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Evaluation of Protein Utilization in Low and High Protein Forage Sources and the Economic Value of Supplementing Field Peas (*pisum sativum*) to Growing Cattle Grazing Crested Wheatgrass Pastures

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Evaluation of Protein Utilization in Low and High Protein Forage Sources
and the Economic Value of Supplementing Field Peas (*pisum sativum*) to
Growing Cattle Grazing Crested Wheatgrass Pastures

by

Braden C. Troyer

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Andrea K. Watson

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Evaluation of Protein Utilization in Low and High Protein Forage Sources
and the Economic Value of Supplementing Field Peas (*pisum sativum*) to
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University of Nebraska, 2019

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Field peas are widely grown in the panhandle of Nebraska; however, markets quickly become saturated. A two year experiment was conducted comparing field peas to dry distillers grains with solubles (DDGS) as a protein supplement. The objective was to establish a price producers could pay for field peas relative to DDGS. There was a significant difference in ADG due to type of supplement ($P = 0.02$). Field pea supplemented heifers had 10% lower ADG compared to DDGS supplemented heifers. Economically, this means if DDGS is priced at or \$124.58/ 909 kg DM a producer could pay \$2.89/ 27 kg, for field peas.

Variable sources of grazed forages are used in cattle backgrounding systems, but in most systems metabolizable protein (MP) is limiting and rumen undegradable protein (RUP) supplement can meet this deficiency. The first objective was to determine if RUP is limiting and the second objective was to determine if highly digestible forages with rapid passage rate allow some rumen degradable protein (RDP) to bypass the rumen. A pooled analysis of growing cattle grazing forages demonstrated that average daily gain (ADG) increased with increased RUP supplement received, with the exception of animals grazing forages that were 17% crude protein (CP) or greater. A metabolism study

evaluating high and low quality forages showed lower intakes and digestibility values for the lower quality forages. Rapid liquid passage rates suggest some degradable protein could be leaving the rumen before degradation. Numerous digestibility markers were used to evaluate markers in forage based diets.

Key words: RUP supplementation, forage digestibility, field peas, beef cattle

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“Brothers and sisters, I do not consider myself yet to have taken hold of it. But one thing I do: Forgetting what is behind and straining toward what is ahead, I press on toward the goal to win the prize for which God has called my heavenward in Christ Jesus.”

Philippians 3:13-14

Table of Contents

Chapter 1. Review of Literature	8
Introduction	8
Metabolizable Protein	10
Rumen Degradable Protein (RDP)	12
Rumen Undegradable Protein (RUP)	15
Bypass Soluble Protein	16
Measuring Rumen Undegradable Protein (RUP)	16
Forage Quality	19
Protein Supplementation	23
Passage Rate	26
Field Peas	28
Field Peas in Cattle Diets	29
Conclusion	31
Literature Cited	33
Chapter II. Economics of Field Pea Supplementation for Cattle Grazing Crested Wheatgrass	39
Abstract	40
Introduction	41
Materials and Methods	42
Animal Management	42
Pasture Management	43
Statistical & Economical Analysis	43
Results	44
Cattle Performance	44
Economic Analysis	45
Implications	46
Literature Cited	47
Chapter III. Utilization of Forage Crude Protein and the Need for Rumen Undegradable Protein Supplementation	52
Abstract	53
Introduction	54
Materials and Methods	55

Experiment 1.....	55
Experiment 2.....	60
Results	63
Experiment 1.....	63
Experiment 2.....	68
Literature Cited	74

List of Tables and Figures

CHAPTER II.

Table 1. Performance of heifers grazing crested wheatgrass pastures supplemented with whole field peas or distillers grains plus solubles.....	49
Table 2. Economic value of field peas compared to DDGS.....	50
Figure 1. Performance of heifers supplemented with field peas or dry distillers grains plus solubles while grazing crested wheatgrass pastures.....	51

CHAPTER III.

