in acetate : propionate predominantly were the result of differences in molar proportions of propionate, as acetate concentrations did not fluctuate with DMI, which suggest a shift in the availability of glucogenic (propionate) versus lipogenic (acetate) precursors.

Although isobutyrate and isovalerate compose relatively small proportion of the total VFA profile of rumen fluid, the fluctuation in their proportions relative to the other VFAs mimics the pattern of plasma ghrelin concentrations more so than do the other VFAs. Previous research, however, demonstrated no relationship between supplementation of isovalerate and isobutyrate and voluntary forage intake (Gunter et al., 1990). Although acetate, propionate, and butyrate have been implicated as potential regulators of DMI, their affects on DMI have been varied. Sheperd and Combs (1998) reported that intraruminal propionate infusion decreased DMI to a greater extent than did acetate in dairy cows, whereas Bahttacharya and Alulu (1975) demonstrated that acetate was more efficacious than propionate in decreasing DMI. In contrast, Quigley et al. (1991) demonstrated no effects of propionate infusion into the portal vein on DMI regardless of whether lambs were in positive or negative energy balance. It is important to note that infusion of VFAs does not completely suppress DMI. Reported suppression of DMI as a result of VFA infusion ranges from 3 to 58% (Bahttacharya and Alulu, 1975; Combs, 1998). Incomplete suppression of DMI with VFA infusion suggests that factors other than VFAs are involved in the regulation of DMI in ruminants.

Pearson correlation and stepwise regression established that significant relationships existed between ghrelin and end-products of ruminal fermentation or hormones and metabolites indicative nutritional status but that the relationships were moderate to weak. Pearson correlations indicated that for 2.4xM steers, plasma GH concentrations (Pearson coefficient = 0.40) and ruminal acetate concentrations (Pearson coefficient = 0.27) were correlated positively ( $P \le 0.01$ ) to plasma ghrelin concentration, whereas plasma NEFA (Pearson coefficient = -0.23) and ruminal propionate (Pearson coefficient = -0.24) concentrations were correlated negatively ( $P \le 0.05$ ) to plasma ghrelin concentrations. Stepwise regression indicated that the fluctuation in ghrelin for 2.4xM steers was explained by ( $P \le 0.01$ ) plasma



**Figure 2**. Ruminal fermentation characteristics during a 12-h feeding interval for cattle consuming energy and protein above or below that necessary for maintenance of BW. TRT = 0.8xM - 80% of the DMI needed to meet the energy requirement for maintenance; 2.4xM - 240% of the DMI needed to meet the requirement for maintenance. TIME = sampling time relative to feeding times of 0800 and 2000h. TRTxTIME= interaction of the main effects.

GH concentrations (partial  $R^2 = 0.15$ ), and to a lesser extent by ruminal acetate concentrations (partial  $R^2 = 0.07$ ) and plasma NEFA concentrations (partial  $R^2 = 0.06$ ). In contrast, the fluctuation in plasma ghrelin concentrations for 0.8xM steers was correlated positively ( $P \le 0.05$ ) to plasma NEFA concentrations (Pearson coefficient = 0.20), and ruminal isovlaerate (Pearson coefficient = 0.25), and valerate (Pearson coefficient = 0.21), but negatively correlated ( $P \le 0.05$ ) to ruminal acetate concentrations (Pearson coefficient = -0.23), acetate : propionate (Pearson coefficient = -0.23) and plasma INS concentrations (Pearson coefficient = -0.53). Stepwise regression indicated that the fluctuation in plasma ghrelin concentration was explained ( $P \le 0.01$ ) by plasma INS concentrations (partial  $R^2 = 0.28$ ) and to a lesser extent by ruminal acetate concentrations (partial  $R^2 = 0.03$ ).

If indeed ghrelin is an orexigenic peptide in ruminant animals, it does not have a strong correlation with end-products of carbohydrate fermentation in the rumen or other hormones and metabolites indicative of nutritional status in ruminants. Data from this experiment are consistent with the hypothesis that, whereas plasma ghrelin concentrations fluctuate with nutritional status of the ruminant animal, fluctuations in plasma ghrelin concentrations within a 12-h feeding interval are not explained completely by fluctuations in plasma GH, NEFA, and INS concentrations or end-products of carbohydrate fermentation in the rumen.

#### Implications

Although ghrelin is responsive to acute and prolonged nutrient restriction, fluctuation in plasma ghrelin concentrations within a 12-h feeding interval is not explained completely by other hormones or metabolites indicative of nutritional status or end-products of carbohydrate fermentation in the rumen. These data imply that ghrelin may serve as a signal for long-term nutrient status in the ruminants.

#### Acknowledgement

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# Plasma ghrelin concentrations of beef cattle consuming a similar amount of dietary energy supplied by different ingredients<sup>1</sup>

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# BEEF 2007-04

# Summary

Previous research demonstrated that restricting nutrient intake by decreasing DMI of a high-grain diet increased plasma ghrelin concentrations. Objectives of this experiment were to determine 1) whether dietary ingredient composition influenced plasma ghrelin concentrations when energy intake was similar, and 2) whether relationships existed between plasma ghrelin concentrations and plasma insulin, NEFA, and GH concentrations or end-products of carbohydrate fermentation in the rumen. Five steers (1290 ± 39.9 lb) were used in a crossover design with dietary treatments of 50% hay-50% concentrate (HAY) offered at an amount that would meet the steer's NEm requirement plus supply an additional 3.5 Mcal of NEg daily, or a diet composed of 10% hay-90% concentrate but limit-fed to achieve an energy intake similar to that of the HAY steers (LFC). Feed was offered in equal aliquots twice daily. Period I: on d 21 following initiation of the dietary treatment, serial blood samples were collected via indwelling jugular catheter at 15-min intervals, and rumen fluid samples were collected hourly throughout a 12-h feeding interval. Following period I, steers were weighed, dietary treatments were switched between steer groups, and intake amounts were recalculated on the basis of period I ending BW. Period II adaptation and sampling was repeated as described for period 1. Plasma samples were assayed for ghrelin, insulin, GH, and NEFA concentrations. Rumen fluid was assayed for VFA concentrations and pH. Net energy for gain was similar between treatment groups  $(3.5 \pm 0.04 \text{ Mcal NEg/d})$ . However, a higher DMI was required by HAY steers compared with LFC steers (20.7 vs.  $15.9 \pm 0.13$  lb) to achieve the same energy intake. Plasma ghrelin concentrations were similar for HAY and LFC steers (115 vs. 107 ± 3.3 pg/mL) despite differences in DMI and incredient composition. Plasma GH, NEFA, and insulin concentrations also were similar regardless of dietary ingredient composition. Strong correlations between plasma ghrelin concentrations and other hormones and metabolites or end-products of carbohydrate fermentation did not result. These data are consistent with the hypothesis that ingredient composition and quantity of DMI do not influence plasma ghrelin concentrations of steers when energy intake is similar and steers are in positive energy balance.

# Introduction

Feed intake decreases and composition of gain shifts toward fat deposition, resulting in slower gains and poorer feed efficiencies in cattle approaching market weight. Understanding of factors that regulate feed intake and composition of gain may improve cattle performance. Ghrelin is a peptide hormone that is involved in the central nervous system regulation of feed intake and body composition (TschÖp et al., 2000). The receptor that binds ghrelin is found on the hypothalamus, adipose tissue, skeletal muscle, and liver, and therefore may influence composition of gain (Wang, et al., 2002). Ghrelin has been reported to altered body composition to favor the deposition of fat accretion (Tschöp et al., 2000; Patel et al., 2006). Because body fat content and distribution influence the value of a carcass marketed in a grid-marketing system, and because rumen VFAs are the primary source of energy and glucose in ruminants, this experiment was designed to determine 1) whether dietary ingredient composition influenced plasma ghrelin concentrations and plasma insulin, NEFA, and GH concentrations or end-products of carbohydrate fermentation in the rumen.

