THE IMPACTS OF SUPPLEMENTING RUMEN DEGRADABLE OR UNDEGRADABLE PROTEIN TO HEIFERS AND COWS ON SUPPLEMENT INTAKE BEHAVIOR, PERFORMANCE, REPRODUCTION, AND NUTRIENT DIGESTION

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2021

DEDICATION

To the two best people I know, my parents: Marko and Linda Manoukian. They exemplify all that I strive to be in this world.

ACKNOWLEDGEMENTS

Thank you to Sweet Pro LLC for the funding and products used in this project. Thank you also to Webb Livestock Co. for the use of their livestock and facilities during the demonstration study, and to the BART farm employees for all their assistance.

I would like to express my gratitude to my committee members. Thank you, Dr. Tom Geary for the help in the lab and for serving on my committee. Thank you, Dr. Tim DelCurto for always answering my questions, and for your never-ending patience and wisdom. Thank you, Dr. Carla Sanford for your advice and encouragement in school and in life. Thank you to my advisor, Dr. Megan Van Emon for all the opportunities you have given me the past five years. Thank you for your expertise, encouragement, and mentorship. I would not be where I am today without it.

Thank you to my classmates for the help with data collection, schoolwork, and for all the support and laughs along the way. A big thank you to our wonderful undergraduate assistant, Janessa Kluth for going above and beyond to help with this project. Thank you also to Dr. Sam Wyffels for all the help with data analysis and interpretation, I really appreciate it.

Thank you to my friends and family for their constant encouragement and support throughout my college career. I would also like to thank my family who has influenced me in the field of animal science. Thank you to my Uncle Steve and Aunt Gwyn for the time I spent at the ranch. Thank you to my brother Troy for the endless support and for inspiring me to always chase my dreams. Last but certainly not least, thank you to my parents. Thank you for teaching me the importance of hard work, for giving me the opportunity to find my passion in agriculture, and for supporting me always. I have been extremely blessed to have so many great people in my life, and I am grateful.

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ABSTRACT

Low-quality forages, often low in protein, are a common feed resource for beef cattle in Montana and the western United States. A supplement intake study, as well as a digestion study were performed to observe the effects of rumen degradable protein (RDP) and rumen undegradable protein (RUP) on supplement intake behavior, performance, reproductive parameters, nutrient digestion, and rumen kinetics. Yearling heifers were used in a completely randomized design and two- and three-year old cows were used in a randomized complete block design for an 84-d supplement intake study. Treatments were: 1) pressed supplement block containing RUP (RUP), and 2) pressed supplement block containing RDP (RDP). Heifer and cow supplement intake displayed ($P < 0.01$) a treatment × period interaction. Cow intake rate and coefficient of variation displayed ($P < 0.01$) a treatment × period interaction. The RUP heifers consumed supplement faster ($P < 0.01$) than RDP heifers. The RDP cows had greater ($P < 0.01$) average daily gains than RUP cows. The RUP cows had greater final pregnancy rates than RDP cows ($P = 0.04$). In conclusion, protein type impacted intake behavior in cows and heifers, and RDP cows had ADG, but protein type did not negatively impact final performance or pregnancy success. Eight two-year old and eight three-year old rumen fistulated cows were used in a 2 × 2 factorial design for a 22-d digestion study. Animals were fed an ad libitum low-quality diet. Supplements included either RDP or RUP and were self-fed (SF) salt-limited pressed blocks or hand-fed (HF) the same loose ingredients without salt resulting in 4 dietary treatments: 1) RUP-SF, 2) RUP-HF, 3) RDP-SF, and 4) RDP-HF. There was a delivery × protein type interaction ($P \leq 0.04$) for both NDF digestibility and water intake. There was an effect ($P = 0.02$) of protein type on fluid flow rate. Ruminal ammonia displayed ($P < 0.01$) a delivery × protein type × hour interaction. Valerate ruminal concentrations were greater in RDP supplemented animals compared to RUP supplemented animals ($P = 0.04$). In conclusion, self-fed supplements containing RDP may enhance the use of low-quality forages.

CHAPTER ONE

GENERAL INTRODUCTION

Due to the importance of beef cattle production in Montana and the integral role replacement heifers play, their development is important. Subsequently, young cows may be difficult to manage. The arid environment of Montana and the western United States causes forages to have seasonal deficiencies, such as protein (DelCurto et al., 2000), which is why supplementing these heifers to meet their growth requirements may be necessary. Furthermore, retaining these heifers within the cow herd and supporting them as they continue to grow and as they enter lactation and gestation, also requires supplemental protein. Producers dependent on forage resources should maximize their cattle production while minimizing input costs. Distillers grain products, by-products of the ethanol industry, can be used as a supplemental feed for ruminant animals. SweetPro® supplements are one of these distillers' grains-based products are commercially available and provide adequate nutrition at critical time points such as during pre-breeding and breeding for both heifers and cows. These products contain protein that is undegradable within the rumen. The protein required by beef cattle can be separated into two specific requirements; the protein need for rumen microorganisms, and the need of the individual animal (Eisemann et al., 2016). Rumen undegradable protein is not hydrolyzed upon entering the rumen and is digested and absorbed in the small intestine. Conversely, rumen degradable protein provides the microorganisms in the rumen with a source of nitrogen and is required for the synthesis of microbial protein. Because of the differences in how these two types of protein are digested and metabolized, there is a potential for differences in intake, nutrient digestion, rumen kinetics, supplement intake behavior, and reproductive parameters.

Previous research regarding the impacts of supplementing rumen degradable and rumen undegradable protein has produced conflicting results. Therefore, more research is needed to evaluate the impacts of these two protein types. This research provides more insight on 1) the impacts of rumen degradable or undegradable protein on supplement intake behavior, performance, and reproductive parameters of yearling heifers and cows grazing dryland pastures with access to automated feed technology and 2) the impacts of rumen degradable or undegradable protein with or without salt on intake, nutrient digestion, rumen kinetics and fermentation in beef cattle fed low-quality forage. The results of this research will give beef cattle producers information to help them best select the supplemental inputs for their animal's needs.

CHAPTER TWO

LITERATURE REVIEW

Most beef production in Montana and the western U.S. is cow-calf production, which relies on heifers for beef cow replacements. According to the National Agriculture Statistics Service (2019), there were 380,000 heifers for beef cow replacements in Montana, which accounts for 15.2% of all the cattle and calves in the state. Cow-calf operations in Montana primarily rely on forages to provide nutrients to these animals (Galyean and Goetsch, 1993). The goal in these production settings is to optimize animal performance while utilizing the forage resources effectively. Pasture and rangeland accounts for most of the land used in farms and ranches at 66.2% in Montana, and the average annual precipitation for this geographic area is 389.6 mm a year, with large variations by year and by location (NASS, 2019; National Atlas, n.d.). These factors combine to place an emphasis on the development of replacement heifers and cow-calf production on a forage-based diet which may be low-quality at times. The challenges faced by cattle production in the western United States results from having to continuously adapt to the ever-changing arid environment that impacts both forage quantity and quality (DelCurto et al., 2000). Short growing seasons, shallow soils, and limited precipitation can result in forages being both limited in quantity and quality throughout the year. This quantity and quality of the forage must be considered in its relationship with the nutrient requirements of the developing heifers as well as the growing, lactating, and gestating young beef cows.

Replacement heifers can be developed either in a drylot or range setting. While developing heifers in an intensive, drylot situation where they are easily provided an increased plane of nutrition may increase pregnancy success, it may not optimize profitability or sustainability of an operation due to increased costs (Funston et al., 2012). When heifers developed in a drylot were compared to those grazing low-quality forage, no differences in pregnancy rates were observed (Funston and Larson, 2011). Similarly, heifers developed on extensive rangeland had greater average daily gains (ADG) and pregnancy rates than heifers developed in a drylot from the time of breeding to pregnancy diagnosis (Mulliniks et al. (2013). This information supports that heifers can successfully be developed in a Montana range setting and that developing them in this extensive range environment can cause them to have similar or improved productivity.

In addition, developing heifers on rangeland may prepare them for future grazing settings and allow them to gain grazing experience (Summers et al., 2018). It has been suggested that animals can use both positive and negative digestive response to form preferences of how they graze (Launchbaugh et al., 1999). When range developed heifers and drylot developed heifers were placed in a drylot for 40 days during estrous synchronization and 5 days following artificial insemination (AI), then were moved to summer pasture, drylot developed heifers had decreased ADG, which could have led to the decrease in conception rates (Summers et al., 2014). Similarly, drylot developed heifers moved immediately to grass after AI had decreased ADG and decreased AI conception rates compared to heifers developed on rangeland (Perry et al., 2013; Perry et al., 2015). Furthermore, supplementing heifers with dried distillers grains with solubles (DDGS) when moved to rangeland following artificial insemination (AI) improved AI conception rates, compared to heifers moved to rangeland and not supplemented (Perry et al., 2015). In addition to this, developing heifers on a range setting compared to in a drylot not only had no differences in body weight (BW) or ADG, range developed heifers also experienced less chronic stress, as measured by plasma cortisol levels, and attained puberty sooner compared to those developed in a drylot (Schubach et al., 2017). Ultimately, developing heifers on rangeland gives them grazing experience and can set them up for success when they enter the mature cow herd and will be grazing the same rangelands that have the potential to be low quality, while decreasing their stress potential and improving their reproductive efficiency. Supplementing these heifers can be beneficial and increase their productivity while grazing rangelands.

Livestock are commonly supplemented when forage is limiting or nutrients are deficient (Galyean and Goetsch, 1993). The nutritional deficiencies provided by forages in arid environments results in the need for supplemental nutrients, commonly protein (DelCurto et al., 2000). Grazing cattle are a challenging group to supplement as they select certain plants and plant parts that can vary in the nutrient content (Kunkle et al., 2000). Individual animals within a group are likely grazing different plants, therefore receiving different nutrients. When developing heifers on these poor-quality forages, supplementation may be needed for heifers to achieve adequate growth targets (Mulliniks et al., 2013). Because of this, it is common to supplement heifers during development, as they are trying to reach target weights and condition.

Replacement Heifer Development

Replacement heifers represent the future of cattle production, as well as the opportunity to further the progress of genetic improvement of a cow herd. Producers spend a substantial amount of capital on developing these replacement females, and because of this, their proper and timely development is crucial. It is critical that replacement heifers breed early in the breeding season, calve at the age of two (Bagley, 1993), and breed back in an appropriate amount of time to optimize economic returns (Engelken, 2008). To meet these expectations, there are certain performance parameters that heifers should meet prior to breeding that will determine reproductive success, including target BW and body condition scores (BCS). Nutrition is closely linked to these factors, which is why a management plan that provides adequate nutrition, in a cost-effective manner, to develop replacement females is necessary for the profitability and sustainability of an operation.

Timing of breeding and subsequently timing of calving for the first calf has large impacts on an operation's profitability. Females that conceive early will also calve early, and continue to maintain early calving, ultimately increasing their overall lifetime calf production (Lesmeister et al., 1973). Analysis of calving records of 16,549 heifers allowed for conclusions that two-year-old heifers that calved in the first 21 d compared to the second or third 21 d remained in the herd longer, were older when diagnosed as open, and had greater pregnancy rates from the second through the sixth breeding seasons (Cushman et al., 2013). Heifers that breed and calve early suggests their superior reproductive efficiency and potential (Lesmeister et al., 1973). Selection and development of such replacements can influence the future longevity and productivity of the entire cowherd (Mulliniks et al., 2013). The traits related to fertility; specifically, longevity, are of low heritability (Roberts et al., 2015; Summers et al., 2018), which places emphasis on the environment having an impact on these traits and subsequently management of factors that can also impact these traits. Developing heifers on rangeland while providing a supplement can reduce input costs of heifer development compared to a drylot setting, while also offering heifers with the nutrition that is needed to increase their productivity and longevity.

Body Weight

Body weight is an important growth parameter and can be indicative of the potential for reproductive success. Previously, it has been recommended that heifers be 60-65% of their mature body weight (MBW) by the time of their first breeding (Patterson et al., 1992), which for heifers of British breeds means they need to gain between 0.5 to 0.8 kg/d from weaning until breeding (Engelken, 2008). For example, a heifer weighing 270 kg at weaning with a MBW of 615 kg, should weigh approximately 370 kg at first breeding. Recent research (Funston and Deutscher, 2004) suggests that it is possible to develop heifers to a lighter BW (53-58% MBW) without having adverse effects on the reproductive performance of the heifers. Similarly, heifers at 50% and 55% of their MBW prebreeding did not differ in pregnancy rates (Martin et al., 2008). Additionally, heifers developed to 55% of their MBW compared to heifers developed to 62% of their MBW had similar reproductive performances (Lardner et al., 2014). There was also a reduction in development costs when heifers were developed to 55% compared to 62% of their MBW. Therefore, developing heifers on rangeland to reduced BWs may decrease costs, while still ensuring reproductive success.

In the past, heifers have been shown to have higher fertility on their third estrus compared to their puberal estrus (Byerley et al., 1987), which places importance on gains being targeted to reach puberty six weeks before breeding. However, these differences may be due to the older age of the heifers bred on their third estrus (375 d old) compared to those bred on their puberal estrus (322 d old) (Endecott et al., 2013). More current research has shown that delayed puberty has less of a negative effect on pregnancy rates. There were no differences in pregnancy rates of heifers with delayed puberty due to nutritional restriction (Lynch et al., 1997). These changes may have occurred over time, such as calving heifers at two years old instead of three years, genetic changes in scrotal circumference and its relation to progeny puberty, and possibly a change in the fertility of the first puberal estrus and those that follow. This research highlights the importance of BW in heifer development. However, BW does not give a description of the fat cover of the animals. This is why BCS is important, as it provides an estimation of energy reserves of the animal.

Body Condition Score

Body condition scores are also important in heifer development. Once pregnant, nutritional management is key as heifers should be managed to maintain a BCS of 5.5-6.0 (Engelken, 2008). If this condition is maintained until calving, heifers will experience less dystocia, as well as higher pregnancy rates in their second breeding season because of a shortened postpartum interval. Heifers with a “moderate” body condition, between 5-6.5, were more reproductively efficient and had a higher probability of successful first-service conception rates (Utter et al., 1994). Similarly, heifers with a BCS of 6 had a higher percentage of both becoming pregnant to artificial insemination and pregnancy rates at the conclusion of the breeding season compared to heifers with BCS of 4 and 5 (Dickinson et al., 2019). Proper condition is necessary for heifers to successfully breed and to continue to be productive within the cowherd.

Rumen Environment

Volatile Fatty Acids

Adenosine triphosphate (ATP) is the energy source of cellular metabolism of microorganisms (Van Houtert, 1993). As only a small percentage of energy from plants a ruminant consumes is transferred to ATP during this process, by-products of ruminal fermentation, such as volatile fatty acids (VFA) are created. The three main VFAs are propionate, acetate, and butyrate, which serve as the major energy sources for the ruminant animal. When cattle are grazing rangeland that have the potential to be deficient in protein, they need to be supplemented with additional protein to be able to produce those necessary VFAs.

Volatile fatty acids are absorbed through the rumen epithelial in free form and enter into the circulatory system through the hepatic portal blood (Van Soest, 1982; Millen et al., 2016) Propionate is the only major gluconeogenic VFA, while acetate and butyrate are metabolized to acetyl-CoA and oxidized in the citric acid cycle (Van Soest, 1982). Measuring VFAs is important in determining the impacts of treatment on rumen microbial fermentation.

Propionate. Propionate can be produced from either starches or cellulose (Van Houtert, 1993). After being produced in the rumen, it is absorbed through the rumen wall, and enters portal circulation. The liver takes up roughly 80% of the propionate within the blood in one pass (Van Soest, 1982). Gluconeogenesis is the main way in which the liver metabolizes propionate (Cridland and Leng, 1985) where propionate is converted to propionyl-CoA, then carboxylated to methylmalonyl-CoA, and rearranged to succinyl-CoA. It then enters the citric acid cycle as succinate, is converted to oxalacetate, and from there glucose is produced (Van Soest, 1982). In both roughage and grain fed ruminants, propionate serves as the major glucose precursor. However, animals fed high concentrate diets have an increase in concentration of propionate, whereas acetate is the main VFA in forage-based diets (Lana et al., 1998; Lage et al., 2017). Diets that produce higher levels of propionate will provide more substrate and increase rates of gluconeogenesis. This increase in available blood glucose is important in both developing heifers and cows in lactation and as both groups enter gestation.

Acetate. Acetate is the dietary component absorbed in the greatest amounts (Van Soest, 1982) and is the only major VFA that reaches the peripheral tissues in considerable amounts (Van Houtert, 1993). Acetate is an important lipogenic precursor (Van Soest, 1982). It can be used for oxidative metabolism to generate ATP, as well for synthesizing acetyl-CoA which is then used to produce lipids (Millen et al., 2016). Within adipocytes, acetate is converted to triglycerides and can be stored as fat (Millen et al., 2016). Acetate is associated with diets high in fiber (Lage et al., 2017). It is likely that grazing animals and animals fed a low-quality roughage diet will have high levels of acetate within the rumen. With this high level of acetate that can be eventually stored as fat, there is a potential to see an increase in fat cover and body condition. This increase in body condition, as previously stated, is important to achieving reproductive success.

Butyrate. Butyrate is produced and accumulated when nonstructural carbohydrates are available and when there is high-fiber degradability (Plöger et al., 2012). A large portion of butyrate within the rumen is utilized by the rumen epithelial (95%), with the remaining amount (5%) becoming long-chain fatty acids and ketones within the bloodstream (Millen et al., 2016). Butyrate is needed for growing and differentiating the rumen, and improving its absorptive capabilities (Plöger et al., 2012). It also enhances the function of the intestinal barrier (Peng et al., 2009; Plöger et al., 2012). However, large amounts of butyrate coupled with low pH within the intestinal lumen may be toxic. Because butyrate is associated with nonstructural carbohydrates, animals consuming supplements that have a high level of nonstructural carbohydrates may have an increase in butyrate levels.

