EFFECTS OF ALTERNATE DAY FEEDING OF DRIED DISTILLER'S GRAINS PLUS SOLUBLES ON DIGESTION AND PERFORMANCE IN FORAGE-FED CATTLE

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Title

Effects of Alternate Day Feeding of Dried Distiller's Grains Plus Solubles and Grass Hay on Metabolism and Performance in Cattle

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MASTER OF SCIENCE

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ABSTRACT

Two studies were conducted to determine the effects of supplementing dried distiller's grains plus solubles (DDGS) on alternate days to forage-fed cattle. We hypothesized that feeding either DDGS at a low percent of body weight or a moderate to low quality hay on alternate days would decrease forage intake without negatively impacting rumen kinetics and digestibility in steers as well as body weight and body composition in gestating cows. Therefore the objective of these research trials was to examine the effects of alternate day feeding of DDGS and grass hay on ruminal and digestion kinetics in Holstein steers (EXP. 1) and performance in gestating beef cows (EXP. 2). In both research experiments a decrease of total forage DMI was observed (EXP. 1; $P = 0.0004$ and EXP. 2; $P < 0.0001$) for cattle allotted to the alternate day feeding of DDGS and grass hay.

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Finally, to those watching from above…I hope I have made you proud. Keep smiling down upon me.

DEDICATION

To Justin and to my sisters, both those in blood and friendship

I never could have done it without you

*****

And especially to my mom:

Because no matter what, you were always there

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Abbreviation	Wording
A:P	acetate:propionate
ADF	acid detergent fiber
ADG	average daily gain
AOAC	Association of Official Analytical Chemists
BCS	body condition score
BUN	blood urea nitrogen
BW	body weight
°C	degrees Celsius
Ca	calcium
Ca:P	calcium to phosphorus ratio
CH_3	methane
cm	centimeter(s)
Co	cobalt
CON	control hay only
CP	crude protein
Cr_2O_3	chromic oxide
d	day
DDGS	dried distillers grains plus solubles
DG3	hay daily and DDGS on alternate days
DG7	hay and DDGS daily
DGA	hay only or DDGS only on alternate days
DIP	degradable intake protein
DM	dry matter

DMI ..... dry matter intake

EBW ..... empty body weight

EDTA ..... ethylenediaminetetraacetic acid

FDR ..... fluid dilution rate

FFR ..... fluid flow rate

g ..... gram(s)

h ..... hour

HC ..... hydrochloric acid

IACUC ..... Institutional Animal Care and Use Committee

IGF_1 ..... insulin like growth factor-1

IU ..... international unit(s)

kg ..... kilogram

L ..... liter(s)

LSMEANS ..... least squares means

mcal/kg ..... megacalories/kilogram

Mg ..... magnesium

min ..... minute(s)

mL ..... milliliter(s)

m ..... meter(s)

m*M* ..... millimolar

n ..... number

NaCl ..... sodium chloride

NDF ..... neutral detergent fiber

NDSU ..... North Dakota State University

NEFA ..... non-esterified fatty acids

Nem ..... net energy for maintenance

Nep ..... net energy for production

NH_3 ..... ammonia

NPN ..... non-protein nitrogen

NRC ..... National Research Council

NSUP ..... non-supplemented days

OM ..... organic matter

P ..... phosphorous

RFV ..... rumen fluid volume

SAS ..... Statistical Analysis Software

SBH ..... soybean hulls

SBM ..... soybean meal

SEM ..... standard error of the mean

SG ..... sorghum grain

SUP ..... supplemented days

TMR ..... total mixed ration

trt ..... treatment

TT ..... turnover time

UIP ..... undegradable intake protein

USDA ..... United States Department of Agriculture

VFA ..... volatile fatty acids

vol ..... volume

vs ..... versus

wk ..... week

wt ..... weight

CHAPTER I. INTRODUCTION AND LITERATURE REVIEW

Introduction

Ruminant animals are a unique species compared with monogastrics both anatomically and in the way that they breakdown and assimilate feed. Ruminants are able to use highly fibrous feeds due to the symbiotic relationship shared between the host animal and the microorganisms that dwell within the ruminal complex. These microorganisms assist in the degradation and nutrient absorption of feeds and are essential in the continued health and survival of the host animal. Due to these differences in anatomy and function, ruminants are also able to utilize and reuse nitrogen (N) and also utilize volatile fatty acids (VFA) as a main energy source. However, the concentrations of N (Owens and Zinn, 1988) and VFA (Owens and Goetsch, 1988) are highly correlated to the feedstuff that is being consumed.

Dried distiller's grains plus solubles (DDGS) which is a by-product of the ethanol industry is a widely used feedstuff for beef cattle. Since the passing of the 2005 energy bill ethanol production and consequently supply for DDGS has increased substantially. The 2005 legislation mandated that by 2012 United States (US) gasoline would be required to be mixed with 7.5 billion gallons of renewable fuel annually (Yacobucci, 2006). Distiller's grains are a very good source of protein (25-30%; Lardy, 2007), energy (NEm, Mcal/kg 1.96-2.20 and NEg, Mcal/kg 1.48-1.70; Lardy, 2007), and digestible fiber (46%; NRC, 1996) for cattle and have been used successfully in creep feeding (Reed et al., 2005), backgrounding (Taylor et al., 2008), feedlot (Gunn et al., 2009), and cow supplementation (Radunz et al., 2010). In situations where supplementation is necessary, DDGS can make an excellent replacement for corn (Ham et al., 1994). The decreased starch concentration of DDGS reduces the likelihood of detrimental effects (ruminal acidosis) associated with corn supplementation (Ham et al., 1994). In addition, the negative associative effects of supplement intake on forage intake observed while feeding high

starch supplements (corn) are not observed when supplements containing DDGS are offered (Ham et al., 1994). This makes distiller's grain an ideal supplementation option for cattle when they are consuming low-quality fiber sources.

However, when feeding cattle it is not always the best option for producers to feed a supplement daily due to labor and equipment costs associated with supplement delivery. In addition to supplement delivery costs, there can also be efficiency issues associated with weight gain and body condition that can be seen from the animals consuming the supplement. Therefore, strategies that decrease supplement feeding frequency may be of great value to producers who are looking to minimize production costs.

Several studies have been conducted that provide varying conclusions regarding differing energy and protein supplementation frequencies as well as varying access time to forage. Supplementing the basal diet with protein concentrates when suitable amounts of forage are available can improve energy status and is usually economically feasible (McCollum and Horn, 1990). Therefore, implementing management strategies for cattle that optimize feed utilization and meet nutritional requirements can be very beneficial for producers.

Even though there is an abundance of information regarding protein and energy supplementation for cattle consuming forage based diets, there is a paucity of information investigating the effects of either feeding only supplement or only forage on alternate days and the effects this may have on forage intake, rumen kinetics, forage digestibility and cattle performance. Therefore, research in these areas is warranted.

Literature Review

Ruminants: Anatomy and Anatomical Function

Ruminant animals such as cattle and sheep are a unique species in that they have a four compartmented stomach (in order of function: reticulum, rumen, omasum, and abomasum), compared with monogastrics such as pigs and horses, which have a single compartmented stomach. The four stomach compartments in ruminants make up approximately 25-28% of body weight (BW), compared to monogastrics whose stomach is about 4% of BW (Kellems and Church, 2010a) and the ruminal complex encompasses approximately three fourths of the total abdominal cavity (Van Soest, 1994).

The four stomach compartments (reticulum, rumen, and omasum) vary in epithelial cover as well as function. The reticulum, rumen, and omasum are lined with keratinized stratified squamous epithelium which is similar tissue to that which shields the exterior surface of most mammals (i.e. skin; Kellems and Church, 2010a). The difference, however, is the permeability of the epithelial wall and the transport mechanisms the cells possess to transfer nutrients from the lumen, as well as its electrical resistance (Van Soest, 1994). These compartments serve to accumulate and suppress the passage of ingested feed allowing for microbial fermentation to take place, which can then be followed by the absorption of fermentation end products through the epithelial surfaces of the ruminal complex (Hofmann, 1988).

The luminal surface of each of the four stomach compartments (reticulum, rumen, omasum) are lined with different types of papillae that serve several functions in the digestive process (Kellems and Church, 2010a). The reticular papillae are arranged in a distinct polygon or honeycomb shape which can temporarily trap coarse food particles that are formed into a bolus and then regurgitated for further mastication, this is also known as rumination. The rumen is the

most spacious of the four compartments, and ruminal papillae are much larger than those of the other sections of the rumen. The papillae in the rumen are often referred to as "tongue-like" projections from the lining of the rumen wall. The rumen is a large vat in which a majority of pregastric microbial fermentation occurs (Kellems and Church, 2010a). Fermentation in the rumen is aided by strong muscular contractions that mix the ingested feed and microbes together, thus increasing the rate at which feed is digested and passed from the rumen and into the omasum. The omasal compartment is comprised of several leaves all of which have small round papillae. These layers are able to hold small particles of digesta, thus enabling minerals and water to be absorbed. The omasum also prevents large feed particles from entering the abomasum (Hofmann, 1988).

The abomasum functions similarly to the monogastric glandular stomach which secretes digestive enzymes (pepsinogen, rennin, and lipase) and hydrochloric acid. As the abomasum contracts, the enzymes and acid are mixed with digesta to help further digestion. Next, the digested chyme is moved into the small intestine via peristalsis where the nutrient digestion and absorption processes continue (Hofmann, 1988).

Ruminant and Microbial Synergy

Ruminants and the microorganisms that dwell within the ruminal complex share a synergetic relationship. The animal provides the various bacteria, protozoa, and fungi within the rumen with substrate and a warm, oxygen free environment in which to thrive and multiply (Van Soest, 1994). In turn the microorganisms aid the ruminant animal to digest and obtain nutrients from highly fibrous feeds that they would not be able to utilize were it not for these organisms. This is due to the microorganisms having digestive enzymes that are not present in the host animal (Yokoyama and Johnson, 1988). There are varying types of ruminal microorganisms that

assist in the degradation of feed within the rumen, which begins with the adhesion of the microorganisms to the ingested substrate. However, the colonization and survival of these microorganisms is highly dependent on the type of feed that the host animal is ingesting and the pH of the rumen environment. These variations in substrate and pH cause shifts in the microbial population within the rumen and thus also changes the end products of the fermentation process from which the animal obtains its main source of energy, VFA, as well as the production and utilization of ammonia (NH_3) from protein degradation (Owens and Goetsch, 1988).

Microbial Attachment

After mastication, consumed feed moves down the esophagus and into the reticulorumen. As feed enters the rumen it is mixed via strong ruminal contractions. This mixing allows the microorganisms that dwell within the ruminal fluid and those that are loosely attached to digesta to be dislodged and adhere to the newly ingested feed. This attachment to the substrate occurs through adhesion, specific receptors, physiochemical forces such as van der Waals forces, or a combination of these (Van Soest, 1994) and can occur as quickly as 5 minutes after the feed is ingested (Bonhomme, 1990). Attachment of the microorganisms (bacteria and protozoa) to the surface of the substrate occurs with the glycocalyx present around the organism. This glycocalyx contains external filaments that can stick to the substrate surface, thus allowing the microorganism to stay in place (Van Soest, 1994). Adherence of microorganisms is dependent on the extent of the damage done to the feed by mastication and if the feed has or has not been processed (e.g., chopped, ground). Mastication and processing decreases particle size of the feed while increasing the surface area or broken edges of the plant surface to which the microbial population can bind to. Very little attachment is done on the outer cutinized or lignified surfaces of the plant material, as there is little substrate that is available for the microorganism to bind to

(Van Soest, 1994). Once adhesion occurs, the microorganisms can begin the fermentation process.

Microbial Fermentation and VFA Production

Particle-bound microbes start breaking down the polymers (carbohydrates, protein, lipids) of the ingested feed into smaller molecules called monomers. These monomers, for example glucose, can be used further for fermentation and microbial growth, absorbed through the rumen wall, or passed through the ruminal complex and into the small intestine where a majority of nutrient absorption occurs (Merchen, 1988).

Volatile fatty acids produced by microbial fermentation are the main source of energy for ruminants. For ruminants consuming forage-based diets, VFA provide approximately 50-85% of the metabolizable energy absorbed by the animal (Owens and Goetsch, 1988). The three main VFA that provide the ruminant animal with energy are acetate, propionate, and butyrate (Fahey and Berger, 1988). As microbial fermentation occurs carbohydrates from the ingested feed are broken down to glucose, a 6-carbon compound. This glucose can then be utilized in glycolysis to make pyruvate, a 3-carbon compound. Pyruvate can then be synthesized into acetate (2-carbon) and propionate (3-carbon). The 2-carbon acetate can then be synthesized into butyrate (4-carbon), which is toxic if it enters the bloodstream and, therefore, is converted to ketones during absorption (Owens and Goetsch, 1988).

As ruminal contractions occur, the mixture of ingested feed and microbiota coat the epithelium of the rumen with fermentation fluids, and the VFA (acetate, propionate, and butyrate) can be absorbed through the ruminal epithelium (Owens and Goetsch, 1988). These end products travel through the blood where they are passed through the liver. The acetate and ketones can be metabolized to acetyl CoA which can be used in the citric acid cycle or stored as

fatty acids for future use. After acetate is absorbed it is converted to acetyl-CoA, an important metabolite that is widely distributed and used for many important metabolic pathways.

In the liver, propionate is converted to glucose via gluconeogenesis which can either be used as energy or stored for future use, or can be converted to pyruvate which can also be used in the citric acid cycle (Owens and Goetsch, 1988). The citric acid cycle is a metabolic pathway that catabolizes carbohydrates and lipids after they have been synthesized into usable parts (Bettelheim et al., 2007). It is in this way that the ruminant animal is able to derive energy from material that is highly indigestible to some species. Therefore, if VFA production is decreased, then the amount of energy the animal receives is also decreased.

Total VFA concentration was altered when supplementing steers with either a undegradable intake protein (UIP; soyPLUS and blood meal) or a soybean meal (SBM) degradable intake protein (DIP) supplement source daily, every third day, or every sixth day (Bohnert et al., 2002b). Total VFA concentrations increased linearly with decreased supplementation frequency on the days that all treatment groups were supplemented (Bohnert et al, 2002b). Similarly, an increase in total VFA concentration was observed when providing forage-fed steers a supplement (46.4% sunflower meal, 30.5% cottonseed meal, 7.5% feather meal, 0.5% alfalfa, 0.5% soybean hulls (SBH), 2.3% limestone, 0.7% salt, 0.5% selenium, 0.6% trace mineral premix, 0.2% grease mix, and 5.0% molasses) 2, 3, 5, and 7 d/wk. The increase in total VFA concentration occurred as supplementation frequency decreased (Farmer et al., 2001). Beef steers consuming moderate-quality hay were supplemented with increasing amounts of DDGS (0, 0.3, 0.6, 0.9, 1.2% of BW daily) and had a linear decrease in the ratio of acetate: propionate (A:P) as the quantity of DDGS increased (Leupp et al., 2009). These authors believed the decrease in VFA concentration was a result of the larger quantity of supplement that

was being fed for treatment groups that were being fed at varying frequencies as they received a greater quantity when they were supplemented. This led to a larger quantity of fermentable substrate for the ruminal microorganisms. The ratio of A: P is important because a decreasing ratio means there is a decrease of acetate, which is an indication of the efficiency of microbial fermentation. As the A: P ratio increases, microbial fermentation and energy usage becomes less efficient as some carbon is being released as methane (CH_3) and carbon dioxide (CO_2). As the A: P decreases more carbon is being utilized by the host animal and is therefore not being expelled into the atmosphere but sequestered and utilized (Van Soest, 1994).

Protein Metabolism

Protein metabolism is a complex process involving the host animal and its microbial population. The process begins with the ingestion of feed protein which can be categorized into three separate classifications: UIP, DIP, and non-protein nitrogen (NPN; NRC, 1996). Undegradable intake protein is considered to be the protein sources that are not broken down or utilized by the microbial population in the ruminal complex and subsequently move into the small intestine where absorption occurs by the animal. Degradable intake protein is considered to be the protein sources that are available to the microbial population of the rumen and are utilized and not passed to the small intestine. Non-protein nitrogen are feeds or circulating metabolites that can be utilized by the microbial population similar to DIP, however it contains non-alpha amino N forms (e. g. urea). Another protein source that can be utilized by the ruminant animal derives from the digestion of microbial protein. Approximately 20 to 60% of the dry matter (DM) of the microbial population is protein and this protein comprises about 40% of the non-NH_3 nitrogen entering the small intestine of the ruminant animal (Owens and Zinns, 1988).

Degradable intake protein and NPN enter the ruminal environment and are either broken down by the microbial population into various peptides and amino acids or in the case of NPN are utilized to synthesize NH_3. The amino acids can then be utilized by the microbial population and used to synthesize NH_3 as well, which a majority of bacteria in the rumen can utilize for protein synthesis or it can be absorbed through the ruminal wall and into the bloodstream (Owens and Zinns, 1988). Having higher concentrations of NH_3 in the rumen, which does not exceed 100 mg/dl, is conducive to maintaining a majority of the microbial growth in the ruminal complex (Owens and Zinns, 1988). If the ruminal NH_3 concentration exceeds 100 mg/dl then the animal will suffer from NH_3 toxicity.

Ammonia travels through the blood and into the liver where it is converted into urea. From the heart, blood passes to the lungs, and then to the rest of the body where the urea can be absorbed at the salivary glands and reused as NPN or it can be reabsorbed at the rumen where the microbial population can utilize the NPN to synthesize amino acids and NH_3. This urea can also be passed to the kidneys where it is flushed out with urine. In grazing steers provided a supplement consisting of 91% cottonseed meal and 9% corn grain, an increase in NH_3 concentration was observed at -3, 0, 1, and 4 h after supplementation compared with unsupplemented steers (Caton et al., 1988). Similarly, in a study where SBM and sorghum grain (SG) were mixed to obtain increasing levels of CP (10, 20, 30, or 40 %) and then fed daily or 3 d per week (wk) to forage-fed steers, ruminal NH_3 was greater with increasing crude protein (CP) concentration in the supplement (Beaty et al., 1994). This increase in ruminal NH_3 concentration is one of the benefits commonly reported when feeding supplemental protein with low-quality forage.

Ruminal pH

It is important for ruminal pH to be maintained at a level that is ideal for microbial health and function. For forage based diets, a pH of approximately 6.2 to 7.0 is the optimal range for microbial adhesion and cellulolytic digestion to occur (Owens and Goetsch, 1988). If a starch based concentrate is fed in addition to the forage a decrease in pH will occur with the increasing amount of concentrate. If the pH drops below 6.2, digestion of cellulose is inhibited because the low pH causes a decrease in microbial attachment to feed particles as well as a decrease in bacterial cell division (Owens and Goetsch, 1988). If the microbial population does not divide at a faster rate than the substrate leaves the rumen, then there will be a decrease in the ruminal population of microorganisms. This would then lead to a decrease in the extent and rate of digestion of the specific substrate that the existing population of microbes colonizes.

Beef heifers consuming grass hay were supplemented with DDGS and dry-rolled corn daily or on alternate days and ruminal pH was not influenced by feeding frequency or supplement type (Loy et al., 2007). However, heifers receiving a control diet of only grass hay had an increased ruminal pH compared with supplemented heifers. In addition, a decrease in rate and extent of hay neutral detergent fiber (NDF) disappearance was observed in supplemented heifers compared with control heifers (Loy et al, 2007). This outcome is logical as the average pH in the supplemented animals was below 6.2, which would cause a decrease in microbial adhesion and cellulolytic digestion of the forage. A similar trend of decreased pH was observed in steers supplemented 2, 3, 5, and 7 d/wk. On days when all treatment groups were supplemented, steers receiving larger quantities of supplement (i.e. those that were supplemented less frequently) mostly exhibited a more rapid decline in pH and a decline of greater magnitude (Farmer et al., 2001). However, this decrease in ruminal pH was not low enough to inhibit fiber

digestion. These findings support other data that show pH below 6.2 can inhibit forage digestibility in ruminant animals.

The process by which ruminants breakdown and assimilate nutrients is a vast and complicated process that involves many components, including microbial colonization of substrate and microbial enzymes. Supplementing protein to cattle consuming forage based diets has many impacts on rumen kinetics. Protein supplementation can cause an increase in VFA production and NH_3 concentration in the ruminal complex. Supplemental protein can also cause a decrease in ruminal pH which can effect microbial adhesion to substrate in the rumen and decrease the rate and extent of fiber digestion in the ruminal complex if the pH decreases to a concentration that is too low for microbial function.

Ethanol Production

For many years ethanol has been manufactured in the US to power motorized vehicles, including Henry Ford's Model T and the machines used in both World War I and II (OAITC, 2012). Within the past 20 years ethanol production has steadily increased as government programs including the Energy Policy Act of 2005, and the Energy Independence and Security Act of 2007 were passed (USDA-FAS, 2011). In 2010 the US manufactured over 13.2 billion gallons of ethanol, 1.2 billion gallons of which was surplus and exported to other countries. As ethanol production increases a subsequent increase in the production of ethanol by-products occurs. By-products of the ethanol industry can be utilized as livestock feeds as most are high in protein, energy, and digestible fiber. Ethanol can be produced utilizing two different methods, dry-milling or wet-milling. The majority of the ethanol produced in the US is manufactured using the dry milling process (Bothast and Schlicher, 2005).

Process of Ethanol Production (Figure 1.1)

To initiate the process of ethanol production, whole corn is passed through a hammer mill and ground into a fine powder called flour. This flour is then transferred to a slurry tank where it is mixed with water and enzymes which break down starch into glucose, this mixture is called mash. Yeast is then added to convert the glucose into ethanol and carbon dioxide and the mash is continually mixed and cooled until the desired ethanol concentration is reached. The fermented mash is pumped through a continuous flow multi-column distillation system where the ethanol is removed from non-ethanol portion (whole stillage) of the fermented mash (Lardy, 2007).

Ethanol is then pushed through molecular sieves where the remainder of the water is removed from the alcohol. This 200 proof ethanol product can now be mixed with gasoline. The whole stillage is transferred to a centrifuge where the liquid and solids are separated into wet grain and thin stillage. The thin stillage is put through an evaporator and condensed resulting in condensed syrup or condensed distiller's solubles. The condensed distiller's solubles is then mixed with the wet distiller's grain to make a product called wet distiller's grains plus solubles which is approximately 35% DM. The wet distiller's grains can then be moved through a rotary dryer and dried until it is about 90% DM subsequently producing dried distiller's grains plus solubles (Lardy, 2007).

Dried Distiller's Grains plus Solubles

Dried distiller's grains plus solubles makes an excellent livestock feed as it is a good source of protein, energy, and digestible fiber. Dried distiller's grain plus solubles on a DM basis contains approximately 34 % CP, 52% undegradable protein, 9% fat, and 101% total digestible nutrients (TDN, NRC, 2000; Leupp, 2008). This nutrient profile makes DDGS an exceptional

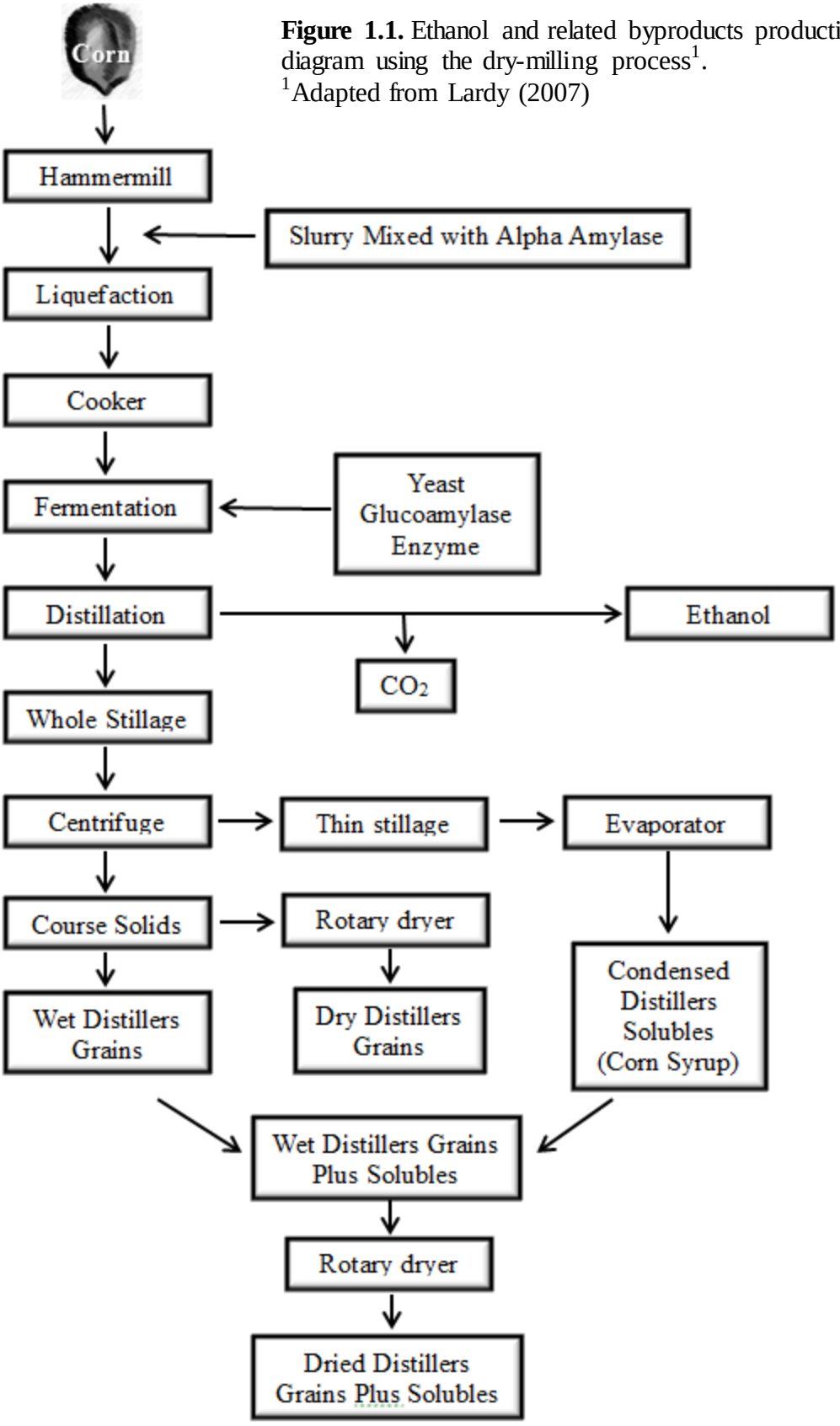

Figure 1.1. Ethanol and related byproducts production diagram using the dry-milling process[1].
[1]Adapted from Lardy (2007)

supplement for cows consuming forage based diets. Table 1.1 demonstrates the differences in nutrient composition of dry-rolled corn and ethanol by-products.