<sup>&</sup>lt;sup>1</sup> This project was funded by National Research Initiative Competitive Research Grant no. 2004-35206-14372.

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#### **Materials and Methods**

**Animals.** Five ruminally-cannulated steers (initial BW 1290 <u>+</u> 39.9 lb) were used in a crossover design. Animal procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

**Dietary treatments.** Steers were adapted to a climate-controlled facility, their respective diet, and a specific feeding schedule during a 21-d pre-treatment period. Steers were offered feed twice daily (0800 and 2000 h). During the adaptation period, it was established that the steer with the lowest DMI on the hay-based diet was consuming enough feed to meet its NEm requirement plus an additional 3.5 Mcal/d NEg. It was assumed that physical fill was limiting intake of this animal, and that intake was therefore used as the benchmark to calculate a common energy intake for all steers, regardless of dietary ingredient composition. Intake of the remaining steers was adjusted such that all steers consumed 3.5 Mcal NEg in addition to that required to meet their NEm requirement, regardless of diet ingredient composition. Dietary treatments differed in ingredient composition and were 50% hav-50% concentrate (HAY) offered at an amount that would meet the steer's NEm requirement plus supply an additional 3.5 Mcal of NEg daily or a diet composed of 10% hay-90% concentrate but limit-fed to achieve a caloric intake similar to that of the HAY steers (LFC; Table 1). To determine the amount of feed intake necessary to meet the NEm requirement (Mcal/d), the equation 0.077 x empty BW(kg)<sup>0.75</sup> was used (NRC, 2000). This NEm requirement (Mcal/d) then was divided by the NEm density of the diet (Mcal/lb) to determine the amount of feed (lb/d) necessary to meet the maintenance requirement of each particular steer based on its own BW. The amount of feed needed to supply the additional 3.5 Mcal/d of NEg was calculated by dividing 3.5 Mcal/d by the NEg density (Mcal/lb) of the diet.

	%, Dry Matter Basis		
Ingredient	HAY	LFC <sup>a</sup>	
Grass hay	50.00	10.00	
Corn	23.12	65.00	
Beet pulp	10.00	10.00	
DDGS <sup>▷</sup>	11.00	8.00	
Soybean meal	5.25	5.67	
Limestone	0.50	1.00	
Trace mineral salt <sup>c</sup>	0.10	0.30	
Rumensin <sup>d</sup>	0.02	0.02	
ZnSO4 <sup>e</sup>	0.007	0.006	
Vitamin E <sup>r</sup>	0.005	0.005	
Vitamin A <sup>g</sup>	0.0004	0.0004	
Vitamin D <sup>h</sup>	0.0001	0.0001	
	Calculated Nutrient Composition, % DMB		
CP, %	13.0	12.3	
NE <sub>m</sub> , Mcal/lb	0.72	0.91	
NE <sub>g</sub> , Mcal/kg	0.44	0.61	

Table 1. Dietary Ingredient Composition

<sup>a</sup> Limit-fed concentrate

<sup>b</sup> Dried distiller's grains with solubles

<sup>c</sup> NaCl 94.0 – 98.5%, Zn 0.35%, Fe 0.20%, Co 0.005%, Mn 0.20%, Cu 0.30%, I 0.007%

<sup>d</sup> Formulated to contain 30 g/Ton

<sup>e</sup> 35.54% Zn

<sup>f</sup> 500 IU/g

<sup>g</sup> 30,000 IU/q

<sup>h</sup> 500,000 IU/g

*Sampling period I.* On d 20 following initiation of the dietary treatment, steers were fitted with an indwelling jugular catheter and allowed a minimum of 12 h to recover prior to initiation of the sampling period. On d 21, blood samples were collected via the indwelling jugular catheter at 15-min intervals from 0700 to 1145, 1300 to 1345, 1600 to 1645, and 1800 to 1845 h. At the beginning of each hour of blood sample collection, a rumen fluid sample also was collected via the rumen cannula.

*Crossover and sampling period II.* Following the initial sampling period, steers were weighed and dietary treatments were switched between steer groups. Feed intake required to meet the NEm requirement plus supply an additional 3.5 Mcal/d NEg was re-calculated as described for period I but on the basis of steer BW at the end of sampling period I. Steers were adapted to the change in dietary ingredients for 20 d at which point an indwelling jugular catheter again was inserted, and the sampling period I was conducted as described for period I.

*Rumen fluid data.* Rumen fluid pH was recorded immediately following collection, and an aliquot of rumen fluid was acidified and centrifuged. The acidified supernatant was frozen and subsequently analyzed for molar proportion of VFA using gas chromatography.

*Hormone concentration data.* Plasma was separated from blood samples by centrifugation, and harvested plasma aliquots were stored at -7° C for subsequent analyses of hormone and metabolite concentrations. Plasma ghrelin and GH are secreted in a pulsatile fashion. Therefore, to minimize variation, ghrelin and GH concentrations were quantified on samples collected at 15-minute intervals and pooled by hour for statistical analyses. Laboratory methodology described by Wertz-Lutz et al. (2006) was used to quantify plasma ghrelin and GH concentrations. Plasma insulin **(INS)** and NEFA concentrations were quantified for samples collected at the beginning of each hour according to the procedures of Wertz-Lutz et al. (2006).

**Statistical analyses.** Plasma ghrelin, GH, INS, and NEFA and ruminal pH and VFA data were analyzed as repeated measures by using the MIXED procedure of SAS. Differences in hormone concentrations that resulted from ingredient composition, sampling time relative to feed offering, or their interaction were separated by using least squares means with the PDIFF option of SAS. The data set then was divided by dietary treatment and Pearson correlation and stepwise regression was performed to characterize the relationship between plasma ghrelin concentrations and plasma hormones and metabolites or rumen fermentation characteristics.

#### **Results and Discussion**

**Performance characteristics.** Steers were in positive energy balance throughout the experiment as indicated by their final BW (1413 ± 26.2 lb) relative to initial BW (1290 ± 39.9 lb). The net change in BW during the 21-d period was 128 ± 44.5 lb and did not differ as a result of treatment. The NEg intake was similar between treatment groups ( $3.5 \pm 0.04$  Mcal NEg/d). However, a higher DMI (P<0.001) was required by HAY steers compared with LFC steers (20.7 vs.  $15.9 \pm 0.13$  lb kg) to achieve the same energy intake.

Previous research from our laboratory demonstrated that plasma ghrelin concentrations were elevated when intake of a high-grain diet was restricted to result in a prolonged moderate energy and protein restriction sufficient to result in loss of BW. Because correlated differences in plasma hormone and metabolite concentrations and ruminal VFA concentrations also resulted with restricted intake of the common ingredient diet, it could not be deciphered whether differences in plasma ghrelin concentrations were attributable to energy intake or altered end-products of carbohydrate fermentation. In the current experiment, plasma ghrelin and GH concentrations fluctuated as a result of time relative to feed offering (P < 0.001) but did not differ as a result of dietary energy source (HAY vs. LFC; Figure 1A and 1B, respectively). Additionally, plasma INS and NEFA concentrations did not differ as a result of dietary energy source or sampling time relative to feed offering (Figure 1C and 1D, respectively). These data are consistent with the hypothesis that plasma ghrelin concentrations are similar for steers for steers in positive energy balance and similar energy intake regardless of dietary energy source or DMI.