Potential Effect of Rumen Degradable Protein (RDP) or Rumen Undegradable Protein (RUP) on VFA Production. Microbes utilize protein available within the rumen to grow (Wickersham et al., 2008). An increase in microbial growth and activity increases the production of VFAs within the rumen (Scott and Hibberd, 1990; Köster et al., 1996). In a study by Wickersham et al. (2008), beef steers were supplemented with an RDP source in increasing amounts of 0, 59, 118, and 177 mg of N/kg of body weight once daily. Overall VFA concentrations increased with increasing amounts of RDP supplementation. However, acetate decreased while propionate increased with increasing amounts of supplement provided. A reduction in acetate concentrations would be expected due to the increase in degradable protein in the diet, which lends itself to more rapidly fermentable energy source for the rumen microbes. This rapid fermentation increases propionate production; therefore, reducing acetate concentrations. Isovalerate, valerate, and isobutyrate increased linearly as RDP supplement increased (Wickersham et al., 2008).

Similarly, in a study by Köster et al. (1996), supplementation of RDP in amounts of 0, 180, 360, 540, and 720 g/d in beef cows increased total VFA concentrations. Similar to Wickersham et al. (2008), acetate decreased with increasing amounts of RDP supplement. Propionate levels were the highest in the 540 g treatment. Additionally, valerate, isovalerate, and isobutyrate amounts increased with increasing supplementation (Köster et al., 1996). However, supplementing steers with a RUP did not affect total VFA, butyrate, or propionate concentrations (Reed et al., 2007). There was, however, an increase in molar percentages of acetate in steers that did not receive the supplement compared to those that did (Reed et al., 2007). Based on these results, it is likely that an increase in RDP will increase VFA production compared to a RUP supplementation; subsequently having the potential to alter gluconeogenesis.

Gluconeogenesis

Glucose is essential for the production of glycoproteins, glycolipids, structural polysaccharides and mucopolysaccharides in ruminant animals (Cerrilla and Martínez, 2003). Beyond this, glucose is the main energy source for the brain, mammary tissue, kidney medulla, and erythrocytes (Cerrilla and Martínez, 2003; Mayes et al., 2003). The majority of the glucose in the diet is consumed by the rumen microbial population. The glucose that does escape the rumen and reaches the small intestine is metabolized by gut tissues, resulting in limited glucose in the bloodstream (Pond et al., 2004). The total glucose absorbed in the gastrointestinal tract accounts for less than 10% of their total glucose needs, which is why ruminants are dependent on a process called gluconeogenesis as their main source of glucose (Yost et al., 1977).

The nonessential amino acids are those that are glucogenic. When there is a decrease in glucogenic precursors such as propionate, carbon skeletons from deaminated amino acids may be used in gluconeogenesis (Millen et al., 2016). Because of the amino acids that will be made available in protein supplementation, there is a potential for differences in gluconeogenesis, and ultimately performance in rumen degradable vs rumen undegradable protein supplemented animals.

Propionate (60-75%), L-lactate (16-26%), valerate and isobutyrate (5-6%), alanine (3-5%), and other amino acids (8-11%) account for the major gluconeogenic precursors (Aschenbach et al., 2010). The substrates’ entry into gluconeogenesis relies on their conversion to oxaloacetate. As previously described, propionate is absorbed through the rumen epithelial wall, and travels via the bloodstream to the liver. Propionate is converted to oxaloacetate from propionyl-CoA through propionyl-CoA carboxylase, which is then converted to succinyl-CoA through methylmalonyl-CoA mutase (Van Houtert, 1993; Aschenbach et al., 2010). Succinyl-CoA can then enter the citric acid cycle, where oxaloacetate is produced. In the cytosol, alanine and lactate are converted to pyruvate, which then is converted to oxaloacetate by pyruvate carboxylase (Aschenbach et al., 2010). Phosphoenolpyruvate carboxykinase metabolizes oxaloacetate to phosphoenolpyruvate and then finally to glucose, or it can also be an acetyl-CoA

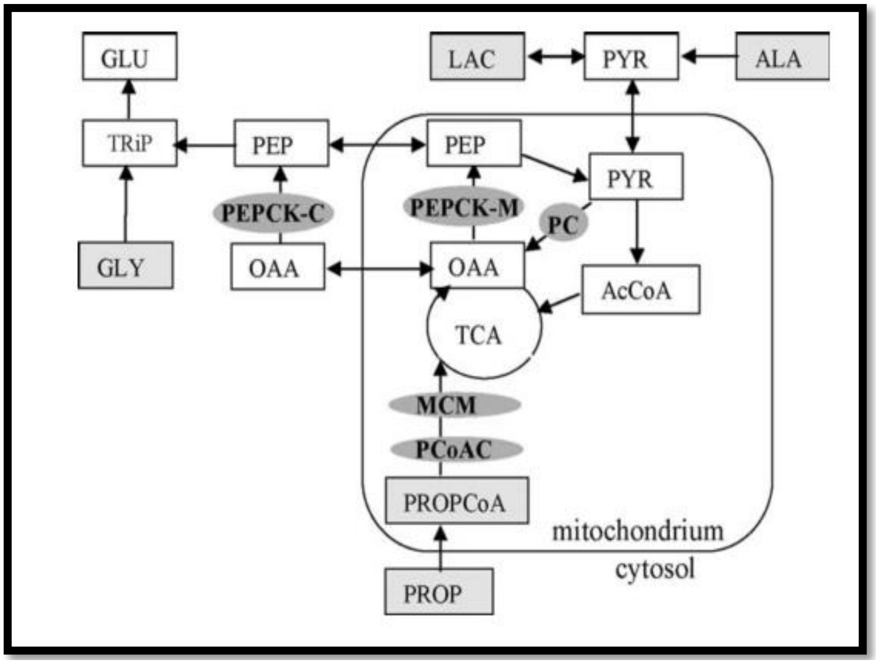

Figure 2.1. Entry points into gluconeogenesis in the bovine liver. Entry of alanine, propionate, and lactate can be regulated by the differential expression of propionyl-CoA carboxylase (PCoAC), pyruvate carboxylase (PC), and phosphoenolpyruvate carboxykinase (PEPCK-C and PEPCK-M) isoforms of the cytosol and mitochondria. Prioprionate entry can also be modified by increasing the availability of coenzyme (vitamin B12) for methylmalonyl-CoA mutase (MCM). Abbreviations: Acetyl COA, AcCoA; alanine, ALA; glucose, GLU; glyercol, GLY; lactate, LAC; oxalaoacetate, OAA, phophoenolpyruvate, PEP; propionate, PROP; proponyl CoA, PROPCoA; pyruvate, PYR; tricarboxulic acid cycle, TCA; triose phosphates, TRiP (Aschenbach et al., 2010).

acceptor in the citric acid cycle. These processes are shown here in Figure 2.1 adapted from Aschenbach et al. (2010).

Phosphoenolpyruvate carboxykinase presence in the mitochondria and cytosol acts as a regulator to the gluconeogenesis precursors, as forming phosphoenolpyruvate depends on NADH production in the cytosol (Aschenbach et al., 2010). Cystosolic phosphoenolpyruvate carboxykinase and pyruvate carboxylate acts as the regulators of amino acids into gluconeogenesis, mitochondrial phosphoenolpyruvate carboxykinase and pyruvate carboxylase regulate lactate going into gluconeogenesis, and cytosolic phosphoenolpyruvate, methylmalonyl-CoA mutase, and propionyl-CoA carboxylase regulate propionate entry into gluconeogenesis (Aschenbach et al., 2010). These regulatory pathways have been proven as research in which additional propionate is supplemented to beef cattle does not impact glucose clearance (Mulliniks et al., 2008).

Rumen Microbiology

The existence of the ruminant animals is due to the symbiotic relationship between the animal and the microorganisms within the gastrointestinal tract (GIT), mainly the rumen/reticulum. This allows for ruminants to consume low-quality forage and produce high-quality protein, fiber, and milk (Ross et al., 2012). The main microorganisms of the rumen/reticulum include bacteria, fungi, and protozoa (Cammack et al., 2018). The rumen/reticulum provides an ideal environment for the microbes to survive with a constant temperature and pH, moistness due to saliva production, and anaerobic conditions (Millen et al., 2016).

Protein that is available to the rumen is metabolized by a mix of rumen microbes, including protozoa and bacteria (Van Soest, 1982). The protein is broken down into large peptides, smaller peptides, and then into amino acids (Nocek and Russell, 1988). These amino acids are absorbed across the cell membrane where they can become ammonia or are consumed by the microbes and increase microbial growth, ultimately becoming microbial crude protein (MCP). Different types of protein and protein consumed in differing amounts has the potential to alter the rate of microbial growth and efficiency.

Bacteria. Of the microbes inhabiting the rumen, bacteria are found in the largest concentration, however they have similar biomasses as protozoa. Bacteria predominate the rumen at more than 95% of the microbial population, which include liquid-associated, solid-associated, eukaryote-associated, and rumen epithelium-associated bacteria (Puniya et al., 2015). The number of bacteria that inhabit the rumen is related to the digestibility of the feed they are consuming, therefore ruminants on a grain-based diets have 10 to 100-fold higher counts of bacteria compared to those on forage-based diets (Millen et al., 2016). Of the bacteria in the rumen, 80-90% of the population is gram-negative, with the number of gram-positive bacteria increasing with increasing amounts of grain. Bacteria distribute throughout the rumen, either floating within the fluid, or attaching to epithelial or protozoal cells, fungal sporangia, or feed particles (Millen et al., 2016). The bacteria within the rumen fluid or adhered to feed particles are involved with digesting the feed the animal consumes. There is limited information on how protein impacts bacterial populations, which is why investigating the impacts of RUP vs RDP on bacterial profiles is important.

Protozoa. Although the overall population of protozoa in the rumen is low, their large size causes them to makeup approximately 50% of the viable biomass within the rumen (Puniya et al., 2015). Protozoa are important to fermentation. They are also important in consuming bacteria and controlling bacteria populations within the rumen (Van Soest, 1982) Ruminal protozoa are classified as cilia or flagella (Millen et al., 2016). Cilia are smaller in length of the two types and allow for digestion of feedstuffs. Flagella are larger in length and thickness than the cilia, fewer in number, digest soluble nutrients, making their contribution to overall fermentation minimal (Millen et al., 2016). Digestion and fermentation of substrates by protozoa does not produce lactic acid (Williams, 1986). This then prevents buildup of lactic acid and thus prevents a drop in pH.

Ciliated protozoa represent the largest number of protozoa and are participants in digestion within the rumen (Millen et al., 2016). The ciliated protozoa can be broken down into two different groups: holotrichs and entodiniomorphs. The holotrichs use mainly soluble sugars and the entodiniomorphs utilize carbohydrates from the cell wall, as well as breaking down starch granules due to the amylase they contain. Entodiniomorphs are also able to digest protein within their cells.

Fungi. Fungi account for approximately 10% of the microbial mass within the rumen (Millen et al., 2016) and some aid in digestion. Within the rumen, fungi can be classified as single celled yeasts or multicellular molds (Millen et al., 2016). The yeast within the rumen does not contribute to ruminal digestion. Within the classification of molds, the aerobic group has no known impact on digestion, and the anaerobic group is important in digestion. Fungi contain many enzymes that can aid in digestion (Puniya et al., 2015), particularly in breaking down plant

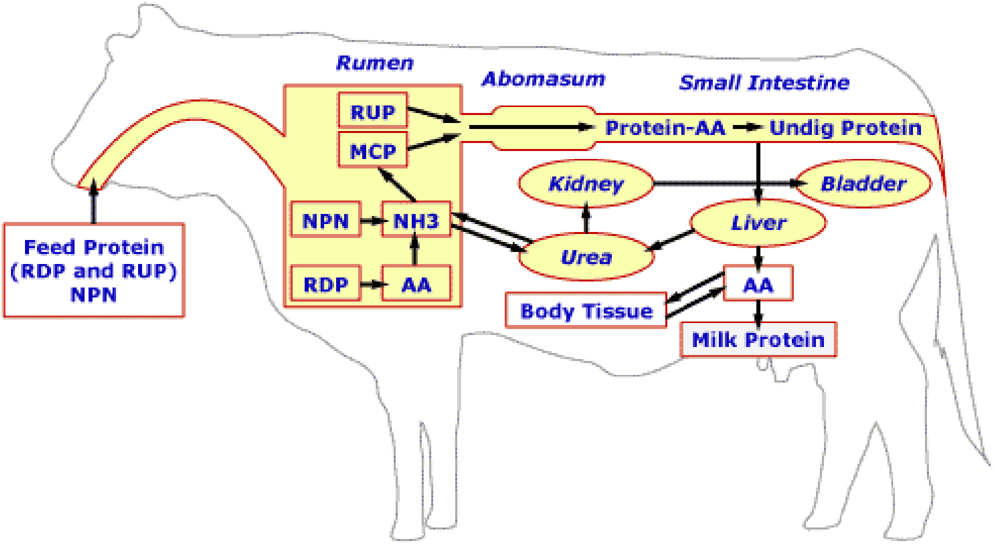

Figure 2.2. Diagram of protein digestion in beef cattle. RDP: rumen degradable protein; RUP: rumen undegradable protein; NH3: ammonia; MCP: microbial crude protein; NPN: non-protein nitrogen; AA: amino acids. (https://courses.ecampus.oregonstate.edu/ans312/seven/cows_2_trans.htm)

components (Millen et al., 2016). These enzymes include proteases, amylases, pectin lyases, cellulases, and hemicellulases.

Ammonia

Ammonia is a product of excess protein in the diet and is ultimately absorbed and released as the waste product urea (Van Soest, 1982). Within the rumen, RDP is broken down into peptides and then amino acids (Millen et al., 2016). These amino acids are deaminated and ammonia is produced. There are a group of bacteria that hyper-produce this ammonia. The microorganisms use ammonia as their main N source for the synthesis of microbial protein (Van Houtert, 1993). Estimates of 50-70% of the N used to create this new bacterial protein comes from ammonia (Millen et al., 2016). High levels of ammonia within the rumen have been shown to increase the efficiency of microbial growth (Mackie and White, 1990). This increase in efficiency could increase efficiency of digestion and increase the amount of microbial protein digested in the small intestine. Ammonia not digested in the rumen is absorbed into the bloodstream (Millen et al., 2016). The absorption of ammonia across the rumen wall is a passive process down a concentration gradient and is therefore dependent on the concentrations of ammonia in the rumen (Parker et al., 1995). All ammonia that is absorbed travels to the liver via the portal system (Tan and Murphy, 2004). Upon entry to the liver, ammonia is converted to urea, which can then be recycled into the small or large intestine, the rumen directly or through saliva, or be excreted through the kidneys and urine, milk, or sweat (Alio et al., 2000; Millen et al., 2016). It is likely that higher levels of rumen ammonia will be observed in animals receiving RDP supplementation, as this protein is available to the rumen to be broken down into ammonia.

Potential Impacts of RDP and RUP on Ammonia Concentrations. Steers supplemented with RUP had increased duodenal ammonia flows compared to those that were not supplemented (Reed et al., 2007), likely due to enzymatic break down in the abomasum and small intestine. Rumen ammonia concentrations increased in RUP supplemented steers compared to steers not supplemented (Reed et al., 2007). Because the protein that is available in the rumen is what is ultimately broken down and producing ammonia, we would expect to see an increase in ruminal ammonia with animals supplemented with RDP. Figure 2.2 represents how RDP and RUP are digested in ruminants.

Rumen ammonia concentrations increase linearly with increasing amounts of RDP supplement in steers (Wickersham et al., 2008). Rumen ammonia concentrations of heifers increased with 25% RUP of CP compared to 40% of CP (Pina et al., 2009). Following supplementation in lambs, ammonia levels decreased with an increase in RUP that replaced RDP

(Atkinson et al., 2007). This supports the hypothesis that we will observe an increase in ammonia with RDP vs RUP supplements.

pH

The conditions within the rumen must be kept at certain levels in order to maintain the microbial population's growth and metabolism (Van Soest, 1982). Maintenance of the microbial population is indicative of the animal's well-being, which is why pH is of such importance. A pH of 6.7 within a range of ± 0.5 is needed for optimum cellulolytic microorganism growth and function (Van Soest, 1982). Cellulolytic microorganisms are important especially for grazing animals that are consuming large proportions of cellulose in their diets. Decreasing rumen pH impacts fermentation (Van Soest, 1982) and specifically, a pH below 6.2 can decrease the rate of digestion in forage diets (Grant and Mertens, 1992). Decreased pH levels are mostly associated with dietary increases of concentrates or starches (Grant and Mertens, 1992). The rumen ability to maintain a fairly constant pH is due to the absorption of fermentation acids across the rumen epithelial, and buffers within the saliva (Van Soest, 1982).

The amount of production and absorption of VFAs impacts pH (Van Soest, 1982). Different supplementation strategies that produce different levels of VFAs have the potential to create differences in rumen pH. Steers supplemented with low levels of RUP had decreases in rumen pH compared to those supplemented with medium and high levels (Reed et al., 2007). The increased levels of RUP supplementation may potentially reduce rumen fermentation by bypassing the rumen fermentation and microbial populations; therefore, increasing rumen pH. In addition to this, Atkinson et al. (2007) also investigated impacts of ruminal characteristics with RDP and RUP supplementation. Rumen pH was not affected by either protein degradability or level of RUP supplementation. As results on the impacts of RDP and RUP supplementation on pH are contrasting, it is possible that we may not only see differences in pH in different protein types, but also with protein types that have such different levels of consumption. However, it is also possible that we will not observe any differences in rumen pH between and within protein groups.