Forage Access and Supplementation

As forage and pasture costs continue to rise, it is becoming more imperative for cattle producers to reduce costs associated with forage processing and handling, and pasture rent and

Table 1.1. Nutrient composition of corn and ethanol by-products[1]

Item	DRC[2]	DDG[3]	WDG[4]	DDGS[5]	WDGS[6]	CDS[7]
DM, %	88.0	89.0	30.0	90.4	34.9	35.5
	----------DM basis----------					
CP, %	9.8	30.0	32.5	33.9	31.0	23.8
UIP, % CP	60.0	55.0	52.0	52.0	52.0	20.0
Fat, %	4.5	9.0	10.0	9.0	10.0	12.0
TDN, %	90.0	82.5	126.0	101.0	112.0	112.0
Phosphorus, %	0.32	0.40	0.65	0.51	0.84	1.38
Sulfur, %	0.12	0.48	0.58	0.42	0.58	0.66

[1]Data are adapted from NRC (2000) and Leupp (2008)

[2]Dry rolled corn

[3]Distillers dried grains

[4]Wet distillers grains

[5]Distillers dried grains with solubles

[6]Wet distillers dried grains with solubles

[7]Condensed distillers solubles

upkeep. For cow-calf production systems the cost of feeding reproducing females accounts for greater than 60% of a beef producers' annual cow costs (Miller et al., 2001). Over half of the feed costs are attributed to the winter feeding period and thus this is one of the main focus areas when determining profitability of an operation (Miller et al., 2001). In order to reduce the expenses associated with feeding, cattle producers should consider management strategies that optimize forage utilization while still meeting nutrient requirements and maintaining desired production levels. Limiting or restricting the amount of hay or forage that cattle receive may

assist in reducing these cost associated with feeding with only minor consequences to cow body condition and weight gain (Cunningham et al., 2005).

Furthermore, these management goals (e.g. decreased production costs) are especially hard to meet when nutrient requirements of cattle are high due to stage of production such as late gestation and lactation, and environmental extremes. In addition, if the available forage being provided is of low quality then gestation and lactation production requirements would not be met. An example would be for gestating and lactating cattle grazing winter range when the forage digestibility and protein content is low. As the growing season progresses forage CP content decreases (Switchgrass; May 18% CP-October 5% CP) and fiber content increases (Switchgrass; May 74% NDF-October 78% NDF (Sedivec et al., 2008). If gestating or lactating cattle are consuming forage into October or later then nutrient requirements would not be met (8-10% CP; Beef NRC, 2000).

Supplementation is necessary for cattle consuming low to moderate quality forage. When protein and energy levels in forage are limited the addition of a supplement will assist in meeting the animal's production requirements. Methods of meeting production requirements include altering the amount of supplement that is fed and the frequency that a supplement is delivered. Decreasing production costs is also dependent on the type of supplement that is being delivered.

Limiting Forage Access

Forage intake can be influenced by access time and amount restrictions. An experiment was conducted to compare the effects of round bale (19.6% CP) access for either 4, 8, or 24 h/d in lactating beef cows (Cunningham et al., 2005). Hay disappearance for cows on the 4 h treatment was approximately 37% less than those cows allowed hay access for 24 h. In a similar experiment utilizing beef cows in late gestation, alfalfa hay (17.7% CP) access was restricted to

3, 6, or 9 h/d resulting in increased hay disappearance with increased time of access (Miller et al., 2007). Furthermore, in a study utilizing beef heifers in a digestion trial, bromegrass hay (9.2% CP) was fed at 30, 60, 90, and 120% of maintenance intake (Scholljegerdes et al., 2004). As would be expected, organic matter (OM) intake increased as maintenance intake increased from 30 to 120% (Scholljegerdes et al., 2004).

Body weight and body condition score (BCS) are other factors that have been assessed when limiting access to forage. Beef cows in the third trimester of gestation were utilized to compare four dietary treatments either ground hay (14.5% CP) fed at 90% of NRC requirements or access to round bales of hay (14.5% CP) for 3, 5, or 7 h/d. Researchers observed that cow BW increased with increased access to hay over the 91 d feeding trial (Cunningham et al., 2005). Additionally, cows allowed hay access for 7 h/d gained approximately 25 kg more than cows that were allowed hay access for 3 h (Cunningham et al., 2005). Similarly, when allowing beef cows in late gestation ad libitum hay access for 3, 6, 9, or 24 h/d, cow BW and BCS were altered (Miller et al., 2007). Both BW and BCS increased with increased time of access to round bales during the 87 d feeding trial (Miller et al., 2007). In a trial conducted with ewes receiving either ad libitum wheatgrass hay (9.7% CP; 48.8 g DM/d per $W^{0.75}$) or a restricted amount of wheatgrass hay (18.0 g DM/d per $W^{0.75}$), BW during the 84 d study was decreased for ewes with restricted intake while BW increased in ewes that were allowed ad libitum intake (Tatman et al., 1991).

As access to forage is altered it would be logical to assume that ruminal pH and VFA concentrations would also be altered with varying time and amount of forage restriction. When heifers were fed chopped grass hay at 30 to 120% of maintenance intake, ruminal pH was not influenced by dietary treatment; however, total ruminal VFA concentrations increased linearly

with increasing maintenance intake (Scholljegerdes et al., 2004). Ruminal pH for wether lambs consuming a concentrate diet (16.7% CP) containing either 25 or 75 % alfalfa hay as a forage at two levels (1,100 or 1,700 g DM/d) was not influenced by level of intake (Merchen et al., 1986). However, ruminal pH was increased for wethers that were consuming the diet containing 75% alfalfa compared with wethers consuming the 25% alfalfa diet (Merchen et al., 1986). Total ruminal VFA concentrations were not influenced by diet, however the molar proportion of acetate was increased in wethers receiving 75% alfalfa in their diets compared with those wethers with only 25% of alfalfa in their diets and molar proportions of propionate were greater in wethers receiving 25% of alfalfa at the increased level of intake compared with all other treatments (Merchen et al., 1986).

Digestibility of various nutrient components in feed can also be influenced by the amount of or access to forage. When comparing feeding either orchardgrass or alfalfa at high (90% of ad libitum intake) and low (60% of the high intake) levels in wether lambs, Varga and Prigge (1982) observed that there were no effects of level of intake or forage type on apparent digestibility of organic matter (OM). However, sheep fed the alfalfa hay had increased digestibility of CP and the apparent digestibility of NDF and acid detergent fiber (ADF) were increased for sheep fed the orchardgrass hay. This is usually the case when comparing fiber digestibility of grass and legume hays and is contributed to the difference in hemicellulose and lignin content of the hays (Varga and Prigge, 1982). Level of intake did not influence fiber digestibility of either the orchardgrass or alfalfa hay. In a study feeding cattle (8 beef heifers and 4 beef steers) bromegrass hay (11.4% CP) at 30, 55, 80, or 105% of maintenance intake as well a UIP supplement (blood, feather, and fish meal) at 2,556, 1,491, 762, or 0 g/d for increasing maintenance intake, OM and NDF digestibility were influenced (Scholljegerdes et al., 2005).

Total tract OM digestibility was decreased linearly as the inclusion of UIP decreased and total tract NDF digestibility decreased linearly with increased hay allotment Scholljegerdes et al., 2005).

Protein Supplementation

Supplementing protein to cattle consuming dormant or low quality forage is a common practice for beef cattle producers. Protein is the most beneficial supplemental nutrient when consumption of low-quality roughages is not limited (Campling, 1970). This is especially true when the protein content of the forage is less than 6 to 8 % CP (Campling, 1970, Kartchner, 1980, DelCurto et al., 1991, Patersen et al., 1994). The amount of protein in the supplement is also important. Usually protein supplements will contain 20% or more CP (Kellems and Church, 2010b). Most often when supplementing cattle consuming a low quality forage source several items can be influenced, including forage intake, forage digestibility, alterations in ruminal kinetics, and cow performance.

Low quality forage (2.67% CP) DM intake was almost doubled for steers receiving three different supplements of SBM and SG, alfalfa hay, and dehydrated alfalfa pellets compared with steers receiving no supplement (DelCurto et al., 1990c). Additionally, steers fed the dehydrated alfalfa pellets consumed 15% more forage than steers supplemented with alfalfa hay or the SBM and SG mix. Steers receiving a moderate CP supplement (25% CP) consumed 50% and 32% more forage than steers receiving low CP (13% CP) and high CP (39% CP) supplements, respectively at 0.5% of BW (DelCurto et al., 1990b). In a similar study utilizing the same dietary treatments, BW was increased in gestating cows receiving medium and high levels of protein, whereas those that were receiving the low protein supplement lost weight. Additionally, cows receiving high and medium levels of protein gained body condition, whereas those cows

receiving low protein lost body condition (DelCurto et al., 1990b). Beaty et al., (1994) observed a tendency for forage intake to increase in beef steers and gestating beef cows as supplemented CP increased from 10 to 40 %. In addition, several other studies have demonstrated an increase in intake of low-quality forages when supplemental CP is added to diets of beef cattle (Guthrie and Wagner, 1988; DelCurto et al., 1990a; Köster et al., 1996; Bandyk et al., 2001).

Many studies have yielded results that demonstrate an increase in forage intake with protein supplementation. Some authors, however, have discovered no differences in low-quality forage intake among cattle receiving protein supplements (Coleman and Wyatt, 1982; Bohnert et al., 2002a; Leupp et al., 2006). According to Lardy (2010) when forage intake is not affected by protein supplementation the forage CP levels are usually greater in the studies that do not see a response compared with those studies that do. However, caution must be used to not oversimplify the mechanisms responsible for the intake responses as forage CP alone is not always indicative of intake response. For example, when steers were fed range hay that was only 3.3% CP no differences were observed for dry matter intake (DMI; Coleman and Wyatt, 1982).

Usually the observed increase in intake of poor quality hay associated with supplemental CP is accompanied by an increase in digestibility. Steers that received a low, moderate, or high protein supplement had greater DM digestibility compared with steers that only received forage (DelCurto et al., 1990a). Additionally, NDF digestibility responded in a quadratic fashion where the steers fed moderate and high amounts of protein had a 30% increase in NDF digestibility compared with the low protein treatment (DelCurto et al., 1990a). Furthermore, a linear increase in DM digestibility was observed in steers as protein level increased from 12 to 41% of a SG and SBM supplement (Beaty et al., 1994). In addition both NDF and OM digestibility were increased

when beef cattle were supplemented with SBM and SG (DelCurto et al., 1990b) and ruminal and postruminal casein infusion (Bandyk et al., 2001).

Forage intake and digestibility can also be altered by the frequency with which a supplement is fed. When daily or three times weekly supplementation strategies were compared, gestating beef cows fed a supplement containing SG and SBM three times/wk had decreased forage intake and similar performance compared with cows supplemented daily (Beaty et al., 1994). In addition, total tract DM digestibility in steers increased linearly with increased CP in the supplement (10-40% CP) and with 3 d/wk supplementation (Beaty et al., 1994). Steers receiving a low-quality forage (4.8% CP) supplemented with mainly a sunflower meal and cottonseed meal mix twice weekly, had a decrease in forage DMI compared with steers supplemented three, five, or seven d/wk and both OM and NDF digestibility increased with increased frequency of supplementation (Farmer et al., 2001).

Additionally, beef cows during late gestation that were fed either a SBM or soyPLUS and blood meal supplement for approximately 78 d had increased body weight and BCS both pre and post calving compared with cows receiving only hay (Bohnert et al., 2002c). When wheat and oat green chop were supplemented daily, on alternate days, or every 4 d, no differences in hay intake were observed among treatments (Coleman and Wyatt, 1982). Other researchers demonstrated similar weight gain results to those of Coleman and Wyatt (1982) with steers fed cottonseed meal every 12, 24, or 48 h (Hunt et al., 1989), with gestating cows receiving cottonseed meal and SG daily, 3, or 1 time/wk (Huston et al., 1999b), and gestating cows receiving cottonseed meal daily or every 6 d (Schauer et al., 2005).

In addition to impacting intake and digestibility, supplement can also affect rumen kinetics. When grazing beef steers were fed a supplement of mainly cottonseed meal and corn to

provide 150% of NRC maintenance requirements for protein no differences were observed in ruminal pH compared with unsupplemented steers (Caton et al., 1988). However, total VFA concentration tended to be greater in supplemented steers compared with unsupplemented steers but butyrate concentration was greater in supplemented steers versus unsupplemented steers (Caton et al., 1988). A similar increase in concentrations of butyrate were observed when beef steers were fed either SG and SBM, long stem alfalfa hay, or dehydrated alfalfa pellets compared with unsupplemented steers (DelCurto et al., 1990c). The A: P ratio decreased linearly and the total VFA concentration tended to increase with increasing levels of a SBM and SG protein supplement (13 to 39% CP) in grazing beef steers, whereas no differences in ruminal pH were observed among treatments (DelCurto et al., 1990b).

Frequency of supplementation can also impact ruminal pH and VFA concentrations. When supplementing steers either daily or 3 d/wk (Monday, Wednesday, and Friday) Beaty et al. (1994) observed that on the day when only the daily supplemented steers received supplement (Tuesday, Thursday, Saturday, and Sunday) ruminal pH was decreased compared with the 3 d/wk supplemented steers. However, on the days that both groups received supplement (Monday, Wednesday, and Friday), those receiving supplement 3 d/wk had a decreased ruminal pH compared with the daily supplemented group (Beaty et al., 1994). When providing a sunflower meal and cottonseed meal supplement to beef steers 2 (Tuesday and Friday), 3 (Monday, Wednesday, and Friday), 5 (Monday through Friday), and 7 d/wk, steers receiving supplement seven d/wk had a decreased ruminal pH on a day only they were supplemented (Sunday) compared with all other treatments (Farmer et al., 2001). However on a day when all groups were supplemented (Friday) steers receiving larger quantities of supplement (those supplemented less frequently) generally had a decreased ruminal pH compared with those steers that were

supplemented daily (Farmer et al., 2001). Additionally, total ruminal VFA concentrations increased in steers as supplementation frequency increased (2-7 d/wk; Farmer et al., 2001). On the day when only the steers supplemented 7 d/wk were supplemented (Sunday) total VFA concentration among all treatments seemed to decrease, however, on the day when all treatments were supplemented (Friday) total VFA concentrations were greater until several hours after feeding (Farmer et al., 2001).

Ruminal NH_3 and BUN concentrations are correlated with protein supply in the diet. As the concentration of UIP in supplement increased in steers consuming low-quality hay (6 % CP), ruminal concentrations of NH_3 increased (Reed et al., 2007). In addition, BUN concentrations in steers fed medium (19.6% UIP) and high (40.6% UIP) levels of UIP were greater than unsupplemented steers, whereas steers fed the low level of UIP (0.8% UIP) were intermediate (Reed et al., 2007). Ruminal NH_3 concentrations increased in beef forage-fed steers as amount of SBM based supplement increased from 0.08 % to 0.50% of BW (Mathis et al., 1999). Similarly, when supplementing steers with increasing amounts of protein in a SBM/SG mix at a low (13% CP), moderate (25% CP), and high (39% CP) level those steers given the high protein supplement had the greatest ruminal NH_3 concentration compared with all other treatments and the low protein supplement group had the least ruminal NH_3 concentrations compared with all other treatments (DelCurto et al., 1990b).

Supplement feeding frequency has an effect on ruminal NH_3 and BUN concentration as well. Ewes with ad libitum access to low-quality bromegrass hay (7.5% CP) were supplemented with SBM once every 24 h or once every 72 h, as a result arterial urea N was doubled by feeding SBM compared with unsupplemented control ewes (Krehbiel et al., 1998). Furthermore, arterial concentration of urea N tended to be greater for ewes receiving supplement every 72 h compared

with ewes supplemented every 24 h (Krehbiel et al., 1998). In a study feeding low-quality forage to wether lambs with a DIP (SBM) and UIP (soyPLUS and blood meal) supplement offered every 1, 3, or 6 d, BUN was less in unsupplemented lambs compared with all other treatments (Bohnert et al., 2002c). In addition, BUN decreased in lambs as frequency of supplementation decreased from 1 to every 7 d (Bohnert et al., 2002c). When supplementing steers consuming a low-quality forage (3.1% CP) 3 or 7 d/wk with increasing amounts of CP (10 to 40%) ruminal NH_3 concentrations on days when only the 7 d/wk treatment received supplement were generally increased with increasing amounts of CP in the supplement (Beaty et al., 1994). In the same trial, on days when all steers received supplement, ruminal NH_3 concentrations for steers fed supplement 3 d/wk peaked at greater concentrations compared with steers supplemented daily even though supplements contained the same amount of CP. This increase in ruminal NH_3 concentration is in all probability due to the increased amount of supplement offered to the steers receiving supplement 3 d/wk (4.62 kg) compared with those supplemented daily (1.98 kg; Beaty et al., 1994). This increased amount of substrate for microbial fermentation and subsequently protein would cause the ruminal NH_3 concentration to increase.

Circulating non-esterified fatty acids (NEFA) are an indicator of variation in the metabolism of fat deposits in the body. This is especially true when caloric intake is low causing an increase in the mobilization of body fat and even though the amount of NEFA in a ruminant bloodstream is relatively low, it is an important aspect of caloric homeostasis of the ruminant's body (Bowden, 1971). When gestating beef cows were supplemented with either a low (0.4% UIP), medium (20.0% UIP), or high (39.0% UIP) level of a UIP supplement, plasma NEFA concentrations were greater in unsupplemented cows compared with UIP supplemented cows during periods of blood collection (Sletmoen-Olson et al., 2000). Additionally, increased NEFA

concentrations were also observed in unsupplemented cattle and sheep compared with those that were supplemented (Krysl et al., 1987; Cheema et al., 1991; Barton et al., 1992). In contrast, adding supplemental DIP (corn gluten meal and blood meal) and UIP (SBM) to a low quality forage diet in beef cows did not affect plasma NEFA concentrations (Rusche et al., 1993).

Energy Supplementation

For cattle consuming low to moderate quality hay, protein is not the only nutrient item that may need to be supplemented. Although energy is not considered to be one of the six key nutrients needed to sustain life, it is still a very important factor to consider when supplying feed to livestock. Energy can be measured in several ways but for the purposes of this thesis I will be discussing the net energy system and how it is used to measure and determine energy requirements for ruminants.

The net energy system factors in several outlets of waste energy retention to obtain an accurate prediction of what energy requirements are at varying stages of production. These factors include losses of feces, urine, gas, and heat from the animal's body. For the sake of simplicity net energy is split into two separate sections; net energy for maintenance (Ne_m) and net energy for production (Ne_p). What an animal needs to maintain itself is considered to be Ne_m. This includes basal metabolism, activity at maintenance, and maintaining body temperature. Net energy of production evaluates what an animal needs above its maintenance energy requirements for tissue growth, fetal growth, milk production, or physical exertion above what is required for maintenance. Approximately 50% of Ne_m is utilized by the liver, digestive tract, heart, and kidney (Ferrell, 1988). Muscle utilizes about 23% of Ne_m, but this may increase substantially in grazing ruminants when you take into consideration the energy expenditures related to the amount of work that is required to consume and process forages (Robbins, 1993).

Low-moderate quality forages may lack sufficient energy for several classes of cattle and thus, producers may need to supply both protein and energy supplementation in order to meet maintenance and production requirements. In general supplementing forage diets with a high energy grain will decrease forage intake as well as digestibility in ruminants (Minson, 1990; Reese et al., 1990; DelCurto et al., 1990a; Pordomingo et al., 1991; Schoonmaker et al., 2003). In most cases decreased digestibility is due to negative associative effects which can occur when grain is used to supplement forage diets (Caton and Dhuyvetter, 1997). Cereal grains, such as corn, barley and SG, are comprised of approximately 90% starch (NRC, 2000). When a ruminant animal consumes grain the readily digestible starch is broken down quickly by rumen microbes causing a decrease in ruminal pH. When ruminal pH falls below 6.2, cellulolytic bacteria attachment and coincidental forage digestion can be decreased (Ørskov, 1982) leading to an increased population of amylolytic bacteria, which are better suited to survive in a low pH environment compared with cellulolytic bacteria (Owens and Goetsch, 1988). As the amount of grain in the diet increases, the shift in the microbial population will be more prominent allowing the colonization of more amylolytic bacteria in the ruminal complex (Owens and Goetsch, 1988). Bacterial populations shifting from a greater proportion of cellulolytic bacteria to a greater proportion of amylolytic bacteria, results in a reduction of forage digestibility (Owens and Goetsch, 1988). In addition, this change in bacterial population will result in an increase in forage retention in the rumen which will cause the ruminant animal to decrease its forage intake (Owens and Goetsch, 1988).

In contrast, several authors reported that energy supplementation resulted in an increase in forage intake (Henning et al., 1980; Matejovsky and Sanson, 1995). However, this research was conducted with sheep and according to Caton and Dhuyvetter (1997) research reporting

increases in forage intake while supplementing with low levels of energy seems to occur more often in sheep than cattle.

Digestibility of diet components can be influenced by the inclusion of an energy supplementation in the ration. When wethers consuming low-quality hay (8% CP) were supplemented with high (20% of ad libitum intake) or low (10% ad libitum intake) amounts of a corn/SBM mix, NDF total tract digestibility was similar among treatments (Howard et al, 1991). Similarly, in a study conducted by Reese and others (1990) ewes were supplemented with a mixed energy supplement (rice bran, molasses, cassava meal, and fish meal) that provided increasing levels of metabolizable energy (Medium, 1% of average BW; low and high 60% and 140% of daily energy intake of medium group). No differences in DM digestibility were observed among treatments (Reese et al., 1990). Apparent OM digestibility increased linearly with increased feeding rate (1.4 kg DM to 2.8 kg DM) of molasses, SBH, or corn in steers consuming ammoniated hay (Royes et al., 2001). Additionally, increasing amount of supplementation with SBH caused a linear increase in NDF and ADF apparent digestibility and increased amount of corn and molasses supplementation caused apparent NDF and ADF digestibility to decrease linearly and quadratically for corn and molasses respectively (Royes et al., 2001). For DelCurto and others (1990a) total tract DM digestibility increased in beef steers with increasing amounts (9.2 and 18.4 ME/kg BW) of an energy supplement consisting of SG and SBM. However, NDF digestibility tended to decrease with increased levels of energy supplementation (DelCurto et al., 1990a).

Many research projects have explored the impact of energy supplementation on ruminal pH and the coincident negative associative effects, however, ruminal pH results vary greatly. When supplementing steers with a mineral mix, a protein feed (cottonseed meal), a high fiber

energy source (wheat middlings and SBH), or a high grain (SG) energy source, those steers receiving the mineral treatment had an increased ruminal pH compared with the protein, fiber, and grain diets (Bodine et al, 2001). Furthermore, steers consuming the fiber and grain supplements had similar ruminal pH, whereas the steers fed protein supplement had a greater ruminal pH compared with the energy supplemented steers (Bodine et al., 2001). When feeding beef steers two levels of energy supplements DelCurto and others (1990a) observed high energy supplemented steers had decreased ruminal pH, 3 h after feeding compared with steers fed low energy supplements. Additionally, at 6 h after supplementation, steers that received a diet containing both high protein and low energy had the greatest ruminal pH compared with all other treatments. Total VFA concentration was not affected by supplemental treatments (DelCurto et al., 1990a). Steers that received a high energy supplement had decreased molar proportions of acetate at 0, 3, 9, and 12 h post-supplementation compared with all other treatments and butyrate concentrations were increased with increasing levels of energy supplement (DelCurto et al., 1990a) When supplementing increasing levels (10, 30, or 50% of diet) of a barley-based supplement to beef steers consuming grass hay (10.3% CP) a decrease in ruminal pH was observed with increasing levels of the supplement (Leventini et al., 1990). This effect is anticipated, however, as the high percentage of grain fed to the steers would likely lead to a decrease in ruminal pH. Additionally, several other experiments have demonstrated varying levels of ruminal pH where there was either a decrease (Chase and Hibberd, 1987; Zorrilla-Rios et al., 1989; Westvig, 1992), or no change (Sime et al., 1990; Ulmer et al., 1990; Carey et al., 1993) in ruminal pH when energy supplements were fed compared with an unsupplemented control in cattle.

Ruminal NH_3 and BUN concentrations can also be altered by the addition of an energy supplement to a ruminant's diet. Recall that BUN and ruminal NH_3 concentration are indicative of circulating N in the ruminants system which is an important aspect of digestion. When ammoniated stargrass hay was supplemented with cane molasses, SBH, or corn, unsupplemented steers and those receiving corn as a supplement had an increased ruminal NH_3 concentration compared with the steers supplemented with molasses, whereas SBH supplemented steers were intermediate (Royes et al., 2001). When supplementing steers consuming timothy silage with molasses, canola meal, and a combination of these at different levels, ruminal NH_3 was decreased in steers fed the molasses based supplements compared with steers supplemented with canola meal, and steers that received no supplement (Petit and Veira, 1994). Lambs fed high energy (2.34 Mcal/kg ME) diets had greater ruminal NH_3 compared with lambs fed low energy (1.82 Mcal/kg ME) diets and BUN concentrations were increased for lambs fed the low energy diet at 4 and 8 h after feeding compared with the lambs fed high energy diets (Sultan and Loerch (1992). Grazing heifers that received a wheat middlings/SBH supplement 3 d/wk had an increase in BUN concentrations compared with heifers supplemented 7 d/wk on the days when only the 7 d/wk treatment group was supplemented (Cooke et al., 2008). Conversely, BUN concentrations were decreased for the 3 d/wk supplemented group compared with the 7 d/wk supplement group on days when both treatment groups were supplemented (Cooke et al., 2008).

As mentioned previously blood NEFA concentrations are indicative of fat mobilization in the body. In a study conducted by Moriel and others (2012) replacement beef heifers were given ad libitum access to low (8% CP) and medium (12% CP) quality hay and supplemented (SBH and wheat middlings) with a high (15.8 kg) and low (7.9 kg) amount of energy daily or 3 d/wk. Plasma NEFA concentrations were similar in heifers supplemented daily on days when all

treatments were supplemented and days when the 7 d/wk heifers were supplemented. For heifers supplemented 3 d/wk, plasma NEFA concentrations were greater on days when only the 7 d/wk heifers were supplemented than on days when all 3 d/wk heifers were supplemented. Hussain et al., (1996) observed differences in NEFA concentrations when feeding gestating goats either hay, or good (silo stored), or poor (aerobically deteriorated) quality silage. Plasma NEFA concentrations in goats fed poor-quality silage were increased compared with goats fed either hay or good-quality silage.

Meeting energy requirements is very important for gestating and lactating cattle. If energy requirements are not met, detrimental effects on animal performance can be observed. Beef cows in late gestation grazing dormant prairie hay (2.1% CP) were supplemented with increasing amounts (0.48, 0.72, and 0.96% BW) of alfalfa hay. Cow BW increased with increasing levels of alfalfa whereas BCS was unchanged for the first month (mo) of supplementation (Vanzant and Cochran, 1994). During the remainder of the time before calving cow weight was unchanged by alfalfa supplementation (Vanzant and Cochran, 1994). Gestating beef cows were used to compare feeding either limit-fed corn with hay or only hay (10.2% CP) as sources of energy when wintering beef cows (Loerch, 1996). Those cows that received limit-fed corn had an increase in BW and BCS compared with cows fed only hay (Loerch, 1996). Additionally, calves born to cows that were limit-fed corn had increased birth weights compared with calves from cows that received only hay (Loerch, 1996). Other research determining the effects of energy supplementation on cow performance have shown similar results to those stated above. Cows receiving energy supplements, regardless of source, have demonstrated improved performance including decreased weight and body condition losses (Anderson et al., 1988; Schoonmaker et al., 2003).