Table 1. Pooled analysis papers, authors, and years.....	77
Figure 1. Pooled response in ADG above the control calves to RUP supplement in 10 studies subdivided by initial BW.....	78
Figure 2. Pooled response in ADG above the control calves to RUP supplement in 10 studies subdivided by grazed forage type.....	79
Figure 3. Pooled response in ADG above the control calves to RUP supplement in 10 studies subdivided by CP of grazed forage.....	80
Table 2. Average dry matter, organic matter, and neutral detergent fiber intake of treatments.....	81
Table 3. Feed nutrient analysis including NDF, ADF, and CP.....	82
Table 4.1. Dry matter digestibility values using in-vitro, AIA, TiO ₂ , and IADF as markers.....	83
Table 4.2. Organic matter digestibility values using in-vitro, AIA, TiO ₂ , and IADF as markers.....	84
Table 4.3. Neutral detergent fiber digestibility values using AIA, TiO ₂ , and IADF as markers.....	85

Chapter 1. Review of Literature

Introduction

Protein is an essential part of every mammalian species. Protein is utilized for muscle deposition and for energy within the animal. Feeding protein in most species is relatively simple compared to feeding protein to ruminants. Most animals break down protein into amino acids in the stomach and small intestine where it can be absorbed into the portal vein and travel to the liver or peripheral tissue to be utilized or stored.

Ruminants, like cattle, have a much more complex system that takes place within the rumen, an anaerobic environment, as well as the stomach and small intestine.

Protein that is broken down by the microbes within the rumen is known as rumen degradable protein (RDP). These microbes utilize the dietary protein and energy to grow and divide, which increases the concentration of microbes within the rumen. Some microbes wash into the lower tract with the digesta and these are broken down in the small intestine and amino acids are absorbed. The microbes that wash out are known as microbial crude protein (MCP), in the NASEM (2016). Between 50 and 100% of MP requirements are provided by digestible MCP (Wilkerson et al., 1993; Owens et al., 2014). The remainder, especially in growing or lactating cattle is rumen undegradable protein (RUP).

Rumen undegradable protein is utilized similar to protein in non-ruminants. This bypass protein escapes the rumen without being degraded and re-synthesized into amino acids by the microbes and is digested in the stomach and some in the small intestine as well. Then RUP is absorbed as amino acids, along with the MCP and the total absorbed amino acids are termed metabolizable protein. Many have debated calculations for RDP,

MCP, and RUP and recommendations have been given and given again, however, even experts within the field do not fully understand how protein is utilized within the ruminant. Many factors affect how dietary protein is digested and absorbed within the digestive tract and research in this area is continuously improving our knowledge.

Forage quality is an important aspect to account for when attempting to maximize performance of growing animals. Plants are comprised of two major parts, a completely digestible core, known as cell solubles, and a partially digestible structural component, known as the cell wall. As plants mature they become less digestible, as a result of lignification of their cell walls. Cattle are very selective animals when they forage, but as the grazing season progresses pastures change and force cattle to adapt. Producers can affect forage quality and yield by managing their pastures with grazing systems and timely fertilization application, however, environmental factors such as light intensity, precipitation, and ambient air temperature also play an important role. An inevitable outcome is a decline in available nutrients from forage for growing cattle late in the grazing season, but this can be alleviated by supplementing protein in order to meet requirements and maintain performance.

Understanding exactly how protein is utilized is crucial when formulating diets or allocating supplements to grazing animals. As forage quality changes growing animals may be deficient in protein. Supplementing the correct type of protein is an extremely important economic decision. Studies have shown benefits of both RDP and RUP supplementation dependent on the time of year and system in place.

Passage rate is another factor that plays a role in nutrient utilization. Ruminants are unique in that they utilize a symbiotic relationship with microbes in their rumens to

extract nutrients from ingested feed. However, protein that can be digested and absorbed in the gastrointestinal tract benefits the host animal to a larger degree. Passage rate impacts how many available nutrients bypass the rumen before the microbes can utilize them. Passage rate is affected by intake, forage:concentrate ratio, particle size and density, and environmental temperature. Accounting for passage rate of different diets is another challenging issue when determining nutrient utilization within a ruminant.