**Figure 1.** Effects of ingredient composition on plasma hormone concentrations for steers fed similar amounts of energy. HAY (50% hay, 50% concentrate) was fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations. LFC (10% hay 90% concentrate) was limit-fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations. LFC (10% hay 90% concentrate) was limit-fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations. Statistical effects are reported as TRT – effects of HAY vs. LFC; TIME effect of sampling time relative to feeding; TRTxTIME is the interaction of the main effects.

We speculated, on the basis of previous research in our lab, that differences in plasma ghrelin concentrations for steers fed different amounts of a common high-grain diet could have resulted from energy intake or could be attributed to differences in rumen distention as the vagus nerve has been implicated as responsive to ghrelin and involved in its release in various animal species (Sugino et al., 2003; Arnold et al., 2006). In the current experiment, plasma ghrelin concentrations did not differ when energy intake was similar but DMI and dietary ingredient composition differed. These data further support the hypothesis that differences in plasma ghrelin concentrations observed for cattle experiencing prolonged nutrient restriction were the result of differences in nutrient intake and not rumen fill.

Ruminal pH differed as a result of time relative to feeding (P < 0.001) and dietary energy source (P < 0.001) (Figure 2A). Ruminal pH was lower (P < 0.001) for LFC steers compared with HAY steers, regardless of sampling time relative to feed offering. Additionally, rumen pH decreased following feed offering and reached a nadir 3 to 4 h post feed consumption and then began to rise regardless of ingredient composition. Dietary energy source influenced ruminal VFA concentrations (P < 0.002) (Figure 2B – 2H). Molar concentrations of acetate were higher (P < 0.001) for HAY cattle at all sampling times. In contrast, propionate, valerate, and isovalerate concentrations were higher for LFC steers (P < 0.001). Ruminal acetate : propionate was higher (P < 0.001) for HAY steers compared with LFC. Other researchers have demonstrated that the increased acetate : propionate that occurs with greater dietary forage content is the result of decreased propionate production (Bauman et al., 1971), as acetate production is constant with a wide variety of diets (Esdale, et al., 1968 and Davis, 1967).



**Figure 2**. Effects of ingredient composition on characteristics of rumen function for steers similar amounts of energy. HAY (50% hay, 50% concentrate) was fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations. LFC (10% hay 90% concentrate) was limit-fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations.

Ruminants generate the majority of their glucose from the metabolism of propionate in the liver, for this reason, plasma glucose concentrations are lower than those of monogastric animals and fluctuates less relative to meal. In rodents, circulating ghrelin concentrations decreased with re-feeding or infusion of glucose but not water (Tschöp et al., 2000). Glucose infusion, however, did not decrease plasma ghrelin concentrations when gastric emptying was prevented, which suggests that glucose absorption is necessary to influence plasma ghrelin concentrations (Williams et al., 2003). Little carbohydrate that can be converted to glucose for absorption reaches the small intestine of the ruminant. Therefore little glucose is absorbed from the intestine of ruminants. If however, propionate in ruminants is analogous to glucose in monogastric animals, we hypothesized that ghrelin concentrations should be lower when propionate concentrations are high (acetate : propionate is low). Because decreased acetate : propionate occurs with a high-grain diet as a result of increased propionate production (Bauman et al., 1971), greater glucogenic precursor is available when a high-grain diet is fed to steers. Despite a lower acetate : propionate for LFC compared with HAY steers in the current experiment, plasma ghrelin concentrations did not differ, suggesting that the ratio of glucogenic VFA to lipogenic VFA does not influence plasma ghrelin concentrations when steers are in positive energy balance and energy intake is similar.

Pearson correlation was used to determine whether significant relationships existed between plasma ghrelin concentrations and other hormones and metabolites indicative of nutritional status or end-products of carbohydrate fermentation. For LFC steers, acetate (Pearson coefficient = 0.29) and isovalerate (Pearson coefficient = 0.44) were correlated positively ( $P \le 0.02$ ) to plasma ghrelin concentrations, whereas ruminal propionate (Pearson coefficient = -0.32) and ruminal valerate concentrations (Pearson coefficient = -0.41) were correlated negatively ( $P \le 0.05$ ) to plasma ghrelin concentrations. Stepwise regression revealed that, although significant ( $P \le 0.05$ ), isovalerate (partial  $R^2 = 0.19$ ), valerate (partial  $R^2 = 0.16$ ), butyrate (partial  $R^2 = 0.11$ ) and isobutyrate (partial  $R^2 = 0.09$ ) explained a small amount of the variation in plasma ghrelin concentrations. For HAY cattle, INS (Pearson coefficient = -0.27) was correlated negatively ( $P \le 0.09$ ) to plasma ghrelin concentrations. Stepwise regression indicated that plasma INS concentrations explained half (partial  $R^2 = 0.51$ ) of the variation in plasma ghrelin concentrations explained half (partial  $R^2 = 0.51$ ) of the variation in plasma  $R^2 = 0.10$ .

These data indicate that plasma ghrelin concentrations are similar when dietary energy intake is similar for cattle in positive energy balance, regardless of ingredient composition or DMI. For steers in positive energy balance, fluctuations in plasma ghrelin concentrations relative to feeding time are not explained completely by differences in plasma GH, INS, or NEFA concentrations or by fluctuations in end-products of carbohydrate fermentation.

## Implications

Similar plasma ghrelin concentrations for cattle with similar energy intake regardless of differences in ingredient composition, resulting differences in VFA or hormone profiles, or amount of DMI implies the fluctuation of plasma ghrelin concentrations are the result of differences in nutrient intake and not the result of end-products of carbohydrate fermentation or rumen fill. Further research is warranted to investigate the role of ghrelin communicating nutrient status in the animal and its potential influence on composition of gain and efficiency of nutrient utilization.

#### Acknowledgement

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# Influence of post-AI nutrition on blood urea nitrogen, progesterone, and pregnancy<sup>1</sup>

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# BEEF 2007-05

# Summary

Research has shown that changes in nutrition can have an effect on reproductive performance. Our objective was to determine the effect of post-AI nutrition on BCS, blood urea nitrogen (BUN), progesterone, and pregnancy rates. Forage-developed Angus-cross bred heifers (n = 336) were synchronized with the Select Synch+ Controlled Internal Drug Releasing device (CIDR) protocol (d -7 100 ug GnRH and CIDR; d 0 25 mg PG and removal of CIDR). Estrus was detected for 72 h and heifers bred by AI 12 h after being detected in estrus; heifers not in detected in estrus were bred by AI and given an injection of GnRH at 72 h. Each breeding period was equally divided into three treatments; 1) heifers returned to feedlot (LOT), 2) heifers were moved to pasture (PASTURE), or 3) heifers were moved to pasture and supplemented with 5 lb/hd/d of dried distillers grains plus solubles (SUPP). Blood samples were collected on d -7, 0, 2, 14 and 42 (pregnancy determination; d 0 = AI). Body condition scores were determined on d -7 and 42. All heifers were in similar BCS (5.4  $\pm$  0.05) on d -7, but on d 42 SUPP (5.9  $\pm$ 0.04) were in better condition than LOT (5.8  $\pm$  0.04) which were in better condition than PASTURE (5.4  $\pm$ 0.04). All treatments had similar BUN concentrations on d -7 (129  $\pm$  1), but on d 2, 14, and 42 SUPP had greater BUN concentrations compared to both LOT and PASTURE. There was no difference in BUN concentrations between pregnant and open heifers. Progesterone concentrations were similar among all heifers on d 0 and 2. On d 14, SUPP had greater progesterone concentrations compared to LOT, and on d 14 and 42 PASTURE had greater progesterone concentrations compared to LOT. Progesterone was similar for open and pregnant heifers on d 0 and 2, but greater in pregnant heifers on d 14 and 42. There was no difference among treatments in pregnancy rates (57, 56, and 59% for SUPP, LOT, and PASTURE; analyzed by chi-square). In summary, supplementing forage-developed heifers after insemination increased BCS and BUN concentrations but had no effect on pregnancy rates.