Distiller's Grains Supplementation

As previously stated, distiller's grains are a good source of protein and energy and therefore make an excellent supplement when cattle production requirements are high and forage quality is low. As amount of supplemental DDGS was increased in steers fed moderate quality forage, OM intake was decreased (Leupp et al., 2009). Additionally, true ruminal OM digestion and total tract OM digestibility were increased with increasing levels of DDGS (Leupp et al, 2009). Frequency of DDGS supplementation can also have an effect on intake and digestibility. Heifers on an alternate day DDGS or dry rolled corn supplementation strategy (0.8% of BW on alternate days) had decreased forage DMI on days they received supplement compared with heifers receiving 0.4% of BW daily (Loy et al., 2007). When supplementing heifers with 0, 0.5, 1.0, 1.4, or 2.0 kg of distiller's grains a linear decrease in forage intake was observed with increasing amounts of DDGS fed (MacDonald and Klopfenstein, 2004). Similarly, when feeding heifers a high (53% total digestible nutrients; TDN) and low (65% TDN) quality forage while supplementing with five different levels of DDGS forage intake was linearly decreased with the increasing (0 to 6 pounds) level of DDGS (Morris et al., 2005). Intake and digestibility can also be influenced when cattle are supplemented DDGS while consuming good quality roughage. Supplementing DDGS at different levels (0.2, 0.4, and 0.6 % BW) to heifers grazing small-grain pasture (17.7% CP) had no effect on forage and total OM, CP, and NDF intake (Islas and Soto-Navarro, 2011). Additionally, OM and CP digestibility were not affected by the inclusion of DDGS, however, total NDF digestibility increased linearly with increasing (0.2 to 0.6% BW) levels of DDGS (Islas and Soto-Navarro, 2011).

Ruminal pH and total VFA concentrations were not altered by the inclusion of increasing levels of DDGS for steers consuming moderate-quality brome hay (Leupp et al., 2009).

However, a linear decrease in the molar proportion of acetate was observed, whereas the molar proportion of butyrate linearly increased quadratically with increasing levels of DDGS in the diet. Additionally, the ratio of A:P linearly decreased with increasing DDGS in the diet (Leupp et al, 2009). Supplementing heifers consuming low-quality hay with daily or alternate days of either DDGS or DRC resulted in alterations in ruminal pH. While ruminal pH was not affected by type of supplement or supplement feeding frequency, average ruminal pH was decreased for all supplemented treatments compared with an unsupplemented control group (Loy et al., 2007). In the same trial, total ruminal VFA concentrations were greater for supplemented heifers compared with non-supplemented heifers and total VFA increased with the alternate day feeding of corn but decreased when supplementing DDGS. Lastly, the A:P was decreased in heifers that were supplemented with DDGS compared with those supplemented with corn (Loy et al., 2007). Conversely, ruminal pH and VFA concentrations for heifers grazing small grain pasture and supplemented with increasing levels of DDGS were not different among treatments (Islas and Soto-Navarro, 2011).

Distiller's grain supplementation as well as frequency of supplementation can also influence ruminal NH_3 concentrations. Ruminal NH_3 concentrations peaked at 2 h post-feeding when supplementing steers consuming moderate-quality brome hay with 0.3 and 0.6% of BW daily of DDGS (Leupp et al., 2009). However, ruminal NH_3 concentrations for all other treatments (0, 0.9, and 1.2% BW DDGS) peaked at 4 h after feeding and by 12 h after feeding ruminal NH_3 concentrations returned to pre-feeding levels (Leupp et al., 2009). Conversely, when supplementing heifers grazing small grain pasture with 0, 0.2, 0.4, or 0.6% BW DDGS there were no differences in ruminal NH_3 concentrations among treatments (Islas and Soto-Navarro, 2011).The lack of differences was more than likely due to the fact that there were no

differences in CP in the diets and there was a surplus of CP available for microbial growth. Supplementing heifers receiving chopped grass hay with 0.4% BW daily or 0.8% BW on alternate days with dry-rolled corn or DDGS resulted in an increased ruminal NH_3 concentration for supplemented heifers compared with heifers receiving only hay (Loy et al., 2007). Moreover, ruminal NH_3 concentrations for heifers supplemented on alternate days tended to be greater compared with heifers that were supplemented daily for both the dry-rolled corn and DDGS supplemented heifers (Loy et al., 2007).

Few authors have published reports evaluating DDGS supplementation that included a sampling protocol for both BUN and NH_3. Authors of the afore-mentioned studies did not collect blood samples to obtain circulating BUN. However, other researchers obtained BUN measurements but did not obtain ruminal NH_3 concentrations. Late-gestation beef cows consuming a DDGS diet had increased BUN concentrations at 0 and 3 h after feeding compared with cows consuming hay and limit-fed corn diets (Radunz et al., 2010). When feeding gestating ewes either ad libitum haylage, limit-fed corn, or limit-fed dried distiller's grains plus solubles, BUN concentrations were increased in ewes fed DDGS immediately prior to feeding (Radunz et al., 2011). Additionally, post-feeding BUN concentrations were greater for ewes fed DDGS and haylage compared with ewes fed corn (Radunz et al., 2011). In the two previous studies mentioned, the increase in BUN in animals receiving DDGS is more than likely due to the intake of CP in the diets, which was greatest in the DDGS diets. A digestion trial comparing the effects of three corn-based total mixed rations (TMR) including a control (10.2% CP), urea (13.3 % CP), and DDGS (14.9% CP) was conducted (Brake et al., 2010). No differences in BUN concentrations among treatments were observed. Overall it would be expected to see an increase

in BUN and NH_3 concentrations when feeding ruminants DDGS due to the high CP content of the feed.

It would also be assumed that due to the high energy content of DDGS, concentrations of NEFA would also be influenced by the inclusion of DDGS. Non-esterified fatty acid concentrations tended to be greater before feeding for cows consuming hay diets and DDGS diets compared with cows consuming corn diets (Radunz et al, 2010). In addition, NEFA concentrations tended to be greater for cows on the hay diets at 3 h after feeding compared with the cows fed corn diets and DDGS diets (Radunz et al., 2010). Concentrations of NEFA were more than likely increased in cows fed hay diets due the fact that the amount of energy in the hay was not sufficient to meet the production energy requirements of the cows and they were mobilizing body fat, whereas the increase in NEFA concentrations in cows fed the DDGS diets were more than likely due to the increased amount of fat in the diet from the DDGS (Radunz et al., 2010).

Feeding ewes during gestation with dietary treatments consisting of either, ad libitum haylage, limit-fed corn, or limit-fed DDGS resulted in increased pre-feeding concentrations of NEFA during mid-gestation for ewes consuming the DDGS and haylage rations compared with those consuming corn and by 6 h after feeding ewes consuming the haylage diet had NEFA concentrations less than both the DDGS and corn rations (Radunz et al., 2011). However, there were no differences in NEFA concentrations among treatments when blood was collected during late gestation (Radunz et al., 2011). Differing results were observed when supplementing gestating heifers consuming grass hay (9.1% CP) with either DDGS or SBH (Engel et al., 2008). A time effect was observed where NEFA concentrations among all treatments were increased from prepartum concentrations from d 4 through d 8 postpartum and then a gradually decrease

was observed through d 20 postpartum, by this time NEFA concentrations were similar to those observed prepartum. Energy requirements were more than likely not being met at this time and therefore blood NEFA concentrations were increased.

Ruminant performance including ADG, BW, and BCS can also be altered by the addition of DDGS in growing heifer and steer rations as well as mature cow rations. In a study where heifer calves consuming low or moderate quality forage were supplemented with 1 of 5 levels of increasing DDGS (from 0 to 2.7 kg DM) a linear increase in ADG was present as levels of DDGS increased in the diet (Morris et al., 2005). Gestating heifers receiving a TMR including DDGS had greater BW change compared with heifers receiving a TMR including SBH, (Engel et al., 2008). Those heifers supplemented with the DDGS had an increased BW at calving compared with all other treatments but BCS, calf birth weights, weaning weights, and ADG were similar among treatments (Engel et al, 2008). In a study comparing grass hay, limit-fed corn, or limit-fed DDGS, cows during late gestation fed DDGS gained approximately 20 kg more BW than cows consuming the hay and corn diets and there were no differences in cow BCS among treatments 3 wk prior to calving (Radunz et al., 2010). Additionally, efficiency of weight gain was greatest for cows fed DDGS and least for cows fed grass hay, while those fed corn were intermediate (Radunz et al., 2010). The increased efficiency of weight gain for cows consuming DDGS was attributed to an energy content that was greater than the other two treatments.

Distiller's dried grains plus solubles is a feed that can be beneficial to include in cattle rations, due to its favorable content of protein and energy. By including DDGS in rations for cattle forage intake can be reduced and animal digestive and metabolic performance can be enhanced making DDGS an optimal supplement in beef cattle systems.

Conclusions

Overall, when cattle are consuming low to mid quality forage it is feasible to supplement the animals with a good quality protein and energy source. By supplementing cattle, forage intake can be reduced while meeting nutritional requirements for production. Therefore, supplementing cattle with a feed such as DDGS can reduce the cost of cattle and forage production. However, these reductions in production and forage costs must also be compared with the added cost of the supplement that is being fed. As demonstrated there are several research studies that have been conducted that delve into the effects of not only level of protein and energy supplementation but also the frequency that the supplement is fed. However, there is a paucity of information about what would occur if one would feed only DDGS, or only a mid to low-quality hay, on alternating days to cattle.

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CHAPTER 2. EFFECTS OF ALTERNATE DAY FEEDING OF DRIED DISTILLER'S GRAINS PLUS SOLUBLES IN FORAGE-FED STEERS

Abstract

The objective of this experiment was to examine effects of feeding either dried distiller's grains plus solubles (DDGS) or grass hay on alternate days on intake, ruminal fermentation, and digestibility in forage-fed steers. Four ruminally and duodenally cannulated Holstein steers (448.8 ± 7.3 kg BW) received each of 4 dietary treatments in a 4 × 4 Latin square: 1) hay only (CON); 2) hay and 0.4% BW DDGS daily (DG7); 3) hay daily and 0.8% BW DDGS on alternate days (DG3); and 4) hay only or 0.8% DDGS only on alternate days (DGA). Treatment periods consisted of 13 d of adaptation and 8 d of collecting ruminal pH, ruminal fluid, digesta, and blood. Supplemented days (SUP) and non-supplemented days (NSUP) were defined as days when DG3 and DGA did or did not receive DDGS, respectively. Over the entire collection period DMI was decreased ($P < 0.01$) for DGA (13.18 ± 0.68 kg/d) compared with CON (16.00 ± 0.68 kg/d), DG7 (15.30 ± 0.68 kg/d), and DG3 (16.15 ± 0.68 kg/d). Immediately after feeding on SUP, ruminal pH of DGA was less than all other treatments, but by the end of the day was greater than all other treatments (treatment × time; $P < 0.001$). At the time of feeding on NSUP, ruminal pH of DGA steers was greater than all other treatments but returned to levels similar (treatment × time; $P < 0.001$) to DG3 and CON by 5 h after feeding. On NSUP, ruminal pH of steers fed DG7 was less ($P < 0.01$) than all other treatments until 9 h after feeding. Total concentrations of VFA were similar ($P = 0.09$) among treatments on SUP; however on NSUP total VFA concentrations were least in DGA from feeding until 4 h post feeding (TRT × Time; P 0.02). Ruminal ammonia was similar ($P > 0.10$) for all treatments at feeding on SUP. Four h post-feeding on SUP, DG7 had greater (TRT × time; $P = 0.002$) NH3 compared with CON and

DGA; whereas DG3 was intermediate. At feeding and 6 h post-feeding on NSUP DGA had increased (TRT × time; $P < 0.001$) NH3 compared with all other treatments. At 4 and 6 h post-feeding on NSUP, DGA had increased (TRT × time; $P < 0.001$) NH3 compared with CON, DG7, and DG3. Steers fed DGA had less ($P < 0.01$) BUN on SUP compared with all other treatments (5.5, 7.9, 8.3, and 7.8 ± 0.4 for DGA, CON, DG7, and DG3; respectively). Total tract DM digestibility did not differ ($P = 0.18$) among treatments (56.9 ± 1.7%). Blood urea nitrogen was greater ($P < 0.01$) for DGA on NSUP compared with CON, DG7, and DG3 (11.8, 8.1, 8.6, 8.9 ± 0.5 m*M* for DGA, CON, DG7, and DG3; respectively). There were no differences ($P > 0.10$) in NEFA among treatments on SUP; however, on NSUP DGA (209.5 ± 12.7 m*M*) steers had increased ($P < 0.01$) NEFA compared with all other treatments (84.4, 88.0, and 77.7 ± 12.7 m*M* for CON, DG7, and DG3; respectively). All treatments had similar ($P > 0.10$) concentrations of IGF_1 throughout the collection period (114.0, 126.3, 128.8, 128.9 ± 6.6 ng/mL for CON, DG7, DG3, and DGA; respectively). The feeding strategy DGA influenced DMI and ruminal kinetics without negatively impacting total tract digestibility. Additionally DGA altered BUN and NEFA concentrations but had no effect on circulating IGF_1.

Introduction

As forage and pasture costs continue to rise, it is becoming more imperative for cow-calf producers to reduce production costs. Feed cost alone accounts for greater than 60% of beef producers annual cow costs (Miller et al., 2001). According to the North Dakota Center for Career and Technical Education (2009), 77% of direct cow-calf expenses are feed related. By maximizing feed efficiency while meeting nutrient requirements of cattle, producers may be able to reduce feed costs. An additional method of reducing cost is to alter supplementation strategies on beef cattle operations.

Dried distillers grains plus solubles (DDGS) is a byproduct of the ethanol industry which is becoming increasingly accessible as ethanol production rises (Wallander et al., 2011). According to the National Research Council, distiller's grains are approximately 29.5 % crude protein, contain 2.18 megacalories (Mcal) of net energy for maintenance, are low in starch, and approximately 40% digestible fiber (NRC, 2000). This nutrient profile makes DDGS an exceptional supplement for cows consuming forage based diets. Supplementing cattle with DDGS can reduce forage intake (MacDonald and Klopfenstein, 2004; Morris et al., 2005). Thus, producers can reduce the amount of hay and incidental input costs associated with harvesting and processing forage by simply adding a supplement to their forage ration. However, this reduction in cost of forage must then be compared with the added cost of the supplement.

This would also be beneficial during years of inclement weather when cow-calf producers need to stretch their hay reserves to meet feeding demands such as times of drought when forage availability is low and when conditions are too wet for forage to be processed or retrieved from fields.

Forage intake can also be altered by the frequency with which the supplement is fed. When daily or 3 times weekly supplementation were compared, cattle fed supplement 3 d/ wk had decreased forage intake and similar performance when compared with cattle supplemented daily (Beaty, 1994). By decreasing supplementation frequency, producers would be able to decrease the amount of time spent feeding, the amount of labor required for feeding, and decrease the use and depreciation of equipment. Research conducted by Schoonmaker et al., 2003, demonstrated that limit-feeding concentrate diets with a moderate-quality hay resulted in a reduction of forage intake. However, there is a paucity of information about what would occur if

one would alternate feeding of either supplement only, or hay only, every other day in beef cattle systems.

Therefore, we hypothesized that alternate day feeding of DDGS with hay would decrease overall hay dry matter intake while still maintaining a healthy ruminal environment. Our objectives were to determine the effects of eliminating forage from diets on alternating days while supplementing steers with DDGS on forage intake, rumen kinetics, digestibility, and serum hormone and metabolite profiles in steers fed moderate quality forage.

Materials and Methods

All research procedures were approved by the North Dakota State University (NDSU) Institutional Animal Care and Use Committee.

Animals and Diets

Table 2.1. Chemical composition of forage and dried distiller's grains plus solubles

	Component[1]					
Item	DM	CP	OM	NDF	ADF	Sulfur[2]
Bromegrass hay	77.8	16.2	85.1	64.1	35.8	---
DDGS[3]	89.6	28.3	94.5	30.6	8.7	10060.4

[1]Expressed as a % of DM
[2]Expressed as parts per million
[3]Dried distiller's grains plus solubles

Four ruminally and duodenally cannulated Holstein steers (448.8 ± 7.3 kg) were used in a 4 × 4 Latin square consisting of 4 periods of 21 d. Steers were housed in a climate controlled room in individual pens (3.0 × 3.7 m) during a 13 d adaptation period and transferred to individual tie stall stanchions (1.0 × 2.2 m) during an 8 d collection period. Diets consisted of combinations of brome grass hay (mainly brome; *Bromus inermis*) and/or DDGS in the following treatments: 1) hay only (CON); 2) hay and 0.4% body weight (BW) DDGS daily

(DG7); 3) hay daily and 0.8% BW DDGS on alternate days (DG3); and 4) hay only or 0.8% DDGS only on alternate days (DGA). Hay was chopped to pass through a 3.8 cm screen fed at 0700 h and 1900 h daily, whereas DDGS was fed at 0630 h daily to DG7 and on alternate days to DG3 and DGA. Nutrient content of the hay and DDGS are listed in Table 2.1. All hay originated from the same cutting and field and all DDGS used in the study originated from the same production lot. Steers were weighed before feeding 2 and 1 d prior to the beginning of each period to determine the quantity of DDGS to be delivered on a BW basis for the entirety of the ensuing period. Steers also had ad libitum access to water and a pressed mineral block (Easylix®, 12-12-12, Mineral pressed block; Hubbard Feeds, Inc., Mankato, MN; 11% Ca, 13% P, 12% salt, 0.5% Mg, 180,000 IU/lb vitamin A, 18,000 IU/lb vitamin D-3, 50 IU/lb vitamin E). To meet magnesium requirements (0.12% DM basis) and maintain the Ca:P ratio requirement of 2:1, 288 g of Ca, and 2 g Mg was added to the DDGS or hay depending on the treatment during each morning feeding. Steers were allowed 30 min to consume the delivered DDGS. At 0700 h, remaining DDGS was weighed and placed back in the bunk and hay was delivered. Steers consumed the allotted supplement within the assigned 30 min.

Sample Collection

The study consisted of four 21 d replicates of a 13 d adaptation period followed by an 8 d collection period. Both hay and DDGS samples were taken daily and composited by week to determine nutrient composition of feed. Orts were collected daily at 0615 h and composited by week to determine nutrient composition of refusals and samples were frozen (-18°C) until sample processing and chemical analysis could be performed. Eight ± 0.2 g of chromic oxide (Cr_2O_3) was dosed through the ruminal cannula intraruminally in gelatin capsules (Torpac Inc.,

Fairfield, NJ) at 0700 and 1900 h to serve as a digesta flow marker, beginning on d 9 until the end of the collection period (d 9-21).

Rumen content and duodenal samples were taken so that every 2 h of a 24 h period were accounted for and to also ensure a compliment of samples for supplemented days (days DG7, DG3, and DGA received supplement; SUP) and non-supplemented days (days CON, DG3, and DGA received hay; NSUP). Ruminal content and duodenal samples were taken at 0800, 1400, and 2000 h on d 14 and 15; 0200, 1000, 1600, and 2200 h on d 16 and 17; 0400, 1200, and 1800 on d 18 and 19; and 0000 and 0600 on d 20 and 21. Ruminal contents (approximately 500 g) were collected at the same time points as duodenal samples. When collecting the ruminal contents, random grab samples were taken from both the ventral and dorsal ruminal sacs and were representative of the liquid and fiber phases. Ruminal grab samples were placed in large heavy plastic bags which were doubled to reduce the risk of losing any sample. Two hundred mL of formalin/saline solution (3.7% formaldehyde/ 0.9% NaCl) was added to each bag at every collection point that the ruminal content samples were taken for isolation of bacterial cells. For each collection period ruminal content samples were composited by week for both SUP and NSUP, resulting in 2 samples per steer per period and a total of 32 separate ruminal content samples. Ruminal content samples were refrigerated (7°C) until being blended on medium speed for 1 min using a Waring commercial heavy duty blender (model 37BL19 CB6, Waring Products division, New Hartford, CT), strained through 4 layers of cheese cloth, and frozen (-18°C) until chemical analyses could be performed for both SUP and NSUP. Duodenal samples (approximately 200 mL) were collected into individual whirl pak bags (Nasco; 532-mL) then composited into a 3 L container for each steer for both SUP and NSUP days resulting in 32 total

composited samples. Composited duodenal samples were then frozen (-18°C) until sample processing and chemical analysis.

Steers were dosed with 200 mL of Co-EDTA (1,734 mg of Co; Uden et al., 1980) at 0430 h on d 18 and 21 to serve as a liquid passage marker. Dosing was accomplished by inserting a 1.0 m piece of polyvinyl chloride pipe directly into the ventral sac of the rumen and pouring the Co-EDTA down the pipe and into the liquid phase of the rumen. Ruminal fluid samples (200 mL) were obtained via a 2 L suction strainer from the ventral ruminal sac immediately prior to Co-EDTA dosing, at feeding, and every 2 h thereafter until 8 h post-feeding (0430 h, 0630 h, 0830 h, 1030 h, 1230 h, and 1430 h). Immediately after collection, rumen fluid samples were poured into individual whirl pak bags(Nasco; 532-mL) and acidified with 2 mL of 6.0 *M* HCl, and frozen (-18°C) until analysis of ruminal volatile fatty acids (VFA), ammonia (NH3), and cobalt concentrations.

Whole rumen pH was obtained using wireless rumen pH and temperature sensors (Kahne Ltd., Auckland, NZ) with a measurement taken every 10 min for the collection periods entirety. Sensors were calibrated with 7.0 and 4.0 pH solutions before being manually inserted into the rumen, through the fiber phase, and placed in the liquid phase of the ventral sac. Recalibration and bolus insertion was performed on the evening of d 13 (1 d prior to the initiation of the collection period). Sensors were retrieved after the completion of each collection period and data were downloaded from the pH boluses using the Kahne Ltd. software. Three consecutive data points for pH were averaged for every 1/2 h giving a total of 48 time points per day. A feed day was defined as beginning at 0630 h with the feeding of the morning DDGS and ended at 0600 h before the subsequent days DDGS feeding.

Total fecal material was scraped into stainless steel pans located directly behind each steer throughout the day to ensure that fecal loss was minimized. Total fecal output was weighed once daily (d 14-21) at 0530 h. Fecal output for each day was mixed by hand and 10% of the total fecal weight (wet basis) was collected from various locations throughout the fecal material to obtain a representative subsample. Each fecal subsample was composited per steer per period for both SUP and NSUP. Fecal samples were refrigerated (7°C) until the end of the collection period when total fecal samples could be mixed for 3 min in a large commercial rotary mixer (Model A- 200, Hobart MFG. Co., Troy, OH). Mixed total fecal samples were subsampled then placed in an aluminum pan (13 × 9 inches) and immediately taken to be dried.

Blood samples were obtained daily (d 14-21) via coccygeal venipuncture into 2 red-top serum separator tubes (BD Vacutainer®; 10 mL) at 1100 h and centrifuged (TJ-6, Beckman, Brea, CA) at 1500 × *g* at room temperature for 25 min. Serum was then pipetted from the tubes into individually labeled micro tubes (VWR International; 2 mL). Samples were placed in the freezer (-18°C) as soon as pipetting was complete until analysis for blood urea nitrogen (BUN), IGF_1, and NEFA.

Laboratory Analysis

Feed, ort, and fecal samples were dried in a Grieve forced-air oven (60° C; The Grieve Corporation) for 48 h then ground to pass a 2-mm screen using a Wiley mill (Thomas-Wiley Lab Mill, Model 4; Thomas Scientific USA). Feed and ort samples were then analyzed for DM, CP, and ash (Procedure numbers: 934.01, 2001.11, and 942.05 respectively; AOAC, 2010) as well as NDF and ADF (Goering and Van Soest 1970, as adapted by Ankom Technology). Neutral detergent fiber and ADF were determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY).

Duodenal samples were lyophilized (VirTis Genesis 25L, SP Scientific, Gardiner, NY). Both the freeze dried duodenal samples and the dried fecal samples were analyzed for DM, CP, ash, NDF, and ADF (Same procedures as above) as well as concentrations of Cr_2O_3. Fecal and duodenal samples were prepared for Cr analysis via the method proposed by Fenton and Fenton (1979) and absorbance was read using a UV-VIS biotech spectrophotometer (DU-640, Beckman Coulter, Brea, CA).

For analysis of VFA concentrations, rumen fluid samples were thawed at room temperature and then centrifuged at 20,000 × *g* for 10 min. After centrifugation the supernatant was mixed with 25% (wt/vol) metaphosphoric acid (1-mL metaphosphoric acid/ 5-mL of rumen fluid) and recentrifuged at 10,000 × *g* for 10 min. The resulting supernatant was used for VFA concentration analysis using the method proposed by Goetsch and Galyean (1983). Determination of VFA concentration was done via gas chromatography (Agilent Technologies, 6890 series gas chromatograph, Santa Clara, CA) using a capillary column and an internal standard of 2-ethylbutyric acid.

To determine ruminal NH_3 concentrations, thawed rumen fluid was centrifuged at 13,800 × *g* for 10 min at 4° C. The resulting supernatant was saved, reagents were added (Chaney and Marback, 1962) and the reaction method was done as outlined by Weichselbaum et al., 1969. Absorbance of NH_3 was measured via a UV-VIS biotech spectrophotometer (DU-640, Beckman Coulter, Brea, CA). Concentrations of cobalt used for determination of liquid passage rate were determined using an atomic absorption spectrophotometer (AAnalyst800, PerkinElmer Inc., Waltham, MA) with an air-plus-acetylene flame.

Thawed, blended ruminal contents were placed in 250 mL centrifuge bottles and centrifuged at 500 × *g* for 20 min to remove protozoa and feed particles. The supernatant was

removed and recentrifuged at 500 × *g* for 20 min. Bacteria isolation was accomplished by centrifuging the resulting supernatant at 30,000 × *g* for 20 min. Separated bacterial cells from the rumen contents and lyophilized duodenal samples were analyzed for purines (Zinn and Owens, 1986) as a microbial protein marker which was used as a component in determining total tract CP digestibility. Rumen content samples were also analyzed for DM and CP (same procedures as above).

Blood samples were thawed at room temperature before being analyzed. Serum NEFA was determined using the acyl-CoA synthetase, acyl-CoA oxidase method (NEFA-HR, Wako Pure Chemical Industries, Richmond, VA). Reagents for BUN analysis; urease, phenol nitroprusside, and alkaline hypochlorite were made in the NDSU ruminant nutrition laboratory (Chaney and Marback, 1962). Absorbencies were read using a UV-VIS biotech spectrophotometer (DU-640, Beckman Coulter, Brea, CA). The method for BUN analysis was based on methods of determination from Fawcett and Scott (1960). Serum IGF_1 values were determined using the procedures specified by Camacho et al. (2012).

Calculations

Parameters for liquid passage rate included fluid dilution rate (FDR), fluid flow rate (FFR), rumen fluid volume (RFV), and turnover time (TT). These parameters were determined using the method proposed by Galyean, (2010). Liquid passage rate was determined by regressing the natural log of the cobalt concentration against time.