Field peas, a highly digestible legume, are grown in excess in parts of western Nebraska. This excess is due to an increase in production and slow utilization due to processing bottlenecks. Production has increased as a result of field pea's popularity in rotational cropping systems. Excess peas are available for cattle producers in that area, which may help alleviate scarce byproduct availability. Peas provide adequate protein and also energy, proving to be viable options in both growing and finishing diets. If economics allow, cattle producers may help alleviate the overabundance of field pea production.

Metabolizable Protein

The best way to express protein requirements has been debated some, but historically requirements have been expressed in terms of crude protein (NRC, 1984). One main flaw of this system was that it assumed equal rumen degradation of all feedstuffs, which led to improper calculations of absorbed protein (NRC, 1996). An alternative system was needed to fix this issue, so the Subcommittee on Nitrogen Usage in Ruminants (1985) proposed expressing protein requirements in terms of absorbed protein which was accepted in the dairy industry in 1989 (NASEM, 2016). The beef industry followed this new expression and it has become known as metabolizable protein.

The new system was designed to help producers feed cattle more efficiently, which would reduce the environmental effects of producing meat and milk products by decreasing excess nitrogen the animal does not retain (Broderick et al., 2010).

Metabolizable protein is the sum of dietary escape protein, also known as digestible RUP, and digestible microbial protein (Wilkerson et al., 1993). Understanding that ruminant animals have different requirements than just that of the host is a foundational part of the metabolizable protein (MP) system. Microbes, which contribute to the protein supply for the animal in the small intestine, have separate protein requirements that allow them to grow (Lardy et al., 2004). However, determining the exact amount of both microbial crude protein (MCP) that will be produced from a feedstuff and RUP in a feedstuff can be challenging. Producers' targeted MP supply may differ depending on the goal of the operation and the particular animal they are attempting to feed. In general, animals that are at or near maintenance can reach MP balance by supplying them with dietary RDP, and thereby MCP in the small intestine. However, animals that are lactating or growing will need to have additional dietary RDP and RUP supplied in order to maintain growth and milk production (Klopfenstein et al., 1996). Determining the amount of RDP and RUP in a diet is an important aspect in feeding cattle.

Several methods have been used for obtaining RDP and RUP values in feedstuffs. The first method is to measure in vivo the amount of dietary protein that has bypassed the rumen and enters the abomasum. The second method is to encapsulate a feedstuff in a bag that is permeable to rumen microbes and measure the amount of protein that remains after in situ ruminal incubation (Orskov et al., 1970). Both methods measure the amount of RUP within a feedstuff. Measuring either RDP or RUP is a good way to determine the

unknown because crude protein (CP) is equal to the sum of RDP and RUP (NASEM, 2016). The in situ method is popular because it takes into account time of incubation, which is correlated to passage rate, a variable factor depending on feedstuff, diet, intake, and type of animal (Orskov et al., 1970).

Rumen Degradable Protein (RDP)

Rumen degradable protein is a source of protein that is degraded in the rumen by the microbes that allows them to grow and divide (Klopfenstein et al., 1996; Lardy et al., 2004). Rumen degradable protein is degraded in the rumen by bacteria, protozoa, and fungi which produce volatile fatty acids (VFA) and ammonia (Kang-Meznarich et al., 1981). These VFA's can supply 70-85% of the energy that is absorbed and used by the animal. The conversion of the diet to MCP is related to degradability of the feed, ruminally available energy, and mean retention time in the rumen (Storm et al., 1983). Microbial crude protein is the microbes that flow out of the rumen in the liquid fraction or attached to feed particles and are available to be absorbed by the host animal. These microbes that pass into the small intestine ultimately provide amino acids as the animal digests and absorbs them across the small intestine epithelium (Stern et al., 2006). Also, MCP can supply between half to nearly all of the MP the body needs to function (NRC, 1996). This is true for cattle that are at or near maintenance, with the exception of growing cattle, which typically need to have some escape protein to supplement total MP in the diet (Klopfenstein et al., 1996). Maximizing the growth of microbes in the rumen can have huge impacts on growth of the host as well. Microbial crude protein is a function of the flow of microbes out of the rumen that can be degraded and absorbed as protein in the small intestine. As microbial activity within the rumen increases, MCP

supply and microbial nitrogen (N) flow to the small intestine both increase as well (Wickersham et al., 2008).