Distillers Grains Plus Solubles

As previously mentioned, heifers may need supplemental feeds when developed on range in order to achieve targeted growths. Distillers grains (DG) can be used as one of these effective feed sources and feed additives for ruminant livestock production. The United States ethanol industry is continuously expanding and broke records in production and exports in 2018 (RFA, 2019). Distillers grains are a by-product of this growing industry (Figure 2.3). Grains, mostly corn, are used to produce DG, which is ground, and the starch is fermented to produce ethanol (Stock et al., 2000). After distillation, a slurry, referred to as whole stillage, remains, which can either be centrifuged or screened and pressed to remove the coarse solid particles. Centrifuging the whole stillage leaves both coarse solids (CDS) and thin stillage. The coarse solids can be dried into dried distillers grains (DDG) while the thin stillage is left to evaporate, leaving condensed distillers solubles (CDS). Both DDGS and CDS have been used as effective feed sources and feed additives for livestock production. The CDS can also be dried with the DDG to produce a different product referred to as DDGS (Stock et al., 2000).

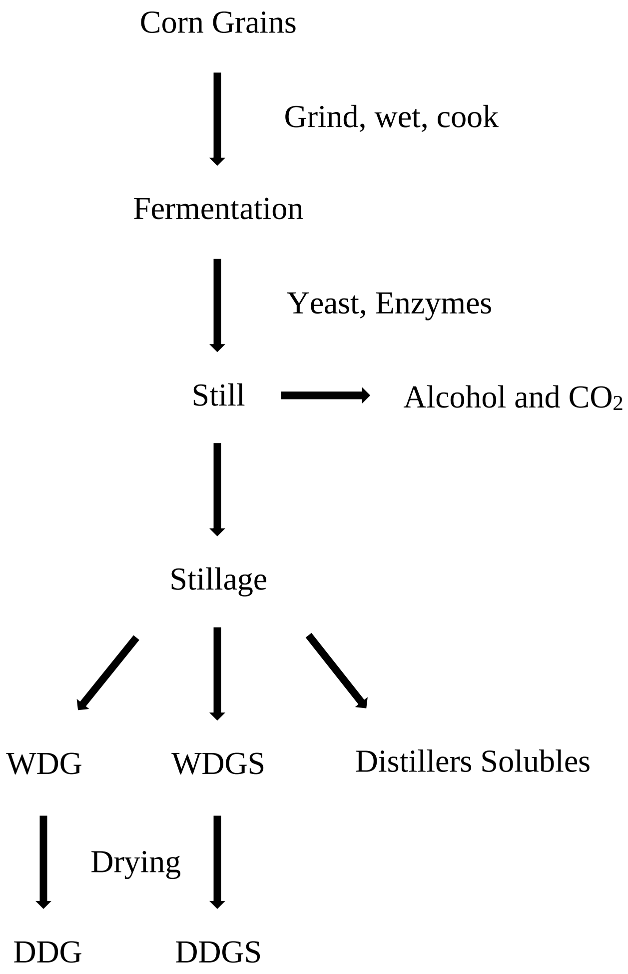

Figure 2.3. Feed products produced from the ethanol dry milling industry. WDG: wet distillers grains; DDG: dried distillers grains, WDGS: wet distillers grains with solubles; DDGS: dried distillers grains with solubles. Adapted from Klopfenstein et al. (2007).

Distillers grains are nutrient dense products (Table 2.1). Corn consists of two-thirds starch on a DM basis, prior to being processed to produce ethanol (Stock et al., 2000). As the starch and water are removed during processing, the concentrations of fiber, fat, protein, and phosphorus increase 3-fold. This high level of protein is primarily RUP, as high as 55% of CP on a DMB (Schingoethe, 2004). The increase in fat could help promote growth, potentially allowing the animal to reach an acceptable BW to successfully breed. Similarly, the increase in phosphorus could also promote growth, as phosphorus is a major component of bone. This concentration of nutrients in DDG make it a valuable feed source.

Table 2.1. Nutrient concentrations of corn gluten meal, distillers solubles, and dried distillers grains with solubles. Adapted from NRC (2016).

Component[1]	Corn gluten meal	Distillers solubles	DDGS[2]
DM, %	90.4 ± 1.54	30.89 ± 6.02	89.99 ± 2.07
Ash, % DM	2.91 ± 0.95	9.11 ± 1.72	5.32 ± 0.88
TDN, % DM	87.8 ± 3.14	98.0 ± 7.79	89.0 ± 4.48
NEm, Mcal/kg	2.17	2.47	2.21
NEg, Mcal/kg	1.49	1.74	1.52
Starch, % DM	15.42 ± 3.47	10.68 ± 5.46	5.88 ± 2.43
Fat, % DM	2.40 ± 0.93	16.58 ± 5.00	10.73 ± 2.05
NDF, % DM	8.07 ± 6.16	4.71 ± 2.74	33.66 ± 3.51
ADF, % DM	4.81 ± 2.80	3.81 ± 2.14	16.17 ± 3.15
CP, % DM	68.21 ± 9.92	18.94 ± 4.92	30.79 ± 2.67
RDP, % CP	29.99 ± 21.81		32.00 ± 6.26
RUP, % CP	69.72 ± 21.66		67.93 ± 6.27
Soluble CP, %CP	14.74 ± 14.05	43.64 ± 28.99	16.53 ± 4.73
ADICP, % DM	15.08 ± 11.53	10.40 ± 6.39	27.85 ± 12.58
Ca, % DM	0.04 ± 0.04	0.11 ± 0.07	0.05 ± 0.04
P, % DM	0.55 ± 0.15	1.52 ± 0.35	0.86 ± 0.11
K, % DM	0.26 ± 0.23	2.34 ± 0.58	1.05 ± 0.18
S, % DM	0.82 ± 0.11	0.82 ± 0.30	0.66 ± 0.16

[1] DM: dry matter; TDN: total digestible nutrients; NEm: net energy of maintenance; NEg: net energy of gain; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; RDP: rumen degradable protein; RUP: rumen undegradable protein: ADICP: acid detergent insoluble crude protein.

[2] Dried distillers grains with solubles.

Due to the nutrient concentrations of DDG products, they can be fed to growing animals. Heifers consuming a low-quality forage and supplemented with DDGS had increased ADG and decreases in dry matter intake (DMI) with increasing amounts of DDGS (Morris et al., 2005). Heifers supplemented with DDGS + CDS had less ADG and DMI, but no difference in BW.

When DDG were utilized to develop beef heifers, final BCS, final BW, and ADG did not differ compared to the control supplement which contained dried corn gluten feed, urea, and whole corn germ (Martin et al., 2007). Heifers grazing high-quality forage had significantly increased ADG when supplemented with DDG compared to corn gluten meal and corn oil (MacDonald et al., 2006). Yearling beef heifers fed a DDGS supplement had increased body weight gains, no difference in blood glucose concentrations, and minimal impact on rumen production and fermentation compared to heifers supplemented with blood and fish meal (McFarlane et al., 2017). When the value of dry distillers grains were investigated while heifers were consuming a high-forage diet, heifers on DDG compared to two other supplements (dry-rolled corn and corn gluten meal) were more feed efficient and had greater gains (Loy et al., 2003).

Dried distillers grains products have also been shown to positively impact reproduction. This was shown in the heifer group that was supplemented with DDG compared to the group supplemented with dried corn gluten feed, urea, and whole corn germ (Martin et al., 2007). Pregnancy rates did not differ between these supplement groups, however, a greater percentage of DDG heifers conceived to AI compared to control heifers (75.0 vs. 52.9%). The heifers on the DDG supplement consumed 267 g RUP/animal/d on average while the other supplement group consumed only 90 g RUP/animal/d on average. There is a possibility that the increase in RUP consumption caused the increase in AI conception rates, and our study has the potential to yield similar results.

While DDGS can effectively be used as a supplemental feed for beef cattle, there are precautions that must be taken. Sulfuric acid is used in distillers grains products to manage fermenter pH levels, which gives the products the opportunity to be both high and also variable in the amount of sulfur (S) they contain (Crawford, 2007). While S is needed in a ruminant as it is a part of some B-vitamins and S-containing amino acids, S in high amounts can be dangerous. Polioencephalomalacia (PEM) is a condition that can occur in ruminant animals that negatively impacts their neurological functions with clinical signs including ataxia, blindness, and recumbency (Gould, 1998). There are conflicting results as to what mechanism actually causes sulfur induced PEM, but S toxicity is a known cause of PEM (Drewnoski et al., 2014). Ultimately, the S level of the feed, as well as the S level of the water must be tested and considered together to ensure that the animal's S maximum tolerable levels are NOT exceeded.

General Supplementation

In the western United States, cow-calf production is largely dependent on rangelands for forage production. Often these rangelands do not meet the nutrient requirements of livestock production, which is when supplemental feeds may be necessary. The goal of producers is to utilize available forage and minimize the cost of these supplemental feeds, while still providing the animals with adequate nutrients and maintaining production (DelCurto et al., 2000). Minimizing the cost of supplements is dependent upon reducing variation of individual animal intake and meeting target supplement intake (Bowman and Sowell, 1997). Supplementation must be strategically managed to best achieve these goals.

Supplements can be delivered in a variety of forms; liquids, loose, cubes, pellets, and blocks, and can be fed either by hand or self-fed (Bowman and Sowell, 1997). Supplements are often delivered in self-fed form. Self-feeding supplementation has been thought to have resulted from the lack of labor during and around the time of World War II (Riggs et al., 1953). These self-fed supplements are still common today. They come in various forms and can be composed of various nutrients. Both the number of non-consumer animals and the variation between individual animal intake are increased by these self-fed supplements (Bowman and Sowell, 1997). Nevertheless, strategic use of self-fed supplements can improve grazing uniformity compared to hand-fed supplements (Bailey and Jensen, 2008), and because utilizing available forage resources is an integral factor in the goal of producers, self-fed supplements may be necessary. However, there is limited information of both comparing hand-fed and self-fed supplements, as well as information on supplement intake behavior. Ultimately, because of these reasons, it is important to measure supplement intake, as well as supplement intake behavior while animals are grazing and compare the impacts of hand-fed versus self-fed supplements to best address the impacts of supplementation.

Protein Supplements

Protein requirements differ based on animal age and stage of production. The protein that is required at certain time points in the year must also be considered with the amount of protein in the available forage. This information is needed to know the appropriate amount of protein, if any, that needs to be supplemented. Lactating and growing animals are the only groups that microbial protein won’t fulfill their requirements when on a high-quality diet and would need supplemental protein (Van Soest, 1982). Although rangelands are of the highest quality in the spring, cows are lactating and heifers are still growing, which would require them to need supplemental protein. As the growing season progresses, forage quality begins to decline. As previously mentioned, the arid environment of the western United States causes rangeland and forages to often be low in protein (DelCurto et al., 2000). As the forage quality decreases, the supplemental protein is of more importance as the forage is likely to be deficient in protein.

Energy Supplements

Cattle on forage diets may not have adequate amounts of energy to achieve targeted animal performance, which is why it may be necessary to provide energy supplements (Moore et al., 1999). Similar to protein requirements, energy requirements differ based on age, breed, stage of production, ambient temperature and other factors. However, energy supplements that are high in non-structural carbohydrates, such as cereal grains, are traditionally used to supplement such cattle on low-quality forages, which can decrease intake and digestibility (Sanson et al., 1990; Bowman et al., 2004). Cattle consuming low-quality forages usually show an increase in performance only when offered energy supplements are in balance with other nutrients (Kunkle et al., 2000), namely protein. As a rule of thumb, when the ratio of total digestible nutrients (TDN) to CP (TDN:CP) is less than 8, there is a balance between TDN and CP, meaning there is enough protein to match the energy that is in the forage (Moore et al., 1991). In contrast, high ratios above 8 imply that there is a protein deficiency in relation to energy (Moore et al., 1991) and animals would not benefit from an energy supplement, but they would benefit from a protein supplement. Thus, if a producer’s goal is to optimize a forage that is low in quality and high in fiber, energy supplementation is discouraged (DelCurto et al., 2000). Low-quality forage is common in the arid environment of the western U.S., which is why protein supplementation is common and profitable.

Frequency of Supplementation

Protein and energy supplementation differ in the frequency at which they can be offered. The information surrounding the frequency of protein supplementation points to infrequent supplementation not having negative impacts on cattle performance (Pope et al., 1963; Wallace, 1988). Infrequent feeding compared to daily feeding may decrease the interruptions in grazing patterns by supplementation. Less frequent supplementation can decrease the variation in supplement and forage intake, as well as the changes in live body weight (Huston et al., 1999). Other benefits of less-frequent supplementation include less labor costs, as well as less competition because of larger quantities of supplement provided at a time (DelCurto et al., 2000). Being able to supplement protein infrequently also highlights the importance of protein recycling in ruminant animals.

Unlike protein, energy supplements should be fed daily instead of less-frequently. Replacement heifers offered a low-starch energy supplement daily compared to three-times per week had increased reproductive development (Cooke et al., 2008; Moriel et al., 2012). These results may be due to a reduction in daily variation of circulating hormones and nutrients. Energy supplements that are high in starch should also be fed daily, as inconsistent feeding can lead to decreases in rumen pH and digestion due to being fed a larger amount of supplement per feeding (Kunkle et al., 2000). Frequency of supplementation can cause negative impacts, which is why self-fed supplements can serve as a beneficial alternative.

Rumen Degradable and Rumen Undegradable Protein

The protein required by beef cattle can be separated into two specific requirements; the protein need for rumen microorganisms, and the need of the individual animal (Eisemann et al., 2016). Rumen degradable protein provides the microorganisms in the rumen with a source of N and is required for the synthesis of MCP. Rumen undegradable protein is not hydrolyzed upon entering the rumen and is digested and absorbed in the small intestine. Rumen degradable protein requirements to satisfy the microorganisms must be met before an addition of RUP may elicit a response (Klopfenstein, 1996). This aligns with the concept of the First Limiting Nutrient where there will not be a response to supplementing a nutrient other than the one that is first limited (Klopfenstein, 1996). Low-quality forages are often limited in the protein that can be degraded in the rumen, which results in a positive relationship of supplementation of RDP and forage utilization (Köster et al., 1996). However, forages that are actively growing contain protein that is degradable in the rumen; therefore, cattle consuming these forages will respond to additional RUP supplementation (Klopfenstein, 1996). We may observe both responses in our study, as early in the growing season when heifers and cows are grazing, the RUP group may respond best, while later in the season when forage quality is declining, the RDP group may respond best.

Impacts of RDP and RUP

Microbial Efficiency. Feeding ruminants forages that are deficient in CP can have implications on insufficient N available for the rumen microbes to be able to grow and develop (Hannah et al., 1991; Köster et al., 1996). A decline in microbial activity will ultimately lead to a decrease in rumen fermentation and the N available for the animal to absorb in the small intestine. Low quality forages are common throughout Montana and the western United States. Low precipitation coupled with shortened growing seasons result in rangelands that can be low in protein. The implications of animals grazing rangelands low in protein may be severe, which is why supplemental CP, and specifically RDP may result in increased performance. A positive relationship exists between RDP supplementation and forage utilization (Köster et al., 1996). The N source that RDP provides rumen microorganisms allows the microbes to grow (Wickersham et al., 2008). This increase in microbial activity increases microbial N flow to the small intestine (Scott and Hibberd, 1990). Forage OM intake increases with an increase of RDP

(Wickersham et al., 2008). Rumen degradable intake protein also increased total NDF and OM digestibilities, N intake, fecal and urinary N excretion, N absorption and retention and microbial efficiency. It is possible that we will see effects of RDP supplementation on microbial populations and activity.

Production Stage. The production stage of an animal dictates their nutrient needs. Therefore, growing cattle require RUP in their diet, while cattle at or near maintenance can sufficiently satisfy their needs with MCP (Klopfenstein, 1996). Heifers that were supplemented with 50% RUP had greater pregnancy rates compared to heifers supplemented with 36% RUP or those developed in the drylot (Mulliniks et al., 2013). Additionally, heifers supplemented with 50% RUP remaining in the herd after the third and fourth breeding seasons, compared to the other two treatments. They concluded that increasing RUP concentrations increases the metabolizable protein supply to heifers, increasing pregnancy success, retention within the cowherd, and ultimately profitability (Mulliniks et al., 2013).

Requirements of cows vary, just as the requirements of heifers do as stages of production change. Beef cattle do not reach their mature body weight until age 5 (American Angus Association, 2013), meaning our young cows still have a growth requirement. During this time, they are also gestating as well as lactating and supporting the calf at side. Because milk is approximately one-third protein on a dry matter basis, lactating cows will have an increase in protein requirements (Van Soest, 1982). This increase in protein requirements could benefit from early summer protein supplementation while they are lactating. The variation in requirements in production stage must also be considered with changes in forage quality throughout the growing season, and how to strategically target the need for supplemental protein.

Digestion. When lambs were fed a low-quality forage and supplemented to meet RDP requirements, or with 50,100, or 150% of RUP requirements; NDF and ADF intakes were not impacted by protein type but tended to increase with increasing amounts of RUP (Atkinson et al., 2007). Intakes of total OM and N were similar for RDP and 100% RUP supplemented lambs and increased with increasing levels of RUP supplementation. True ruminal OM, ADF, and NDF and ADF digestibilities were not impacted by protein degradability. True N ruminal digestibility was greater for RDP supplemented lambs compared to lambs supplemented to meet 100% of RUP requirements (Atkinson et al., 2007). Total tract OM, NDF, and ADF digestibilities were greater, however, for lambs supplemented to meet 100% of RUP, compared to RDP supplemented lambs. These results indicate that RUP supplemented lambs were recycling N to make up for their RDP deficiency (Atkinson et al., 2007). In addition, digestibility of ADF and OM intakes were not impacted by either RDP or RUP supplementation when first calf heifers were supplemented with RDP or RUP postpartum (Anderson et al., 2001). Nitrogen digestibility was increased by RDP supplementation, with N intake being higher for both RDP and RUP supplemented groups. Similarly, steers not supplemented or supplemented with low, medium, and high levels of RUP had no differences in forage OM intake (Reed et al., 2007). Due to ruminants' abilities to recycle N, it is possible that there will be no difference in intake or fiber digestibility in animals consuming RUP supplementation compared to RDP supplementation.