Apparent ruminal digestibility was calculated for DM, OM, and CP. Apparent DM digestibility was determined by taking [1-(diet Cr_2O_3/ duodenal Cr_2O_3)], which yielded apparent ruminal DM percent. Apparent OM percent digestibility was calculated by taking [1- (Ruminal OM flow/ diet OM intake)]. Lastly, apparent ruminal CP digestibility was determined by [(Diet

CP intake – ruminal CP flow)/(Diet CP intake)], to yield apparent ruminal CP percent digestibility.

Total intestinal digestibility was calculated for DM, OM, and CP. Total intestinal DM digestibility was calculated by [(Ruminal DM flow – fecal DM sum)/ final average DM intake]. The percent of OM digestibility for the total intestine was calculated by taking [(Ruminal OM flow – fecal OM sum)/ (diet OM intake)]. Total intestine CP digestibility was calculated by [(Ruminal CP flow - fecal CP sum)/ (diet CP intake)].

To determine total tract nutrient digestibility, the mean ort DM, organic matter (OM) from the ash analysis, CP, NDF, and ADF values were deducted from the mean hay DM, OM, CP, NDF, and ADF values; respectively, to obtain final forage intake of these variables. Percentage of organic matter was calculated by taking 100 minus the mean ash percent for each steer for each period. The final hay nutrient values (DM, OM, CP, NDF, and ADF) were then added to the final DDGS nutrient values to obtain the final DM, OM, CP, NDF, and ADF intake values. To determine the final digestibility percentages, final fecal DM, OM, CP, NDF, and ADF values were subtracted from the final intake values. This number was then divided by the final intake values to obtain the final digestibility percentages of DM, OM, CP, NDF, and ADF.

The ratio of acetate to propionate (A:P) was calculated as [acetate + (2 × butyrate)/ propionate]. This ratio was calculated so that a majority of the carbon loss in the rumen could be calculated, therefore making this calculation more accurate when trying to determine ruminal fermentation efficiency than when only comparing acetate to propionate.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS version 9.2 (SAS Institute, Inc., Cary, NC) as a 4 × 4 Latin square. The class statement included steer, period, treatment, and in

cases when data were analyzed over time or days, time or day was included as well. The Satterthwaite method was used to determine degrees of freedom and the best fit covariate structure was chosen based on the lowest Akaike, Akaike with small sample size adjustment, and the Bayesian information criterion statistics. Means were separated using the LSMEANS option of SAS and were considered significant when $P \leq 0.05$.

The model for hay, DDGS, and total intake included treatment, day, and the respective interaction. Random variables included steer and period, treatment by steer, and day by period. Day was analyzed as a repeated measure and the subject was steer by treatment. For the three feed intake parameters the best fit covariate structure chosen was the simple (VC) structure. Additionally hay, DDGS, and total DMI were divided into SUP and NSUP. The model for separated SUP and NSUP was similar to the feed intake model used to analyze intake daily.

Ruminal pH data was separated for SUP and NSUP. The model for ruminal pH included treatment, time point (1 through 48), and their interaction. Random variables included steer, period, and treatment by steer. Ruminal pH was analyzed as a repeated measure and the subject included steer by treatment and the covariate structure chosen was the 1st order autoregressive (ar 1).

Ruminal VFA and NH3 concentrations were separated to represent SUP and NSUP. The model for VFA and ruminal NH3 included treatment, time, and treatment by time. Random variables included steer, period, treatment by steer, and time by period. Volatile fatty acids and ruminal NH3 were analyzed as repeated measures. The model for acetate, propionate, butyrate, and total concentration of VFA contained treatment, time (0-8 h after feeding) and their interaction and the random variables included steer, period, and treatments by steer. The best fit covariate structure for the above mentioned VFA was ar (1).

Ruminal liquid dilution parameters i.e. FDR, FFR, RFV, and TT, were analyzed separately to represent both SUP and NSUP. The model included the effect of treatment. Random variables included steer, period, and treatment by steer. Digestibility parameters DM, OM, CP, NDF, and ADF were analyzed separately and each model included treatment. Data for blood hormones and metabolites was separated for SUP and NSUP. The model for BUN, NEFA, and IGF_1 included treatment, day, and treatment by day and were analyzed as repeated measures. The best fit covariate structure chosen for each of these were compound symmetry (CS), ar (1), and CS for BUN, NEFA, and IGF_1 respectively. Random variables included steer, period, treatment by steer, and day by period.

Results

Mean daily hay intake and DMI were lower ($P < 0.01$) in DGA compared with DG7 and DG3, which were lower ($P < 0.01$) compared with CON (Table 2.2). By design DDGS intake was similar ($P \geq 0.42$) in DG7, DG3 and DGA (Table 2.2). Hay intake was greatest in DGA

Table 2.2. Total intake per day of bromegrass hay, dried distiller's grains plus solubles, and dry matter in steers fed varying frequencies of supplement

	Treatment[1]					*P*- Value
Item	CON	DG7	DG3	DGA	SEM	Trt
Hay intake, kg/d	13.0[c]	10.5[b]	11.2[b]	8.7[a]	0.96	0.0004
DDGS[2] intake, kg/d[2]	0.0[a]	2.2[b]	2.1[b]	2.2[b]	0.10	< 0.0001
Total DMI, kg/d	13.0[c]	12.7[b]	13.3[b]	10.9[a]	0.95	0.0037

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on alternate days, DGA = Hay only or DDGS only on alternate days
[2]Dried distillers grains plus solubles
[ab]Means within row lacking common superscripts differ ($P \leq 0.05$)

on NSUP but least on SUP compared with all other treatments (trt × feed day; $P < 0.0001$; data not shown). Total DMI showed an analogous trend, where DMI was greatest ($P < 0.001$) for

Figure 2.1. Ruminal pH on SUP starting at feeding (0630 h) in forage-fed steers supplemented with various frequencies of dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). † DGA differs from all other treatments ($P \leq 0.05$). ‡ DG7 differs from all other treatments ($P \leq 0.05$).

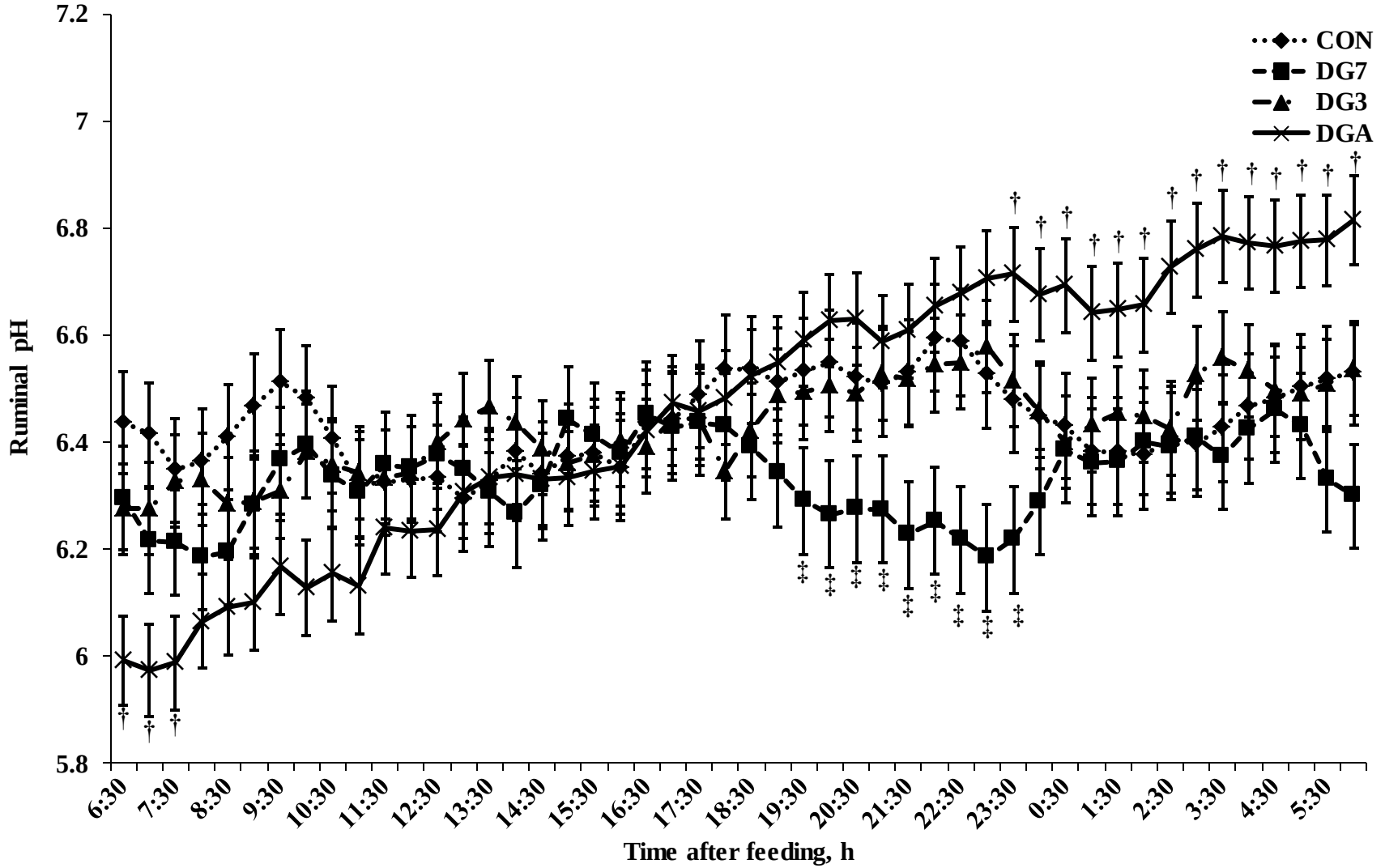
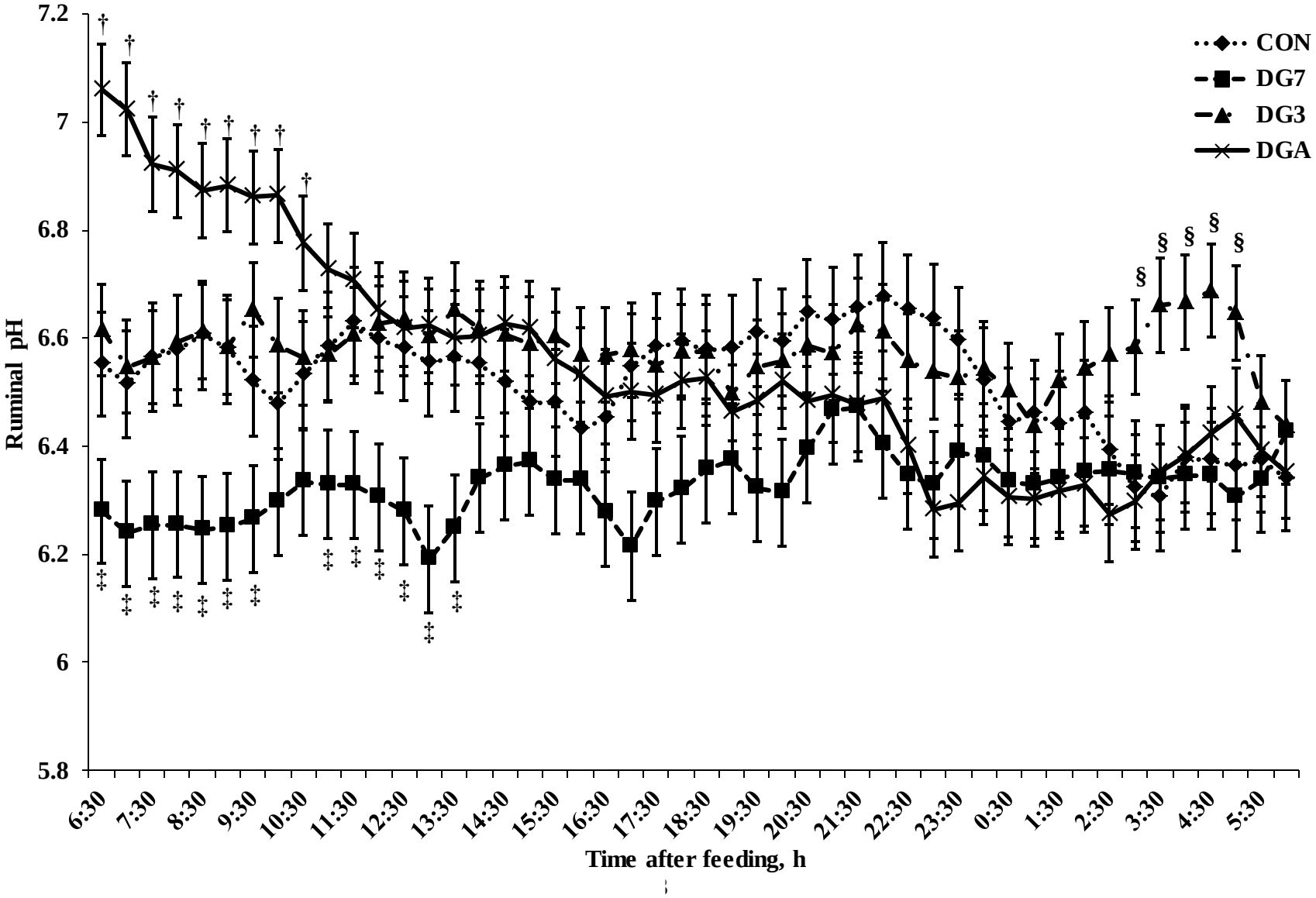


Figure 2.2. Ruminal pH on NSUP starting at feeding (0630 h) in forage-fed steers supplemented with various frequencies of dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). † DGA differs from all other treatments ($P \leq 0.05$). § DG3differs from all other treatments ($P \leq 0.05$). ‡ DG7 differs from all other treatments ($P \leq 0.05$).



DGA on NSUP, and least ($P < 0.0001$) on SUP compared with DG7, DG3, and DGA, respectively.

A treatment × time interaction ($P < 0.0001$) was present on SUP for ruminal pH (Figure 2.1). At feeding on SUP, ruminal pH was lower ($P \leq 0.05$) in DGA compared with CON, DG7, and DG3 (Figure 2.1). However, from 0230 h until the end of the feed day on SUP ruminal pH for DGA was greater ($P \leq 0.05$) than all other treatments. Ruminal pH for DG7 was lower ($P \leq 0.05$) from 2 ½ to 4 h after the 1900 h feeding compared with all other treatments, but was similar ($P \geq 0.10$) to ruminal pH of CON and DG3 for the remainder of the SUP feed day. A treatment × time interaction ($P < 0.0001$) was present on NSUP for ruminal pH (Figure 2.2). At feeding on NSUP, ruminal pH was greatest ($P \leq 0.05$) for DGA and least ($P \leq 0.05$) for DG7; whereas both CON and DG3 were intermediate until 3 h after feeding. Ruminal pH for DG7 continued to be lower ($P \leq 0.05$) than other treatments until 7 ½ h after feeding. At 8 h after the 1900 h hay feeding (0230-0600 h) on SUP ruminal pH was greater ($P \leq 0.05$) for DGA compared with all other treatments.

Treatment × time interactions ($P < 0.0001$) were present for concentrations of acetate on SUP (Figure 2.3) and NSUP (Figure 2.4). At 2, 4, and 6 after feeding on SUP, concentrations of acetate were greater ($P \leq 0.01$) in CON compared with DG7, DG3, and DGA. Conversely, at 4, 6, and 8 h after feeding on SUP, DGA had lower ($P \leq 0.01$) concentrations of acetate compared with all other treatments. At feeding on NSUP, concentrations of acetate were lower ($P \leq 0.01$) in DGA compared with all other treatments, whereas concentrations of acetate were similar ($P \geq 0.07$) among all treatments 8 h post feeding. In addition, concentrations of acetate for DG7 were lower ($P \leq 0.05$) at 0, 2, 4, and 6 h after feeding compared with CON (0 h), CON and DG3 (2 h), CON, DG3 and DGA (4 h), and CON, DG3, and DGA (6 h) respectively.

Figure 2.3. Acetate concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). *****CON differs from all other treatments ($P \leq 0.01$). † DGA differs from all other treatments ($P \leq 0.05$).

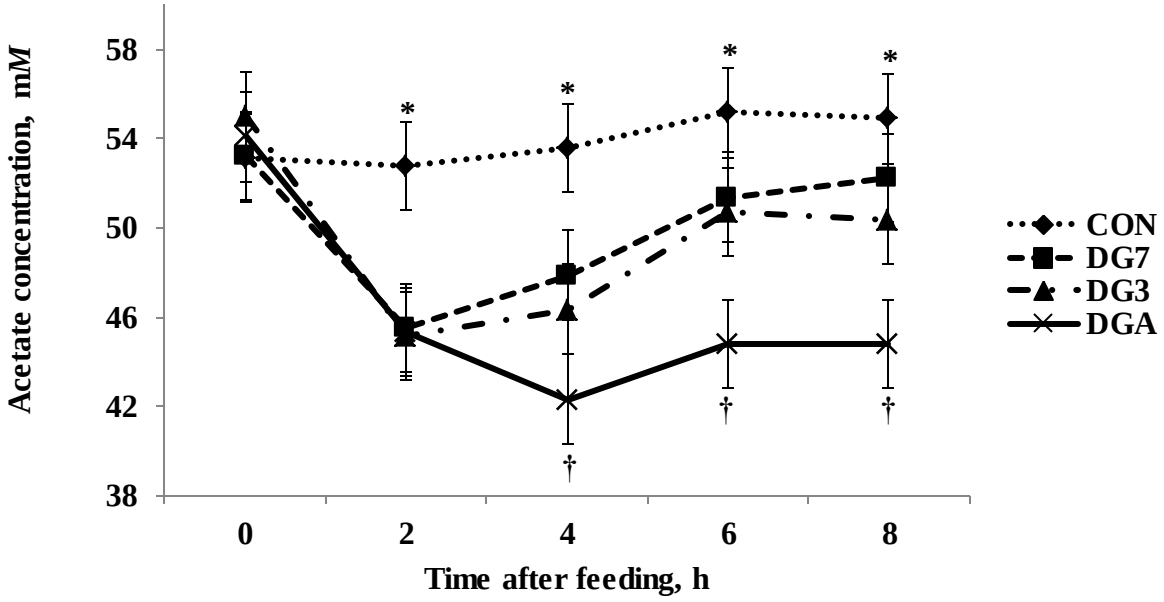
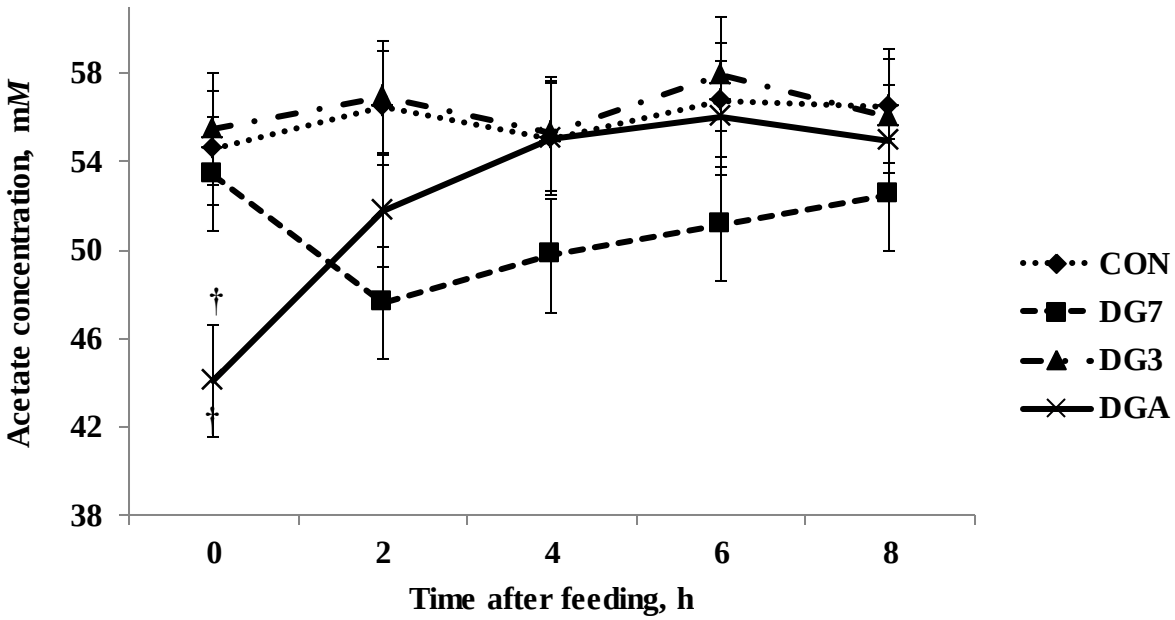


Figure 2.4. Acetate concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). † DGA differs from all other treatments ($P \leq 0.0002$).



A treatment × time interaction ($P = 0.01$) was present for concentrations of propionate on SUP (Figure 2.5). Ruminal concentrations of propionate were greater ($P \leq 0.05$) for DGA from 2 to 8 h after feeding compared with all other treatments on SUP. A treatment × time interaction ($P = 0.02$) was present for concentrations of propionate on NSUP (Figure 2.6). At feeding on NSUP, all treatments had similar ($P \geq 0.06$) propionate concentrations. However, 2, 4, and 6 h post-feeding DG7 had greater concentrations of propionate ($P \leq 0.05$) compared with all other treatments.

A treatment × time interaction ($P < 0.0001$) was observed for concentrations of butyrate on SUP (Figure 2.7) and NSUP (Figure 2.8). Butyrate concentrations were greatest ($P \leq 0.01$) in DGA from 4 to 8 h after feeding compared with CON, DG7, and DG3. Moreover on SUP, CON had lower ($P \leq 0.05$) concentrations of butyrate at 2, 4, and 6 h post-feeding compared with all other treatments. Butyrate concentrations were greatest ($P \leq 0.05$) in DG7 and least ($P \leq 0.01$) in DGA from 2 to 8 h after feeding on NSUP, and CON and DG3 were intermediate.

On SUP a treatment × time interaction was present for the ratio of A:P ($P = 0.03$). At feeding and 2 h post feeding DGA had a lower ($P \leq 0.05$) A:P compared with DG7 and DG3. In addition A:P for DGA was lower ($P \leq 0.05$) compared with all other treatments at 8 h post feeding on SUP. On NSUP (Figure 2.10), a treatment effect ($P = 0.01$) occurred for A:P concentrations, which were lower ($P \leq 0.01$) in DGA compared with CON and DG3 and similar ($P \leq 0.10$) to DG7. Furthermore the ratio of A:P was greater ($P \leq 0.05$) for DG3 on NSUP compared with DG7 and DGA and similar ($P \leq 0.24$) to CON.

For isobutyrate concentrations on SUP, a treatment × time interaction ($P = 0.0001$) was observed (Figure 2.11). Concentrations of isobutyrate were lower ($P \leq 0.01$) in DGA compared with all other treatments and greater ($P \leq 0.01$) in DG7 compared with CON and DGA at

Figure 2.5. Propionate concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.01$). †DGA differs from all other treatments ($P \leq 0.05$). £CON differs from DG3 ($P \leq 0.05$).

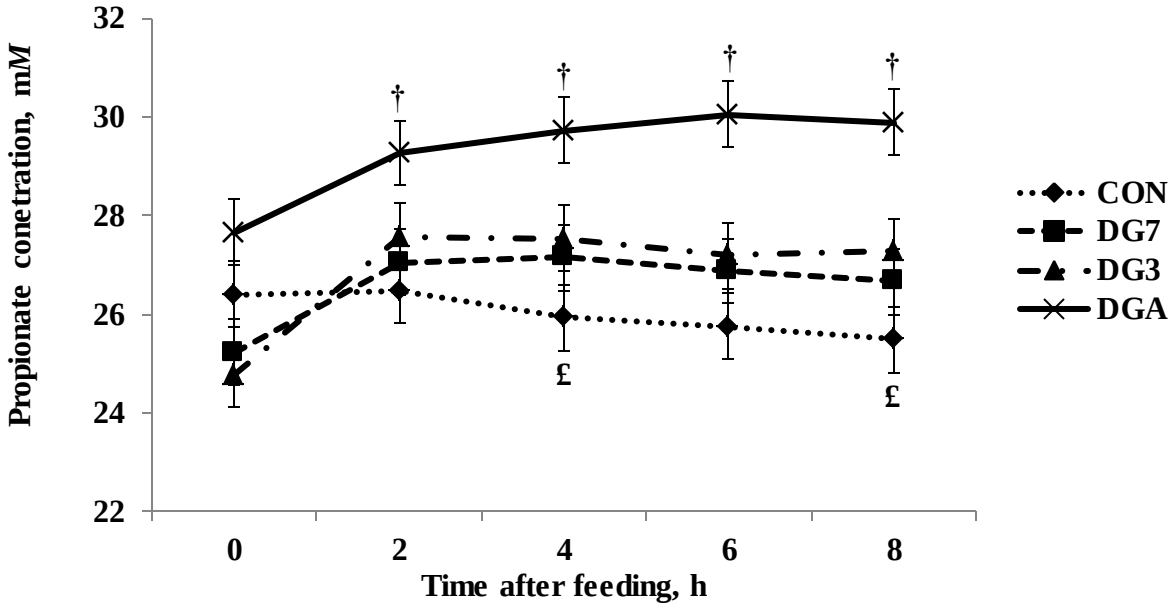


Figure 2.6. Propionate concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.02$). ‡DG7 differs from all other treatments ($P \leq 0.03$).

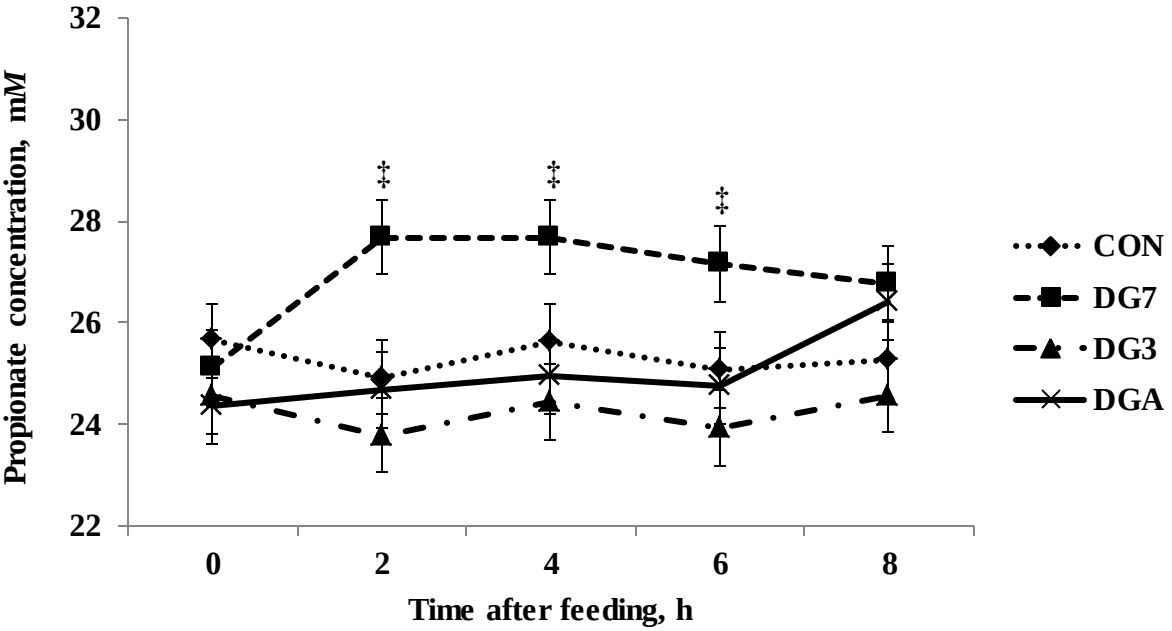


Figure 2.7. Butyrate concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). †DGA differs from all other treatments ($P \leq 0.007$). *CON differs from all other treatments ($P \leq 0.03$).