Microbes need RDP in order to grow and reproduce in the rumen (Orskov et al., 1970). The need for RDP by the microbes is a function of the amount of rumen digestible energy. Energy actually drives the production of MCP as long as there is a sufficient amount of RDP present (Klopfenstein et al., 1996). This means that synchronizing the supply of nitrogen and energy (TDN) in the rumen will result in maximum RDP utilization and optimal microbial growth rate (Chanjula et al., 2004); however, synchrony is well coordinated by the ruminant animal. Ruminants have the ability to recycle nitrogen throughout the body, which is evident due to the appearance of N between the mouth and the small intestine (Wickersham et al., 2008). This process begins in the liver where urea is produced from ammonia via the urea cycle and enters the blood to be reused or is excreted into the urine (Wickersham et al., 2008). Depending on the demand for recycled nitrogen, between 40-80% of the urea produced in the liver can reenter the blood supply (Lapierre et al., 2001). When fermentable energy is present in the rumen the urea can pass through the epithelial tissue or enter through the saliva and be converted into ammonia (Reynolds et al., 2008). This ammonia can be used as a substrate by the microbes along with energy to produce VFA's and increase microbial activity. This process is what allows synchronization of nitrogen and fermentable energy within the rumen, which would normally not be present. This also allows producers to supplement cattle infrequently and expect similar results while decreasing supplementation costs dramatically (Bohnert et al., 2002). One issue with recycling is calculating if RDP supplementation is needed to maximize MCP production.

Calculating MCP that is produced within the rumen is challenging because it deals with total digestible nutrients (TDN) and RDP. Other factors also influence the efficiency of the microbes such as passage rate, how fermentable the energy source is, and where the digestion is taking place (Klopfenstein et al., 1996). An original model was based off of a 10.4% efficiency multiplied by total digestible nutrients (TDN) concentration. This model assumes that 52% of TDN is actually digested in the rumen, of that 52% only one quarter produces MCP when sufficient nitrogen is available, and 80% of the MCP is true protein, of which 80% is digestible and available to the host (Burroughs et al., 1975). The 1996 NRC calculated MCP by assuming 13% of the TDN was converted to MCP for diets containing over 40% forage. Diets containing under 40% forage used a 2.2% reduction in MCP for each 1% decrease in effective neutral detergent fiber (NDF) in the diet below 20% effective NDF (NRC, 1996). Research utilizing 66 treatment means from previously published papers led to a new equation. Forty papers were used to develop the model and 26 were used to evaluate the model (Galyean and Tedeschi, 2014). The Galyean and Tedeschi model and the 1996 NRC model appear to model similar efficiency for low-quality forage diets and high concentrate diets, but they were significantly different for growing diets. Currently the Beef Cattle Nutrient Requirements Model (2016) uses the Galyean and Tedeschi (2014) equation of $MCP = 0.087 TDNI + 42.73$ or $MCP = 0.096 FFTDNI + 55.33$ (TDNI- total digestible nutrient intake; FFTDNI- fat free total digestible nutrient intake). Overall, MCP is hard to measure effectively and has significant consequences when measured incorrectly. The consequence of using an incorrect efficiency when calculating MCP is under or overestimating MP supply (NRC, 1996). This can lead to supplementing cattle when it is

unnecessary or failing to supplement when needed, both of which are costly to operations raising cattle.