In contrast, Pina et al. (2009) observed that level of RUP supplementation did not impact DMI, digestibility of DM, OM, NDFcp, or TDN when Nellore heifers were fed RUP at either 25 or 40% of CP (Pina et al., 2009). Periparturient cows supplemented with RUP compared to a control, non-supplemented group had greater total OM intake during gestation (Sletmoen-Olson et al., 2000b). Control cows also had lower OM and NDF digestibility, with no differences in these two categories among supplemented groups. Because the supplements had the same amounts of RDP, this suggests that control cows may have been RDP deficient. These results indicate that supplementing animals consuming low-quality cool-season hay can be beneficial to fiber digestion. There is a potential that supplementing beef cows with RDP vs RUP will have differing results, as previous supplementation in ruminants is conflicting.

Performance. Although fiber and protein digestion has varied based on previous research, growth and performance may be altered depending on the protein source. First calf heifers supplemented with RDP postpartum had greater weight change compared to cows not supplemented, with RDP having the greatest impact in the first 30 d compared to all 60 d (Anderson et al., 2001). However, supplementing additional RUP postpartum did not impact weight. Similarly, RDP supplementation increased BCS and RUP did not (Anderson et al., 2001). In a consecutive study, RUP supplementation decreased cow weight loss postpartum with RDP not impacting weight change, and BCS were similar between groups (Anderson et al., 2001). Nursing calf weights were not impacted by supplementation.

Non-supplemented control periparturient cows compared with supplemented RUP cows had lower BW for most of the study, while the supplemented groups did not differ in BW (Sletmoen-Olson et al., 2000b). In terms of BCS, there were no treatment differences in BCS during months 7 and 8 of gestation or months 1 and 2 of lactation. In the last month of gestation and month 3 of lactation, the control cows did have lower BCS. The medium and high RUP supplemented groups increased their BCS during gestation. These results indicate that supplementing animals consuming low-quality cool-season hay can be beneficial to cow condition. There is a potential that cows grazing low-quality cool-season forages in late summer supplemented with RUP could have similar impacts.

Wiley et al. (1991) found that 2-year-old heifers supplemented with RUP compared to RDP postpartum and before their second breeding had higher gains compared to those supplemented with only RDP. An increase in amount of RUP in 2-year-old heifers tended to increase their ADG; however, BCS did not show much of a response to an increase in amount of protein (Rusche et al., 1993). These increases in gain as well as the decrease in weight loss could be due to the excess protein from the higher levels of RUP being available to support tissue growth.

Blood Metabolites. Contents of the blood such as metabolites and hormone levels can help indicate both the nutritional and physiological status of an animal (Sletmoen-Olson et al., 2000a). To identify the impacts of RUP supplementation on this, cows either received no supplement, or one of three increasing levels of RUP supplement that all contained the same amount of RDP. They were offered low-quality prairie hay (5.8% CP). The low level of RUP supplement cows had higher plasma glucose for the majority of the study compared to the medium and high levels, which they concluded may be due to increased gluconeogenic precursors. The medium RUP had lower plasma glucose levels compared to the higher RUP supplemented cows. In general, levels of plasma glucose decreased during the last 100 d of gestation, which can be attributed to exponential fetus growth, and then increased during lactation. These results indicate that supplementing protein to animals consuming low-quality forage results in an increase in blood glucose, indicating a positive impact to the animal’s nutritional status.

Similarly, an increase in amount of RUP in 2-year-old heifers decreased plasma glucose levels, although an increase in overall protein tended to increase glucose levels (Rusche et al., 1993). This reduction of plasma glucose in the animals fed high levels of escape protein may be due to increased lactose in the milk. In contrast, steers supplemented with low, medium, and high levels of RUP had increased levels of blood glucose compared to the non-supplemented control group (Reed et al., 2007). Additionally, medium and high RUP supplemented groups had higher blood glucose levels compared to the low level of RUP supplemented steers. Furthermore, Wiley et al. (1991) found that 2-year-old heifers supplemented with RUP compared to RDP postpartum and before their second breeding had no differences in glucose concentrations between treatment groups. Based on these results, we would expect to see an increase in blood glucose levels among animals that are consuming are large amount of RUP supplement, however we may not see differences in RUP vs RDP supplemented animals.

In addition to blood glucose levels, blood urea nitrogen (BUN) may be impacted by RUP supplementation. Range beef cows fed medium-quality hay and a 50% RUP supplement prior to breeding had less weight loss compared to cows fed only 25% RUP supplement (Dhuyvetter et al., 1993). An increase in BUN was observed in the 50% RUP supplemented cows. These results indicate that excess protein is being broken down and deaminated.

Insulin-like growth factor-1 (IGF-1) is a chemical mediator that is important in mammalian reproduction and has been shown to be needed for ovulation, luteal function, embryo survival, and normal estrous cycles (Velazquez et al., 2008). This factor also signals to the reproductive axis the nutritional status of the animal (Zulu et al., 2002), therefore differences in nutrition could impact IGF-1 concentrations, and therefore impact reproduction. Dairy cows fed diets that differed in protein degradability, low metabolizable protein and high metabolizable protein, had no differences in IGF-1 concentrations (Blouin et al., 2002). Similarly, IGF-1 concentrations were not impacted by level of RUP supplementation in puberal dairy heifers (Silva et al., 2018).

Progesterone is a steroid hormone produced by the corpora lutea, which is formed in place of an ovulated dominant follicle (Senger, 1999). Presence of normal estrous cycles are determined by progesterone concentrations ≥ 1 ng · mL^{-1} (Kane et al., 2004). Heifers supplemented to meet RDP requirements and 30%, 38%, and 46% RUP of CP had no impacts on progesterone concentrations (Kane et al., 2004). Similarly, puberal dairy heifers fed RUP at 38, 44, 51 and 57% of CP, had no impacts of treatment on progesterone concentrations (Silva et al., 2018). High intakes of RDP associated with inadequate intakes of energy may cause reduced reproductive efficiency (Kenny et al., 2002). However, RDP supplemented with or without fermentable energy had no impacts on progesterone concentrations. Therefore, it is unlikely that RUP or RDP supplementation would impact progesterone concentrations.

Distillers Grains and RUP

Distillers grains plus solubles serve as an adequate source of RUP, digestible fiber, minerals, and fat to ruminant animals (Cao et al., 2009). The concentration of RUP in DDGS can vary based on sources and processing. Castillo-Lopez et al. (2013) estimated DDGS RUP to average 63.0 ± 0.64%. This value is greater than that found by Kelzer et al. (2010), who determined DDGS RUP was 56.3% when fed to dairy cattle. Kleinschmit et al. (2007) suggested that this difference of RUP in DDGS could be due to the heat application during the drying step in processing. The heat has the potential to reduce the protein that is available to be degraded in the rumen, which can vary by processing plant. The addition of CDS in increasing amounts within DDG did not affect the RUP concentration (Cao et al., 2009). In contrast, with increasing levels of CDS in DDG, RDP increased.

RUP and Reproduction

The economic success of a cow-calf operation depends in large part on the reproductive efficiency of the operation (Rusche et al., 1993). Supplementing young beef cows with RUP prepartum may enhance reproduction (Engel et al., 2008). Dried distillers grains plus solubles supplemented heifers had greater final pregnancy rates compared to the soybean hull supplemented heifers. (Engel et al., 2008). This could be attributed to multiple factors, including a MCP deficiency with the soybean hulls (SBH) supplement, or the greater amount of fat in the DDGS. Similarly, Wiley et al. (1991) found that 2-year-old heifers supplemented with RUP compared to RDP postpartum and before their second breeding had a greater percentage of heifers bred in the first 21 days of the breeding season. Likewise, 2-year-old heifers and mature cows receiving a medium (56.27%) RUP compared to a low (29.13%) RUP had greater first-service conception rates (Triplett et al., 1995). Medium and high (75.60%) RUP groups tended to have higher overall pregnancy rates than the low group, as well. It is possible that we could witness the similar increases in reproductive efficiency in heifers during their first breeding season as observed in the second and consecutive breeding seasons (Wiley et al., 1991; Triplett et al., 1995).

In contrast, range beef cows fed medium-quality hay and a 25% or 50% RUP supplement prior to breeding had no differences in the percentage of cows serviced in the first 21 days of the breeding season and subsequent pregnancy rates did not differ between the supplement groups

(Dhuyvetter et al., 1993). Similarly, an increase in amount of RUP in limit-fed 2-year-old heifers had no impacts on luteinizing hormone (LH) or progesterone levels. (Rusche et al., 1993). There was also no statistical difference found between conception rates between groups. Because of this, it can be concluded that when quantity of forage is limiting, reproduction can still be maintained when additional CP or sources of RUP are fed.

Intake Variation

Intake and consumption of feed is critical to determine the amount of nutrients consumed and available to the animal (Van Soest, 1982). Variation in individual supplement intake can potentially impact an individual animal's nutrient status and subsequent performance (Wyffels et al., 2020). Knowledge of this variation in intake and its impacts are needed to develop a cost-efficient program for developing heifers (Wyffels et al., 2020) and for appropriate supplementation of the cow herd.

Bowman and Sowell (1997) reviewed differing methods of supplement delivery and how that impacted intake while animals are grazing. The form of a supplement can have a major impact on consumption levels. Self-fed supplements such as liquid and blocks should decrease the number of non-feeders (Bowman and Sowell, 1997). Coombe and Mulholland (1983) found that when sheep were supplemented with blocks, liquid, and lick tanks, the targeted intake was never met with sheep consuming the block supplement. However, the total number of non-feeders was less for those consuming the block (2.5%) compared to those consuming the liquid supplement (22.5%) or the lick tanks (30%). Kendall et al. (1980) found similar results, as there was a trend in increased variation of intake with supplement in block form compared to cubed form fed in troughs.

Other supplement factors can impact individual intake such as supplement characteristics, number of animals, dominance, and familiarity with delivery devices. The nitrogen content or hardness can impact supplement intake variation (Bowman and Sowell, 1997). Intake of a DDGS supplement decreased as the hardness of the supplement increased (Zhu et al., 1991). Intake variation tends to be lower in animals that are fed in groups even though they consume less feed compared to individually fed animals (Bowman and Sowell, 1997). More dominant animals will prevent other animals from consuming supplement. The hierarchies and social dominance within groups of cattle play a significant role in how rangeland cattle consume supplement (Sowell et al., 1999). Animals that are unfamiliar with supplement delivery equipment may have increased variation (Chapple et al., 1987). The variation that can exist among individual animals can explain the variation in response each animal has to the supplement (Bowman and Sowell, 1997). An example of this being that there may be a relationship between cattle BCS and supplement intake, as animals in lower condition consume more compared to animals with higher condition (Wyffels, 2019). There is a potential that we could observe similar results with animals in lower condition may consume more supplement.

Salt as an Intake Limiter

Because of the high cost of supplements and the variation in self-fed supplement intake, ingredients are often added to supplements to try to limit intake. Intake-limiters are used to reduce the number of animals over consuming the supplement. Salt is the most often used intake limiter because it is available and usually safe (Kunkle et al., 2000). Salt has been shown to be effective as a self-intake limiter (Riggs et al., 1953; Schauer et al., 2004). However, salt-limited supplements have been shown to have a high intake variation across individual animals, within specific time periods, and between years (White et al., 2019b).

Level of salt within supplements can have variable impacts on beef cattle. Steers consuming a fescue hay diet had no impacts of level of salt within the supplement on feed efficiency or weights gains but steers consuming silage and high levels of salt had decreases in both feed efficiency and gains (Harvey et al., 1986). We would expect that animals consuming large amounts of the salt-limited supplement would respond similar to steers that were on the fescue diet and not decrease BW gains, as they consumed cool season grasses, as well. High levels of salt within supplements have been shown to reduce rumen VFA and ammonia concentrations and increase liquid digesta flow rates in steers (Harvey et al., 1986). Similarly, beef cattle grazing tropical pastures and supplemented with a 30% salt-limited supplemented showed a depression in overall VFA production (Chicco et al., 1971). Increases in salt fed to beef cows did not impact total VFAs, however, there was an increase in acetate molar concentrations, as well as the acetate:propionate ratio (White et al., 2019a). In addition, isobutyrate and butyrate levels and ammonia concentrations decreased as salt levels increased (White et al., 2019a). There is a potential that individuals consuming large amounts of the salt-limited supplement will also exhibit differences in VFA and ammonia concentrations.

Measuring Intake

As previously mentioned, intake variation exists between individual animals. Previously, it has been difficult to get accurate measures of supplement consumed by individual animals. Several techniques exist, one such being using digestive flow markers (Schauer et al., 2005). Limitations of using a digestive flow marker include not being able to sample all animals in the experiment and only provides an estimate of the amount of supplement consumed by each animal. Self-fed and hand-fed supplements have previously been measured by estimating disappearance (Lardy and Kerley, 1994; Sowell et al., 2003). When measured in a herd, disappearance does not measure individual animal intake and when disappearance is measured when individuals are hand-fed supplements, it is not possible to observe feeding patterns.

Recent technological developments allow for better measurement of individual intake. One such system is an electronic radio-frequency-identification-based system that measures feeding behavior and intake (GrowSafe System Ltd., Airdrie, Alberta, Canada). An electronic identification tag (EID tag) is placed in an animal’s ear and is read when the animals eat out of the GrowSafe bunk, which records bunk visits, frequency, duration, and intake. This system has been validated to report these values accurately measured (Mendes et al., 2011). However, there are limitations to the GrowSafe system.

Limitations of the GrowSafe system including the inability to measure intake while animals are grazing on pasture, limiting supplement intake, and non-supplemented animals. Animals on different supplements must be maintained in different pastures, which may influence supplement intake and grazing behavior. Non-supplemented animals cannot be housed with supplemented animals because there isn’t an available mechanism to stop animals from entering the GrowSafe feed bunk. Additionally, if supplement intake needs to be limited to a specific amount, GrowSafe bunks cannot accomplish this. However, GrowSafe technology is highly effective in feedlot environments, where individual intake may be measured in a pen setting, which adds statistical power to the study.

SmartFeed Pro trailers (C-Lock, Inc., Rapid City, SD) offer a solution to these problems. These trailers are mobile units that also read EID tags, measure number and duration of visits, as well as intake. One distinct advantage the SmartFeed Pro system has with four separate feed bunks per trailer, animals can be assigned a specific treatment and be allowed or restricted access to certain bunks based on their programmable EID tag. The ability to control and measure individual animal intake can enhance design flexibility (Reuter et al., 2016). The SmartFeed systems have been used successfully to measure individual supplement intake (Williams et al., 2016; Wyffels et al., 2018). Additionally, this technology allows for multiple treatments to be applied in a single pasture, which removes the potential for a pasture effect in those studies.

Cow Age and Intake

Age of cattle can have an impact on supplement intake variation. Previously, older cows consumed more supplement (Bowman et al., 1999; Sowell et al., 2003; Kincheloe et al., 2004) and have more visits to the supplement feeder compared to younger cows. This is inconsistent, though, as feeding time, intake rate, and total daily supplement intake decreased as the age of cows increased (Wyffels et al., 2018). In a consecutive year, intake rate and visits per day decreased with increasing cow age and no relationship between cow age and supplement intake (Wyffels, 2019). These results may be conflicting due to differences in nutrient delivery method. Supplementing different age groups of cattle results in variations in supplement intake between groups. It is important to be mindful of this variation when choosing how to supplement cattle in both separate and mixed age groups.

Rationale for Research

Cow-calf production is important in Montana and the western United States, resulting in the development of heifers for replacement also being important. The large range resources of the West and the arid environments of the region emphasize the important of developing these replacements on forage-based diets that have the potential to be low-quality at certain points in the year. In order for these replacement heifers to successfully enter and remain in the cowherd, there are certain parameters they must meet. They need to breed early in the breeding season, calve at the age of two (Bagley, 1993), and breed efficiently to optimize economic inputs (Engelken, 2008). To ensure this, heifers must have a BW of 53-60% of their MBW prior to their first breeding (Patterson et al., 1992; Funston and Deutscher, 2004), and maintain a BCS of 5.5-6 once pregnant (Engelken, 2008) which are both closely dependent on nutrition. The need for supplemental inputs, namely protein, for grazing cattle and those consuming low-quality forage has been recognized (DelCurto et al., 2000). This need is especially important when considering developing these replacement females in the western United States.

Rumen environment including microorganisms, VFAs, ammonia, and pH are important to the successful development and maintenance of ruminant animals. Volatile fatty acids are the main energy sources for ruminants (Van Houtert, 1993). The microorganisms within the rumen utilize protein within the rumen to grow (Wickersham et al., 2008). An increase in growth of these microbes will cause an increase in VFA production (Köster et al., 1996). Ammonia in the rumen is produced by RDP being broken down into peptides and amino acids, which are then deaminated into ammonia (Millen et al., 2016). Ammonia, in turn, can increase the efficiency of microbial growth (Mackie and White, 1990). Rumen conditions must be kept at certain levels to optimize microbial growth and metabolism, and a decrease in pH can have impacts on fermentation (Van Soest, 1982). The pH is also impacted by VFA production. Because the rumen characteristics are intercorrelated and dependent upon one another, it is important to consider these factors and when measuring the effects of supplemental inputs, including protein.

Dried distillers grains plus solubles can be supplemented to heifers (McFarlane et al., 2017). Providing these supplements in a self-fed form can decrease labor costs. However, self-fed supplements can increase the number of non-consumers, as well as the variation between individual animal supplement intake (Bowman and Sowell, 1997). Salt can be used as an effective intake limiter (Schauer et al., 2004), but can also cause high intake variation (White et al., 2019b). Because of these factors, it is important to measure individual animal supplement intake, as well as intake behavior such as intake rate to characterize the impacts of providing these supplemental inputs and if they are cost-effective.