## Introduction

Embryonic development is a complex process involving genome activation, compaction, blastocyst formation, elongation, maternal recognition of pregnancy, and attachment. A disruption in any of the preceding processes may result in embryonic mortality. Data on the incidence of embryonic mortality in cattle is limited, but the majority of embryonic loss in cattle is reported to occur before day 42 of pregnancy (Ayalon, 1978; Maurer and Chenault, 1983; Peters, 1996). Nutrition can influence embryonic survival through many mechanisms, but the most likely possibilities include direct or indirect regulation of the uterine environment. Nutritionally mediated changes to the uterine environment can occur by changing components of uterine secretions or by influencing the circulating concentrations of progesterone that regulate uterine environment (Foxcroft, 1997). Nutrition can directly influence the uterine environment through protein intake. Heifers fed 85% of their energy and protein maintenance requirements had reduced numbers of cleaved ova on day 3 and morula on day 8 compared to heifers fed 100% maintenance (Hill et al., 1970) indicating decreased embryonic growth. Heifers fed excess protein (25% excess of UIP or DIP) had altered the pH of the uterus on day 7 (Elrod et al., 1993). Cool season grasses grazed throughout the upper Midwest can vary greatly in crude protein throughout the summer months, and the majority of this variability occurs in degradable intake protein (Patterson, 2000).

<sup>&</sup>lt;sup>1</sup> This project was funded by the South Dakota Corn Utilization Council.

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Therefore, the objective of this experiment was to determine if supplementing energy and protein post-AI influences BCS, blood urea nitrogen (BUN), progesterone, and pregnancy rates.

## **Materials and Methods**

# Experimental Design

This experiment was conducted on 336 foraged-developed heifers at one location. Over the previous winter, heifers were developed on range and supplemented as conditions required. Seven days prior to the start of the breeding season all heifers were brought into a feedlot and synchronized with the Select Synch + Controlled Internal Drug Releasing device (CIDR) protocol (d -7 100  $\mu$ g GnRH and CIDR; d 0 25 mg PG and removal of CIDR). Estrus was detected for 72 h and heifers bred 12 h by AI after detection in estrus; heifers not detected in estrus were bred by AI and given an injection of GnRH at 72 h. Estrus was detected by visual observation with the aid of EstroTech (Rockway Inc., WI) patches. Approximately 12 h following the initiation of standing estrus animals were Aled by a single technician to a single sire.

Following insemination, heifers were immediately placed in one of three treatment groups (Figure 1): 1) heifers were returned to the feedlot (LOT), 2) heifers were moved to pasture (PASTURE), or 3) heifers were moved to pasture and supplemented with 5 lb/hd/d of dried distillers grains plus solubles (DDGS; SUPP). Heifers remained in treatment groups for 42 days until pregnancy was determined by transrectal ultrasonography. Bulls were placed with heifers 11 days following the final AI for a 28-d breeding season. Pregnancy rates were determined by transrectal ultrasonography on day 42 and 60 days following bull removal.



**Figure 1.** Animals were randomly allotted to one of three post-AI dietary treatments following synchronization with the Select-Synch plus CIDR protocol and detection in standing estrus.

## Blood Collection and Radioimmunoassays

Blood samples were collected by venipucture into 10 mL vacutainer tubes (Fisher Scientific, Pittsburgh, PA) on day -7, 0, 2, 14 and 42. Blood was allowed to clot for 1 h at room temperature, stored at 4°C for 24 hours, and centrifuged at 1200xg for 30 minutes to harvest serum. Serum was stored at –20°C until assayed for progesterone and blood urea nitrogen (BUN). Intra- and interassay coefficients of variation for progesterone assays were 9.6% and 4.7% respectively, and assay sensitivity was 0.4 ng/mL of serum. Intra- and interassay coefficients of variation for BUN assays were 5.0% and 2.5% respectively, and assay sensitivity was 5 mg/dL of serum.

## Statistical Analysis

Differences between treatments in BUN and circulating concentrations of progesterone were determined by analysis of repeated measures. Differences in BCS and pregnancy rates were determined by chi-square analysis.

## **Results and Discussion**

At the initiation of the trial all heifers were in similar BCS (P = 0.78; 5.4 ± 0.05), and all treatments had similar BUN concentrations (P > 0.14; 12.9 ± 1 mg/dL). Following the treatment period (day 42 after AI)

SUPP heifers (5.9 ± 0.04) were in better BCS (P < 0.01) than LOT (5.8 ± 0.04) which were in better BCS (P < 0.01) than PASTURE (5.4 ± 0.04; Table 1). On day 2, 14 and 42 after AI supplemented heifers had greater (P < 0.01) BUN concentrations compared to both LOT and PASTURE heifers (Figure 2). However, there were no differences in BUN concentrations between pregnant and open heifers (P = 0.37; Figure 3). The increased protein being supplemented with the DDGS likely caused the increase in BUN. However, the increase in BUN was not to the level that has been reported to change uterine environment and decrease pregnancy rates (Elrod and Butler, 1993).

	Feedlot	Pasture	Pasture & Supplement
Day -7	$5.4 \pm 0.05$	$5.4 \pm 0.05$	$5.4 \pm 0.05$
Day 42	$5.8\pm0.04^a$	$5.4\pm0.04^{b}$	$5.9\pm0.04^{\rm c}$

 Table 1. Influence of treatment on body condition score

Columns with different superscripts are different ( $^{abc}P < 0.01$ ). Data reported as mean  $\pm$  SE



**Figure 2.** Influence of treatment on circulating concentrations of blood urea nitrogen. \* indicates P < 0.01. Day -7 is day of CIDR insertion.



**Figure 3.** Influence of pregnancy on circulating concentrations of blood urea nitrogen. Day -7 is day of CIDR insertion.

Progesterone concentrations were similar among all heifers ( $P \ge 0.05$ ) on d 0 (day of AI) and 2. However, the supplemented heifers had greater progesterone on d 14 (P = 0.02) compared to LOT, and on day 14 and 42 PASTURE had greater progesterone (P < 0.02) compared to LOT (Figure 4). Progesterone was similar (P > 0.16) for open and pregnant heifers on d 0 and 2, but greater (P < 0.04) in pregnant heifers on d 14 and 42 (Figure 5) as expected. There were no differences among treatments in pregnancy rates (Table 2). This confirms that the increased BUN from supplementing extra protein was not to the level to effect pregnancy rates.



**Figure 4.** Influence of treatment on circulating concentrations of progesterone. \* indicates P < 0.01. Day -7 is day of CIDR insertion.



**Figure 5.** Influence of pregnancy on circulating concentrations of progesterone. \* indicates P < 0.01. Day -7 is day of CIDR insertion.

Table 2.	Influence	of treatment of	on preg	gnancy	rates
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			Pasture &
	Feedlot	Pasture	Supplement
Day 42	56%	59%	57%
Final	86%	89%	88%

Bulls introduced 11 days after AI for a 28 day period.

In conclusion, heifers that were developed on pasture throughout the winter prior to breeding had increased BCS and increased BUN when supplemented after AI, but supplementation had no benefit on pregnancy rates. Results may differ if heifers had been developed in a feedlot from weaning to breeding.