The amount of MCP that contributes to MP that is absorbed is another opportunity for error in calculating the protein the animal can utilize. The first main point is to understand that feeding different sources of RDP will not result in large changes in the protein quality that is absorbed as MCP. Furthermore the digestibility of the MCP is constant regardless of source of RDP (Bergen et al., 1968). The value used for MCP digestibility is 80% and only 80% of the nitrogen in MCP is true protein as well (Burroughs et al., 1975). This means that of the MCP that makes it to the small intestine only 64% ($0.8 \times 0.8 = 0.64$) is actually converted into MP that is absorbed for use. This means that ruminants fed low quality forages or non-protein nitrogen benefit from MCP because the nutritive value is increased. However, if ruminants are fed high quality RDP they actually are receiving less nutritive value relative to intake, which makes them less efficient compared to non-ruminants (Dewhurst et al., 2000).

Rumen Undegradable Protein (RUP)

Rumen undegradable protein is protein that is not degraded in the rumen and is available to be broken down in the small intestine (NASEM, 2016). This source of protein may be a smaller portion of total MP, but can have impacts above and beyond that of just supplying the host with MCP from the microbes (Klopfenstein et al., 1996). Cattle that are growing or lactating show increasing performance from a diet containing RUP (Klopfenstein et al., 2001; Buckner et al., 2013). RUP content of feeds, especially forages, is not well established and, historically, digestibility of the RUP in the small intestine has been overestimated (Mass et al., 1999; Buckner et al., 2013). Forages

typically have been known to provide mostly RDP and very little RUP to the diet (Klopfenstein et al., 2001; Buckner et al., 2013). RUP in forages has been estimated to be 10-40% of the total CP (NRC, 1996). However, understanding the amount of RUP that forages provide and the digestibility of the RUP is crucial to formulating a diet that maximizes desired performance as the nutrient profile of the grass changes (Buckner et al., 2013).

Bypass Soluble Protein

High quality forages are rapidly digested and have rapid rates of passage. They are also high in CP, most of which is soluble and rapidly degraded in the rumen. However some of the soluble protein may pass from the rumen due to rate of passage and poor microbial utilization (Volden et al., 2002). Peptides, amino acids, and ammonia that have been broken down by the microbes may flow out of the rumen prior to utilization by the microbial population (Volden et al., 2002). This protein fraction is absorbed in the small intestine similarly to RUP but is digested like RDP within the rumen. Volden et al. (2002) conducted a study using pulse doses of long chain peptides (PLP), short chain peptides (SP), and free fatty acids (FFA). Measurements of PLP, SP, and FFA showed that they were not completely degraded in the rumen. This means that some soluble protein was flowing out of the rumen prior to use by the microbes. Overall, predicting MP supply is heavily dependent on correctly calculating the amount of soluble protein that bypasses the rumen.

Measuring Rumen Undegradable Protein (RUP)

The ability to estimate total RUP in different forage sources is needed because values are not well known or well estimated. The difficulty of estimating RUP is that microbial N is also entering the small intestine and can falsify the N amounts that are

sometimes accounted for as RUP (Mass et al., 1999). Methods such as using total CP or in-vitro dry matter digestibility (IVDMD) are a poor proxy of RUP (Buckner et al., 2013). The method that has been previously accepted is using neutral detergent insoluble nitrogen (NDIN) to estimate RUP. This method used first order disappearance, which was thought to be incorrect due to the buoyancy of forage and the lack of constant passage of material (Lamothe et al., 2003). Mass et al. (1999) set out to test if measuring NDIN on forages that had been incubated in the rumen could correctly predict RUP with or without microbial correction. Also their study was designed to improve the overall procedure in order to be more exact with RUP predictions. The study looked at different pools of nitrogen that included total residual nitrogen, microbial nitrogen, and NDIN. Additionally it determined the effect of using different types of in situ bags, which resulted in a non-significant difference. Lastly it took into account the degradability of NDIN, which has been proven to be less than 100%. Results concluded that NDIN is the best estimate of RUP and that correcting for digestibility of NDIN will give accurate measures of RUP (Mass et al., 1999). Another aspect of RUP predictions takes into account retention time in the rumen. This is affected to a great degree by a lag in passage, due to buoyancy of the forage. This lag time is crucial to account for because it allows the bacteria to attach to the forage, and if it isn't accounted for it will cause RUP values to be overestimated (Orskov et al., 1970.) The estimated lag time is 10 hours. Allowing bags to incubate in the rumen for the total mean retention time (TMRT) will underestimate RUP. This is related to the first order disappearance, which means that forage passes out of the rumen at different time points as fermentation is occurring. A 75% TMRT produced RUP values that were similar to fractional rates of degradation and passage, so that method has

been adopted (Lamothe et al., 2003). Once the RUP fraction of a forage or feedstuff is determined the digestibility of RUP present needs to be calculated.