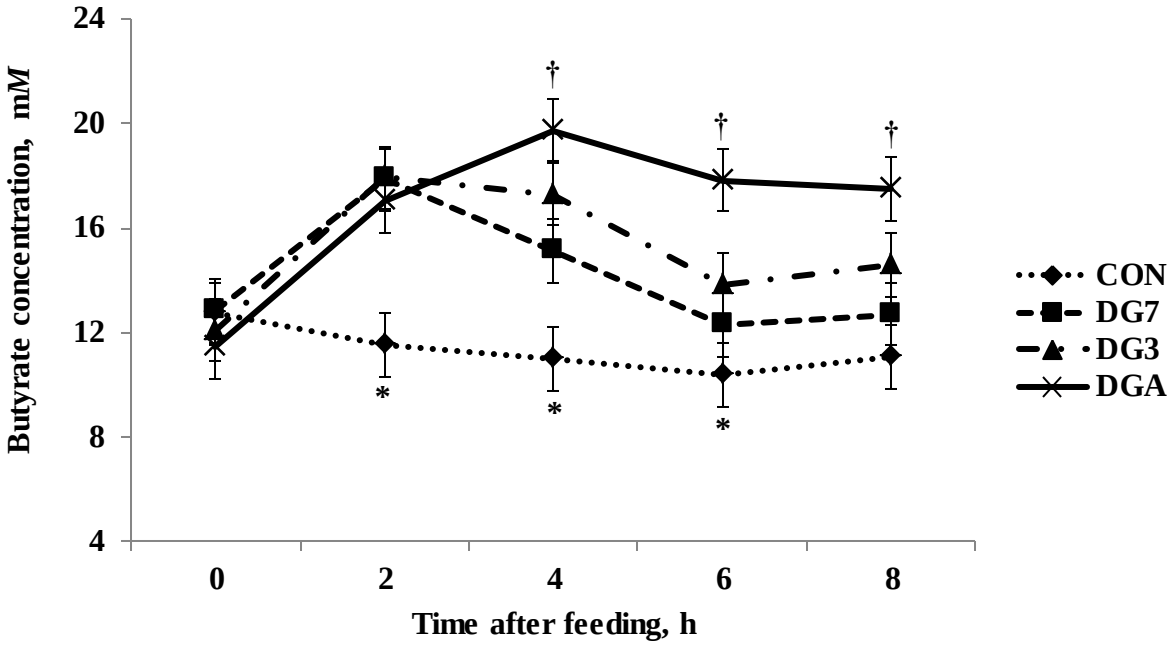


Figure 2.8. Butyrate concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). †DGA differs from all other treatments ($P \leq 0.01$). ‡DG7 differs from all other treatments ($P \leq 0.02$).

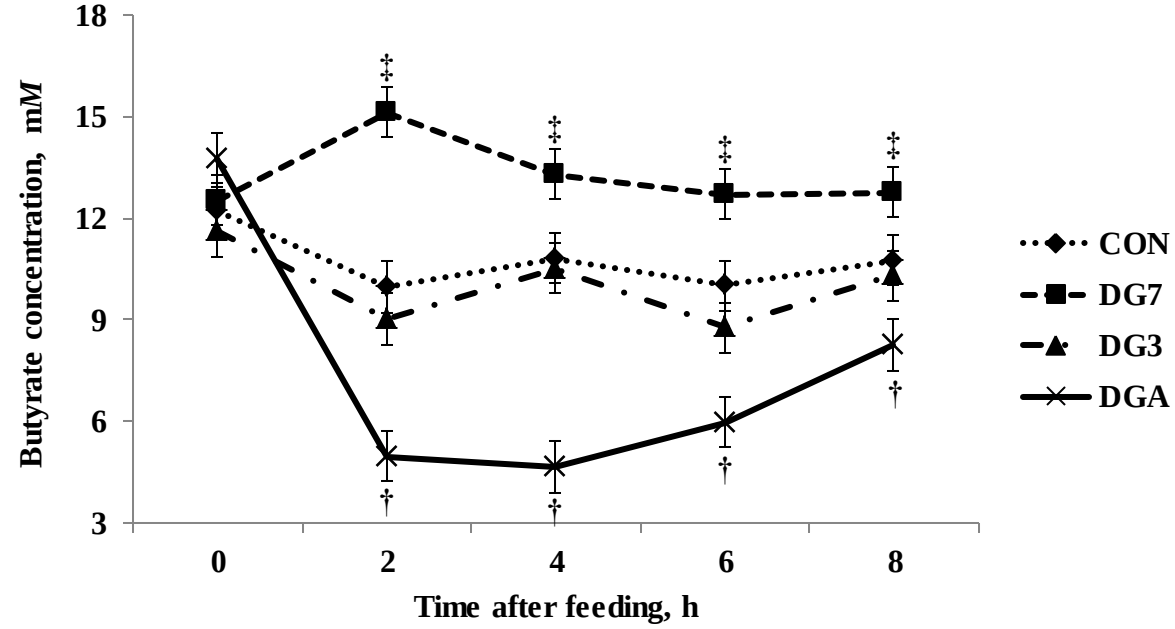


Figure 2.9. A:P concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.03$). £DGA differs from DG7 and DG3 ($P \leq 0.005$). †DGA differs from all other treatments ($P \leq 0.04$).

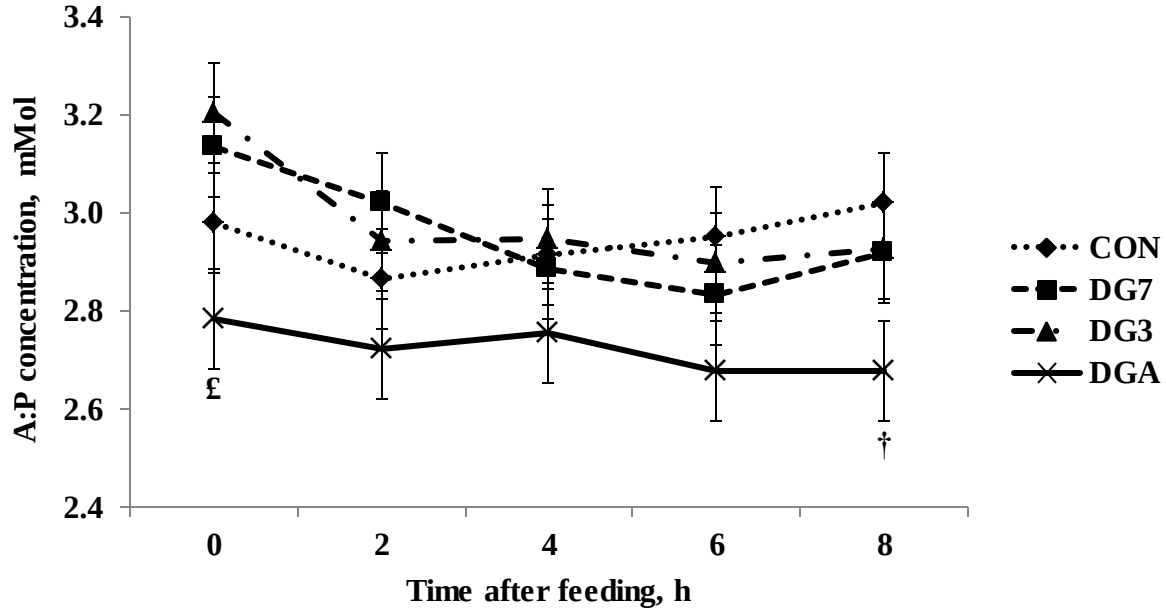
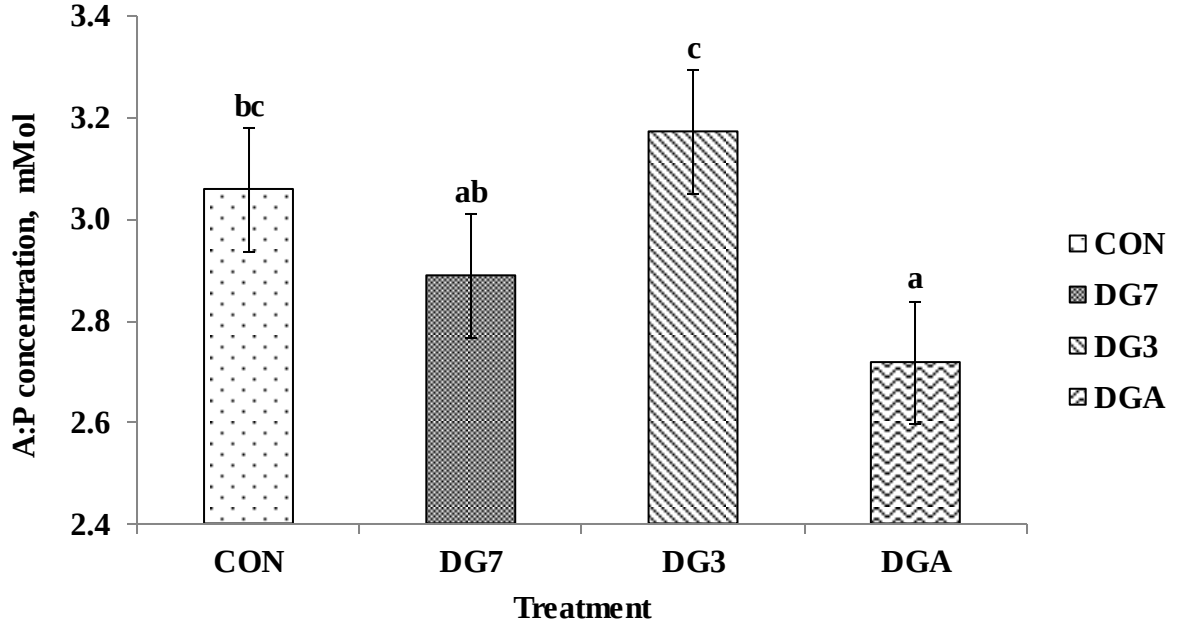


Figure 2.10. A:P concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment ($P = 0.01$). [abc]Treatment means differ ($P \leq 0.02$).



feeding. Two hours post feeding isobutyrate concentrations were lower ($P \leq 0.01$) in DGA compared with CON and DG7 and greater ($P \leq 0.05$) for CON compared with DG3. At 4 h after feeding isobutyrate concentrations were greater ($P \leq 0.05$) for CON compared with DG7. Additionally at 4, 6, and 8 h post feeding isobutyrate concentrations were lower ($P \leq 0.01$) for DG3 and DGA compared with CON and DG7. On NSUP (Figure 2.12) a treatment × time interaction ($P = 0.002$) was present for isobutyrate concentrations, which were greater ($P \leq 0.01$) in DGA compared with all other treatments from feeding until 6 h after feeding.

In addition, concentrations of valerate were greater ($P \leq 0.05$) on SUP for DG7 compared with CON and DGA and were similar ($P \geq 0.34$) to DG3. On SUP concentrations of valerate were similar ($P \geq 0.21$) for CON and DG3. On NSUP valerate concentrations were least ($P \leq 0.05$) in CON and greatest ($P \leq 0.03$) in DGA while DG7 was intermediate. Additionally on NSUP valerate concentrations were similar ($P \geq 0.07$) for DG3 compared with CON and DG7.

On SUP a treatment × time interaction ($P = 0.0003$) was observed for isovalerate (Figure 2.15). However, there were no differences ($P = 0.07$) among treatments at 0, 2, 4, 6, and 8 h relative to feeding. On NSUP there was a treatment × time interaction ($P = 0.01$) for ruminal isovalerate as well. On NSUP, concentrations of isovalerate were greater ($P \leq 0.03$) in DGA from feeding until 4 h after feeding compared with CON, DG7, and DG3, but returned to similar ($P \geq 0.12$) concentrations at 6 and 8 h after feeding, respectively.

On SUP (Figure 2.17) there were no differences among treatments ($P = 0.09$) for total concentrations of VFA. However, on NSUP a treatment × time interaction ($P = 0.0003$) was observed for total VFA concentrations. The total concentration of VFA on NSUP (Figure 2.18) was lower ($P \leq 0.01$) in DGA from feeding until 4 h after feeding compared with all other treatments.

Figure 2.11. Isobutyrate concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.0001$). £DG7 differs from all other treatments ($P \leq 0.03$). †DGA differs from all other treatments ($P \leq 0.01$). §CON differs from DG3 and DGA ($P \leq 0.02$). ‡DG7 differs from DG3 and DGA ($P \leq 0.03$). *CON differs from DG3 ($P \leq 0.01$)

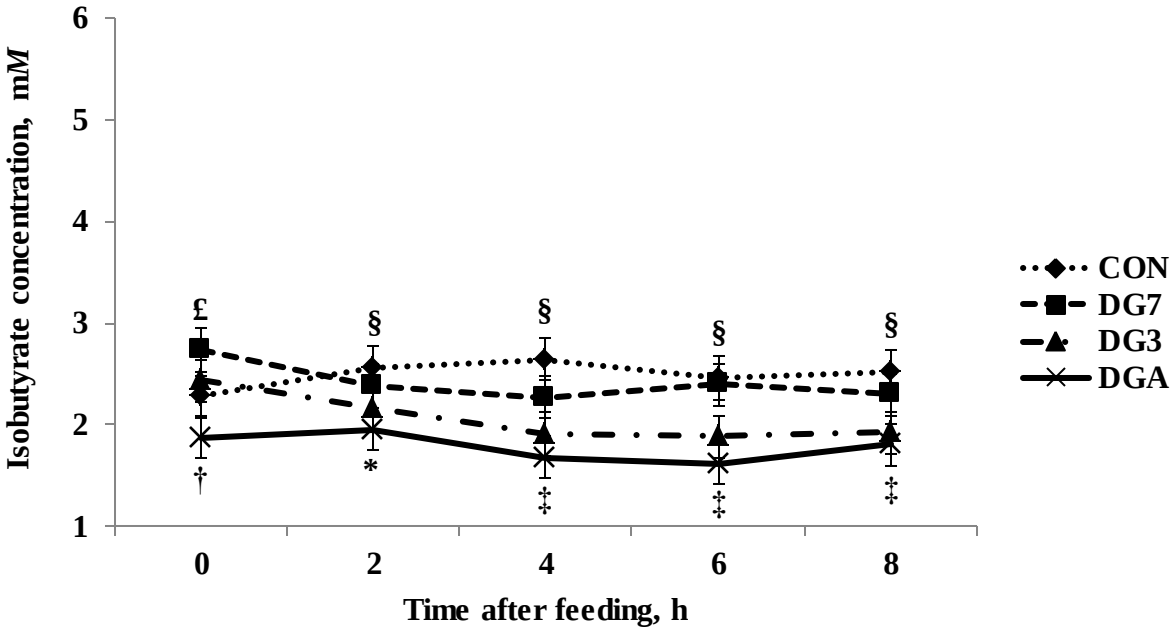


Figure 2.12. Isobutyrate concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.002$). †DGA differs from all other treatments ($P \leq 0.002$).

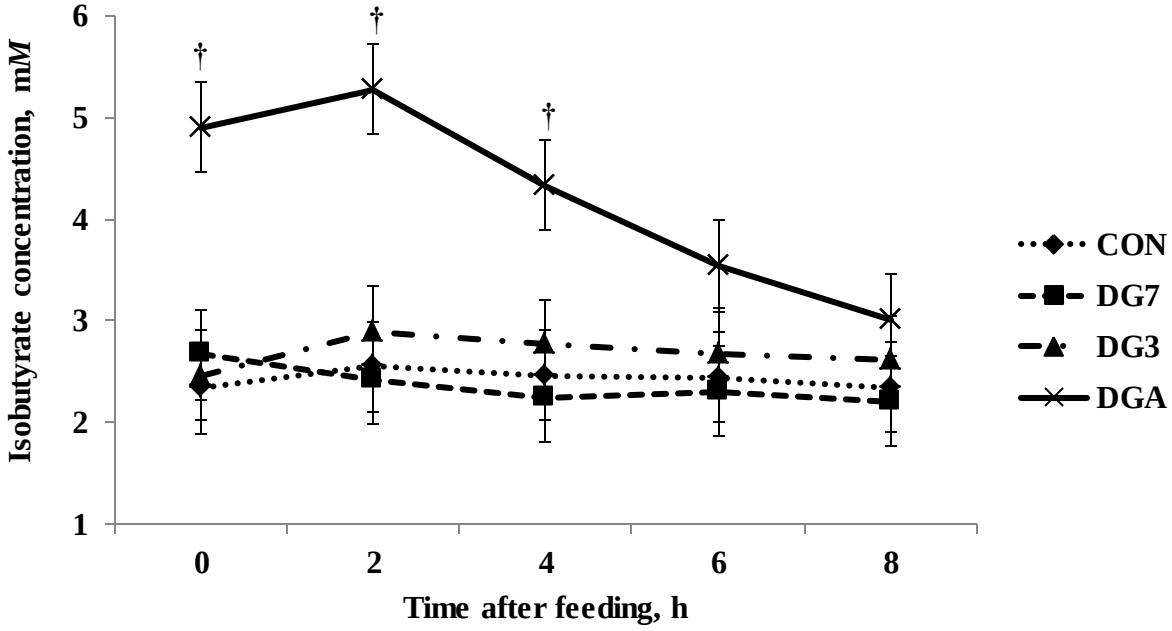


Figure 2.13. Valerate concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment ($P = 0.03$). [abc]Treatment means differ ($P \leq 0.05$).

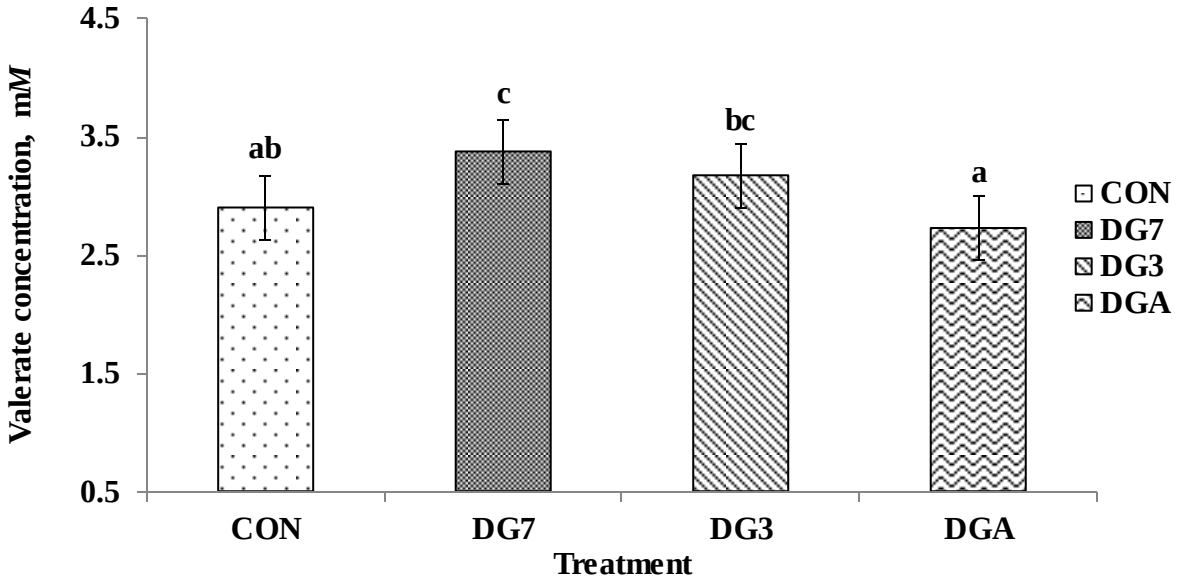


Figure 2.14. Valerate concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment ($P = 0.01$). [abc]Treatment means differ ($P \leq 0.05$).

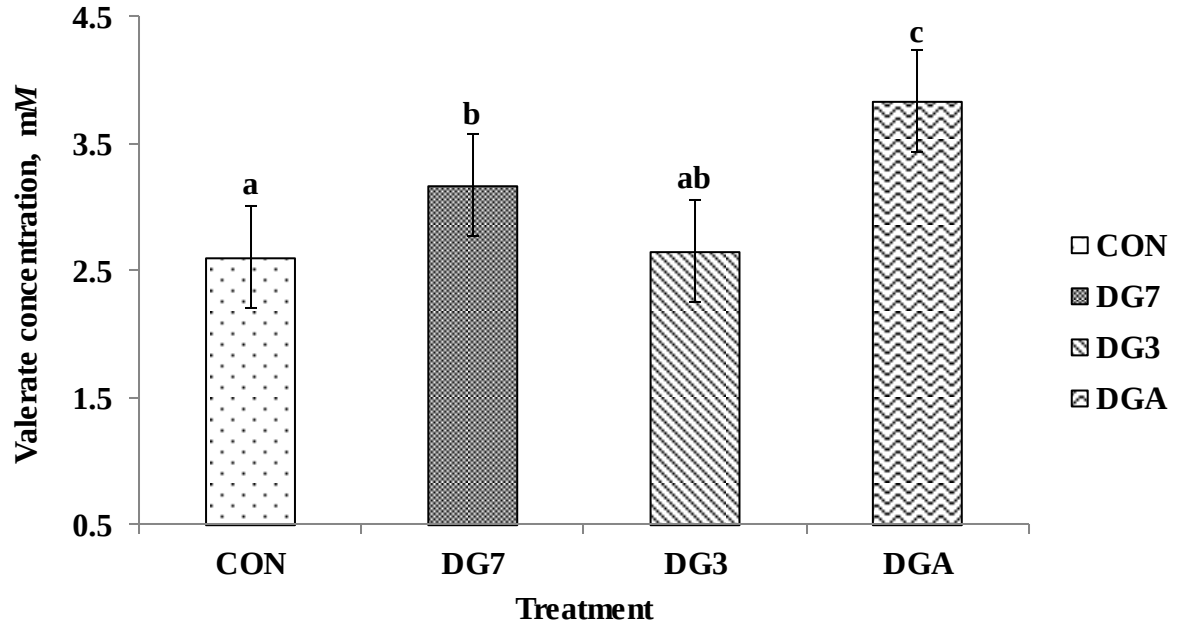


Figure 2.15. Isovalerate concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.0003$).

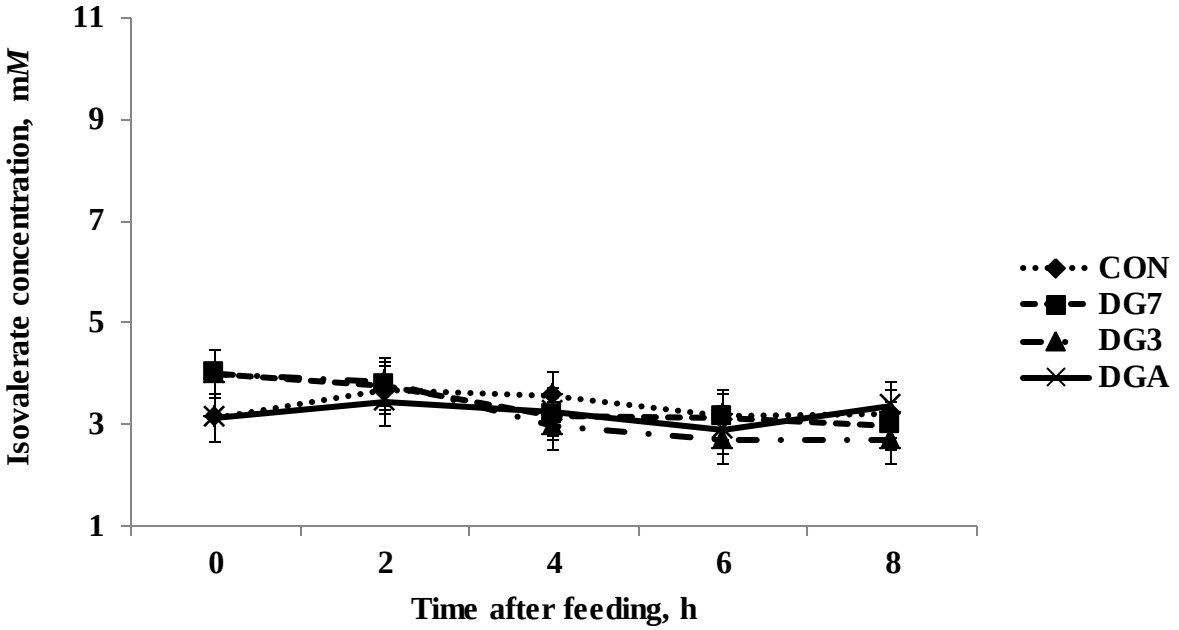
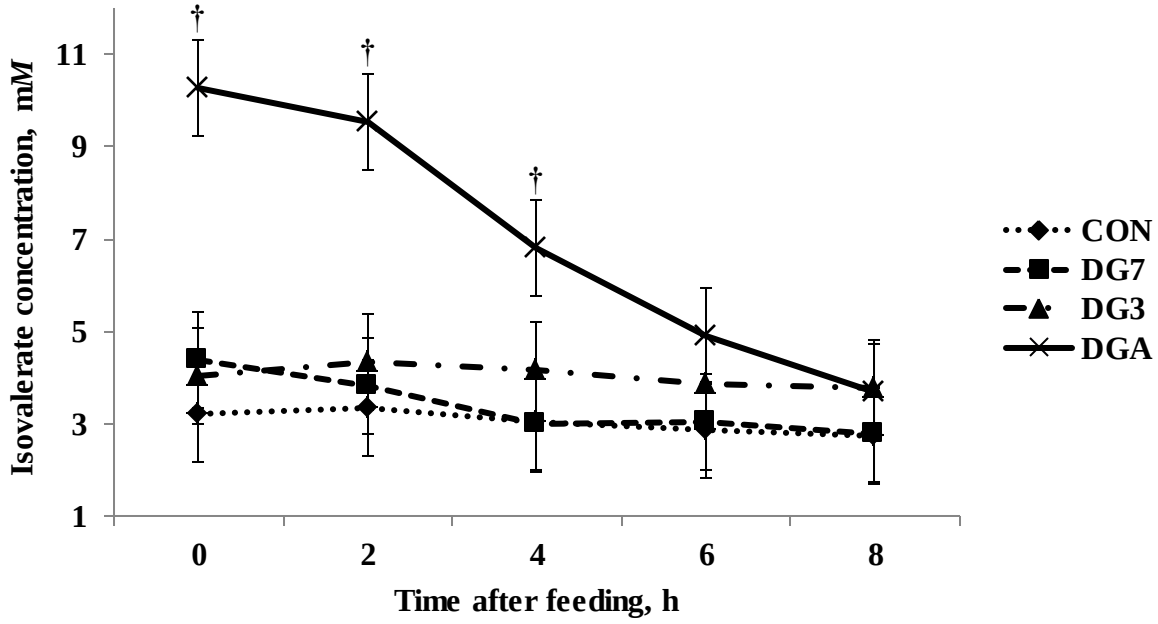


Figure 2.16. Isovalerate concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.01$). †DGA differs from all other treatments ($P \leq 0.03$).