The protein needs of cattle can be identified as either the protein needed by the ruminant's microorganisms and the protein needs of the individual animal (Eisemann et al., 2016). Because of this, protein can be classified as either RDP or RUP. Rumen degradable protein is hydrolyzed in the rumen, while RUP is digested and absorbed in the small intestine. Growing forage contains RDP which is why grazing animals may benefit from RUP supplementation early in the growing season (Klopfenstein, 1996), while low-quality forage is limited in RDP (Köster et al., 1996) and animals consuming such forage may benefit from RDP supplementation.

This information brings into question the impacts of supplementing heifers and cows with RDP vs RUP and our research determines the impacts on nutrient digestion, rumen kinetics, supplement intake, growth, and reproductive parameters to beef cattle consuming low-quality forages. In Chapter 3, we evaluate the impacts of RUP vs RDP salt-limited, self-fed supplement on supplement intake behavior and animal performance with yearling heifers and two and three-year-old cows grazing dryland pastures. In Chapter 4, we evaluate the impacts of RUP vs RDP supplement either in hand-fed, loose form or in salt-limited, press-block form on intake, digestion, and rumen fermentation characteristics with beef cows consuming low-quality forage diets. Finally, in Chapter 5 we offer a complete conclusion of our research and discuss how our research will support further research of strategic supplementation of beef cattle on low-quality forage diets or grazing rangelands in the western United States.

CHAPTER THREE

IMPACTS OF RUMEN DEGRADABLE OR RUMEN UNDEGRADABLE PROTEIN SUPPLEMENT ON SUPPLEMENT INTAKE BEHAVIOR, PERFORMANCE, AND REPRODUCTIVE PARAMETERS WITH YEARLING HEIFERS AND COWS GRAZING DRYLAND PASTURES

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Manuscript Information

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Journal of Animal Science

Status of Manuscript:
_X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-reviewed journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

Running head: Supplement intake behavior and performance

Impacts of rumen degradable or rumen undegradable protein supplement on supplement intake behavior, performance, and pregnancy success with yearling heifers and cows grazing dryland pastures[1]

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[1] The authors would like to thank SweetPro LLC for the funding of this project and for the products used in this project. Appreciation is also expressed to the Nancy Cameron Endowment, and to the employees of MSU BART Farm for their assistance with this project.

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ABSTRACT: The objectives of this study were to evaluate the differences between rumen degradable protein (RDP) and rumen undegradable protein (RUP) on supplement intake behavior, animal performance, and reproductive parameters. Angus and Red Angus-based yearling heifers (n = 40) were used in a complete randomized design and two-year old cows (n = 36) and three-year old cows (n = 24) were used in a randomized complete block design and stratified by weight and body condition score and within stratum randomly assigned to two treatments: 1) pressed supplement block containing RUP (RUP), and 2) pressed supplement block containing RDP (RDP). Individual supplement intake variables were measured using the SmartFeed Pro trailers (C-Lock Inc., Rapid City, SD). Body weight (BW) and body condition score (BCS) were taken on days 0 and 84 following a 16 hour shrink. Statistical analysis was completed in R. Heifer and cow supplement intake displayed ($P < 0.01$) a treatment × period interaction. The RUP heifers and RDP cows consumed more in Periods 2 than Period 1, whereas RDP heifers and RUP cows consumed more in Period 1 than Period 2. Intake rate demonstrated ($P < 0.01$) a treatment effect for heifers, with RUP consuming supplement faster compared to the RDP treatment in both periods. Intake rate for cows demonstrated a ($P < 0.01$) a treatment × period interaction with RUP cows in Period 1 have faster intakes than Period 2, and RDP cows having the inverse. Cow intake variation also displayed ($P < 0.01$) a treatment × period interaction with RUP cows having more variation in Period 2, while RDP cows had less variation in intake in Period 2. In terms of performance, there was no difference ($P \geq 0.40$) in heifer or cow initial and final BW or BCS. There was no difference ($P \geq 0.23$) in heifer ADG or final pregnancy rates. Cows supplemented with RDP had greater ($P < 0.01$) ADG than RUP supplemented cows. Final pregnancy rates of RUP supplemented cows were greater ($P < 0.05$)

compared to RDP supplemented cows. In conclusion, RDP and RUP impacted intake behavior of cows and heifers but had minimal impacts on performance or reproductive parameters.

Key words: Beef cattle, supplement intake behavior, self-fed supplement, RUP, RDP

INTRODUCTION

Beef cattle production is important in the western United States, and because of the integral role replacement heifers play, their development is important to ranching systems. The arid environment of the western United States causes forages to have seasonal deficiencies and often be low in protein (DelCurto et al., 2000), which is why supplementing heifers to meet their growth requirements may be necessary. Furthermore, retaining these heifers within the cowherd and supporting them as they continue to grow as well as during lactation and gestation is a challenge and may also require supplemental protein. Producers who depend on forages must optimize their cattle productivity while minimizing input costs. Self-fed salt-limited supplements are often used to offset seasonal nutrient deficiencies and increase both animal performance and forage intake (Bowman and Sowell, 1997).

The economic success of a cow-calf operation depends in large part on the reproductive efficiency of the operation (Rusche et al., 1993). Supplementing young beef cows with rumen undegradable protein (RUP) prepartum may enhance reproduction (Engel et al., 2008). Range beef cows fed medium-quality hay and a 50% RUP supplement prior to breeding had less weight loss prebreeding compared to cows fed only 25% RUP supplement (Dhuyvetter et al., 1993). Two-year old heifers and mature cows receiving a medium RUP (56.27%) compared to a low RUP (29.13%) supplement had greater first-service conception rates (Triplett et al., 1995). Additionally, medium and high RUP (75.60%) supplemented cows tended to have greater overall pregnancy rates than the low RUP cows (Triplett et al., 1995). It is possible that we could observe a similar increase in reproductive efficiency in heifers during their first breeding season as observed in the second and consecutive breeding seasons (Wiley et al., 1991; Triplett et al., 1995).

However, it is important to consider supplement intake behavior when measuring the impacts of RUP vs rumen degradable protein (RDP) supplementation. Intake and consumption of feed is critical to determine the amount of nutrients consumed and available to the animal (Van Soest, 1982). Variation in individual supplement intake can potentially impact an individual animal's nutrient status and subsequent performance (Wyffels, 2019). Knowledge of this variation in intake and its impacts are needed to develop a cost-efficient program for developing heifers (Wyffels, 2019) and for appropriate supplementation of the cow herd. Therefore, the objectives of this study were: 1) to evaluate the differences between RUP and RDP supplementation on supplement intake behavior and animal performance, and 2) to evaluate the differences between RUP and RDP supplementation on reproductive parameters of beef heifers and cows.

MATERIALS AND METHODS

Experimental procedures described herein were approved by the Agriculture Animal Care and Use Committee of Montana State University (#2020-AA05). All animals used in this study were provided by the Montana Agricultural Experiment Station. This study was conducted at Fort Ellis (45°39'36.5"N 110°58'10.9"W) and the Bozeman Agriculture Research and Teaching (BART; 45°39'41.1"N 111°04'24.9"W) farms at Montana State University in Bozeman, MT. The average precipitation is 46.9 cm and of that, snow represents 59.3%. The average temperature is 9.74°C and there are an average of 113 growing season days.

For this study, Angus and Red Angus-based yearling heifers (n = 40) were used in a complete randomized design and two-year old cows (n = 36) and three-year old cows (n = 24) were used in a randomized complete block design and stratified by weight and body condition score, and within stratum randomly assigned to two treatments: 1) pressed supplement block containing rumen undegradable protein (n = 51; RUP), and 2) pressed supplement block containing rumen degradable protein (n = 50; RDP). Animals were housed on pasture from 4 June 2020 to 26 August 2020 for an 84-d supplement intake trial with supplement intake being recorded beginning on d 4. The 84-d trial was separated into two periods: 1) d 4 – d 45, and 2) d 46 – d 84.

Each animal was considered an experimental unit. Animals were equipped with an electronic identification tag (Allflex USA, Inc., Dallas-Ft. Worth, TX) attached to the exterior of their left ear so individual intake of supplement, number and length of visits, and intake rate could be recorded. One SmartFeed Pro trailer (C-Lock Inc., Rapid City, SD) was housed with the heifers and a second trailer was housed with the two and three-year old cows to provide the treatments. The SmartFeed Pro units can limit animal access to the feed bunk, which allowed us to have multiple treatments within a single pasture. Each trailer has four feeding units, resulting in two feeding units to supply the RUP (Table 3.1) and two feeding units to supply the RDP (Table 3.1) treatments within each trailer. Supplements were provided ad libitum to cattle grazing improved dryland pastures.

Blood samples via coccygeal venipuncture were collected 10-d prior to the start of the trial and on d 0 to determine progesterone concentrations for yearling heifers and 3-year-old cows. Body weight (BW) and body condition scores (BCS) were collected at the beginning (d 0) and end (d 84) of the trial following a 16 hour shrink. Animal body condition was judged independently by two observers using a 9-point scale (1 = extremely emaciated, 9 = extremely obese; (Whitman, 1975) with the same technicians measuring body condition at both timepoints. Blood samples collected via coccygeal venipuncture were also collected at the beginning and end of the trial to determine metabolite concentrations. The blood samples were collected in 10 mL red/gray marble-top tubes (Covidien #8881302015) for serum collection. Immediately following blood collection, tubes were placed on ice for approximately 4 hours to allow clotting for serum tubes. Tubes were centrifuged at 2,700 × *g* for 30 minutes at 4°C. Serum samples were decanted into 2 aliquots, frozen at -20°C, and stored for metabolite analysis.

Individual pasture production was measured by clipping a 0.25 m^2 plot at 5 sites prior to animals entering the pasture and following their exit from the pasture. All clipped pasture samples were composited by pasture and pasture enter/exit and sent to a commercial laboratory (Dairy One, Ithaca, NY) for complete proximate analysis and mineral concentrations (Table 3.2 and 3.3).

Although not a primary objective, animal reproductive performance was measured. The Select Synch + CIDR protocol (EAZI-BREED CIDR; 1.38 g progesterone; Zoetis Animal Health, Parssipany, NJ) was used to synchronize estrus followed by heat detection and timed artificial insemination (TAI). Briefly, animals received 100 µg of gonadotropin-releasing hormone (GnRH; Factrel; Zoetis Animal Health., Parssipany, NJ) and administered a CIDR on d

38 of the trial. On Day 45, the CIDR was removed and prostaglandin $F_{2\alpha}$ (PGF; Estrumate; 250 mcg cloprostenol; Merck Animal Health, Madison, NJ) was administered. Concurrent with CIDR removal, aids for detection of estrus (Estrotect; Estrotect Inc., Spring Valley, WI) were applied. Cows and heifers that expressed estrus were artificially inseminated (AI). The remaining females were fixed-time inseminated (TAI) 72 – 84 h post CIDR removal. Bulls were placed with the heifers and cows 1 d following TAI for 62 d. On d 84, 36 days following TAI, pregnancy was determined using transrectal ultrasonography (ReproScan XTC with a 4.0-MHz convex-array transducer; ReproScan, Winterset, IA) and again 83 days following the completion of the study.

Serum was analyzed for progesterone concentrations using radioimmunoassay test kits with ^{125}I-marked progesterone (ImmuChem™ Double Antibody Progesterone ^{125}I RIA Kit; Costa Mesa, CA, USA), in accordance with the instructions of the manufacturer. Cyclicity was determined if progesterone concentrations equaled or exceeded 1.0 ng · mL^{-1} either 10 d prior to the study or on d 0.

For both cow and heifer data, the effects of protein type on animal BW change, BCS change, calf weight, calf ADG were analyzed using an analysis of variance (ANOVA) with a generalized linear model including treatment as a fixed effect. Daily individual supplement intake (g · day^{-1} and g · kg of body $weight^{-1}$ · day^{-1}), intake rate (g · $minute^{-1}$), and the coefficient of variation (%CV) of supplement intake were analyzed using ANOVA with a generalized linear mixed model including treatment, period, and a treatment × period interaction as fixed effects, and individual animal as the random effect. Beef cattle reproduction characteristics eliciting binomial response (e.g., conception rate) were analyzed using generalized linear models following a binomial distribution in an ANOVA framework. Data were plotted and transformed if needed to satisfy assumptions of normality and homogeneity of variance. An alpha ≤ 0.05 was considered significant. Means were separated using the Tukey method when $P < 0.05$. All statistical analyses were performed in R (R Core Team, 2020).

RESULTS

Heifer average daily supplement intake expressed as $g \cdot cow^{-1} \cdot d^{-1}$ and $g \cdot kg\ BW^{-1} \cdot d^{-1}$ both displayed ($P < 0.01$; Table 3.4) a treatment × period interaction. Period intake in $g \cdot cow^{-1} \cdot d^{-1}$ ($P < 0.01$) for the RUP supplemented group was 22% greater in Period 2 compared to Period 1, while RDP supplemented group had 45% less intake in Period 2 than Period 1. Intake in $g \cdot kg\ BW^{-1} \cdot d^{-1}$ increased ($P < 0.01$) 17.5% for the RUP group from Period 1 to Period 2 and decreased 49% in the RDP group from Period 1 to Period 2. Intake rate demonstrated ($P < 0.01$) a treatment effect, with RUP heifers consuming supplement 86% faster compared to the RDP heifers. The CV for heifer intake tended ($P = 0.10$) to display a treatment × period interaction, as the RUP heifers tended to have more variation in Period 1, while RDP heifers tended to have more variation in Period 2.

The yearling heifers on this study had no differences ($P \geq 0.48$; Table 3.5) in initial and final BW and BCS, ADG, or in heifers exhibiting estrus prior to TAI in RUP vs RDP supplemented animals. There was also no difference in heifers cycling prior to the initiation of the trial or final pregnancy rates ($P \geq 0.23$).

Cow average daily supplement intake expressed as $g \cdot cow^{-1} \cdot d^{-1}$ and $g \cdot kg\ BW^{-1} \cdot d^{-1}$ both displayed ($P < 0.01$; Table 3.6) a treatment × period interaction with RDP cows having greater intakes compared to the RUP cows in both periods. In terms of intake in $g \cdot cow^{-1} \cdot d^{-1}$, cows on the RUP treatment consumed ($P < 0.01$) 51% more supplement in Period 1, while cows on the RDP treatment consumed 80% more supplement during Period 2. Intake rate displayed ($P < 0.01$) a treatment × period interaction with RUP animals consuming supplement 20% faster during Period 1, while RDP animals consumed supplement 42% faster during Period 2. Intake CV also displayed ($P < 0.01$) a treatment × period interaction with RUP cows having 9% more variation in Period 2, while RDP cows had 30% less variation in intake in Period 2.

In terms of cow performance, there was no difference ($P \geq 0.71$; Table 3.7) in cow initial and final BW or BCS, nor were there any differences in cows exhibiting estrus prior to TAI. There was a significant difference ($P < 0.01$) in cow ADG in which RDP supplemented cows had greater ADG ($0.75\ \text{kg} \cdot \text{d}^{-1}$) than RUP supplemented cows ($0.56\ \text{kg} \cdot \text{d}^{-1}$). In terms of reproductive parameters, we did not measure estrous cyclicity prior to allotting treatments, therefore we were not able to allot based on cyclicity. There was a difference in final pregnancy rates ($P < 0.05$) as RUP supplemented cows had greater pregnancy rates (100%) compared to RDP supplemented cows (90%). There tended ($P = 0.09$) to be more RDP 3-year-old cows (75%) cycling at the initiation of the trial compared to RUP 3-year-old cows (42%). There was a tendency ($P = 0.10$) for calves of RDP supplemented cows to have greater ADG than calves of RUP supplemented cows.

DISCUSSION

Research regarding individual supplement intake for animals in rangeland and pastures settings have only been reported recently. Mixed aged cows with access to a salt-limited supplement pre-calving during the winter months had variation in individual supplement intake across different forage qualities and quantities, as well as animal age (Wyffels et al., 2018). In the first year, supplement intake decreased as age increased. However, in the second year, the 2- and 3-year-old group consumed more supplement than the yearlings, 4-5, and 6-7-year-olds. Similarly, in the current study, the 2- and 3-year-old cows consumed more supplement than the yearling heifers.

Both White et al. (2019b) and McClain et al. (2020) reported supplemented intake behavior with yearling heifers on the same paddocks as our study in years previous. This has provided additional information on supplement intake variation. Yearling heifers provided salt-limited protein supplements had greater intakes in Period 2, compared to Period 1 (White et al., 2019b). While the cows on our study showed similar supplement intake behavior, the yearling heifers showed the opposite with greater supplement intakes in Period 1 than Period 2. This may be due to the poor-quality pasture that the cows were located on during their third rotation, while the heifers remained on moderate quality pasture during this time frame. In terms of intake rate, yearling heifers that were provided the protein blocks all had decreased intake rates in Period 2 compared to Period 1 (McClain et al., 2020). In the current study, RUP supplemented cows had decreased intake rates in Period 2, while RDP supplemented cows had increased rates. The increased rates of RDP consumption may have been due to the decline in pasture quality later in the study. Additionally, SmartFeed Pro trailers were always available, and animals did not have to travel long distances to consume supplement in the current study. Yearling heifers provided salt-limited protein supplements had decreased variation in supplement intake in Period 2 compared to Period 1 (White et al., 2019b), as did yearling heifers provided protein blocks (McClain et al., 2020). Cows on the current study had mixed supplement intake behavior results, as RUP supplemented cows had increased variation in Period 2 compared to Period 1, while

RDP supplemented cows had decreased variation in Period 2 compared to Period 1. Yearling heifers on the current study had differing results compared to previous research on the same pastures with as RUP heifers had more variation in Period 1, and RDP heifers had more variation in Period 2. In part, this may be due to differences in forage quality compared to the previous studies.