Rumen undegradable protein digestibility is extremely important to measure because feed that is high in RUP does not always correlate to high MP from RUP. Some forages that are high in RUP and low in RUP digestibility actually have very little MP supplied to the animal (Buckner et al., 2013). Digestibility varies, but the 1996 NRC used an assumption that all RUP was 80% digestible (NRC 1996). Haugen et al. (2006) hypothesized RUP digestibility was overestimated for forages and conducted research to test that hypothesis. Two forages were used and RUP was isolated in in situ bags by using the previously discussed NDIN techniques. These bags were then placed in the duodenum and digestibility of RUP was calculated by looking at indigestible protein that is collected in the bags after passage in feces. These bags are washed in NDF solution to remove microbial nitrogen. The nitrogen left in the bags was used to calculate digestibility. Digestibility of the RUP in smooth brome grass and birdsfoot trefoil were between 21.0-38.6% which proves that RUP contributing to MP was being overestimated assuming 80% digestibility formerly used (Haugen et al., 2006). Another study that was conducted looked at a wider array of forages and concluded that forage RUP digestibility usually ranges between 25-60% of RUP (Buckner et al., 2013). Understanding the amount of RUP in a feed and the digestibility in the small intestine is critically important in determining the amount of MP that is supplied. Another important point is determining how both of these change as forage matures throughout the growing season.

A study was conducted with smooth brome grass, subirrigated meadow, upland native range, and warm-season grasses throughout the growing season. Samples were

collected from esophageally cannulated cows or ruminally cannulated steers throughout the growing season and lab work was conducted to determine RUP and RUP digestibility. Results showed that RUP increased and RUP digestibility decreased throughout the year. This is a function of the plant maturing. These changes led to the total digested RUP as a % of dry matter (DM) decreasing as forage matured. One experiment sampled subirrigated meadow and upland native range using esophageally fistulated cows monthly from May to September. Analysis showed RUP as a % of CP increasing from 13.6% to 20.0% over the sampling period, however, digested RUP as a % DM decreased from 0.75% to 0.21% due to a reduction in RUP digestibility from 41.8% to 10.8% (Buckner et al., 2013). In another study, results showed a decrease in total MP supply from May to September on range and meadow samples (Lamothe et al., 2003). This would indicate that less protein would be degraded in the rumen and would lead to increased RUP, however the maturity would decrease how digestible this escape protein is in the small intestine as well.

Forage Quality

Forages represent the predominant class of feed for beef cattle operations.

Mekonnen et al. (2019) estimated that 81% of beef cattle feed comes from either pasture or harvested forages. However, differences in plant variety, maturity, and management cause DM digestibility, CP, and palatability to vary greatly across forage types (Bohnert et al., 2011). Plants are comprised of cells that contain two major parts. The outer portion is known as the cell wall and is comprised of cellulose, hemicellulose, and lignin. This section of the plant helps with structure and also protects the second portion known as the cell contents or solubles. This inner portion is comprised of protein, minerals, soluble carbohydrates, and starch which the plants use for growth and nutrition. Generally the

cell contents are almost completely digestible so differences in digestibility between forages depend on amount and digestibility of the cell wall (Deinum, 1981). Lignin is thought to be the main factor within the cell wall that reduces digestibility. Lignin is considered indigestible and as plants mature lignification increases, therefore, plant maturity and an increased lignin content lead to lower digestibility in forages (Van Soest, 1994).