At feeding on SUP (Figure 2.19), NH3 concentrations were similar ($P \geq 0.17$) among all treatments. At 2 h post feeding on SUP, concentrations of NH3 were lower ($P \leq 0.05$) in steers fed CON compared with DG3 and DGA, whereas DG7 was intermediate. Furthermore on SUP, concentrations of NH3 were greater ($P \leq 0.03$) in steers fed DG7 compared with CON and DGA at 4 h after feeding. Additionally, ruminal NH3 concentrations were greater ($P = 0.04$) in DG7 compared with DGA at 6 h after feeding. At 8 h after feeding ruminal concentrations of NH3 were similar ($P \geq 0.18$) among treatments. A treatment × time interaction was present for concentrations of ruminal ammonia on NSUP ($P = 0.0002$; Figure 2.20). At feeding on NSUP, ruminal concentrations of NH3 were greater ($P \leq 0.04$) for DGA compared with all other treatments. At 2 h post feeding ruminal concentrations of NH3 were greatest ($P \leq 0.05$) for DG7 compared with all other treatments. In addition, ruminal concentrations of NH3 in steers fed DGA were greater ($P \leq 0.0004$) compared with all other treatments at 4 and 6 h after feeding and greater ($P = 0.01$) than CON at 8 h post feeding.

Liquid passage rate parameters on SUP (Table 2.3) including FDR, FFR, RFV, and TT were similar ($P \geq 0.08$) among all treatments. On NSUP (Table 2.3), FDR, RFV, and TT were similar among treatments ($P \geq 0.08$) and FFR was decreased ($P < 0.05$) for DGA compared with CON, DG7, and DG3.

Intake of DM per day was decreased ($P < 0.01$) in DGA compared with all other treatments. Both OM and CP intake were similar ($P \geq 0.07$) among all treatments. Neutral detergent fiber intake and ADF intake were least ($P < 0.01$) in DGA and greatest ($P < 0.01$) in CON compared with all other treatments. Furthermore, both NDF intake and ADF intake were similar ($P \geq 0.18$) for DG7 and DG3.

Figure 2.17. Total VFA concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment ($P = 0.09$).

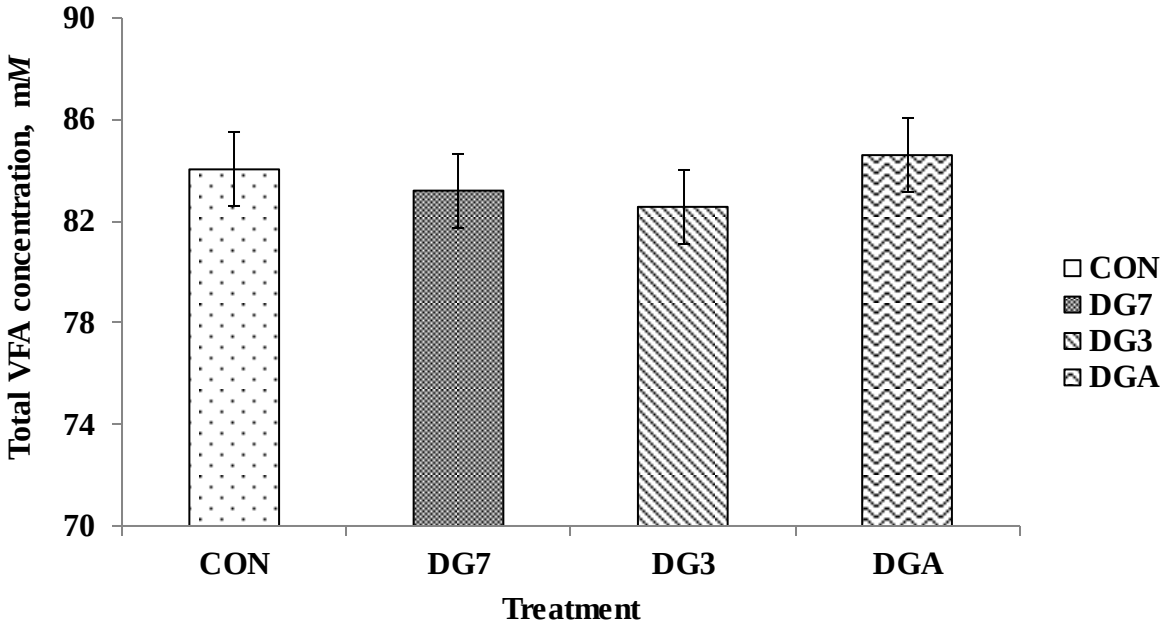


Figure 2.18. Total VFA concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.02$). †DGA differs from all other treatments ($P \leq 0.05$).

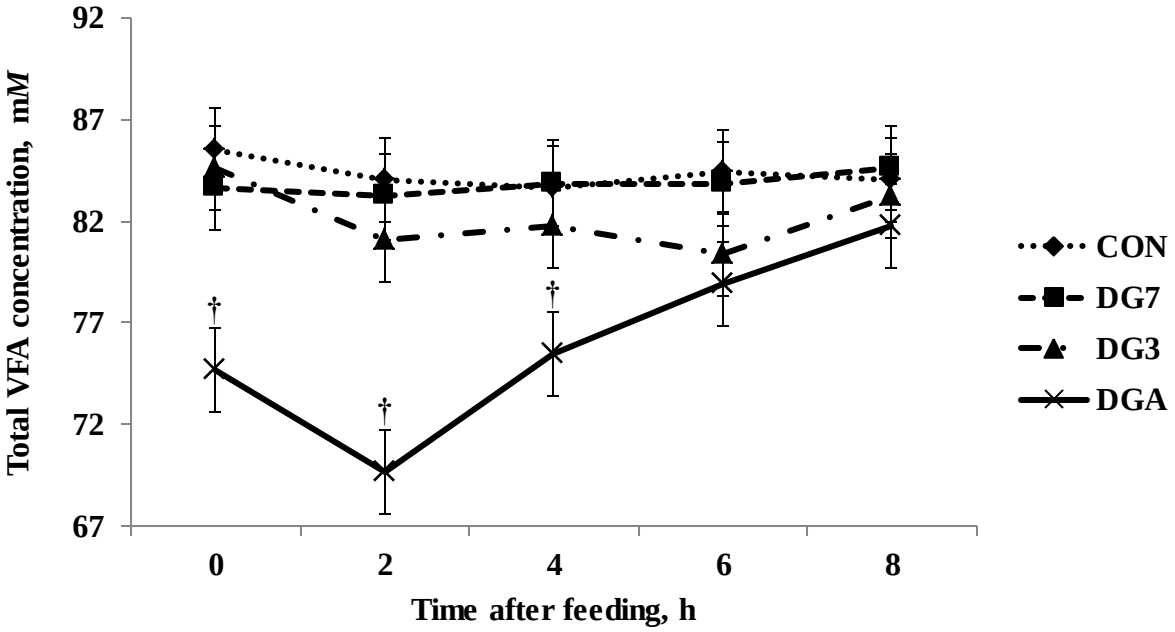


Figure 2.19. Ammonia concentration on SUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.01$).

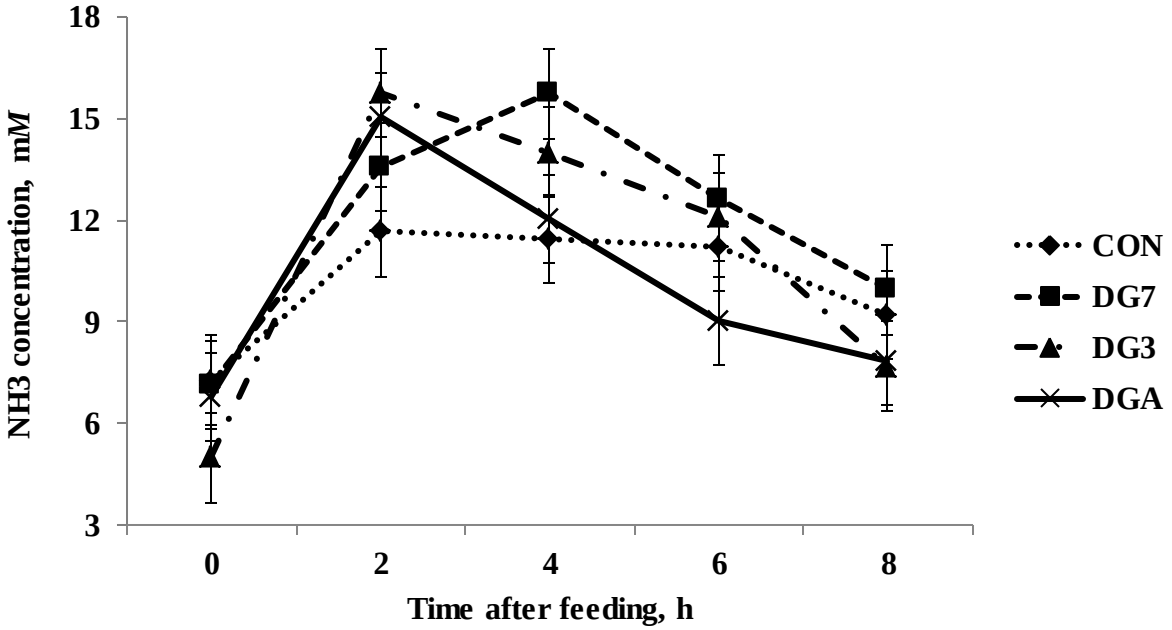
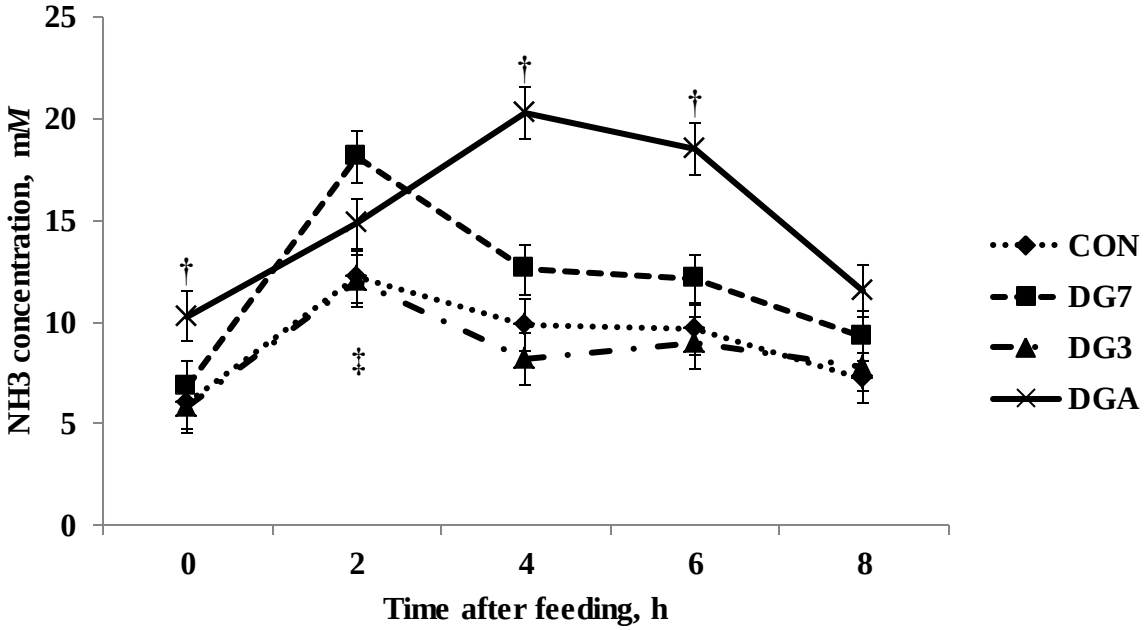


Figure 2.20. Ammonia concentration on NSUP for steers fed varying frequencies of dried distiller's grains plus solubles. Treatment × Time ($P = 0.0002$). †DGA differs from all other treatments ($P \leq 0.05$). ‡DG7 differs from all other treatments ($P \leq 0.05$).



Apparent ruminal digestibility and total intestinal digestibility of DM, OM, and CP were all similar ($P \geq 0.20$) among treatments. Total tract digestibility was similar ($P \geq 0.31$) among all treatments for DM, OM, CP, NDF, and ADF.

Concentrations of BUN on SUP were decreased ($P < 0.01$) in steers fed DGA compared with CON, DG7, and DG3. Conversely on NSUP, concentrations of BUN were greater ($P < 0.01$) in steers fed DGA compared with all treatments. There were no differences ($P = 0.36$)

Table 2.3. Liquid passage rate factors of steers consuming hay and dried distiller's grains plus solubles at varying frequencies

	Treatment[1]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
SUP[2]						
Fluid dilution rate, %/h	10.0	12.6	12.5	9.7	1.2	0.08
Fluid flow rate, L/h	6.0	5.6	5.6	5.3	0.8	0.70
Rumen fluid volume, L	62.4	45.2	47.0	53.6	7.1	0.27
Turnover time, h	11.7	8.0	8.1	10.5	1.7	0.21
NSUP[3]						
Fluid dilution rate, %/h	11.7	12.1	11.9	12.8	0.5	0.54
Fluid flow rate, L/h	5.0[b]	4.8[b]	4.6[b]	3.9[a]	0.6	0.02
Rumen fluid volume, L	42.8	40.9	39.2	31.4	5.8	0.08
Turnover time, h	8.6	8.4	8.5	7.9	0.4	0.61

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on alternate days, DGA = Hay only or DDGS only on alternate days

[2]SUP = Supplemented days (days when DG7, DG3, and DGA received DDGS)

[3]NSUP = Non-supplemented days (days when CON, DG3, and DGA received hay)

[ab]Means within row lacking common superscripts differ ($P < 0.05$)

among treatments for NEFA on SUP. However, on NSUP, NEFA concentrations were greater ($P \leq 0.01$) in DGA compared with all other treatments. No differences ($P \geq 0.64$) were detected among treatments in concentrations of IGF_1 on either SUP or NSUP, respectively.

Table 2.4. Intake and digestibility of diet components of steers consuming hay and dried distiller's grains plus solubles at varying frequencies.

	Treatment[1]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
Intake, kg/d						
DM[2]	13.0[b]	12.7[b]	13.3[b]	10.9[a]	0.8	0.004
OM[3]	11.1	10.6	11.2	9.5	0.7	0.07
CP[4]	2.1	2.2	2.3	2.0	0.2	0.28
NDF[5]	8.3[c]	7.3[b]	7.7[bc]	6.2[a]	0.6	0.004
ADF[6]	4.6[c]	4.0[b]	4.2[b]	3.3[a]	0.3	0.001
Apparent ruminal digestibility, %						
DM	46.1	49.6	45.6	44.5	2.3	0.22
OM	52.7	56.4	51.9	53.4	1.9	0.20
CP	23.3	30.6	19.9	25.3	5.0	0.30
Total intestinal digestibility, %						
DM	21.3	19.5	21.9	24.8	2.8	0.31
OM	17.8	16.1	17.7	20.0	2.3	0.37
CP	47.4	40.6	48.1	47.5	4.1	0.53
Total tract digestibility, %						
DM	67.6	68.3	69.3	69.5	0.8	0.31
OM	70.9	70.6	72.0	73.6	1.3	0.34
CP	69.2	70.0	71.6	73.0	1.9	0.45
NDF	79.7	77.7	78.0	78.6	1.4	0.47
ADF	76.8	74.9	74.9	73.6	1.0	0.21

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on alternate days, DGA = Hay only or DDGS only on alternate days
[2]DM = Dry matter
[3]OM = Organic matter
[4]CP = Crude protein
[5]NDF = Neutral detergent fiber
[6]ADF = Acid detergent fiber
[ab]Means within row lacking common superscripts differ ($P < 0.05$)

Table 2.5. BUN, NEFA, and IGF_1, concentrations in steers consuming hay and dried distiller's grain plus solubles at varying frequencies

	Treatment[1]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
SUP[2]						
BUN, m*M*	7.9[b]	8.3[b]	7.8[b]	5.5[a]	0.4	<0.01
NEFA, m*M*	72.6	81.1	84.1	75.3	7.0	0.36
IGF_1, ng/mL	110.6	123.1	127.3	126.2	12.0	0.64
NSUP[3]						
BUN, m*M*	8.1[a]	8.6[a]	8.9[a]	11.8[b]	0.5	<0.01
NEFA, m*M*	85.4[a]	88.0[a]	77.7[a]	209.5[b]	12.7	<0.01
IGF_1, ng/mL	117.4	129.5	130.2	131.7	11.9	0.73

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on alternate days, DGA = Hay only or DDGS only on alternate days

[2]SUP = Supplemented days (days when DG7, DG3, and DGA received DDGS)
[3]NSUP = Non-supplemented days (days when CON, DG3, and DGA received hay)

Discussion

As hypothesized, steers receiving the DGA supplementation strategy had reduced overall hay intake compared with steers not receiving supplement. Though data evaluating a single day of ad libitum hay access followed by restricted supplement intake are lacking, several projects have evaluated the effect of restricting access to hay within a single day. When the effects of allowing round bale access for either 4, 8, or 24 h/day were compared in lactating beef cows, hay disappearance was reduced by 37% in cows restricted to 4 h of hay access (Cunningham et al., 2005).

Additionally, when DDGS is offered as a supplement hay intake is usually reduced because of substitution. Heifers consuming high (65% total digestible nutrients; TDN) and low (53% TDN) quality forage that were supplemented with five different levels of DDGS had intake linearly decrease with the increasing (0-2.7kg) DDGS intake (Morris et al., 2005). In addition, when beef steers fed a moderate-quality (10.6% CP) hay were supplemented with increasing

amounts of DDGS (0, 0.3, 0.6, 0.9, 1.2% of BW daily), forage intake decreased linearly with increasing supplemental DDGS (Leupp et al., 2009). Taken together, these data suggest that the reduced forage intake observed with one day of ad libitum access to hay followed by one day of restricted access to DDGS was not unexpected. Our observation was more than likely due to a combined effect of restricted access to forage as well as the amount and type of supplement offered.

Our finding that DG7 and DG3 treatments had similar forage intake is in contrast to several reports. Loy et al. (2007) found that forage-fed heifers supplemented with dry-rolled corn or DDGS daily (0.4% of BW) had greater DMI compared with heifers supplemented on alternate days (0.8% of BW). In addition, forage-fed beef steers that were fed a sunflower and cottonseed meal supplement (43% CP) 2, 3, 5, or 7 d/wk had increased forage DMI with increased frequency of supplementation (Farmer et al., 2001). This difference in intake could be a result of the differing hay quality as the previous two studies had hay that was of lower quality than our study.

Although intake was influenced by dietary treatment the only component of digestibility that was affected by treatment was total tract CP, which was increased in steers receiving the DG7, DG3, and DGA treatments compared with CON. Our finding that all treatments receiving DDGS had greater total tract CP digestibility compared with the unsupplemented treatment is a logical outcome as the amount of CP of DDGS fed was greater than CP fed from the delivered hay. Additionally, CP digestibility is not in agreement with all other findings. No differences were observed in OM and CP digestibility when heifers grazing small-grain pastures (17.7% CP) were supplemented with DDGS at 0.2, 0.4 or 0.6 % BW (Islas and Soto-Navarro, 2011). Total NDF digestibility, however, increased linearly with increasing (0.2 to 0.6% BW) levels of DDGS

(Islas and Soto-Navarro, 2011). Differences among reports in quality of forage used as a basal diet may explain why differences in digestibility are observed in some, but not all reports. When wethers consuming low-quality hay (8% CP) were supplemented with high (20% of ad libitum intake) or low (10% ad libitum intake) amounts of a corn/SBM mix, NDF total tract digestibility was similar among treatments (Howard et al, 1991). Similarly, no differences in DM digestibility were observed among ewes supplemented with a mixed energy supplement (rice bran, molasses, cassava meal, and fish meal) providing medium (1% of BW), low (60% of medium), or high (140% of medium) amounts of metabolizable energy (Reese et al., 1990).

The lack of differences in liquid dilution rate parameters on SUP in the current study is similar to results from a study that evaluated increasing level of CP (10, 20, 30, or 40 %) from a SBM and SG which was fed daily or 3 d/wk to forage-fed steers (Beaty et al., 1994). Liquid dilution rate in the aforementioned study was unaffected by both the protein concentration of the supplement and the frequency the supplement was fed. Other studies reported no difference in liquid dilution parameters in supplemented forage-fed cattle (Gilbery et al., 2006; Islas and Soto-Navarro, 2011). Furthermore, particulate passage rate was not affected in steers receiving grass hay that received supplemental cottonseed meal every 12, 24, or 48 h (Hunt et al., 1989).

In our study we observed a reduction in fluid flow rate on NSUP in steers fed DGA compared with all other treatments but did not see any other differences in liquid passage rate parameters. In contrast to our study, ruminal particulate passage increased in grazing steers receiving a cottonseed based supplement compared with an unsupplemented control receiving only forage (Caton et al., 1988). This increase in particulate passage rate was likely caused by the pairing of the low CP (6%) forage with the high CP (45.5%) supplement (Caton et al., 1988). We possibly did not see similar results because our hay was of a better quality than that

mentioned by Caton and others, (1988). Ruminal liquid volume, liquid dilution rate, and liquid flow were all increased in beef steers fed low (12.4% CP), moderate (27.2% CP), and high (41.3% CP) protein supplements compared with non-supplemented steers (DelCurto et al., 1990a). The increase in passage rate factors observed by DelCurto et al., 1990a was likely due to the very low quality hay being fed and may not accurately reflect the effects of a moderate quality hay, such as in the current study. It has been well documented that supplementing cattle consuming low quality hay can increase passage rate (McCollum and Galyean 1985, Caton et al., 1988; Bohnert et al., 2002b). Perhaps, since our hay was of moderate instead of poor quality this explains why we did not observe an increase in our passage rate parameters.

Increasing ruminal pH throughout the feed day when only DDGS was delivered (DGA treatment) was likely due to a decrease in substrate available in the rumen for ruminal fermentation and VFA production as the feed day progressed. As VFA production decreased, ruminal pH increased, which was also probably facilitated by saliva production (which is a natural buffer) and water consumption throughout the day. The following morning at feeding ruminal pH was still greater for DGA compared with all other treatments but began a steady decline after consumption of hay ensued. The steady decline observed was likely due to increased microbial fermentation as substrate in the rumen was digested.

Although data evaluating the pH implications of a feeding strategy such as DGA are lacking, patterns of ruminal pH throughout the feeding day for CON, DG7, and DG3 dietary treatments were comparable to previous reports. For example, no differences were observed in ruminal pH in grazing beef steers supplemented with cottonseed meal and corn to provide 150% of NRC maintenance requirements for protein compared with unsupplemented steers (Caton et

al., 1988). Furthermore, no differences in ruminal pH were observed among treatments when SBM and SG protein supplement levels increased from 13 – 39% (DelCurto et al., 1990b).

The pattern of ruminal pH observed on non-supplemented days for steers receiving the DG7 treatment (lower than all other treatments) was expected, as the steers fed DG7 was the only treatment receiving DDGS on this day. Similar results were observed when steers received either a supplement daily or 3 d/wk; however, ruminal pH was lower in steers receiving supplement 7 d/wk compared with those receiving supplement 3 d/wk on days when only the 7 d/wk group were supplemented (Beaty et al., 1994). Furthermore, ruminal pH for wether lambs consuming a concentrate diet (16.7% CP) containing either 25 or 75% alfalfa hay as a forage at two levels (1,100 or 1,700 g DM/day), was not influenced by level of intake (Merchen et al., 1986). However, ruminal pH was increased for wethers that were consuming the diet containing 75% alfalfa compared with wethers consuming the 25% alfalfa diet (Merchen et al., 1986). As is demonstrated, when ruminants consume diets that are increased in digestibility such as concentrates or high quality forage, reductions in ruminal pH are usually observed due to an increase in volatile fatty acid concentration in the rumen.

The increase in acetate for CON from 2 to 8 h after feeding was expected as this treatment was only receiving hay. Acetate is one of the main fermentation products when only forage is being fed (Owens and Goetsch, 1988). Molar proportions of acetate were increased in wether lambs receiving diets containing 75% alfalfa compared with wethers receiving 25% alfalfa in their diets (Merchen, 1986). The decrease in acetate from 4 to 8 h after feeding on SUP for steers receiving DGA was likely due to this treatment not receiving hay on these days, and therefore acetate production would be decreased. The rational from reduction in acetate concentrations on NSUP for steers fed DG7 compared with all other treatments can be

contributed to the inclusion of DDGS in the diet of steers on this day. Several research groups have previously documented similar results where the inclusion of a supplement in a forage based diet resulted in decreased acetate concentrations in ruminants (Merchen et al., 1986; Bohnert et al., 2002b; Doranalli et al., 2011).

Increased concentrations of propionate in steers fed DGA on SUP were likely caused, by the lack of hay in the DGA diet on this feed day. However on NSUP propionate concentrations were increased in DG7 compared with all other treatments for the majority of the day. Beef steers consuming moderate-quality hay supplemented with increasing amounts of DDGS (0, 0.3, 0.6, 0.9, 1.2% of BW daily) had a quadratic increase in the molar proportion of propionate as level of DDGS increased (Leupp et al., 2009). This relates to the concentration of propionate on NSUP as this was the only treatment that was receiving DDGS on this day.

Results of the current study where steers fed CON had lower concentrations of butyrate from 4 to 8 h after feeding on SUP compared with all other treatments were anticipated. Similarly, concentrations of butyrate were increased in grazing beef steers supplemented with cottonseed meal and corn to provide 150% of NRC maintenance requirements for protein compared with unsupplemented steers (Caton et al., 1988). In addition, concentrations of butyrate were increased when beef steers were fed either SG or SBM, long stem alfalfa hay, or dehydrated alfalfa pellets compared with unsupplemented steers (DelCurto et al., 1990c). Interestingly, on SUP an increase in butyrate was observed for DGA compared with all other treatments from 4 to 8 h after feeding. This was likely due to the DGA treatment only receiving DDGS on this day.

Butyrate concentrations were greatest in DG7 and least in DGA compared with CON and DG3 from 2 to 8 h after feeding on NSUP. DelCurto et al. (1990a) demonstrated a similar

increase in butyrate concentrations when supplementing forage-fed steers with a low (12% CP), moderate (28%) or high (41% CP) protein source consisting of SBM and dry-rolled SG. Those steers that were supplemented had an increase in butyrate concentrations compared with the unsupplemented steers only consuming forage (DelCurto et al., 1990a).