#### Acknowledgements

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# The effect of GnRH at time of insemination on initiation of LH pulses and subsequent progesterone<sup>1</sup>

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# Summary

Research has indicated that luteinizing hormone (LH) pulses play a vital role in corpus luteum (CL) formation and subsequent progesterone concentrations. Therefore, our objectives were to determine: 1) when LH pulses begin following onset of estrus, 2) the effect an injection of gonadotropin releasing hormone (GnRH) would have on initiation of LH pulses, and 3) the effect LH pulse initiation had on subsequent plasma progesterone concentrations. Cows were synchronized with the Select Synch + Controlled Internal Drug Releasing device (CIDR) protocol (d -7 100 µg GnRH and CIDR; d 0 25 mg prostaglandin (PG) and removal of CIDR; estrus detected with HeatWatch). Following detection in estrus, a jugular catheter was inserted in each cow (n = 10). Based on initiation of estrus, cows were allotted into two treatments: 1) GnRH given 12 h (12.5  $\pm$  1.2 h) after the initiation of estrus (n = 5; 100  $\mu$ g) and 2) Control (n = 5). Blood samples were collected at 15-min intervals for 6 h at 12 h (bleed 1), 26 h (bleed 2), 40 h (bleed 3), 54 h (bleed 4), and 68 h (bleed 5) after the onset of estrus. The interval from onset of estrus to bleed 1 and ovulation was similar between treatments. The GnRH cows tended to have a greater area under the LH curve for bleed 1 compared to control cows. No differences were detected in bleeds 2, 3, 4, or 5. Average concentration of LH for GnRH cows in bleed 1 tended to be greater than control. No differences were detected in bleeds 2, 3, 4, or 5. No differences were detected in pulse frequency between treatments in bleeds 1, 3, 4, or 5, but in bleed 2, control tended to have more pulses than GnRH ( $2.5 \pm 0.5$  vs  $1.4 \pm 0.4$ ). The GnRH-treated cows tended to have greater subsequent progesterone concentrations; however, GnRH-treated cows that had no LH pulses during bleed 2 had lower progesterone concentrations than cows with pulses (control or GnRH). In summary, injecting cows with GnRH approximately 12 h after the onset of estrus tended to reduce LH pulses 26-32 h following initiation of estrus, and elimination of LH pulses between 26-32 h resulted in decreased concentrations of progesterone during the subsequent cycle.

## Introduction

A single injection of GnRH results in the release of LH. A surge of LH results in ovulation and the formation of a CL which produces progesterone. Progesterone is essential for embryo development and maintenance of pregnancy. It has been proposed that giving an injection of GnRH at the time of insemination could increase CL function; thereby, increasing subsequent concentrations of progesterone and embryo survival. Unfortunately, researchers have had conflicting results. In dairy cows, Mee et al. (1993) reported that an injection of GnRH given at the time of insemination caused an increase in subsequent concentrations of progesterone. However, Lucy et al. (1986) reported that an injection of GnRH at time of insemination decreased subsequent concentrations of progesterone. Furthermore, a study done in our laboratory with beef heifers also resulted in a decrease in subsequent concentrations of progesterone (Perry, 2006). Therefore, the question arises, why have different studies reported conflicting results?

Release of LH from the pituitary has been reported to be necessary for CL formation and function (Peters et al., 1994). Furthermore, pituitary LH content returned to normal within 1 d following an ovulatory surge of LH (Nett et al., 1986). Therefore, it can be expected that LH pulses return to normal within 1 d of an ovulatory surge of LH. However, Peters et al. (1994) found that utilizing a luteinizing hormone releasing hormone (LHRH) antagonist to inhibit LH pulses following an ovulatory surge of LH resulted in decreased

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CL function; thereby, resulting in decreased subsequent concentrations of progesterone. Based on these studies, we hypothesized that GnRH would delay the initiation of LH pulses, and by changing the timing of the initiation of LH pulses, would influence subsequent concentrations of progesterone. Our objectives were to determine: 1) when LH pulses begin following ovulation, 2) the effect an injection of GnRH would have on the initiation of LH pulses, and 3) the effect the timing of LH pulse initiation would have on subsequent concentrations of progesterone.

#### **Materials and Methods**

Thirty-two non-lactating, open, mature cows were synchronized with the Select Synch + CIDR protocol. An injection of GnRH (100  $\mu$ g as 2 mL of Ovasynch i.m.; IVX, St. Joseph, Missouri) was given at the time of the CIDR insertion. The CIDRs were left in for 7 days. All cows were given an injection of prostaglandin (PGF<sub>2a</sub>; 25 mg as 5 mL of Prostamate i.m., IVX, St. Joseph, Missouri) at the time of CIDR removal. The Heat Watch estrous detection system was used to determine when the cows initiated standing estrus. Onset of estrus was determined as the first of 3 mounts within a 4-h period of time lasting 2 s or longer in duration. After 10 cows were determined in standing estrus within a 6-h period of time, indwelling jugular catheters were inserted into each of the cows. At an average of 12 h after the onset of estrus, 5 of the cows were given an injection of GnRH (treatment group) and 5 did not receive treatment (control group).

Blood samples were collected via jugular catheters every 15 min for 6 h from 12-18 (bleed 1), 26-32 (bleed 2), 40-46 (bleed 3), 54-60 (bleed 4), and 68-74 (bleed 5) h after the onset of estrus. Cows then were bled daily for 15 d until the  $18^{th}$  d of their estrous cycle. Blood was allowed to coagulate at room temperature then stored at 4° C for 24 h. Samples were centrifuged at 1,200 x *g* for 30 min, and the serum was harvested and frozen at -20° C until analyzed. Serum samples from the intensive bleeds were analyzed for LH concentrations, and daily blood samples were analyzed for serum concentrations of progesterone using radio immunoassays (RIA).

Transrectal ultrasonography was used to determine ovulation using an Aloka 500V ultrasound with a 7.5 MHz linear probe (Aloka, Wallingford, CT). Ovulation was defined as the disappearance of a dominant follicle from an ovary.

Cluster was used to determine LH total content, average concentration of LH, and LH pulse frequency. Differences between the treatment groups in total content of LH, average concentration of LH, LH pulse frequency, and subsequent concentrations of progesterone were determined by analysis of repeated measures in SAS.

#### Results

There were no differences between treatments for the interval from the onset of estrus to bleed 1 (P = 0.82; Table 1). The GnRH treated group tended to have a greater total content of LH (P = 0.075) and greater average concentration of LH (P = 0.068) compared to the control group (Table 1). There was no difference (P = 0.65) in LH pulse frequency. During bleed 2, there were no differences in LH total content (P = 0.48) or average concentrations of LH (P = 0.53). However, the control group tended (P = 0.095) to have a greater LH pulse frequency compared to the GnRH-treated group (Table 2). All animals ovulated by 32 h after the onset of estrus. During bleeds 3, 4, and 5 there were no differences (P > 0.10) in LH total content, average concentration of LH, or LH pulse frequency (Tables 3, 4, and 5).

Table 1. Influence of an injection of GnRH on LH concentration and pulse frequency12 - 18 h after the onset of standing estrus.

		0	
	GnRH	Control	P-value
Interval from onset of estrus	12.5 ± 1.2	12.1 ± 1.2	0.82
Total LH content ng/6 h	2091.2 ± 158.4	1647.6 ± 182.9	0.075
Average concentration of LH;			
ng/mL	$5.6 \pm 0.4$	$4.4 \pm 0.5$	0.068
LH pulse frequency; pulses/6 h	2.0 ± 0.5	$2.3 \pm 0.6$	0.65

		<u> </u>	
	GnRH	Control	P-value
Total LH content ng/6 h Average concentration of LH;	1259.5 ± 141.6	1411.5 ± 158.4	0.48
ng/mL	$3.5 \pm 0.4$	$3.8 \pm 0.4$	0.53
LH pulse frequency; pulses/6 h	1.4 ± 0.4	$2.5 \pm 0.5$	0.095

Table 2. Influence of an injection of GnRH on LH concentration and pulse frequency 26 - 32 hours after the onset of standing estrus.