Many factors influence the relative value of forages as a feed source for ruminants, but two of the largest factors are forage species and stage of growth (Beever et al., 1986). Three common categories of plants utilized by ruminants are legumes, C₃ grasses, and C₄ grasses. Overall C₃, or cool season, grasses are more nutritious than C₄, or warm season, grasses because they have greater CP levels and lower carbon:nitrogen (C:N) ratios (Barbehenn et al., 2004). As the grazing season progresses the plant matures and CP levels and in vitro organic matter digestibility (IVOMD) decrease significantly (Kirby and Parman, 1986). This decrease in CP and IVOMD is largely due to the shift in leaf to stem ratio. Leaf blades can contain up to twice as much CP as the stem within the same plant. Legumes have an increased leaf to stem ratio compared to other plant species that cattle consume making them more nutritious throughout the grazing season (Buxton, 1996). As the plant begins to transition from a vegetative state to a reproductive state the leaf to stem ratio declines. Also, as the plant matures CP declines more rapidly in the stem than in the leaves leading to a lower overall nutrient supply for the animals consuming mature plants later in the grazing season (Griffin and Jung, 1983). Maturation of plants also leads to a decrease in digestibility of both stems and leaves. Leaf digestibility decreases at a slower rate than stem digestibility, likewise legumes are more

digestible compared to grasses even when they are mature (Buxton, 1996). Grazing animals are very selective on which plants they eat, often they choose to eat the more vegetative plants because they benefit the animal due to an increased nutrient intake (Anderson et al., 1988). Selectivity of animals is due in part to nutrient uptake and digestibility, but animals also learn to be selective based on palatability, which isn't well understood, but is somewhat correlated to more nutrient rich feed sources (Baumont et al., 2000). Availability of forage also affects selectivity. Cattle select forage based on available biomass of individual grass types within a pasture. This response was observed by Willms and Rode (1998) when cattle selected taller standing grass over foraging for shorter grass types, especially during times of snow fall.

Forage quality and yield can be affected by many management and environmental factors. Maturity of the plant influences quality more than any other single factor; however, environmental factors also play a key role (Buxton, 1996). Grazing systems can influence nutritive value of grasslands for years to come. More intensive grazing systems lead to increases in both CP and digestibility of forage resources because plants remain in the vegetative state throughout a longer period of the grazing season. Also intensive grazing can lead to a more productive piece of land in terms of total forage yield (Pavlu et al., 2006). Fertilization is another way that both CP and yield can be increased in forage sources (Buxton, 1996). Water stress affects both the yield and the quality of plants. Quality of plants increases during times of drought, but yield decreases (Deinum, 1981). The increase in digestibility and CP of the stems of the plant is believed to be attributed to a slower maturation of the plant itself and also a reduction in cell wall formation. Stem to leaf ratio is also increased in water deficient plants mostly due to a

decrease in stem length, which plays a role in the lower yield (Halim et al., 1989). Light intensity also plays a key role in forage yield and quality, but has no significant effect on digestibility. Greater light intensity increases yield and soluble carbohydrates within the forage, but it also decreases total CP. This relationship is explained by an increase in photosynthesis, which is caused by the high light intensity, resulting in increased growth of the forage and more storage of carbohydrates. The plant uptake of nitrogen is limited by the available nitrogen in the soil and causes the CP of the forage to decrease due to more total yield of forage with relatively little change in uptake of nitrogen. Low light intensity causes the opposite change in forage sources yield and CP amounts. These forages yield less total mass, but are known for increased nutrient densities due to the lower distribution across the forage and relatively unchanged uptake from the soil (Deinum, 1981). Temperature may have the largest impact on forage quality and influences both yield and digestibility significantly (Buxton, 1996). Greater temperatures lead to a decrease in digestibility due to increase in lignification. Stems are affected at a greater rate than leaves, which are not structural in nature, and also grasses have a greater decrease in digestibility compared to legumes. The increased temperature leads to an increase in photosynthesis and causes the cellular components to be converted to structural components at a greater rate. This leads to an increase in forage yield, but decreased forage quality due to lower protein in the entire plant (Van Soest, 1994). As a whole environmental factors are often highly correlated and play a large role in the quantity and quality of forage available for use by cattle. For instance, high light intensity, high temperatures, and water stress frequently occur simultaneously and can all influence growth and characteristics of the plants that are available. It is difficult in

nature to point out what exactly is impacting the forage when all of these factors are playing a role (Deinum, 1981). One thing that is well known is that as available nutrients in forage decline during the grazing period animals may need additional supplementation in order to meet nutrient requirements for growth and lactation.