Acetate and butyrate are both synthesized from acetyl-CoA (Owens and Goetsch, 1988). This relationship may explain the higher concentration of butyrate for DGA on SUP and the lower concentrations of butyrate observed in DGA on NSUP. As acetate concentrations increased or decreased an opposite effect was observed for butyrate.

The similarity in A:P among CON, DG7, and DG3 on SUP conflicts with several reports that document differences in A:P among supplemented and unsupplemented cattle. The ratio of A:P decreased linearly as amount of supplemental DDGS increased from 0, 0.3, 0.6, 0.9, 1.2% of BW daily in beef steers consuming moderate quality hay (Leupp et al., 2009) Due to the scarcity of information pertaining to our DGA treatment it is unclear why the ratio of A:P is decreased on SUP in this treatment. However, we speculate that in order to acclimate to the DGA treatment, ruminal microorganisms were becoming more efficient at utilizing substrate in the rumen. On NSUP the ratio of A:P was least in DGA and greatest in DG3. It is well documented that in ruminants A:P generally decreases with the inclusion of a supplement in a forage based diet (Horn and McCollum, 1987). Since the DGA treatment was not receiving DDGS on this day it is unclear why the ratio of A:P was decreased in DGA compared with CON and DG3 as all three of these treatments were only receiving hay on this day.

Total ruminal VFA concentrations were similar among all treatments. The similarities among our treatments for total concentrations of ruminal VFA on SUP are contradicted by other work. Total VFA concentrations increased linearly as frequency of UIP (soyPLUS and blood

meal) and DIP (SBM) supplementation decreased from daily to every third day, to every sixth day in steers on the days that all treatment groups were supplemented (Bohnert et al, 2002b). The rationale for decrease in concentrations of total VFA on NSUP until 4 h after feeding compared with all other treatments could possibly be explained by factors discussed previously in this paper. Recall that at the end of the feed day on SUP (0600) it had been several hours since the animal last received feed, thus, ruminal substrate and microbial population were likely limited. Due to the time between feedings and lack of substrate in the rumen, the concentration of total VFA were decreased which may be correlated with a decrease in microbial population for DGA prior to feeding on NSUP (Owens and Goetsch, 1988). Therefore, when hay was delivered at 0630 there was a large influx of substrate into the rumen that would not begin to ferment until the microbial population was able to multiply, attach to, and colonize the new substrate. This process lead to a 6 h interval before total VFA production in DGA increased to levels similar to those of CON, DG7, and DG3.

Ruminal NH3 concentrations on SUP were similar among treatments for the majority of the feed day, with the exception of 2 h after feeding where ruminal NH3 concentrations were decreased for CON compared with DG3 and DGA. This decrease is ruminal NH3 is likely related to the fact that the CON treatment did not receive supplement. In steers supplemented with a ration of 91% cottonseed meal and 9% corn grain, an increase in NH3 concentration was observed at -3, 0, 1, and 4 h after supplementation compared with unsupplemented steers (Caton et al., 1988).

Nitrogen recycling likely played a role in the increased ruminal NH3 observed at feeding, and 4 and 6 h post-feeding on NSUP in steers fed DGA compared with all other treatments. Ruminal NH3 concentrations in steers consuming low-quality meadow hay (5.3% CP) and

supplemented with a DIP or UIP source daily, or every 3 or 6 d were greater in supplemented compared with a non-supplemented steers (Bohnert et al., 2002a). These researchers suggested that ruminal NH3 concentrations can be increased on days between supplementation due to N recycling. This conclusion was supported by data from a companion paper (Bohnert et al., 2002b) as well as data from Farmer et al., (2001), in which forage-fed steers given a supplement (46.4% sunflower meal, 30.5% cottonseed meal, 7.5% feather meal, 0.5% alfalfa, 0.5% SBH, 2.3% limestone, 0.7% salt, 0.5% selenium, 0.6% trace mineral premix, 0.2% grease mix, and 5.0% molasses) 2, 3, 5, and 7 d/wk had an increase in ruminal NH3 on days between supplementation compared with steers not fed supplement.

Circulating BUN was least on SUP and greatest in NSUP for DGA compared with all other treatments. Concentration of circulating BUN is positively correlated to protein supply in the diet and levels of ruminal ammonia. Although there is a shortage of information to draw comparisons to BUN for our strategy of alternate day feeding of DDGS only or hay only, the effects of alternate day feeding of supplement in steers consuming forages daily have been evaluated. Wether lambs fed low-quality forage that received a DIP (SBM) and UIP (soyPLUS and blood meal) supplement every 3 and 6 d had increased BUN on days after supplementation compared with wethers supplemented daily.(Bohnert et al., 2002c). Similarly, when grazing (pasture quality 8.8% CP) gestating heifers supplemented (wheat midds, SBH, molasses, and cottonseed meal; 22.2%CP) 3 d/wk had an increase in BUN concentrations compared with heifers supplemented 7 d/ wk on the days when only the 7 d/wk treatment group was supplemented (Cooke et al., 2008). Conversely, BUN concentrations were decreased for the 3 d/wk supplemented group compared with the 7 d/wk supplement group on days when both treatment groups were supplemented (Cooke et al., 2008). In both the aforementioned studies

and in our current study, the increase in BUN was likely observed for treatment groups supplemented less frequently due to maintenance of the ruminal N supply due to recycling of urea.

This increase in NEFA in NSUP in steers fed DGA is indicative of mobilization of body fat in the animal and suggests that steers were in a negative energy balance (Erfle et al., 1974). Since the steers in DGA were only receiving a limited amount of DDGS without forage, the increase in NEFA for DGA reflects that their energy requirements (Ne_m 9.8 Mcal/d, receiving; 8.7 Mcal/d on SUP) were being met by mobilizing body fat stores. Similar results were obtained when gestating beef cows supplemented with either a low (0.4% UIP), medium (20.0% UIP), or high (39.0% UIP) level of a UIP supplement had lower concentrations of NEFA compared with unsupplemented cows (Sletmoen-Olson et al., 2000). Additionally, greater NEFA concentrations were observed in unsupplemented cattle and sheep compared with those that were supplemented (Krysl et al., 1987; Cheema et al., 1991; Barton et al., 1992). In contrast, adding supplemental DIP (corn gluten meal and blood meal) and UIP (SBM) to a low quality forage diet in beef cows did not affect plasma NEFA concentrations (Rusche et al., 1993).

Circulating insulin-like growth factor I is an important mediator in growth, reproduction, and health (McGuire et al., 1992; Roberts et al., 1997). Furthermore, circulating IGF_1 can be influenced by nutritional status in ruminants, however in our study there were no differences among treatments for circulating IGF_1. Pre-pubertal beef heifers that were supplemented daily with a SBH and wheat middlings mix typically had greater concentrations of IGF_1 compared with heifers that were supplemented 3 d/wk, (Cooke et al., 2008). Differences in IGF_1 were believed to be correlated with increased ADG and attainment of puberty in heifers receiving supplement 7 d/wk (Cooke et al., 2008). In contrast, no differences were observed in

concentrations of IGF_1 in nonlactating, nonpregnant crossbred cows fed grass hay and two separate diets of either molasses and CSM or citrus pulp and CSM 3d/wk, (Cooke et al., 2007b).

Conclusions

This study is the first to evaluate a strategy of alternating days of feeding DDGS only or grass hay only. Feeding of the dietary treatment DGA decreased hay and total dry matter intake without negatively impacting ruminal kinetics, liquid dilution rate, and digestibility. However, increased concentrations of NEFA in steers on the DGA treatment on days when only DDGS was delivered may indicate possible changes in body composition over a feeding period of longer duration. Though results of this initial project look promising, additional research is warranted to determine whether this novel supplementation scheme will have deleterious effects over an extended period of time.

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CHAPTER 3. EFFECTS OF ALTERNATE DAY FEEDING OF DRIED DISTILLER'S GRAIN PLUS SOLUBLES ON FORAGE-FED BEEF COWS IN MID-LATE GESTATION

Abstract

Forty-six non-lactating, gestating beef cows were used to examine effects of feeding dried distiller′s grains plus solubles (DDGS) or grass hay on alternate days during mid-late gestation on performance, feeding behavior, and body composition. Cows were arranged in a completely randomized design and dietary treatments included: 1) ad libitum hay daily (CON; n = 12); 2) hay and 0.4% BW DG daily (DG7; n = 12); 3) hay daily and 0.93% BW DG on Monday, Wednesday, and Friday (DG3; n = 11); and 4) hay only on Tuesday, Thursday, Saturday, and Sunday and 0.93% BW DG only on Monday, Wednesday, and Friday (DGA; n = 11). Cows during mid to late gestation were fed diets for 84 d with BW, BCS, and carcass ultrasound data collected every 28 d. Feed intake was continuously monitored and intake data collected daily using the Insentec B. V. roughage intake control feeding system. Hay DM intake was least ($P < 0.01$) in DGA (7.4 ± 0.3 kg/d) compared with all other treatments. Additionally hay DM intake for CON (10.3 ± 0.3 kg/d) was similar ($P > 0.10$) to DG7 (9.6 ± 0.3 kg/d) and greater ($P < 0.01$) than DG3 (9.1 ± 0.3 kg/d). Hay DMI for DG7 and DG3 was also similar ($P > 0.10$). By design DG intake was similar for DG7 (3.0 ± 0.1 kg/d), DG3 (2.9 ± 0.1 kg/d), and DGA (2.8 ± 0.1 kg/d). Total DMI was less ($P < 0.01$) for DGA and CON compared with DG7 and DG3. Body weight was similar ($P > 0.10$) among treatments at each weigh date, however CON (0.3 ± 0.06 kg/d) had lower ($P < 0.01$) ADG compared with all other treatments (0.73, 0.75, and 0.60 ± 0.06 kg/d for DG7, DG3, and DGA, respectively). Likewise, the G: F ratio was lower ($P < 0.01$) for CON compared with all other treatments (0.03, 0.06, 0.065, and 0.06 ±

0.005 kg of gain/kg of feed for CON, DG7, DG3, and DGA, respectively). Change in REA from d 1 to d 84 was greater ($P < 0.05$) in DG7 compared with all other treatments. However there were no differences ($P > 0.24$) among treatments for intramuscular fat, rib fat, and rump fat throughout the trial. On days when CON, DG3, and DGA received hay (Tuesday, Thursday, Saturday, and Sunday) number of hay meals per day were similar ($P = 0.23$) among treatments and both hay intake per meal and time per hay meal was greater ($P \leq 0.05$) in DGA compared with all other treatments. Additionally, on days when CON, DG7, and DG3 received hay, cows on DGA spent an increased ($P \leq 0.05$) amount of time eating hay compared with DG7 and DG3 whereas CON was intermediate ($P \geq 0.30$). When consuming DDGS, number of meals per day, time spent eating, intake per meal, time per meal, and rate of intake were decreased ($P \leq 0.05$) in DG7 compared with both DG3 and DGA. The feeding strategy DGA altered hay intake, total DMI, REA, and feeding behavior, but did not alter other performance and body composition characteristics compared with other supplementation methods.

Introduction

In cow calf production systems, feeding reproducing females accounts for greater than 60% of a beef producers annual production costs (Miller et al., 2007), and is one of the main factors assessed when determining profitability of an operation. Therefore, in order to reduce costs associated with feeding, cattle producers may be able to implement management strategies that optimize feed utilization, while meeting nutrient requirements. One such strategy that can be utilized by producers is to supplement mid to low quality forage with high protein and or energy feeds. One such feed that can be utilized as a supplement is dried distiller's grains plus solubles which is a byproduct of the ethanol industry. Dried distillers grains plus solubles is approximately 29.5% CP, contains 2.18 Mcal Ne_m, is low in starch and contains approximately

40% digestible fiber (NRC, 2000). This nutrient profile makes DDGS a useful supplement for cattle producers to utilize and in situations that supplementation is necessary DDGS also can make an excellent replacement for corn (Ham et al., 1994).

When feeding supplements like DDGS, there are a variety of strategies that can be utilized to optimize production, which include altering the amount and frequency that the supplement is fed. For example, by increasing the quantity of DDGS delivered forage intake can be reduced without losses to cattle performance (MacDonald and Klopfenstein, 2004; Morris et al., 2005). Additionally, when daily or 3 times weekly supplementation were compared, cattle fed supplement 3 times/wk had decreased forage intake and similar performance when compared with cattle supplemented daily (Coleman and Wyatt, 1982; Beaty et al., 1994). Farmer et al. (2001) showed similar results when supplementing cattle 2, 3, 5, and 7 d/ wk. Those animals that were supplemented twice weekly had a decrease in forage dry matter intake when compared with all other treatments. By decreasing supplementation frequency, producers would be able to decrease the amount of time they spend feeding, the amount of labor required for feeding, decrease the use of their feeding equipment, as well as fuel inputs.

Our research group recently summarized a metabolism study in which this type of alternate day supplementation strategy was utilized which was discussed in the previous chapter. To reiterate, this report found that hay DMI was decreased in DGA compared with all other treatments, however total tract digestibility was similar among all treatments. To date, there have been several feeding trials that have been conducted that evaluate varying amounts and frequencies of supplement delivery. However, reports evaluating the long term effects of feeding only supplement or forage on alternate days are lacking. Therefore, we hypothesized that the alternate day feeding of DDGS with low quality grass hay would alter forage intake and feeding

behavior without causing detrimental loss of body composition characteristics. Our objective was to determine the effects of eliminating forage from diets on alternating days while supplementing cows during mid-late gestation with DDGS on forage intake, feeding behavior, body condition and composition characteristics, and serum metabolite profiles in gestating beef cows fed low quality forage.

Materials and Methods

All research procedures were approved by the NDSU Institutional Animal Care and Use Committee.

Animals and Diets

Prior to study initiation all cows were evaluated for a viable fetus and day of gestation was determined via transrectal ultrasonography (Aloka, 500v, Wallingford, CT). Animals were chosen for the study based on stage of pregnancy which ranged from d 110 to d 175 of gestation. Upon arrival at the NDSU Beef Cattle Research Complex (BCRC), 51 non-lactating, pregnant beef cows had a single radio-frequency identification button tag inserted into an ear, BW and BCS collected, and cows evaluated for the presence of a viable fetus via transrectal ultrasonography (Aloka, 500v, Wallingford, CT).

Cows were placed in a three pen (Pen numbers 1-3) area (15.2 × 56.4 m; 55.9 m^2/cow) that had the dividing gates removed so that the animals were allowed to roam freely throughout all pens. Each pen contained 8 individual Insentec B. V. roughage intake control system feeders (Insentec Marknesse, the Netherlands). All cows had access to 24 feed bunks (bunk numbers 1-24) during the initial 14 d acclimation period, in which they received grass hay ad libitum beginning at 0800 h and again at approximately 1200 h, 1600 h, and 2000 h.

When training the cattle to eat from the new feeding system, steps were taken so that cattle could become accustomed to the system gradually so as not to be frightened by the moving gates and sound associated with pneumatic cylinders that opened and closed the feed bunks. During the first week feeder gates were shut off and remained down and animal intake was monitored daily so that the cows could get accustomed to the small space that they needed to put their head through to eat. Cows (n = 2) that did not acclimate to the facility (did not eat from bunks) during the first 7 d were removed from the study. During the second week feeder gates were raised halfway so that the animals could get accustomed to the sounds of decompressing air accompanying the feed gates lowering and also learn to associate moving their heads into the bunk with the corresponding movement of the feed gates depressing. After the two weeks of acclimation time feeder gates were raised completely at the 0800 h feeding on the day previous to study initiation.

Of the 51 cows that began the project, 46 were utilized (Angus; n = 4, Commercial; n = 23, Shorthorn; n = 3, and Simmental; n =16) which were evenly distributed among treatments when assigned. Two cows did not acclimate to the feeding system and were removed from the study, one cow aborted her calf due to a protozoal infection (Neospora sp.), one cow calved during the 11th week of the study, and one cow that tested positive for Mycobacterium paratuberculosis became symptomatic and expired. The remaining 46 cows were blocked by expected calving date (mid-early to mid-late gestation) stratified by rib fat, then randomly assigned to 1 of 4 dietary treatments: 1) hay only Monday through Sunday (CON; n = 12); 2) hay and 0.4% BW DDGS Monday through Sunday (DG7; n = 12); 3) hay daily and 0.93% of BW DDGS on Monday, Wednesday, and Friday (DG3; n = 11); and 4) hay only on Tuesday,

Thursday, Saturday and Sunday or 0.93% of BW DDGS only on Monday, Wednesday, and Friday (DGA; n = 11).

Bunk numbers 1-6 and 15-24 were only used for hay delivery. Bunks 7-14 were used for both hay (Tuesday, Thursday, Saturday, and Sunday) and DDGS (Monday, Wednesday, Friday) delivery depending on the feed day. All animals had access to hay bunks on the days that they were to receive hay. Changes in animal access to bunks were made daily before the morning feeding at 0800 h to ensure appropriate diet consumption. For a one week period animals in DG7 were placed in a separate pen to acclimate to consuming DDGS from bunks 7 and 8 whereas animals in DG3 and DGA were allowed access to DDGS in bunks 9-14 on Monday, Wednesday, and Friday.

Nutrient composition of forage and DDGS is provided in Table 3.1. The basal diet consisted of brome hay chopped to pass through a 15.2 cm screen and fed at 0800 h and approximately every 4 h afterward until 2000 h. Feeding multiple times per day was necessary to

Table 3.1. Chemical composition of forage and dried distiller's grains plus solubles

	Component[1]					
Item	DM	CP	OM	NDF	ADF	Sulfur[2]
Bromegrass hay	85.8	5.9	91.8	76.4	46.6	---
DDGS[3]	90.3	27.8	91.7	31.8	9.2	10060.4

[1]Expressed as a % of DM
[2]Expressed as parts per million
[3]Dried distiller's grains plus solubles

accommodate ad libitum intake as a full feed bunk only held approximately 12 kg of chopped hay. Distiller's grains were fed in two equal portions, once at 0800 and again at 0830. Delivering two portions of DDGS was done to decrease the incidences of animals consuming more than their allotted amount of DDGS. Average analyzed composition of the grass hay and

DDGS is listed in Table 3.1. In order to keep the Ca:P ratio balanced (2:1) , 34.5 kg of limestone was added and mixed with every 909.1 kg DDGS fed (DM basis). Hay was delivered using a truck mounted feeding box, whereas the DDGS/Ca mix was shoveled by hand directly into the allotted feed bunks based on percent of cow's BW and the number of bunks being utilized. All hay originated from the same field and the same cutting and all DDGS used in the study originated from the same production lot. Cows also had ad libitum access to water and traced mineralized salt blocks (American Stockman, Trace mineralized pressed salt block; North American Salt Company, Overland Park, KS; 95.5-98.5% NaCl, 3500 ppm Zn, 2000 ppm Fe, 1800 ppm Mn, 280-420 ppm Cu, 100 ppm I, 60 ppm Co).

Sample Collection

Cows were weighed on d 0 and 1 after a 16 h period without feed access. These weights were averaged and used as the initial BW for the trial and for the determination of DDGS allotment. Body weight, carcass ultrasound, and BCS were taken every 28 d (d 1, 28, 56, and 84) in the morning prior to feeding. Ultrasound measures included rump fat, rib-eye area, and rib fat, and percentage intramuscular fat (%IMF) collected according to guidelines set forth by the Ultrasound Guidelines Council (Bozeman, MT). From the saved images taken from these scans, percent of intramuscular fat (IMF), ribeye area in inches squared (REA), rib fat in inches (RBFT), and rump fat in inches (RMFT) were determined. Therefore after the completion of the study change in body composition scan measurements, BW, and BCS were calculated by subtracting the final parameters measured on d 84 from the measurements taken on d 1. To determine final body weights, all treatments were given access to only hay up to 1.5% of their BW on d 82 and 83 the ensure consistent gut fill at the time of weighing on d 83 and 84. The average of day 83 and 84 weight for each cow was used as the final BW.

Intake data for both hay and DDGS were continuously monitored and data collected daily for the duration of the 84 d feeding trial using the Insentec B. V. roughage intake control feeding and computer system. Data were divided into feed days (1 through 84) which began at the 0800 h feeding and ended on the subsequent day immediately prior to the 0800 h feeding. Total hay and DDGS DMI were determined for each of the 84 d. Before data were evaluated and feeding behavior calculations were determined, data were composited and outlying data were removed including negative intakes and feeding events that lasted longer than 30 min. Feeding behavior parameters evaluated included feed intake, number of meals per day and time spent eating. Meals were determined based on the beginning and end time of a feed event. If the end time of a feed event was within 7 min of the following feed event it was considered to be a meal. Time spent eating was defined as the sum of each eating event length over a 24 h period. Additional calculations made to evaluate daily feeding behavior for both hay and DDGS intake included:

1) Intake per meal (total daily intake in kg divided by number of meals),
2) Minutes per meal (total minutes per day spent eating divided by intake in kg), and
3) Rate of intake (kg of intake divided by min spent eating).

Hay and DDGS samples were taken every morning at feeding, composited by wk and frozen (-18°C) until analysis of DM, CP, ash, NDF, and ADF. Blood samples were obtained via jugular venipuncture into red top serum tubes (BD Vacutainer; 10 mL) at 1200 h on d 80, 81, and 82 (Friday, Saturday, and Sunday, respectively). This arrangement of sample collection represented a day that the cows in the DG3 and DGA treatments were supplemented, a day that the cows in the DG3 and DGA treatments were not supplemented, and the 2nd consecutive day cows in the DG3 and DGA treatments were not supplemented. Blood samples were centrifuged (ST16R, Thermo Scientific, Waltham, MA) at 20,000 × *g* for 15 min and serum was pipetted

into individually labeled micro tubes (VWR International; 2 mL). Samples were then frozen (-18°C) until analysis for concentrations of blood urea nitrogen (BUN) and NEFA.

Calf birth weights were obtained after the completion of the 84 d feeding trial at the NDSU beef cattle research and extension unit. Cows began to calve approximately 1 mo after the conclusion of the feeding trial.

Laboratory Analysis

Hay and DDGS samples were dried in a Grieve forced-air oven (60° C; The Grieve Corporation) for 48 h then ground to pass a 2-mm screen using a Wiley mill (Thomas-Wiley Lab Mill, Model 4; Thomas Scientific USA). Feed samples were then analyzed for DM, CP, and ash (Procedure numbers: 934.01, 2001.11, and 942.05 respectively; AOAC, 2010) as well as NDF and ADF (Goering and Van Soest 1970, as adapted by Ankom Technology). NDF and ADF concentrations were determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY).

Blood samples were thawed at room temperature before being analyzed. Serum NEFA was determined using the acyl-CoA synthetase, acyl-CoA oxidase method (NEFA-HR, Wako Pure Chemical Industries, Richmond, VA). Reagents for BUN analysis; urease, phenol nitroprusside, and alkaline hypochlorite were made in the NDSU ruminant nutrition laboratory (Chaney and Marback, 1962). Absorbencies were read using a UV-VIS biotech spectrophotometer (DU-640, Beckman Coulter, Brea, CA). The method for BUN analysis was based on methods of determination from Fawcett and Scott (1960).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS version 9.2 (SAS Institute, Inc., Cary, NC) as a complete randomized design. The class statement included cow and treatment,

and when data were analyzed over time, day, weekday, or feed day it was included. The method used for the determination of degrees of freedom was the Satterthwaite method and the best fit covariate structure was chosen based on the lowest Akaike, Akaike with small sample size adjustment, and Bayesian information criterion statistics. Means were separated using the LSMEANS option of SAS and were considered significant when $P \leq 0.05$.

The model used to evaluate hay, DDGS, and total intake over the duration of the feeding period included treatment. This model was analyzed as a repeated measure and the subject was cow nested within treatment. The best covariate structure chosen was the simple (VC) structure. The model used to evaluate patterns of hay intake among days of the week treatment, weekday, and respective interaction. Weekday was analyzed as a repeated measure and the subject was cow nested within treatment. The best fit covariate structure chosen was the simple (VC) structure. Data were analyzed to represent a full week of intake data and data were analyzed separately to represent days CON, DG3, and DGA received hay (T, Th, Sa, and Su) and days DG7, DG3, and DGA received DDGS (M, W, and F).

The model for ADG, G:F, change in body composition parameters, and calving data included treatment. Fixed effects were considered to be cow and treatment and the random effect was cow.

The model for BUN and NEFA contained treatment, feed day (80, 81, and 82, respectively) and their interaction in the model. Feed day was repeated and the subject was cow nested within treatment. The best fit covariate structure for BUN was compound symmetry; whereas the best fit covariate structure for NEFA was auto regressive (1).

The model for feeding behavior parameters included treatment, week day and the interaction. Day was repeated, subject was cow nested within treatment, and the covariate

structure chosen was compound symmetry. Data were also analyzed separately to represent days CON, DG3, and DGA received hay (T, Th, Sa, and Su) and days DG7, DG3, and DGA received DDGS (M, W, and F).

Results and Discussion

There have been few research projects that have evaluated feeding behavior parameters for beef cows consuming forage and supplement. This report is the first to separately quantify feeding behavior patterns of cows consuming each DDGS supplement and hay at different frequencies making this data beneficial for those looking for information regarding this topic.

Cow BW was similar at the initiation and conclusion of this study ($P \geq 0.77$; Table 3.2). Total hay DM intake per day was lower ($P \leq 0.02$) for DGA compared with all other treatments. In addition, hay DMI was lower ($P \leq 0.01$) for DG3 compared with CON; whereas DG7 was intermediate. This decrease in hay intake for DGA was similar to results in chapter two in which

Table 3.2. Intake and efficiency characteristics of gestating beef cows fed ad libitum grass hay and varying frequencies of supplemental dried distiller's grains plus solubles.