Table 3. Influence of an injection of GnRH on LH concentration and pulse frequency 40 - 46 h after the onset of standing estrus.

	GnRH	Control	P-value
Total LH content ng/6 h	1316.3 ± 141.6	1296.5 ± 141.6	0.92
Average concentration of LH;			o o=
ng/mL	$3.5 \pm 0.4$	$3.4 \pm 0.4$	0.87
LH pulse frequency; pulses/6 h	$2.0 \pm 0.4$	2.2 ± 0.4	0.74

Table 4. Influence of an injection of GnRH on LH concentration and pulse frequency 54 - 60 h after the onset of standing estrus.

	GnRH	Control	P-value							
Total LH content ng/6 h Average concentration of LH;	1306.7 ± 141.6	1352.7 ± 158.4	0.83							
ng/mL	$3.5 \pm 0.4$	$3.6 \pm 0.4$	0.83							
LH pulse frequency; pulses/6 h	1.8 ± 0.4	$2.3 \pm 0.5$	0.49							

Table 5. Influence of an injection of GnRH on LH concentration and pulse frequency 68 - 74 h after the onset of standing estrus.

	GnRH	Control	P-value
Total LH content ng/6 h Average concentration of LH:	1595.3 ± 141.6	1697.5 ± 158.4	0.63
ng/mL	$4.3 \pm 0.4$	$4.5 \pm 0.4$	0.67
LH pulse frequency; pulses/6 h	1.6 ± 0.4	$2.3 \pm 0.5$	0.32

For subsequent concentrations of progesterone, the GnRH-treated group tended (P = 0.07) to have greater concentrations than the control group, but there was not a treatment x time interaction (Figure 1). Two of the GnRH-treated animals did not have any LH pulses during bleed 2, while 3 animals did have LH pulses. All of the control animals had LH pulses. The GnRH-treated animals that did have LH pulses during bleed 2 had greater (P < .0001) concentrations of progesterone compared to animals that did not have LH pulses. The control group falls intermediate between the two (Figure 2).



Figure 1. Influence on an injection of GnRH 12 hours after the onset of standing estrus on subsequent concentrations of progesterone. (Treatment P = 0.07; Time P < 0.0001; Treatment x time P = 0.72).



Figure 2. A comparison among the GnRH animals which had LH pulses during bleed 2, GnRH which did not have LH pulses during bleed 2, and control. (Treatment P < 0.0001; Time P < 0.0001; Treatment x time P = 0.014).

#### Discussion

Adequate production of progesterone by the CL must occur in order to establish and maintain pregnancy; therefore, it is important to have a well formed CL. In this study, GnRH was given at the time of insemination and tended to increase LH content and average concentrations from 12 to 18 h after the onset of estrus. However, it tended to reduce LH pulse frequency during bleed 2 (26 to 32 h after the onset of estrus). This may be due to the increased dumping of LH from the pituitary, down regulation of GnRH receptors in the pituitary, or another unknown mechanism. Bleed 2 may be an important time point for LH pulses because it is during the time that ovulation occurs. Ovulation occurs at about 30 h after the onset of estrus, and bleed 2 was 26-32 h after the onset of estrus. Animals that pulsed during that time period had greater subsequent concentrations of progesterone compared to animals that did not have LH pulses. Furthermore, the control animals had concentrations of progesterone intermediate between the other two groups. This may explain why sometimes research has indicated increases in subsequent concentrations of progesterone intermediate between the other two groups. This may explain why sometimes research has reported decreases in subsequent concentrations of progesterone when given GnRH at the time of insemination.

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# Evaluation of mixing characteristics of diets containing modified distillers grains<sup>1</sup>

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# Summary

Six mixes of feed were manufactured and analyzed to determine how sequence of ingredient addition into a feed mixer influences mixing characteristics when modified distillers grains (mDG) was used as a feed ingredient. Five mixes were manufactured using a 3-bar rotor mixer and one mix was manufactured using a staggered-rotor mixer. There were three diet types evaluated: 1) high-forage receiving diet; 2) high-grain finishing diet with ground grass hay (GH) as the roughage source; and 3) high-grain finishing diet with silage as the roughage source. Five samples were collected from each mix and were analyzed for particle size and nutrient composition to determine within load coefficient of variation (CV). Based on these data mDG should be added before GH. The within load CV for particle size, CP, ADF, and sulfur were lower for a diet containing silage and mixed in a staggered-rotor mixer compared with a diet containing GH mixed in a 3-bar rotor mixer.

# Introduction

The ability to consistently mix what has been formulated by a nutritionist to supply feedlot cattle with the appropriate nutrients for expected growth is a daily expectation at all feedlots. As discussed by Pritchard and Stateler (1997), there are multiple characteristics of feed processing and mixing that influence cattle performance. Properties of feed such as particle size, particle shape, density, hygroscopocity, static charge, and adhesiveness can influence how a diet mixes (Behnke, 2005). Those ingredient characteristics along with mix time, mixer overload, worn/broken mixing components, ingredient build up, and/or improper sequence of ingredient addition can lead to non-uniformity within a mix of feed (Behnke, 2005). Using data from the South Dakota State University Feedlot Shortcourse, Wagner (1995) demonstrated that the sequence that hay is added to a mixer influences the mix quality. Additionally, Wagner (1995) reported that the length of time to obtain an adequate mix can differ dependent on the type of mixer being used. Daily diligence such as following the management practices outlined by Turgeon (Turgeon, 2006) is critical to prevent or at least minimize inconsistencies in the diets prepared and distributed.

# Materials and Methods

*Mixers:* Six diets were evaluated to determine how sequence of ingredient addition affects uniformity of a mix of feed. Five of the diets were mixed in a ROTO-MIX 184-10 wagon-mounted mixer (RM184). This mixer wagon has two augers and a 3-bar rotor and is listed to have 180 ft<sup>3</sup> mixing capacity. One diet was mixed in a ROTO-MIX 620-16 truck-mounted mixer (RM620). This mixer has two augers and a staggered-rotor and is listed to have 620 ft<sup>3</sup> mixing capacity.

*Mixing procedures:* Three types of diets were mixed. Listed in Table 1 are the ingredients and the sequence they were added for each of the six mixes that were manufactured. Mixes 1 and 2 were receiving diets and mixes 3, 4, and 5 were finishing diets. These five mixes all contained ground grass hay as the roughage source and were mixed in the RM184. Mix 6 was a finishing diet that contained corn

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silage as the roughage source and was mixed in RM620. For each mix of feed, the mixer was on continuously as ingredients were loaded into the mixer. The mixer ran for three minutes after the last ingredient was added.