An increase in supplement intake variation can exist among animals that are unfamiliar with the supplement delivery equipment (Chapple et al., 1987). Yearling heifers on the current study were not familiar with the SmartFeed Pro trailers, while the 2- and 3-year-old cows had been exposed to the SmartFeed Pro trailers in previous studies. This may explain the yearling heifers having higher CV values than the cows on this study. Additionally, this may explain the increased intakes of the 2- and 3-year-old cows. Loose supplements may be easier for animals to consume, as yearling heifers grazing the same pastures as the current study had greater intakes when supplement was in a loose or pelleted form compared to blocks (White et al., 2019b; McClain et al., 2020). Therefore, unfamiliarity with pressed supplement blocks and how to efficiently consume them may also explain the variation in supplement intakes in the yearling heifers.

In a study comparing RDP and RUP supplementation to first calf heifers, RDP supplemented postpartum did not impact weight change (Anderson et al., 2001), which was similar to the current study. However, ADG was greater for RDP cows compared to RUP cows in the current study. Additionally, the suckling calf weights in the study were not impacted by supplementation (Anderson et al., 2001), but calf ADG tended to be greater in RDP cows compared to RUP cows in the current study. Although milk quality or quantity was not measured in the current study, milk quality or quantity may have been impacted in the RDP cows, which lead to an increase in ADG of their calves. Two-year old heifers and mature cows receiving greater amounts of RUP tended to have higher pregnancy rates (Triplett et al., 1995). Similarly, in the current study RUP cows had greater pregnancy rates than RDP cows.

There was a potential that the RUP treatment may have increased cyclicity in the cows, leading to the increase in pregnancy rates. An increased in absorption of essential amino acids in the small intestine from RUP supplementation could stimulate reproductive processes. However, the insertion of the CIDRs may have led to anestrus cows returning to estrus, but because we did not measure cyclicity throughout the trial, we are unsure due to lack of sampling. The improved pregnancy rates of the RUP supplemented cows may not be due to increased reproduction efficiency with RUP, but rather a reduction in pregnancy success in the RDP supplemented group compared to the RUP group. Dairy cows supplemented with 50% excess RDP requirements were reported to have an increased early embryonic loss due to a decrease in uterine pH (Elrod and Butler, 1993). Because the early embryo is dependent on the uterine environment for survival (Senger, 1999), an alteration in pH would cause complications and could compromise the pregnancy. However, decreases in uterine pH is not specific to only RDP fed in excess. Both RDP and RUP when fed in excess of 25% has also been shown to decrease uterine pH (Elrod et al., 1993). However, it must be considered that while RUP cows had greater pregnancy rates, RDP cows had acceptable pregnancy rates, as well, and that there was no difference in pregnancy rates of RUP and RDP supplemented heifers. Small sample sizes must be taken into consideration when interpreting pregnancy success data. During this trial, yearling heifers were older than typical yearling heifers at time of first breeding, and cows had greater postpartum intervals due to a change in calving date. This must be considered while interpreting results, as there could be implications. These results must also be considered with the forage quality over the course of the study.

IMPLICATIONS

Our results suggest that salt-limited supplements have high degrees of intake variation, including variation among animals, over periods of time, and between types of protein. Rumen degradable protein supplementation has the potential to increase cow ADG and calf ADG, while RUP supplementation has the potential to increase pregnancy rates. This research will contribute to the efforts to improve strategic supplementation of beef heifers and cows.

Table 3.1. Nutrient analysis of supplement offered ad libitum to yearling, and 2- and 3-year- old cows in Smart Feed Pro Trailers while grazing improved dryland pasture.

	RUP[1]	RDP[1]
DM, %	77.3	86.6
Chemical composition, %DM		
Crude protein	27.8	37.0
ADICP[2]	2.5	1.2
Soluble protein	13	15
Degradable protein	30	63
Acid detergent fiber	6.4	5.5
Total digestible nutrients	57	60
Ca	1.98	2.77
P	2.04	1.91
Mg	2.96	0.8
K	1.84	2.5
Na	6.15	4.3
S	1.23	0.73
Chemical composition, mg · kg^{-1}		
Fe	852	1,060
Zn	1,300	1,530
Cu	848	222
Mn	1,060	726
Mo	1.5	8.1

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Acid detergent insoluble crude protein.

Table 3.2. Heifer pasture (1-5) forage quality and production at the time of entry and exit. Montana State University Fort Ellis research farm.

Rotation	1	2	3	4	5
Production, kg · ha^{-1}					
Enter	1453.9	2906.8	2620.6	2497.3	1572.6
Exit	1280.4	1300.9	1526.1	870.5	1644.2
DM, %	90.7	90.8	91.7	91.3	92.9
Chemical composition, % DM					
CP[1]	14.8	9.0	7.3	8.7	6.4
ADF[2]	35.7	36.0	38.3	38.1	40.8
NDF[3]	58.7	57.9	57.2	54.2	66.2
TDN[4]	61.0	61.0	61.0	62.0	59.0
Ca	0.41	0.42	0.44	0.54	0.51
P	0.41	0.29	0.28	0.23	0.23
Mg	0.11	0.17	0.16	0.18	0.19
K	2.54	2.03	1.79	1.73	1.61
Na	0.011	0.013	0.008	0.006	0.006
Chemical composition, mg · kg^{-1}					
Cu	16	15	16	12	15
Fe	172	139	132	96	82
Mn	51	70	105	85	73
Mo	4.9	1.7	2.1	3.2	3.9
Zn	29	19	15	18	15

[1] Crude protein.

[2] Acid detergent fiber.

[3] Neutral detergent fiber.

[4] Total digestible nutrients.

Table 3.3. Cow pastures (1-4) forage quality and production at the time of entry and exit. Montana State University Fort Ellis research farm.

Rotation	1	2	3	4[1]
Production, kg · ha^{-1}				
Enter	2683.3	3553.4	3203.1	4958.0
Exit	831.1	1388.8	1371.8	1982.9
DM, %	91.5	91.4	91.8	93.0
Chemical composition, % DM				
CP[2]	9.0	7.9	6.5	10.9
ADF[3]	38.8	36.5	35.2	35.3
NDF[4]	59.9	61.3	56.5	58.0
TDN[5]	60.0	60.0	61.0	61.0
Ca	0.37	0.33	0.43	0.52
P	0.26	0.23	0.17	0.28
Mg	0.12	0.11	0.14	0.18
K	1.97	1.58	1.80	2.50
Na	0.009	0.006	0.011	0.011
Chemical composition, mg · kg^{-1}				
Cu	12	15	16	9
Fe	232	122	70	58
Mn	80	40	112	47
Mo	2.3	2.7	3.1	2.5
Zn	17	18	15	17

[1] The 4th rotation was at the Bozeman Agriculture Research and Teaching farm.

[2] Crude protein.

[3] Acid detergent fiber.

[4] Neutral detergent fiber.

[5] Total digestible nutrients.

Table 3.4. Intake, intake rate, and % coefficient of variation (CV) data of yearling heifers supplemented with rumen undegradable or degradable protein while grazing improved dryland pastures.

	Treatment[1]			*P*-Value[3]		
Item	RUP	RDP	SEM[2]	Treatment	Period	Trt × Period
Intake, $g \cdot cow^{-1} \cdot d^{-1}$				0.06	<0.01	<0.01
Period 1	286.31	151.87	50.82			
Period 2	349.00	83.44	51.06			
Intake, $g \cdot kg\ BW^{-1} \cdot d^{-1}$				0.06	<0.01	<0.01
Period 1	0.80	0.43	0.14			
Period 2	0.94	0.22	0.14			
Intake rate, $g \cdot min^{-1}$	84.38	11.52	10.44	<0.01	0.57	0.29
CV, %	187.84	201.42	17.79	0.43	0.14	0.10
Period 1	204.20	176.35	24.76			
Period 2	172.79	230.05	25.72			

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Pooled standard error of the means presented.

[3] *P* – values of main effects of treatment, period, and treatment × period.

Table 3.5. Performance and reproductive parameters of yearling heifers supplemented with rumen undegradable or degradable protein while grazing improved dryland pastures.

	Treatment			
Item	RUP[1]	RDP[1]	SEM[2]	*P*-Value
Initial body weight[3], kg	334.1	335.6	9.77	0.91
Initial BCS[4]	4.89	4.95	0.06	0.48
Final body weight, kg	449.6	446.8	8.65	0.82
Final BCS	5.63	5.65	0.06	0.83
Average daily gain, kg · d^{-1}	1.38	1.32	0.09	0.66
Cycling[5] at d 0, %	47	58	11.0	0.52
Bred to estrus[6], %	50	50	11.0	1.00
Final pregnancy rate, %	95	100	3.0	0.23

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Pooled standard error of the means presented.

[3] Body weight.

[4] Body condition score.

[5] Corresponds to the percent of heifers confirmed to have reached concentrations of progesterone > 1 ng/mL (Stevenson et al., 2015).

[6] Heifers exhibiting standing estrus and bred prior to time artificial insemination.

Table 3.6. Intake, intake rate, and % coefficient of variation (CV) data of cows supplemented with rumen undegradable or degradable protein while grazing improved dryland pastures.

	Treatment[1]			*P*-Value[3]		
Item	RUP	RDP	SEM[2]	Treatment	Period	Trt × Period
Intake, $g \cdot cow^{-1} \cdot d^{-1}$				<0.01	<0.01	<0.01
Period 1	337.44	836.19	74.07			
Period 2	164.93	1503.02	75.21			
Intake, $g \cdot kg\ BW^{-1} \cdot d^{-1}$				<0.01	<0.01	<0.01
Period 1	0.72	1.76	0.16			
Period 2	0.33	3.00	0.16			
Intake rate, $g \cdot min^{-1}$				0.10	<0.01	<0.01
Period 1	30.41	36.97	2.80			
Period 2	24.08	52.65	5.70			
CV, %				0.03	<0.01	<0.01
Period 1	136.79	112.14	8.07			
Period 2	149.19	78.37	7.37			

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Pooled standard error of the means presented.

[3] *P* – values of main effects of treatment, period, and treatment × period.

Table 3.7. Performance of cows and calves and reproductive parameters of cows supplemented with rumen undegradable or degradable protein while grazing improved dryland pastures.

	Treatment[1]			
Item	RUP	RDP	SEM[2]	*P*-Value
Initial body weight[3], kg	474.3	472.5	11.26	0.91
Initial BCS[4]	4.85	4.83	0.06	0.80
Final body weight, kg	521.2	535.2	11.67	0.40
Final BCS	5.28	5.37	5.33	0.46
Average daily gain, kg · d^{-1}	0.56	0.75	0.04	< 0.01
Calf average daily gain, kg · d^{-1}	1.07	1.12	0.02	0.10
3-year-old cows cycling[5] at d 0, %	42	75	13.0	0.09
Bred to estrus[6], %	41.94	46.67	8.98	0.71
Final pregnancy rate, %	100	90	3.0	0.04

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Pooled standard error of the means presented.

[3] Body weight.

[4] Body condition score.

[5] Corresponds to the percent of heifers confirmed to have reached concentrations of progesterone > 1 ng/mL (Stevenson et al., 2015).

[6] Cows exhibiting standing estrus and bred prior to time artificial insemination.

CHAPTER FOUR

IMPACTS OF RUMEN DEGRADABLE OR RUMEN UNDEGRADABLE PROTEIN SUPPLEMENT WITH OR WITHOUT SALT ON NUTRIENT DIGESTION AND VFA CONCENTRATIONS

Contribution of Authors and Co-Authors

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Contributions: Main author and lead scientist responsible for data collection, data analysis and interpretation, and drafting of this thesis.

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Manuscript Information

M.K. Manoukian, J.A. Kluth, S.A. Wyffels, T. DelCurto, A. Scheaffer, and M.L. Van Emon

Journal of Animal Science

Status of Manuscript:
_X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-reviewed journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

Running head: Protein source, salt on nutrient digestion

Impacts of rumen degradable or rumen undegradable protein supplement with or without salt on nutrient digestion, and VFA concentrations[1]

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[1] The authors would like to thank SweetPro LLC for the funding of this project and for the products used in this project. Appreciation is also expressed to the Nancy Cameron Endowment, and to the employees of MSU BART Farm for their assistance with this project.

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ABSTRACT: Eight two-year old and eight three-year old rumen fistulated cows were used in a 2 × 2 factorial design for a 22-d digestion study. Shrunk weights were collected on d 1. Dietary treatments included: 1) self-fed, salt-limited pressed supplement block containing rumen undegradable protein (RUP-SF; SweetPro® Premium supplements, Walhalla, ND), 2) hand-fed, loose supplement containing RUP (RUP-HF; SweetPro® Premium supplements, Walhalla, ND), 3) self-fed, salt-limited pressed supplement block containing rumen degradable protein (RDP-SF), and 4) hand-fed, loose supplement containing RDP (RDP-HF). Statistical analysis was complete in R. There were no protein type or delivery method effects ($P \geq 0.24$) on dry matter intake expressed in g · kg BW^{-1} · d^{-1}, forage intake expressed as both kg · $d^{-1}$and g · kg BW^{-1} · d^{-1} or DM volume. There was a delivery effect ($P = 0.04$) on NDF intake as the SF animals consumed more NDF than HF animals. The RDP-SF animals had greater NDF digestibility ($P = 0.04$), and water intake ($P = 0.03$) compared to the other three treatment groups. Animals on the SF method had increased ($P = 0.01$) supplement intake in kg · d^{-1}compared to HF animals. Supplement intake in g · kg BW^{-1} · d^{-1} displayed a protein type effect ($P = 0.03$) as RDP supplemented animals consumed more supplement on a g · kg BW^{-1} · d^{-1} basis than RUP animals did. There was an effect ($P = 0.02$) of protein type and an effect ($P = 0.03$) of delivery method on fluid flow rate, with HF and RUP treatments having higher liquid flow rates. There were no protein type or delivery method effect on acetic, propionic, butyric, or isobutyric acids, acetate:propionate ratio or total VFAs ($P \geq 0.19$). Ruminal pH displayed a protein type × hour interaction ($P < 0.01$), where RDP had lower pH than RUP at all hours. Ruminal pH also displayed a delivery × hour interaction ($P < 0.01$), however, the only difference of ruminal pH between HF and SF was at h-23 ($P = 0.02$). Ruminal ammonia displayed ($P < 0.01$) a delivery ×

protein type × hour interaction as RDP-SF animals had the greatest concentrations. There was a delivery × hour interaction for valeric acid ($P = 0.04$; Figure 2), however, there was no difference ($P \geq 0.12$) between delivery methods within each hour. Valerate ruminal concentrations were greater in RDP supplemented animals compared to RUP supplemented animals (2.39 ± 0.06 vs 2.20 ± 0.06; $P = 0.04$). In conclusion, self-fed supplements containing RDP may enhance the use of low-quality forages.

Key words: Beef cattle, nutrient digestion, rumen undegradable protein, rumen degradable protein

INTRODUCTION

Beef cattle production is important in the western United States. The arid environment of the region causes forages to have seasonal deficiencies and often to be low in protein (DelCurto et al., 2000), which is why supplementing cattle consuming these low-quality forages with protein is important. The protein required by beef cattle can be separated into two specific requirements; the protein need for rumen microorganisms, and the need of the individual animal (Eisemann et al., 2016). Rumen degradable protein (RDP) provides the microorganisms in the rumen with a source of N and is required for the synthesis of microbial crude protein. Rumen undegradable protein (RUP) is not hydrolyzed upon entering the rumen and as a result, is digested and absorbed in the small intestine.

A positive relationship exists between RDP supplementation and forage utilization (Köster et al., 1996). The N source that RDP provides rumen microorganisms allows the microbes to grow (Wickersham et al., 2008). This increase in microbial activity increases microbial N flow to the small intestine (Scott and Hibberd, 1990). The differences in how RDP and RUP are digested may result in a difference in microbial populations among our treatments.

Because microbes utilize protein available within the rumen to grow (Wickersham et al., 2008), an increase in microbial growth and activity increases the production of VFAs within the rumen (Scott and Hibberd, 1990; Köster et al., 1996). In a study by Wickersham et al. (2008), beef steers were supplemented with increasing amounts of RDP. Overall VFA concentrations were increased with increasing amounts of RDP, specifically with acetate declining and propionate increasing. It is probable that we could see similar results of changes in VFA concentrations. Additionally, ammonia is a product of excess protein in the diet and is ultimately absorbed and released as the waste product urea (Van Soest, 1982). Within the rumen, RDP is broken down into peptides and then amino acids (Millen et al., 2016). It is likely that increases in protein available to the rumen will results in an ammonia increase.

The impacts of RUP and RDP supplementation on fiber digestion vary. Digestibility of ADF was not impacted when first calf heifers were supplemented with RDP or RUP postpartum (Anderson et al., 2001). Similarly, Pina et al. (2009) observed that level of RUP supplementation did not impact dry matter intake (DMI), digestibility of DM, OM, NDFcp, or TDN when Nellore heifers were fed RUP at either 25 or 40% of CP (Pina et al., 2009). In contrast, non-supplemented periparturient cows compared to cows supplemented with RUP had lower NDF digestibilities (Sletmoen-Olson et al., 2000b).

These differences in how each type of protein is digested have the potential to cause differences in nutrient digestion, VFA concentrations, ammonia concentrations, and rumen microbiology profiles. Therefore, the objectives of this study were: 1) to evaluate the differences of treatments on rumen dynamics, and 2) evaluate the differences of treatments on nutrient digestion.