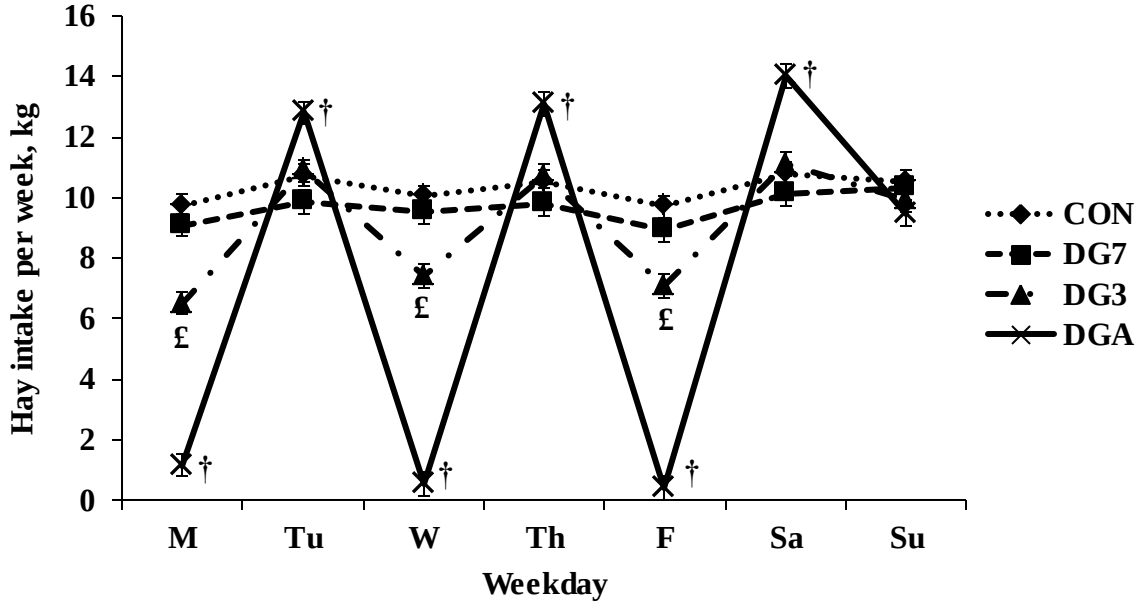
	Treatment[1]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
Intake, kg/d						
Hay	10.3[c]	9.6[bc]	9.1[b]	7.4[a]	0.33	<0.0001
Dried distillers grains plus solubles	0.0[a]	3.0[b]	2.9[b]	2.8[b]	0.09	<0.0001
Total dry matter intake	10.3[a]	12.6[b]	12.0[b]	10.2[a]	0.35	<0.0001
Performance						
Initial body weight, kg	657.1	645.4	653.8	651.8	17.5	0.97
Final body weight, kg	685.5	707.3	717.7	701.5	22.2	0.77
Average daily gain, kg	0.3[a]	0.7[b]	0.8[b]	0.6[b]	0.1	<0.0001
Gain to feed, kg of feed/kg of BW gain	0.03[a]	0.06[b]	0.06[b]	0.06[b]	0.005	0.007

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on M, W, and F, DGA = Hay only on T, Th, Sa, Su or DDGS only on M, W, and F

[abc]Means within row lacking common superscripts differ ($P < 0.05$)

there was a decrease in hay intake for forage-fed steers supplemented with the DGA strategy compared with steers supplemented daily, on alternate days with ad libitum hay intake, or unsupplemented steers. However, the similar forage intake observed for DG7 and CON in the current study is in contrast to our previous study. This increase in forage intake for DG7 for the current study may be due to the quality of hay (5.9% CP and 76.4 % NDF) in this experiment as it is of reduced quality compared with the previous experiment discussed in chapter 2 (16.1 % CP and 77% NDF). It is well documented that when a protein supplement is provided in conjunction with a low-quality hay forage intake increases in beef cattle (Guthrie and Wagner, 1988; DelCurto et al., 1990a; Köster et al., 1996; Bandyk et al., 2001). This is usually due to an increase in digestibility of the low-quality forage with the inclusion of the protein in the supplement.

Figure 3.1. Daily mean dry matter hay intake pattern for gestating beef cows fed ad libitum hay and varying frequencies of supplemental dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). †DGA differs from all other treatments ($P \leq 0.001$). £DG3 differs from all other treatments ($P \leq 0.001$).



When looking at hay DMI throughout the week, hay DMI was least on SUP (Monday, Wednesday, and Friday) but greatest on NSUP (Tuesday, Thursday, and Saturday) for DGA compared with all other treatments (Trt × Time, $P < 0.0001$; Figure 3.1). On the second consecutive day that DGA and DG3 received hay (Sunday), hay DMI was similar among treatments ($P \geq 0.06$) Additionally, cows fed DG3 consumed less ($P \leq 0.001$) hay on SUP compared with CON and DG7 fed cows (Figure 3.1). We believe that this decrease in hay intake for DGA fed cows is likely caused by limited hay access time. This pattern in intake was similar to that seen in a study conducted by Cunningham et al., (2005) where lactating beef cows were allowed access to round bales (19.6% CP) for 4, 8, or 24 h. Hay disappearance for cows on the 4 h treatment was approximately 37% less than those cows allowed hay access for 24 h (Cunningham et al., 2005). Other researchers have observed similar results when limiting access or quantity of forage (Scholljegerdes et al., 2004; Miller et al., 2007). Hay intake for the DG3 treatment were likely decreased compared with CON due to the substitution effect. Where amounts of the forage portion of the diet was being replaced by supplemented nutrients (Caton and Dhuyvetter, 1997). Similar substitution parameters were observed for forage intake in gestating beef cows (Huston et al., 1999), nursing calves consuming forage (Cremin et al., 1991; Faulkner et al., 1994; Tarr et al., 1994).

As per experimental design, DDGS DM intake per day was similar ($P \geq 0.15$) for DG7, DG3, and DGA; and all were greater than CON (Table 3.2). In addition, total DMI per day was greater for DG7 and DG3 compared with CON and DGA ($P < 0.0001$; Table 3.2). These data for total DMI are similar to results of Loy et al. (2007) who supplemented beef heifers consuming grass hay with DDGS or dry-rolled corn daily or on alternate days. Heifers that were not supplemented and only receiving hay had a decreased total DMI compared with heifers that were

supplemented both daily and on alternate days (Loy et al., 2007). This is similar to total DMI for CON fed cows in our study which had decreased total DMI compared with cows on the DG7 and DG3 treatments. When providing a sunflower meal and cottonseed meal supplement to beef steers 2 (6.37 kg/d), 3 (4.24 kg/d), 5 (2.55 kg/d), or 7 (1.82 kg/d), steers that received the larger quantity of supplement 2 d/wk had decreased forage DMI compared with all other treatments but total DMI increased linearly with increased frequency of supplementation (Farmer et al., 2001). We did not observe an increase in forage intake with increasing supplementation frequency between DG3 and DG7; however an increase in forage DMI was observed from DGA to DG7.

Average daily gain (ADG) was greater ($P \leq 0.01$) in DG7, DG3, and DGA compared with CON (Table 3.2). Similarly, the gain to feed ratio (G:F) was lower in CON compared with all other treatments ($P = 0.007$; Table 3.2). Our findings of all supplemental treatments (DG7, DG3, and DGA) having similar values for ADG conflicts with other reports. Grazing heifers that received a wheat middlings and SBH supplement 7 d/wk had greater ADG compared with heifers supplemented 3 d/wk with the same ration (Cooke et al., 2008). These increases in ADG are similar to those observed by Kunkle et al. (2000) and Cooke et al. (2007), when forage-fed beef cattle were supplemented 3 or 7 d/wk saw an increase in ADG with increased supplementation frequency. The inconsistencies among the current study and previous reports for ADG among supplement frequencies may be due to the stage of production of the cattle being evaluated. In the case of Cooke et al. (2007; 2008), growing heifers were the experimental units, whereas we utilized gestating beef cows in the current study. Nutrient utilization is different among growing heifers and gestating cows, thus, effects that are masked in gestating cows may be manifested in growing heifers. For growing forage-fed heifers ADG was decreased when supplemented 3 d/wk with either dry rolled corn, DDGS, or dry rolled corn with corn

gluten meal compared with heifers that were supplemented daily and there were no differences in G:F among dietary treatments (Loy et al., 2008). Similar results to those of Loy et al. (2008) have also been observed when supplementing forage-fed gestating beef cows at varying frequencies (Kartchner and Adams, 1982; Beaty et al., 1994). In the current study, DGA, DG7, and DG3 all had similar ADG even though DGA had a lower hay and total DMI. This inconsistency may point out a fundamental opportunity for cattle in the DGA treatment to utilize feed more efficiently. However, the fact that no differences in gain to feed ratio were observed and, in a previous report, no difference in digestibility were observed among DGA, DG3, and DG7 treatments (Klein et al., 2012) leaves questions about why cattle fed DGA can perform similarly compared with cattle fed DG3 and DG7.

Change in BCS, IM, RBFT, and RMFT were all similar among treatments ($P \geq 0.24$; Table 3.3). However, REA in cows fed with the DG7 strategy had a greater ($P \leq 0.05$) increase in REA compared with all other treatments (Table 3.3). In an experiment utilizing beef cows fed

Table 3.3. Performance change for gestating beef cows fed ad libitum grass hay and varying frequencies of supplemental dried distiller's grains plus solubles.

	Treatment[1]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
Performance change[2]						
Body weight, kg	28.9[a]	61.6[b]	63.0[b]	50.7[b]	5.3	<0.0001
Body condition score[3]	-0.29	0.25	-0.14	-0.32	0.23	0.26
Intramuscular fat, %	-0.12	-0.32	-0.52	-0.49	0.18	0.35
Rib fat, cm	-0.08	0.08	-0.08	-0.05	0.05	0.73
Rump fat, cm	-0.08	0.10	0.08	0.05	0.20	0.24
Rib eye area, cm^2	-2.41[a]	2.49[b]	-0.36[a]	-0.41[a]	0.84	0.002

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on M, W, and F, DGA = Hay only on T, Th, Sa, Su or DDGS only on M, W, and F
[ab]Treatment means with varying superscripts differ ($P < 0.05$)
[2]Performance change = measurement on d84 – measurement on d28
[3]Body condition score scale 1(emaciated) to 9 (obese)

either grass hay (CP 12.1%) with added vitamins and minerals to meet maintenance requirements (control) or cows were fed millet hay (9.9%CP) with half of the control diet vitamins and minerals restricted (Miller et al., 2004). Those cows on the restricted diet had decreased RBFT, IMF, and REA by d 59 of gestation compared with control. In the current study there were no changes in BCS, IM, RBFT, and RMFT which agrees in part with the findings of the previous studies. However, in our study we did see a decrease in REA for the CON, DG3, and DGA cows (Table 3.3). This decrease in REA for DG3 and DGA was unexpected since these two treatment groups had similar BW, BCS, IMF, RBFT, and RMPFT measurements compared with DG7.

A treatment × day interaction was present for concentrations of NEFA among treatments from d 80-82 (Figure 3.2). On d 80 circulating concentrations of NEFA were greater ($P \leq 0.05$) in CON compared with all other treatments. However, on d 81, concentrations of NEFA were greater ($P < 0.01$) in CON and DGA compared with DG7, whereas DG3 was intermediate. On d 82 concentrations of NEFA were lower ($P \leq 0.001$) for DG7 compared with all other treatments Our observation of unsupplemented cows having greater concentrations of NEFA on days when other treatment received supplement was expected. Similarly, NEFA concentrations were greater in unsupplemented cows compared with cows supplemented with low (0.4% UIP), medium (20.0% UIP), or high (39.0% UIP) levels of a UIP (Sletmoen-Olson et al., 2000). Additionally, increased NEFA concentrations were also observed in unsupplemented cattle and sheep compared with those that were supplemented (Krysl et al., 1987; Cheema et al., 1991; Barton et al., 1992). In contrast, adding supplemental DIP (corn gluten meal and blood meal) and UIP (SBM) to a low quality forage diet in beef cows did not affect plasma NEFA concentrations (Rusche et al., 1993). In general, NEFA concentrations usually decrease in ruminants when they are only consuming low-quality forage without supplement. This pattern can be observed in our

data as concentrations of NEFA increase when any of our treatments received hay only. As caloric intake was low it caused an increase in the mobilization of body fat.

The greater concentrations of NEFA on d 82 in the DG3 group compared with DG7 observed in the current study are similar to other research. In a study conducted by Moriel et al. (2012), beef heifers were given ad libitum access to low (8% CP) and medium (12% CP) quality hay and supplemented with a high (15.8 kg) and low (7.9 kg) amount of energy daily or 3 d/wk, resulting in plasma NEFA concentrations which were similar in heifers supplemented daily on days when all treatments were supplemented and days when the 7 d/wk heifers were supplemented. For heifers supplemented 3 d/wk, plasma NEFA concentrations were greater on days when only the 7 d/wk heifers were supplemented compared with days when all 3 d/wk heifers were supplemented (Moriel et al., 2012).

A treatment × day interaction was present for concentrations of BUN among treatments from d 80-82 (Figures 3.3). On d 80 BUN concentrations were decreased ($P \leq 0.01$) for CON compared with all other treatments and concentrations of BUN were decreased ($P \leq 0.05$) for DGA compared with DG3 and DG7. On d 81 BUN was greater ($P \leq 0.01$) in DG3 and DGA compared with DG7, which was greater ($P < 0.01$) than CON. On d 82, concentrations of BUN were decreased ($P \leq 0.01$; Figure 3.3) in CON compared with DG7 and DG3 but were similar ($P = 0.16$) to those of DGA. Additionally, DG7 had greater ($P < 0.01$) concentrations of circulating urea compared with DGA, but had similar ($P = 0.11$) circulating urea concentrations to those of DG3, while DG3 and DGA were similar ($P = 0.16$) on d 82 (Figure 3.3).

Concentrations of BUN are correlated with amount of protein in the diet (Reed et al., 2007). The peak concentrations of BUN observed for the DGA and DG3 treatments occurred on a day when no supplement was delivered to these respective treatments. The peak on a non-

Figure 3.2. Concentrations of NEFA on day 80, 81, and 82 for gestating beef cows fed ad libitum hay and varying frequencies of supplemental dried distiller's grains plus solubles. Treatment × Time ($P = 0.0012$). *****CON differs from all other treatments ($P \leq 0.05$). ‡DG7 differs from all other treatments ($P \leq 0.01$). **£**DG7 differs from CON and DGA ($P \leq 0.01$).

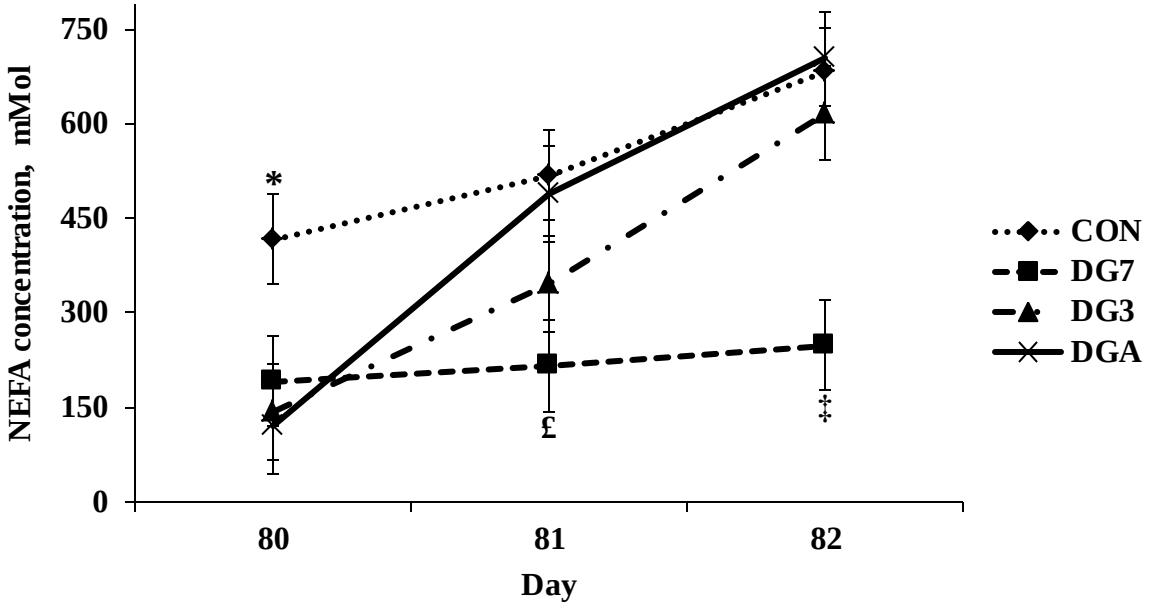
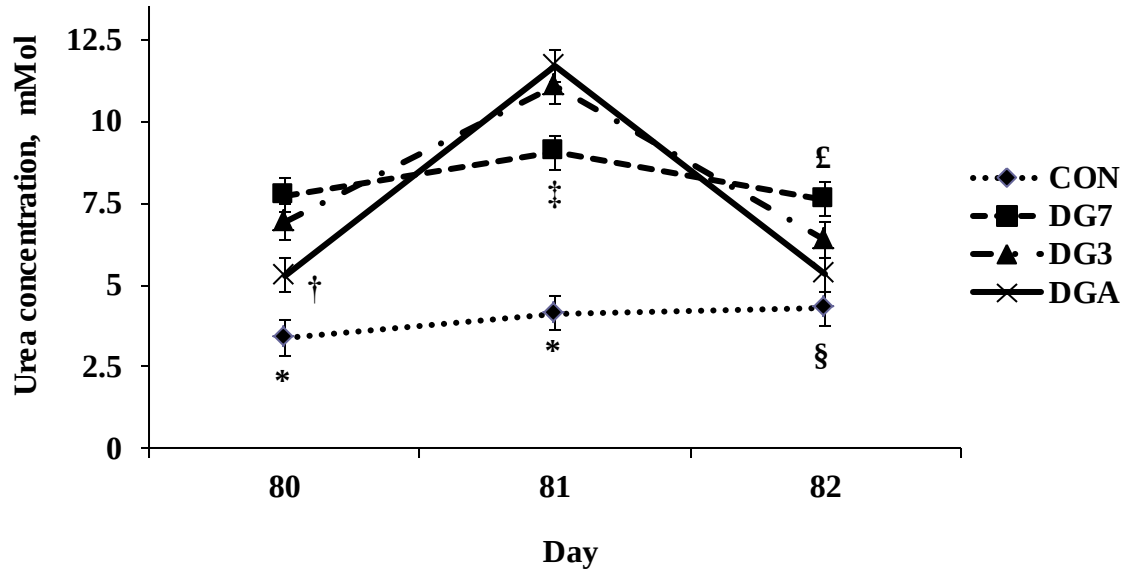


Figure 3.3. Concentrations of BUN on day 80, 81, and 82 for gestating beef cows fed ad libitum hay and varying frequencies of supplemental dried distiller's grains plus solubles. Treatment × Time ($P < 0.0001$). *****CON differs from all other treatments ($P \leq 0.01$). ‡DG7 differs from all other treatments ($P \leq 0.01$). †DGA differs from all other treatments ($P \leq 0.05$). **£**DG7 differs from CON and DGA ($P < 0.01$). **§**CON differs from DG3 ($P \leq 0.01$).



supplemented day was likely due to the recycling of N originating for DDGS consumed on the previous day. Similarly, grazing heifers that received a wheat middlings/SBH supplement 3 d/wk had greater BUN concentrations compared with heifers supplemented 7 d/wk on the days when only the 7 d /wk treatment group was supplemented (Cooke et al., 2008). Conversely, BUN concentrations were lower for the 3 d/wk supplemented group compared with the 7 d/wk supplement group on days when both treatment groups were supplemented (Cooke et al., 2008). Authors of the previous report postulated that the increase in BUN for heifers supplemented 3 d/wk on non-supplemented days may have negatively influenced performance by causing excess conversion of ammonia at the liver. Excessive concentrations of ruminal ammonia require additional energy from the liver to be metabolized into urea. In the current study, greater BUN was observed the day after cows fed DG3 and DGA were supplemented with DDGS. Though no difference in ADG were observed in the current study the increased concentrations of BUN and NEFA are an indication that both DG3 and DGA are altering blood metabolites in a way that is not altered when strategies of consistent (DG7) supplementation are employed.

Table 3.4. Feeding behavior of gestating beef cows consuming hay ad libitum[1]

	Treatment[2]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
Hay						
Meals, #/d	10.4	10.3	11.1	9.1	0.7	0.23
Time spent eating, min/d	212.9^{bc}	184.4^{a}	198.5^{ab}	227.9^{c}	8.7	0.02
Intake per meal, kg/d	1.3^{a}	1.2^{a}	1.2^{a}	1.7^{b}	0.1	0.002
Time per meal, min/kg/d	22.8^{a}	19.3^{a}	20.3^{a}	27.4^{b}	1.6	0.005
Rate of intake, kg/min/d	0.06	0.07	0.07	0.94	0.4	0.36

[1] Days that CON, DG7, and DG3 received hay
[2]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on M, W, and F, DGA = Hay only on T, Th, Sa, Su or DDGS only on M, W, and F
[abc]Means within row lacking common superscripts differ ($P < 0.05$)

Feeding behavior data were categorized by number of meals per day, time spent eating per day, intake per meal, time spent eating per meal, and rate of intake separately for hay and

DDGS intake. Data presented for hay feeding behavior represent days when CON, DG3, and DGA received hay (Tuesday, Thursday, Saturday, and Sunday). No differences ($P \geq 0.23$) among treatments were present for number of hay meals per day or rate of hay intake (Table 3.4). However, cows fed DGA spent more ($P \leq 0.05$) time eating compared with DG7 and DG3. In addition, cows fed CON spent more ($P \leq 0.05$) time eating compared with DG7; whereas DG3 were intermediate. Both hay intake per meal and time per hay meal were greater ($P \leq 0.05$) in cows fed DGA compared with all other treatments, while all other treatments were similar ($P \geq 0.13$).

Data presented for feeding behavior of cows consuming DDGS represent days when DG7, DG3, and DGA received DDGS (Monday, Wednesday, and Friday). Number of DDGS meals per day, time spent eating DDGS, DDGS intake per meal, time per DDGS meal, and rate

Table 3.5. Feeding behavior of gestating beef cows consuming supplemental dried distiller's grains plus solubles.

	Treatment[1]					*P*-value
Item	CON	DG7	DG3	DGA	SEM	Trt
DDGS						
Meals, #/d	0.1[a]	2.9[b]	5.1[c]	4.4[c]	0.3	<0.0001
Time spent eating, min/d	0.5[a]	7.5[b]	26.1[c]	22.8[c]	2.7	<0.0001
Intake per meal, kg/d	0.0[a]	1.3[b]	1.8[c]	1.9[c]	0.1	<0.0001
Time per meal, min/kg/d	0.0[a]	2.7[b]	5.5[c]	5.5[c]	0.7	<0.0001
Rate of intake, kg/min/d	0.1[a]	0.7[b]	0.5[c]	0.5[c]	0.1	<0.0001

[1]CON = Hay only, DG7 = Hay and DDGS 7 d/wk, DG3 = Hay 7 d/wk and DDGS on M, W, and F, DGA = Hay only on T, Th, Sa, Su or DDGS only on M, W, and F
[abc]Means within row lacking common superscripts differ ($P < 0.05$)

of DDGS intake were greater ($P \leq 0.05$) for DG3 and DGA compared with DG7, which was greater than cows fed CON (Table 3.4). There have been few research projects that have evaluated feeding behavior parameters for beef cows consuming forage and supplement. However, some work has been done in beef steers and heifers where a significant correlation was

observed among the time spent eating and average daily intake (Schwartzkopf-Genswein et al., 2002). Additionally, frequency of bunk visits was not correlated with feed intake, indicating that duration of bunk attendance is more indicative of animal intake than frequency of visits (Schwartzkopf-Genswein et al., 2002). This was observed in the current study as time spent eating on NSUP (Table 3.4) was greatest in DGA and this treatment group also had greater hay intake on NSUP (Tuesday, Thursday, and Saturday) during the week (Figure 3.1).

Other projects have evaluated feeding behavior but differences in experimental design, data collection and evaluation techniques make drawing direct comparisons to our work difficult. For example, no differences were present among dairy cows for time spent eating, meals per day, and rate of intake among treatments groups fed high concentrate (300 g concentrate/ kg forage) or high forage (100 g concentrate/ kg forage) diets of grass silage and a barley-based concentrate in a total mixed ration from calving to 156 d postpartum (Tolkamp et al., 2002). However it's hard to make inferences between Tolkamp and others research and the current study, as, the feeding behavior data was collected for TMR not individual feed components. An additional report found that beef heifers consuming ad libitum grass hay and receiving either DDGS or dry-rolled corn supplement daily ate smaller meals compared with heifers supplemented on alternate days regardless of supplement type (Loy et al., 2007). The discrepancies in feeding parameters among previous research and the current report may be due to different methods of calculating feeding behavior. Whereas, Loy et al. (2007) calculated total feeding parameters for each diet, the current report evaluated feeding behavior characteristics while cows were eating specific ingredients (hay and DDGS, respectively).

Lastly, mean calf birth weights were not affected ($P = 0.44$) by treatment (CON, 41.1 ± 1.4 kg; DG7, 44.2 ± 1.4 kg; DG3, 41.6 ± 1.4 kg; DGA, 42.5 ± 1.4 kg) . These results are similar

to those observed by several other researchers limiting access to forage (Cunningham et al., 2005), supplementing grazing heifers with soybean hulls or DDGS (Engel et al., 2008), and cows receiving a protein supplement while grazing winter range (Patterson et al., 1999). Our current results differ from those of Loerch, 1996, who observed an increase in calf birth weights when limit feeding corn compared with cows receiving ad libitum grass hay (10.2% CP; 75% NDF). This difference may be attributed to the fact that those cows receiving only ad libitum grass hay were not receiving the same amount of energy as those cows consuming the limit-fed corn diet. However, if this were the case then we too might have seen a difference in calf birth weights as our CON group was only receiving a low quality hay, but we did not.

Conclusions

Alternate day feeding of DDGS and grass hay every other day decreased forage intake compared with all other treatments without negatively influencing body composition characteristics except for REA, which was reduced compared with animals receiving supplement daily. As there were no differences among treatments for the majority of body composition parameters, delivering supplement to cattle three days per week on Monday, Wednesday, and Friday is a relatively simple way to ensure that animals receiving low quality forage meet their nutritional requirements. Few reports have evaluated feeding behavior parameters for beef cows consuming supplement at varying frequencies. Within the confines of the current study, implementing the strategy of alternate day feeding of DDGS and grass hay every other day to mid-late gestation beef cows was done without causing deleterious effects to overall cow performance and subsequent calf birth weights.

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CHAPTER 4. SUMMARY AND CONCLUSIONS

This study is the first, to our knowledge, that has evaluated a strategy of alternating days of feeding dried distiller's grains plus solubles only or grass hay only. This fact led to difficulty identifying reports in the literature that contain appropriate comparisons or offered insight into the potential implications of a feeding strategy of alternate day feeding of distiller's grains only or hay only. Therefore, two experiments were conducted and the previous chapters of this thesis elaborate on experimental details and coincident results. Results of the projects conducted will be useful tools for cattle producers and researchers alike.

The first experiment evaluated the effects of feeding DDGS and hay on alternate days on intake, ruminal pH and VFA and NH_3 concentrations, liquid passage rate, digestibility and blood hormones and metabolites. Experiment two evaluated the effects of feeding beef cows in mid-late gestation on intake, body composition change, feeding behavior, and blood metabolites.

Overall the alternate day feeding of DDGS decreased forage dry matter intake compared with cattle that were only receiving hay without affecting total tract digestibility. Additionally, novel pH and VFA concentration data were obtained, outlined, and used to compare, and contrast with what is already known about ruminal fermentation kinetics, as well as the specific effects of our novel supplementation strategy. These data may assist in the further understanding of intake and digestibility, ruminal kinetics, and body composition and feeding behavior in years to come.

Ultimately, the alternate day feeding of DDGS or grass hay may be beneficial to producers in specific scenarios where forage costs are increased but cost of supplement is decreased. This benefit will likely be more pronounced during years when droughts or flooding are prevalent as forage availability will likely decrease. Additionally, reducing the feeding

frequency of supplying supplement daily to three days a week (Monday, Wednesday, Friday) will be beneficial to reduce cattle producers time, labor, and equipment costs associated with feeding supplement daily.

Though data in the current reports are encouraging, future efforts should identify the effects of the supplement strategy using varying supplemental types (e. g. soybean hulls, soybean meal, cottonseed meal, corn etc.) while applying the same dietary scheme. Specific items to evaluate in these efforts include forage intake, ruminal kinetics, body composition traits, and feeding behavior.