	High-for	age diet		High-9	I-grain diet			
Item	MIX 1	MIX 2 <sup>b</sup>	MIX 3	MIX 4 <sup>c</sup>	MIX 5 <sup>°</sup>	MIX 6		
Ingredient level of ir								
Dry-rolled corn	33.50 (2)	(3)	43.73 (2)					
Pelleted supp.	6.50 (1)	(2)	6.50 (1)	(1)	(2)	_		
Liquid supp.	_	_	_	_	_	3.33 (1)		
Silage	-	-	-	_	-	33.84 (3,5) <sup>d</sup>		
Ground hay	35.00 (4)	(1)	10.00 (4)	(3)	(4)	_		
mDG <sup>e</sup>	25.00 (3)	(4)	25.00 (3)	(4)	(1)	19.11 (4)		
Formulated diet con	nposition							
DM	74.85		75.03			47.82		
			DM	basis				
Crude protein, %	14.29		14.45			15.19		
ADF, %	18.28		-					
Sulfur, %	0.33		0.31			0.28		
Monensin, g/ton	27		27			26		

Table 1. Formulated diet composition and sequence of ingredient addition into feed mixers<sup>a</sup>

<sup>a</sup> Mixes 1 – 5 mixed in a 184 ft<sup>3</sup> 3-bar rotor ROTO-MIX; Mix 6 mixed in a 620 ft<sup>3</sup> staggered-rotor ROTO-MIX <sup>b</sup> Similar composition as diet used in MIX 1

<sup>c</sup> Similar composition as diet used in MIX 3

<sup>d</sup> Approximately 1/3 of the corn silage was added after the dry-rolled corn with the remainder added after the mDG

<sup>e</sup> Modified distillers grains

For RM184, the pelleted supplement was weighed on a stationary balance and placed in the bucket of the loader along with a partial scoop of dry-rolled corn (DRC). The liquid suspension supplement in the RM620 mix was added to an empty mixer. The liquid pooled in the front of the mixer compartment. Approximately 1/3 of the corn silage was added after the DRC with the remainder of the silage added after the modified distillers grains (mDG).

Sampling procedures: There were five samples collected from each mix of feed. The samples from the RM184 were collected on the discharge spout. Each load of feed from the RM184 weighed 1,500 lb. The first sample was obtained with <100 lb discharged from the mixer. Each subsequent sample was obtained after approximately 375 lb of feed was discharged. An 18,000 lb load of feed was prepared in the RM620. The samples from the RM620 were collected from the feed in the bunk at equally spaced intervals.

Sample analysis: Particle size was determined for each sample using the Penn State Forage Particle Separator following the procedures and calculations of Heinrichs and Kononoff, 2002. Crude protein (analyzed as nitrogen; AOAC 990.03), ADF (AOAC 973.18), and sulfur (AOAC 923.10) concentrations were determined in the Olsen Biochemistry laboratory. Chloride was measured using Quantab® titrators (Environmental Test Systems, Elkhart, IN). Monensin concentration was determined for 10 samples (two mixes) at the Eurofins Animal Health Testing Laboratory (Memphis, TN) a service provided by Elanco Animal Health (Greenfield, IN).

#### **Results and Discussion**

The first objective of this experiment was to evaluate how the sequence of ingredient addition affected the uniformity of particle size and nutrient distribution within a mix of feed. Mixes 1 and 2 were diets similar in composition to a receiving cattle diet. Ground hay was added either as the first or last ingredient. The

two diets were mixed on separate days explaining the dissimilar diet particle size (Table 2). There were differences in the particle length of the ground hay between days. Because of this it is difficult to ascertain whether the increase in variation of the distribution of particle size is due to the sequence of ingredient addition or to the change in particle length of the hay. In this case, the authors believe that both factors influenced the outcome. When hay was added first, the top auger pushed enough hay to the back of the mixer that the hay was trickling over the side of the mixer wagon. The heterogeneous composition of ground GH leads to variation in how well diets can be mixed.

		High-for	age die	t			High-grain dietHigh-grain diet					
Item	M	ΙXĪ	MI	X 2	Μ	MIX 3		MIX 4		IX 5	MIX 6	
Particle size, mm (CV, %)												
	4.7	5 (8.5)	6.7	9 (19 )	5.54	4 (6.0)	4.72 (7.9)		4.71 (6.0)		8.69	9 (2.9)
Distribution of particles on each sieve or pan, % on sieve						sieve or	<sup>-</sup> pan (C	V, %) <sup>a</sup>				
Upper	6.9	(37)	31	(39)	3.8	(32)	6.7	(37)	8.0	(13)	5.1	(8.9)
Middle	35	(6.8)	20	(42)	48	(6.5)	39	(9.3)	34	(6.4)	66	(3.1)
Lower	37	(7.5)	29	(21)	32	(6.5)	30	(3.3)	32	(3.9)	26	(7.5)
Pan	21	(9.3)	20	(14)	16	(8.1)	25	(10)	24	(7.2)	3.2	(11)
Cumulative pa	articles	under ea	ach siev	e, % un	lder							
Upper	93	(2.8)	69	(18)	96	(1.3)	93	(2.7)	92	(1.2)	95	(0.5)
Middle	58	(6.5)	49	(13)	49	(6.6)	54	(5.8)	56	(4.5)	29	(7.3)

Table 2. Effect of ingredient addition sequence on particle size and particle size distribution within mixes of feed

<sup>a</sup> Within a column the percent retention on the sieves and pan are means of five samples and may not equal 100%

Mixes 3, 4, and 5 were prepared in the RM184. The variation in particle size of those diets was similar among mixes. Comparison of these mixes with Mix 6 (prepared in RM620), demonstrates the decrease in variation of particle size when silage is used rather than ground GH.

The most variation in particle size distribution for diets containing hay occurred for the larger particles which was nearly all attributed to hay. For the diet containing silage, though there was much less variation, the majority of the particles were retained on the middle two sieves. Most variation occurred for the large particles (primarily cob) and for the fines. In all diets, the fine particles that sifted to the pan were primarily mDG.

Table 3 contains the DM and nutrient distribution of the six mixes. In comparison of Mixes 1 and 2, the most variation occurred for ADF within each mix. The increase in ADF variation in Mix 2 vs Mix 1 was again due in part to the particle length of the hay and the addition sequence with most variation due to differences in hay. Of note is the concentration of ADF between diets relative to the formulated value (Table 1). Within Mix 1 and 2, the ADF content generally increased from the first sample to the last sample, more than doubling from the first sample to the last sample within Mix 2. Additionally the protein and sulfur content had similar fluctuations within Mix 1 and 2 demonstrating non-uniform mixing (or discharge) of mDG.

Table 3. Effect of ingredient addition sequence on nutrient distribution within mixes of feed

	High-forage diet				High-grain diet								
Item	MD	MIX 1 MIX 2		M	Х З	MIX 4		MIX 5		M	X 6		
		Means of nutrients							nutrients DM basis (CV, %)				
Dry matter, %	69.2	(1.3)	72.1	(1.5)	71.3	(8.2)	71.8	(1.4)	71.4	(0.55)	48.8	(1.2)	
Crude protein, %	16.0	(2.8)	14.9	(5.5)	15.2	(1.6)	15.4	(2.8)	15.2	(2.7)	14.5	(1.2)	
ADF, %	10.7	(13)	18.6	(23)	8.7	(6.9)	8.5	(12)	9.3	(5.4)	12.7	(3.8)	
Sulfur, %	0.41	(9.2)	0.44	(6.2)	0.34	4 (4.2)	0.38	3 (7.1)	0.38	3 (2.0)	0.34	(2.6)	
Monensin, g/ton	-	-	-	-	19	(14)	-	_		_	31	(15)	
Quantab Cl <sup>-</sup> , %	91.0	(8.5)	197	(12)	133	(29)	149	(16)	137	(9.3)	-	_	

In Mixes 3 and 4 mDG and GH switched places in the loading sequence, with mDG charged before GH in Mix 3 and the opposite in Mix 4; whereas, in Mix 5 mDG was the first ingredient charged into the mixer and GH was the last. On the basis of nutrient analysis, ADF concentration was the most variable when mDG was added last in the sequence. This also caused the most fluctuations in sulfur. The second sample obtained from Mix 4 was lowest in crude protein, sulfur, and chloride. The variation in DM and chloride in Mix 3 were due to those variables being the most concentrated in the first sample from that mix.