MATERIALS AND METHODS

Experimental procedures described herein were approved by the Agriculture Animal Care and Use Committee of Montana State University (#2020-AA05). Eight two-year old (BW= 477.86 ± 35.28 kg) and eight three-year old (BW = 607.59 ± 48.11 kg) rumen fistulated cows were used in a 2 × 2 factorial design for a 22-d digestion study. Shrunk weights were collected on d 1. Cows were stratified by BW within age and within stratum assigned to 1 of 16 pens (1 cow per pen) and one of four dietary treatments (Table 4.1): 1) self-fed, salt-limited pressed supplement block containing rumen undegradable protein (RUP-SF; SweetPro® Premium supplements, Walhalla, ND), 2) hand-fed, loose supplement containing RUP (RUP-HF; SweetPro ® Premium supplements, Walhalla, ND), 3) self-fed, salt-limited pressed supplement block containing rumen degradable protein (RDP-SF), and 4) hand-fed, loose supplement containing RDP (RDP-HF). Self-fed supplements were formulated to contain 25% salt, as salt has been shown to be an effective intake limiter (Schauer et al., 2004). These supplements were also formulated as press blocks, as this form of supplement has been utilized to control intake, as well. The 22-d period included a 14-d adaptation period, 7-d total dry matter intake and total fecal collection, and 2-d collection of rumen fluid samples for ruminal and microbial profiles, and rumen dry matter content.

The experimental period was previously described by and was modified from Bohnert et al. (2002). Low quality forage (7.2% CP; Table 4.2) was fed at 120% of the average previous 3-d intake. Supplement blocks were fed ad libitum and intake measured daily by weight disappearance. Loose supplements were hand-fed at a rate of 0.91 kg · cow^{-1} · d^{-1}. Water was offered ab libitum to all 16 cows. Water was offered in GrowSafe units (GrowSafe Systems Ltd., Airdrie, AB Canada) to measure individual water intake of the 3-year-old cows. Water was offered in metal stock tanks for the 2-year-old cows and intake was measured by weight disappearance. All forage, supplement, and water were provided at 0800 each day. Indwelling, wireless data transmission boluses (smaXtec animal care, GmbH, Graz, Austria) were placed in the reticulorumen and used to monitor pH hourly during the 7-d collection period.

During the 7-d collection period, orts were collected daily from d 16 to d 22 for determination of DMI. Total fecal output was measured for determination of DM digestibility. Forage, supplement, orts, fecal, and water samples were collected from d 15 to d 21. Forage, orts, and supplement samples were dried at 55°C for 48 hours, ground to pass a 1 mm screen (Wiley Mill Model 4, Thomas Scientific, Swedesboro, NJ, USA), and stored for further analysis. Fecal samples were dried at 55°C for 96 h, ground to pass a 1 mm screen, and stored for further analysis. Forage, supplement, orts, and fecal samples were analyzed for NDF (AOAC, 2005) using the Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Total tract dry matter digestibility and NDF digestibility was determined.

On d 21, each cow was intraruminally pulse-dosed with 286.25 mg · mL^{-1} of Cr-EDTA in a 250-mL aqueous solution (Udén et al., 1980), prior to feeding at hour 0. The Cr marker was administered throughout the rumen. Rumen fluid (approximately 115 mL) was collected by a suction strainer (Raun and Burroughs, 1962; 19-mm diameter, 1.6-mm mesh), immediately prior to dosing and at 4, 8, 12, 18, and 24-h post-dosing. Rumen fluid was stored in 50- and 15-milliliter vials at -20°C for Cr analysis, VFA, and ammonia analyses. Rumen fluid samples were analyzed for ammonia concentrations using methods similar to those described by Sigma Technical Bulletin #640, Chaney and Marbach (1962), Horn and Squire (1967), and Weichselbaum et al. (1969). Rumen fluid samples were also analyzed for individual VFA concentrations using a gas chromatography procedure similar to that described by Baumgardt (1964), Supleco Inc. bulletin 749E (1975), Byers (1979), and Fritz and Schenk (1987). Chromium concentrations were analyzed with atomic absorption using an air/acetylene flame. Ruminal liquid volume and liquid dilution rates were estimated by regressing the natural logarithm of Cr concentrations against sampling time (Warner and Stacy, 1968). On d 20, an additional subsample of rumen fluid was collected immediately prior to dosing of Cr-EDTA. This rumen fluid was frozen immediately at -20°C for rumen microbiota analysis.

Additionally, on d 22, ruminal DM and undigestible NDF (uNDF) were determined by manually removing reticulorumen contents 5 h post-feeding. Total rumen contents were weighed, mixed by hand, and subsampled in duplicate. The remaining ruminal contents were immediately replaced in the animal. Rumen samples were weighed, dried in a forced-air oven at 55°C for 96 h and reweighed for DM. Dried rumen samples were composited and ground to pass a 1 mm screen in a Wiley Mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA). Samples were sent to a commercial laboratory (Dairy One, Ithaca, NY) and analyzed for uNDF. Undigestible NDF values were utilized to determine passage rates.

The effects of protein type and delivery method on daily intake, water consumption, and digesta kinetics were analyzed using an analysis of variance (ANOVA) with a generalized linear model for a 2 × 2 factorial design. The effects of protein type and delivery method on volatile fatty acid (VFAs), pH, and ammonia were analyzed using ANOVA with generalized mixed models for a repeated measure analysis. Individual cow was considered a random intercept for VFAs and ammonia as there were repeated measurements for each individual. Individual cow nested within day was considered a random intercept for pH, as ruminal pH was collected hourly for each individual over the course of the 7-d collection period. Data were plotted and log-transformed if needed to satisfy assumptions of normality and homogeneity of variance. An alpha of ≤ 0.05 was considered significant, and tendencies were considered at alpha ≤ 0.1. Means were separated using the Tukey method when $P \leq 0.05$. All statistical analyses were performed in R (R Core Team, 2020).

RESULTS

Intake and Digestibility

There were no protein type or delivery method effects ($P \geq 0.24$; Table 4.3) on dry matter intake expressed in g · kg BW^{-1} · d^{-1}, or forage intake expressed as both g · kg^{-1} · d^{-1} and g · kg BW^{-1} · d^{-1}. There were no protein type or delivery method effects ($P \geq 0.12$) on uNDF fill in kg or uNDF fill in g · kg BW^{-1}, nor uNDF retention or passage. There was a delivery effect (P = 0.04) on NDF intake as the SF animals consumed more NDF than HF animals. There was a delivery × protein type interaction ($P \leq 0.04$) for both NDF digestibility and water intake, where RDP-SF had greater NDF digestibility ($P = 0.05$) and water intake ($P < 0.01$) than RDP-HF, however there was no differences in NDF digestibility ($P = 0.32$) or water intake ($P = 0.98$) between RUP-SF and RUP-HF. As we would expect due to our delivery system, animals on the SF method had increased ($P = 0.01$) supplement intake in kg · d^{-1} compared to HF animals that were limit-fed supplement. Supplement intake in g · kg BW^{-1} · d^{-1} displayed a protein type effect ($P = 0.03$) as RDP supplemented animals consumed more supplement on a g · kg BW^{-1} basis than RUP animals did. Delivery method tended (*P* = 0.07) to effect dry matter intake in kg · d^{-1}, as SF consumed more compared to HF. Dry matter digestibility tended (*P* = 0.08) to display a delivery × protein type interaction in which RDP-SF had greater DM digestibility compared to RDP-HF while RUP-HF had improved DM digestibility compared to RUP-SF.

There were no protein type or delivery method effects (*P* ≥ 0.25; Table 4.4) on ruminal DM volume. There was an effect (*P* = 0.02) of protein type on fluid flow rate, as RUP supplemented animals had greater rates compared to RDP supplemented animals, 2.83 and 2.03 L · h^{-1}, respectively. There was also an effect (*P* = 0.03) of delivery on fluid flow rate, however, post-hoc means separation analysis showed no difference between delivery methods. Protein type tended (*P* = 0.07) to have an effect on ruminal fluid volume as RUP supplemented to have increased volume compared to RDP animals.

Rumen Dynamics

There were no protein type or delivery method effect on acetic, propionic, butyric, or isobutyric acids, acetate:propionate ratio or total VFAs (*P* ≥ 0.19; Table 4.5). Ruminal pH displayed a protein type × hour interaction (*P* < 0.01; Figure 4.1), in which RDP produced lower pH than RUP at all hours. Ruminal pH also displayed a delivery × hour interaction (*P* < 0.01; Figure 4.2), however, the only difference of ruminal pH between HF and SF was at h-23 (*P* = 0.02). Ruminal ammonia displayed a protein type × delivery × hour interaction (*P* < 0.01; Fig. 4.3), with RDP-SF having greater (*P* < 0.01) ammonia concentrations at 18-h post-feeding than RDP-HF, with a tendency (*P* = 0.09) for RUP-HF to have greater ammonia concentrations than RUP-SF at 24-h post-feeding. However, the RDP cows had greater (*P* ≤ 0.04) ammonia concentrations than RUP, regardless of delivery method, at each hour. There was a delivery ×

hour interaction for valeric acid (*P* = 0.04; Fig. 4.4), however, there was no difference ($P \geq 0.12$) between delivery methods within each hour. There tended to be a delivery × protein type interaction (*P* = 0.10) for isovaleric acid, where RDP-SF tended to have greater isovaleric concentrations than RDP-HF with no effect of delivery method for RUP.

DISCUSSION

Intake was greatest for SF animals, as HF animals were given an allotted amount of supplement, therefore increasing their total intake. However, SF supplements may cause variation in individual intake based on previous research (White et al., 2019b; Wyffels, 2019; McClain et al., 2020). The tendency for RDP-SF to increase DM digestibility may be contributed to RDP being a protein source for microbes, promoting their growth and efficiency (Wickersham et al., 2008) and therefore increasing digestibility. When lambs were fed low-quality forage and supplemented to meet RDP requirements or to meet 50, 100, or 150% of RUP requirements; NDF intakes were not impacted by protein type but tended to increase with increasing amounts of RUP (Atkinson et al., 2007). Similarly, RUP-SF animals had increased NDF intakes, however, so did RDP-SF animals, which is indicative of the greater SF intakes. Nellore heifers supplemented with RUP at 25 or 40% of CP had no impacts on DMI, or DM and NDF digestibility (Pina et al., 2009). Similarly, in the current study, DM digestibility was not impacted by treatment, however, NDF digestibility was greater in RDP-SF animals than in RDP-HF animals. Periparturient cows supplemented with RUP compared to a control, non-supplemented group had greater NDF digestibilities compared to the control group (Sletmoen-Olson et al., 2000b). Because the supplements had the same amounts of RDP, this suggests that control cows may have been RDP deficient. These results suggest that RDP, provided in self-fed form, may enhance fiber digestion of low-quality forages.

Beef cows consuming low-quality forages had increased water intakes when fed increasing amounts of salt (White et al., 2019a). Likewise, in the current study, RDP-SF animals had greater water intakes. Animals consuming SF supplements had higher supplement intakes and because the supplement was salt-limited, they consumed more salt which is likely why RDP-SF had increased water intakes. However, animals with increased salt intakes that had increased water intakes have also been repeated to have increased rumen liquid fill (Croom et al., 1982; White et al., 2019a). In contrast, in the current study the animals that had increased water intakes did not have increased rumen liquid volume, likely because there were no differences in fluid flow rates between treatments.

In steers supplemented with RDP, ammonia concentrations increased with increasing amount of RDP (Wickersham et al., 2008). Similarly, in the current study, RDP-SF cows had the greatest ruminal ammonia concentrations. Animals consuming high levels of salt-limited protein supplements have been reported to have decrease ruminal ammonia and VFA concentrations (Harvey et al., 1986). In the current study, SF animals had increased supplement intakes and because the supplement was salt-limited, they had increased salt intakes. However, SF animals did not exhibit decreases in ruminal ammonia or VFA concentrations.

Both cows and steers supplemented with increasing amounts of RDP had increasing amounts of valerate, isovalerate, and isobutryrate (Köster et al., 1996; Wickersham et al., 2008). Likewise, in the current study, RDP supplemented animals had greater levels of valeric acid and tended to have greater levels of isovaleric acid compared to RUP supplemented animals. In contrast, there were no differences in isobutyrate, or total VFA concentrations as Köster et al. (1996) had reported. Wickersham et al. (2008) reported that steers also had increased propionate amounts as RDP amount increased, and a decrease in acetate. There were no effects of protein type on propionate or acetate amount in the current study. Propionate is the major gluconeogenic precursor (60-75%) while valerate and isobutyrate collectively contribute 5-6% (Aschenbach et al., 2010). Because we observed an increase in valeric acid in RDP supplemented animals, this may lead to an increase in gluconeogenesis, however, since there was no difference in propionate levels, it is highly unlikely gluconeogenesis increased.

When comparing the impacts of RDP and RUP supplementation on rumen characteristics, Atkinson et al. (2007) reported no impacts of protein degradability on rumen pH. However, when steers were supplemented with low amounts of RUP, they had a decrease in pH when compared to steers supplemented with medium and high levels of RUP. In the current study, there was a protein type by hour interaction, where RDP had higher pH than RUP at all time points. This may be due to the RDP group having which produced greater levels of ruminal ammonia at all time points, as ammonia indicates free hydrogen ions and lower pH (Bartley et al., 1976). There was also a delivery type by hour interaction, where SF animals had lower pH values compared to HF animals at 23-h, likely due to the availability of the SF supplements to be consumed throughout the day, unlike the HF supplements.

IMPLICATIONS

Self-fed supplements tend to increase DMI due to increases in supplement intake. Self-fed supplements also increase fiber intake, valeric acid, and tended to increase isovaleric acid. Valeric acid is a gluconeogenic precursor (Aschenbach et al., 2010) and this increase in concentrations has the potential to increase gluconeogenic rates. Self-fed RDP supplements increase the efficiency of utilization of low-quality forages. Results from this research provides additional information on how RDP and RUP can impact nutrient digestion in beef cows consuming low-quality forage.

Table 4.1. Supplement nutrient analysis of supplements offered to fistulated 2- and 3-year-old beef cows.

	RDP-HF[1]	RDP-SF[1]	RUP-HF[1]	RUP-SF[1]
Dry matter, %	87.7	86.6	77.4	77.3
Analyzed nutrient composition, % DM basis				
Crude protein	37.7	37.0	27.6	27.8
ADICP[2]	2.4	1.2	1.7	2.5
Soluble protein, % CP	27	15	16	13
Degradable protein, % CP	54	63	35	30
Acid detergent fiber	18.7	5.5	8.1	6.4
Neutral detergent fiber	30.1	8.8	11.7	8.8
Lignin	7.6	1.1	3.4	2.6
Non-fiber carbohydrates	21.4	22.1	16.4	16.6
Starch	3.0	0.9	3.0	2.5
Crude fat	3.64	1.17	7.42	7.20
Ash	14.91	28.0	35.0	35.3
Total digestible nutrients	68	60	57	57
Ca	2.68	2.77	2.07	1.98
P	1.52	1.91	2.25	2.04
Mg	0.62	0.80	3.10	2.96
K	1.34	2.5	2.10	1.84
Na	0.26	4.30	5.84	6.15
S	0.85	0.73	1.49	1.23
Chemical composition, mg · kg^{-1}				
Fe	485	1,060	1,000	852
Zn	680	1,300	1,460	1,530
Cu	216	222	788	848
Mn	303	726	1,000	1,060
Mo	1.3	8.1	2	1.5

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP) and delivery method of hand-fed (HF) or self-fed (SF).

[2] Acid detergent insoluble crude protein.

Table 4.2. Nutrient analysis of hay offered to fistulated 2- and 3-year-old beef cows.

Dry matter, %	85.16
Analyzed nutrient composition, % DM basis	
Crude protein, %	7.21
ADICP, %	1.07
Soluble protein, % CP	39.94
Acid detergent fiber, %	31.23
Neutral detergent fiber, %	61.01
Lignin	7.13
NDFD[1], % NDF	40.8
uNDF240h[2], % NDF	38.1
Fat	1.99
Ash	7.22
Total digestible nutrients	57.58
Ca	0.26
P	0.14
Mg	0.16
K	1.62
Na	0.03
Cl	0.44
S	0.12

[1] Neutral detergent fiber digestibility.

[2] Undigestible neutral detergent fiber based on a 240 hour *in vitro*.

Table 4.3. Impacts of supplement delivery method and protein type on intake and fiber digestion of 2- and 3-year-old beef cows fed low-quality forage.

	RDP[1]		RUP[1]			*P*-value[4]		
Item	HF[2]	SF[2]	HF	SF	SEM[3]	Delivery	Protein	Delivery × Protein
Dry matter intake, kg · d^{-1}	11.61	13.25	12.11	12.91	0.59	0.07	0.56	0.49
Dry matter intake, g · kg BW^{-1} · d^{-1}	21.53	24.75	22.54	24.54	1.82	0.24	0.70	0.74
Supplement intake, kg · d^{-1}	0.83	2.34	0.76	1.40	0.36	0.01	0.89	0.24
Supplement intake, g · kg BW^{-1}· d^{-1}	1.54	3.91	1.41	2.20	0.58	0.12	0.03	0.82
Forage intake, kg · d^{-1}	10.78	10.91	11.35	11.52	0.60	0.89	0.52	0.97
Forage intake, g · kg BW^{-1} · d^{-1}	19.98	20.24	21.11	21.72	1.27	0.89	0.54	0.89
NDF intake, kg · d^{-1}	6.63	7.81	6.89	7.32	0.37	0.04	0.63	0.33
Dry matter digestibility, %	49.10	51.17	51.37	48.05	1.44	0.32	0.28	0.08
NDF digestibility, %	42.03^{a}	50.27^{b}	46.86^{ab}	43.11^{ab}	2.61	0.05	0.20	0.04
Water intake, L· d^{-1}	48.38^{a}	72.60^{b}	52.61^{ab}	52.81^{ab}	4.87	<0.01	0.55	0.03
uNDF Fill, kg	4.47	5.11	4.89	4.12	0.47	0.35	0.54	0.15
uNDF Fill, g · kg BW^{-1}	8.22	9.29	9.28	7.7	0.90	0.38	0.42	0.15
uNDF Retention, h	62.98	73.7	68.34	56.52	6.82	0.29	0.59	0.12
uNDF Passage, % · h^{-1}	1.64	1.42	1.49	1.84	0.17	0.38	0.55	0.12

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Delivery method of hand-fed (HF) or self-fed (SF).

[3] Pooled standard error of the means presented.

[4] Delivery: delivery method of HF vs. SF; Protein: type of protein fed (RDP vs. RUP); and the interaction of delivery method and protein type.

[a,b] Means that lack common superscripts differ for delivery × protein ($P < 0.05$).

Table 4.4. Impacts of supplementation on rumen kinetics of 2- and 3-year-old beef cows fed low quality forage.

	RDP[1]		RUP[1]			*P*-value[4]		
Item	HF[2]	SF[2]	HF	SF	SEM[3]	Delivery	Protein	Delivery× Protein
Fluid flow rate, L · h^{-1}	1.89	2.72	2.75	2.92	0.23	0.03	0.02	0.18
DM volume, L	12.09	13.53	13.69	12.23	1.19	0.41	0.36	0.25
Fluid volume, L	81.06	84.77	99.14	90.92	6.31	0.69	0.07	0.36

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Delivery method of hand-fed (HF) or self-fed (SF).

[3] Pooled standard error of the means presented.

[4] Delivery: delivery method of HF vs. SF; Protein: type of protein fed (RDP vs. RUP); and the interaction of delivery method and protein type.

Table 4.5. Impacts of supplement deliver method, protein type, and hour on ruminal pH, ammonia, and VFA concentrations in 2- and 3-year-old beef cows fed low quality forage.

	RDP[1]		RUP[1]			*P*-value[4]						
Item	HF[2]	SF[2]	HF	SF	SEM[3]	Delivery	Protein	Hour	Delivery ×Protein	Delivery ×Hour	Protein ×Hour	Delivery ×Protein ×Hour
Bolus pH	6.47	6.51	6.65	6.58	0.02	0.82	< 0.01	< 0.01	0.07	0.18	0.03	0.43
Ammonia, mg · dL^{-1}	4.57	6.99	1.52	1.26	0.89	0.33	0.02	< 0.01	0.21	< 0.01	< 0.01	< 0.01
Volatile fatty acids, mol · 100 mol^{-1}												
Acetic acid	66.06	65.73	67.40	66.13	0.68	0.87	0.22	< 0.01	0.85	0.57	0.93	0.41
Propionate	18.14	17.94	17.51	18.47	0.44	0.47	0.44	< 0.01	0.32	0.96	0.35	0.40
Isobutyrate	1.40	1.67	1.29	1.30	0.10	0.21	0.40	< 0.01	0.18	0.12	0.80	0.17
Butyrate	9.86	9.51	10.10	10.47	0.29	0.19	0.90	< 0.01	0.32	0.63	0.59	0.71
Isovalerate	1.69	2.38	1.40	1.27	0.19	0.18	0.31	< 0.01	0.10	0.30	0.39	0.13
Valerate	2.03	2.12	1.84	2.02	0.08	0.53	0.04	< 0.01	0.72	0.04	0.73	0.17
Acetate: propionate	3.59	3.67	3.87	3.59	0.12	0.57	0.35	<0.01	0.44	0.95	0.55	0.45
Total VFA, mM	89.51	91.21	89.67	88.77	3.03	0.21	0.75	< 0.01	0.67	0.74	0.99	0.90

[1] Protein type of rumen degradable protein (RDP) or rumen undegradable protein (RUP).

[2] Delivery method of hand-fed (HF) or self-fed (SF).

[3] Pooled standard error of the means presented.

[4] Delivery: delivery method of HF vs. SF; Protein: type of protein fed (RDP vs. RUP); and possible interactions of hour, delivery method and protein type.

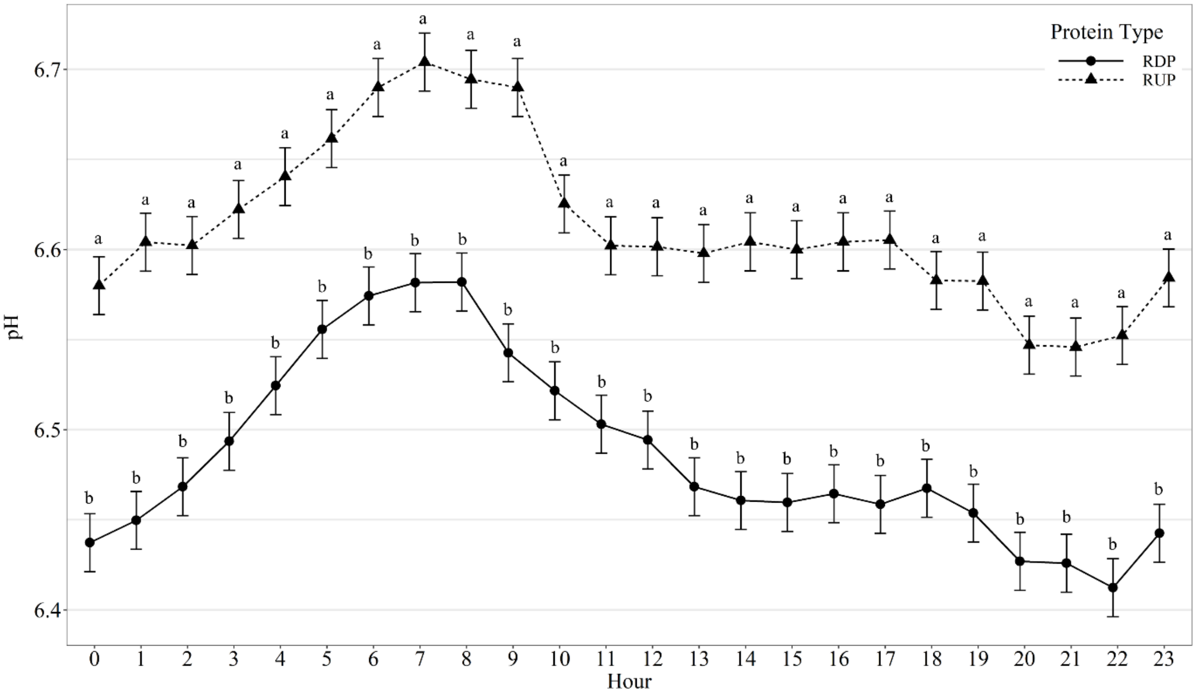

Figure 4.1. Impacts of protein type and hour on ruminal pH over 24-h. Rumen degradable protein (RDP) (●) and rumen undegradable protein (RUP) (▲) protein types represented, respectively. Protein × Hour: $P < 0.01$. a-b: timepoints without a common letter are different within hour ($P < 0.05$).

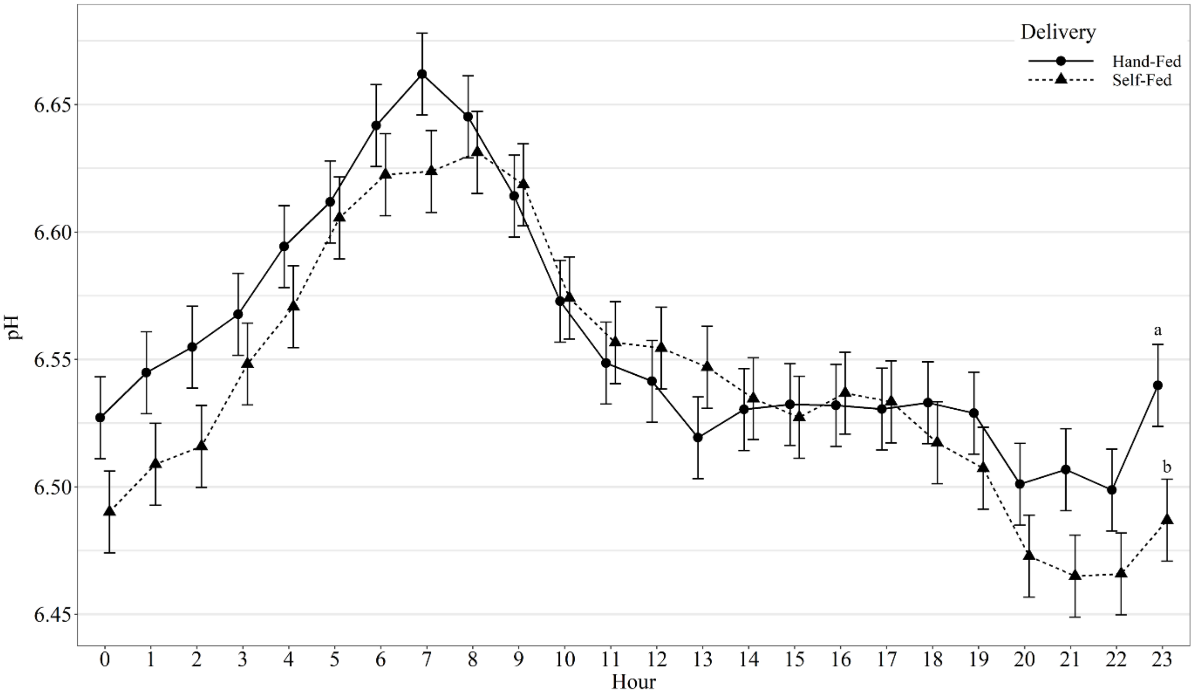

Figure 4.2. Impacts of hand-fed (●) or self-fed (▲) delivery method and hour on ruminal pH over 24-h. Delivery × Hour: $P < 0.01$. a-b: timepoints without a common letter are different within hour ($P < 0.05$).

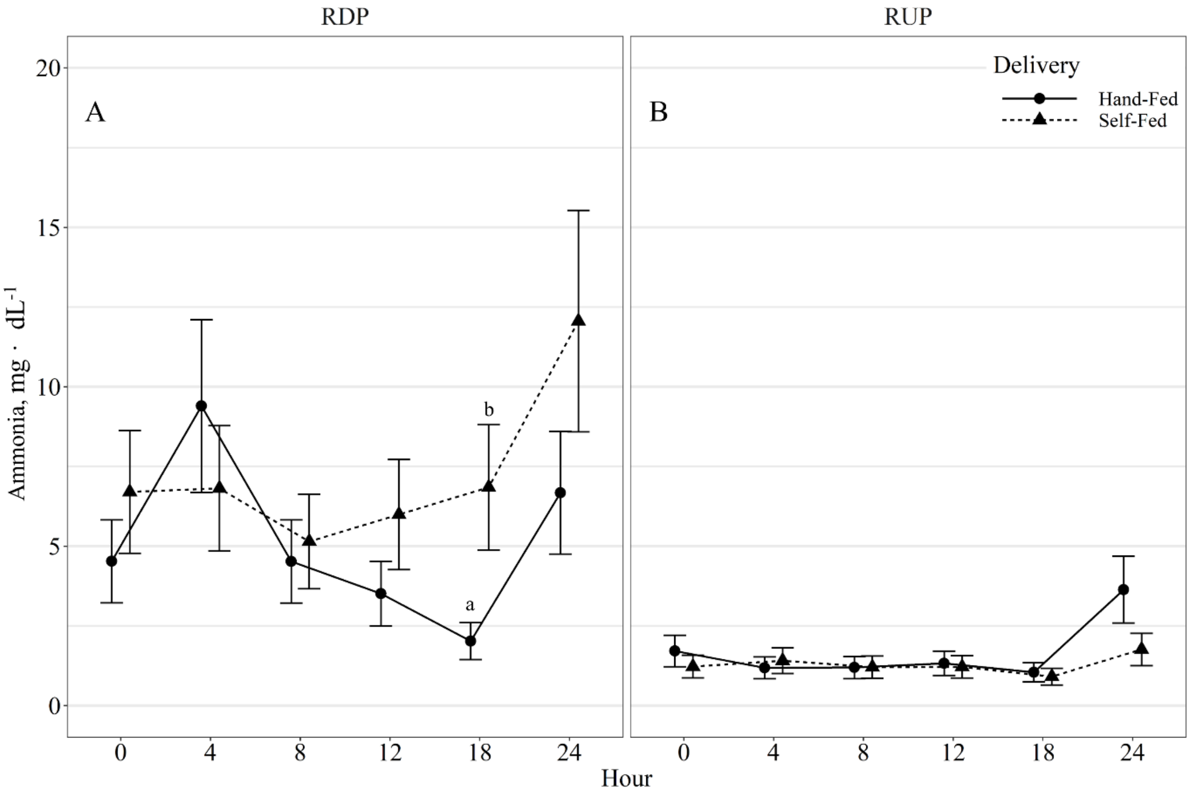

Figure 4.3. Impacts of protein type, delivery method, and hour on ammonia concentrations (mg · dL^{-1}). Hand-fed (●) self-fed (▲) with rumen degradable protein (RDP) and rumen undegradable protein (RUP) represented in panels A and B, respectively. Delivery × Protein × Hour: $P < 0.01$. a-b: timepoints without a common letter are different within hour ($P < 0.05$).

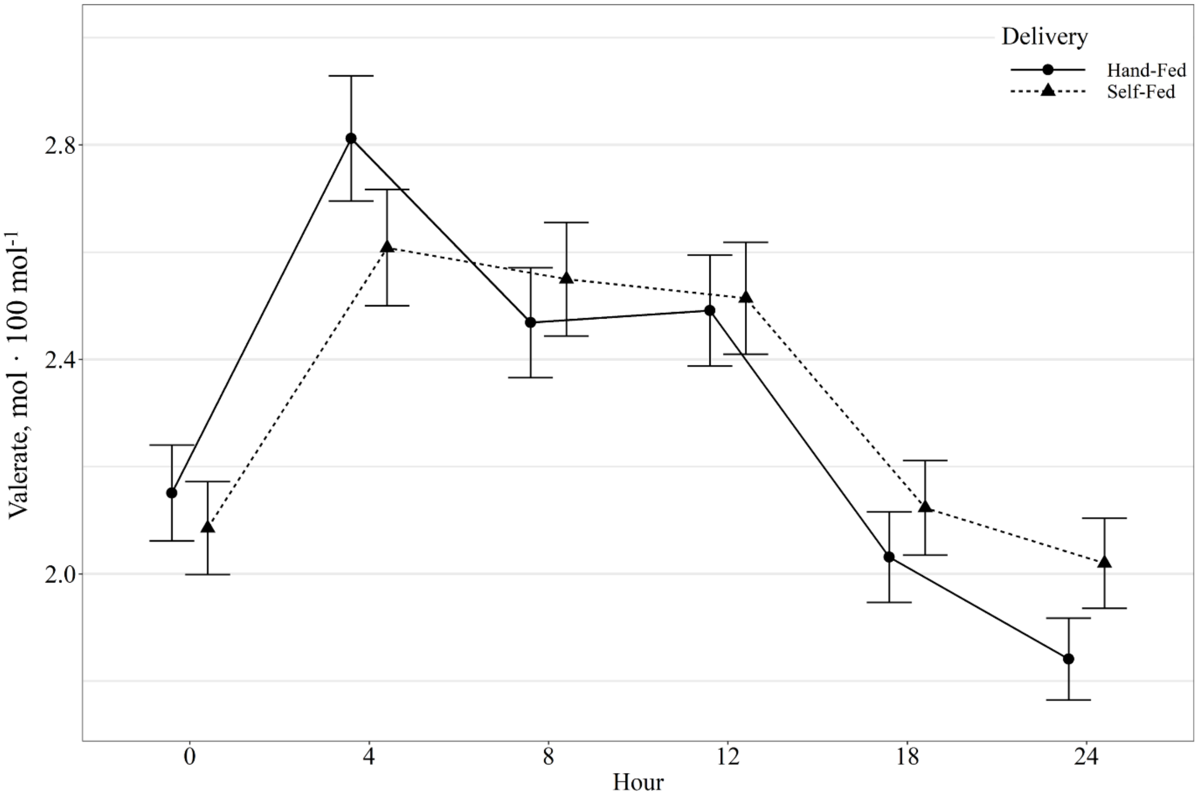

Figure 4.4. Impacts of hand-fed (●) or self-fed (▲) delivery method and hour on valerate (mol · 100 mol^{-1}) concentrations. Delivery × Hour: $P = 0.04$.

CONCLUSIONS

The importance of the beef cattle industry in Montana and the western United States is affected by the arid environment that causes seasonal protein deficiencies (DelCurto et al., 2000). Therefore, to develop heifers and maintain them within the cow herd, supplemental protein may be necessary. Forage-based beef cattle operations must maximize their production while reducing their input costs, thus finding a protein supplement that fits this need is critical.

The protein required by a ruminant animal can be divided into two different requirements; the protein the microorganisms within the rumen need, and the protein the individual animal needs (Eisemann et al., 2016). Rumen degradable protein (RDP) is digested within the rumen, which feeds the microorganisms, while rumen undegradable protein (RUP) is digested and absorbed in the small intestine. Previous research on how these types of protein impact intake, digestion, growth, and reproductive parameters are conflicting. The research projects described in chapters 3 and 4 sought to evaluate the impacts of supplemental RDP and RUP on nutrient supplement intake behavior, digestion, intake, rumen kinetics, performance, and reproductive parameters.

Supplement intake behavior for both yearling heifers and cows consuming RDP and RUP is variable. Heifer performance and reproductive success were not impacted by treatment, while RDP supplemented cows had greater ADG, and RUP cows had greater pregnancy rates. It must be considered, however, that RDP supplemented cows also had acceptable pregnancy rates. Additionally, NDF digestibility was improved, and ammonia concentrations increased in cows fed a self-fed RDP supplement. This indicates that RDP self-fed supplements may enhance the use of low-quality forages.

From this we can conclude that animals consuming low-quality forages may increase their use of such forages when offered RDP in self-fed form, while providing RUP has the potential to increase pregnancy rates in cows. Overall, because ruminants have the unique ability to utilize microbial protein and recycle nitrogen, either RDP or RUP may help maximize production. Producers must evaluate their operation’s goals, resources, and product pricing to help determine which supplement option would be beneficial and economically viable for their operation.

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