Mix 6 was prepared in a staggered-rotor mixer. Though the composition of this diet was quite different than the diets prepared in the 3-bar rotor mixer, the low CV demonstrate consistency within this load of feed.

Of all variables measured, analysis of monensin is the most sensitive due to its content being confined to one feed ingredient. In Mixes 1 through 5 the pelleted supplement contained monensin and in Mix 6 the liquid contained monensin. Monensin concentration was measured in Mixes 3 and 6. Both mixes had high CV, 14 and 15% for mixes 3 and 6, respectively. For example, if each of the five samples obtained from Mix 3 represented a sample taken from feed delivered to five pens of cattle, the amount of monensin delivered would have ranged from 174 to 241 mg/head. For Mix 6, it would have ranged from 284 to 400 mg/head.

Another point to consider is the comparison between formulated diet nutrient composition and analyzed nutrient composition. In this experiment, the formulated vs analyzed values were not as close as they should be.

From these data, the authors recommend that in diets containing mDG and ground grass hay, mDG should be added before the hay and the diet should be mixed no less than 3 minutes after the last ingredient has been added.

#### Implications

Consistent inconsistencies in feed mixing will result in feed deliveries that do not have the formulated nutrient content. This should result in altering cattle performance from what was expected or predicted. As previous authors have indicated, analysis of an ionophore is the most sensitive measurement to use when evaluating mix quality. Another analytical approach is to measure two nutrients that are concentrated in one ingredient allowing insight into which ingredient is creating inconsistencies in the mix. Use of the Penn State Forage Separator or other similar tools can give a quick determination of mix quality.

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# Cottonwood and Antelope Range Livestock Research Stations Unit Report

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# Summary

Two research stations, the Cottonwood and Antelope Range Livestock Research Stations, are located in western South Dakota that allow research projects focused on needs of range livestock producers in that region. The stations are comprised primarily of native rangeland that is grazed by cattle at both stations, and also by sheep at Antelope. The philosophy of the research efforts has been focused on conducting applied research to solve problems and address rangeland and livestock management opportunities relevant to the livestock producers and land mangers of the region.

# Introduction

Two research stations are operated by the Animal and Range Sciences Department that are used to conduct research applicable to the semiarid, rangeland environment of the West River portion of South Dakota. The Cottonwood Station, located near Philip, comprises about 2640 acres of rangeland. It is utilized for range management and beef cattle research. Besides facilities for grazing cattle research, there is also a 12-pen feedlot at Cottonwood. The Antelope Station, located near Buffalo, comprises about 8165 acres of rangeland. Livestock at this station include both sheep and cattle. Again, it is used for both range and livestock research.

Historically, these stations were operated as separate entities with each having its own cow herd. A few years ago, the two herds were merged and then the cattle were separated among the two stations by cow age. The Cottonwood Station became the location for replacement heifers and young cows to be housed. The Antelope Station became the site for the mature cows. This allowed grouping of cows that allowed research to focus on heifers, young cows, or mature cows. With all cows of a given age group combined in one location, the number of cows or heifers of the appropriate age that could be devoted to any given project was increased. This has been particularly useful for research with heifers and young cows because the number of animals in each of these groups had been too small for valid research projects when each station had a separate cow herd. The Cottonwood Station has been the ideal location for this effort because the combination of rangeland pastures and feedlot facilities has allowed the flexibility to use these young cattle in a variety of projects that required the various facilities to meet experimental objectives involving the combined nutritional demands of maintenance, reproduction, and growth that challenge the productivity of heifers and young, growing cows.

The larger numbers of mature cows in the combined herd on the ranch-scale sized area of land at the Antelope Station has allowed the ability to evaluate alternative management strategies in a total production-system setting. With the carrying capacity for 200-plus cows at this station, adequate numbers of cows can be allocated across four or more alternative management strategies and still maintain an adequate number of cows and land allocated to each treatment to be representative of livestock industry production standards and to allow adequate statistical power to compare management treatment responses. The sheep at the Antelope Station have been utilized in a similar philosophy to evaluate the influence of management alternatives in a system setting.

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#### **Current Research Projects**

Research activities conducted during 2006 at each station includes:

#### Cottonwood.

- 1. Livestock Water Quality: A project funded by NCR-SARE that focuses on methods to mitigate the toxic effects of high-sulfate water for cattle. Investigators are Pat Johnson, Roger Gates, Ken Olson, Cody Wright, and Mindy Hubert.
- 2. First-Calf Heifer Management: A project funded by the South Dakota Corn Utilization Council to evaluate the utilization of dried distillers grains or other byproduct feeds to meet the nutrient requirements of young, growing cows. The principal investigator is George Perry.
- 3. Summer by Winter Forage Production Clipping Study: A project to evaluate the response of rangeland forage production after winter grazing. The principal investigator is Sandy Smart.
- 4. Long-Term Range Production and Stocking Rate Study: A project that has continued for over 50 years to document differences in vegetation production and cattle gains associated with controlled stocking rates. The principal investigators are Pat Johnson, Roger Gates, Mundy Hubert, and Ken Olson.
- 5. Additionally, the Cottonwood Station hosts an Acid Rain Deposition Site for NOAA and a weather station for the South Dakota State Climatological Office.

#### Antelope:

- Beef Cattle Systems-Effects of Early Weaning and Winter Feeding Strategy: A project funded by the Four-State Ruminant Consortium to evaluate the combined effects of weaning date (early or normal) and winter feeding strategy (limited or full) on livestock performance, rangeland forage utilization, and economic response. Investigators are Pat Johnson, Roger Gates, Ken Olson, Mary Beutler, Scott Fausti, Mindy Hubert, Sandy Smart, George Perry, and Robin Salverson.
- Sheep Grazing to Control Sagebrush: A project to evaluate the use of sheep grazing in spring or fall to reduce the amount of sagebrush on rangeland. Investigators are Sandy Smart and Jeff Held.
- 3. Yellow-Flowered Alfalfa: A project funded by CSREES to evaluate the adaptation and value of yellow-flowered alfalfa for rangelands. Investigators include Roger Gates, Arvid Boe, Xu Lan, Pat Johnson, and Mindy Hubert.
- 4. Summer by Winter Forage Production Clipping Study: A project to evaluate the response of rangeland forage production after winter grazing. The principal investigator is Sandy Smart.
- 5. Additionally the Antelope Station hosts a Meteorological Monitoring Site for NOAA and a weather station for the South Dakota State Climatological Office.

In addition to these research activities, both stations have been used for Extension and teaching activities. Extension educator training has been conducted at Antelope and the Cottonwood has been used as a laboratory setting for Range 325, a course entitled Range Measurements.

# Animal and Range Sciences Research and Extension Units



1 Brookings: SDSU campus, Agricultural Experiment Station, Cooperative Extension Service

- **2** Beresford: Southeast South Dakota Research Farm Beef cattle nutrition Swine nutrition and management
- **3** Rapid City: West River Ag Research and Extension Center Professional research and Extension staff in Animal Range Sciences, Plant Science, Economics, 4-H, and Extension administration
- 4 Buffalo: Antelope Range Livestock Station Beef cattle breeding and range beef herd management Sheep nutrition, management, and breeding
- 5 Phillip: Range and Livestock Research Station Range beef nutrition and herd management Range management
- 6 Miller: Gerdes Ranch Research Station Research, extension and teaching activities

These research and Extension units are geographically located in South Dakota to help solve problems, bring the results of livestock and range research to users, enhance the statewide teaching effectiveness of the Animal & Range Sciences Department staff, and maintain a close and productive relationship with South Dakota producers and the agribusiness community.

## The state of South Dakota is • our campus • our research lab • our classroom