Protein Supplementation

Backgrounding systems often use grazed forages to produce yearling cattle, target higher prices at the sale barn, and also ensure a constant supply of finished cattle for packing plants (Gillespie-Lewis et al., 2016). Providing protein supplementation in differing amounts is one way to target different end points, which allows producers to maximize profits. Protein supplementation has been shown to enhance forage utilization and increase livestock performance while grazing (Beaty et al., 1994). Delivering supplements in cubes, on the ground, or in troughs, with or without ample room for animals, changes the amount individual animals consume (Bowman and Sowell, 1997).

The majority of the protein associated with forages is broken down rapidly and utilized by rumen microbes for growth. This protein is known as RDP. This rapidly degraded protein found in growing forages is often not enough to meet the MP requirements of growing calves that are grazing forages (Creighton et al., 2003). Small amounts of protein escape the rumen and are available for digestion in the small intestine, however digestibility of this escape protein, or RUP, differs among plants (Klopfenstein et al., 2001). Understanding that very little RUP is available in forages that are being grazed means that RUP may be the first limiting nutrient that calves need to increase growth (Creighton et al., 2003). Anderson et al. (1988) set up an experiment with 0.11, 0.23, and 0.34 kg/d of RUP. The supplement was an equal-protein-basis mix of corn

gluten meal and bloodmeal and 15% molasses. A linear response in average daily gain (ADG) was observed above an energy control supplement when steers were grazing smooth brome grass pastures ranging from 10.4-13.4% CP. Cattle that received no supplement gained 0.89 kg/d compared to cattle receiving 0.34 kg/d of RUP gained just over 1.00 kg/d. Karges et al. (1992) conducted a study near Whitman, NE looking at both RDP and RUP supplementation to cattle grazing summer native range. Supplements included a negative control that received no supplement, an energy control that received no protein but equal amounts of energy and 3 treatments of RDP and RUP. Low, medium, and high RDP treatments provided 0.15, 0.27, and 0.37 kg/d of RDP, respectively, and consisted of cornstarch, molasses, corn steep liquor, and urea. Low, medium, and high RUP treatments provided 0.07, 0.14, and 0.21 kg/d of RUP, respectively, and consisted of cornstarch, molasses, corn steep liquor, urea, soybean meal, and feather meal. These treatments contained 75% RUP and 25% RDP. This forage ranged from 9.0-14.1% CP and results of this study showed a linear improvement in ADG from 1.02 to 1.10 kg/d with greater amounts of RUP supplementation, which agreed with the Anderson et al. (1988) work. Cattle that received no supplement gained 0.96 kg/d, cattle on the energy control gained 1.02 kg/d, RDP supplementation created a quadratic response with 1.06, 1.04, and 0.96 kg/d as supplementation increased. Smooth brome grass is a cool season grass and summer native range is primarily warm season grasses which demonstrates that both forage types may be unable to provide the total MP that growing cattle require. Utilizing RUP sources such as distillers grains is a great way to make up the balance that the forage source cannot provide. However, as supplement increases animals will begin to replace forage intake with supplement intake. Griffen et

al. (2012) observed a 0.5 to 1.0 kg reduction in forage intake for every kg of dry distillers grains plus solubles that was supplemented. Supplementing distillers grains to growing cattle grazing forages, on average, will increase both final body weight (BW) and ADG.

Supplementing RDP sources, especially on low quality forages, has been evaluated as well. Hafley et al. (1993) found that RUP supplementation resulted in no difference in performance of yearlings grazing 12.0% CP and 48.6% DMD forage source compared to an energy control treatment. However, supplementing RDP tended to increase gains over the energy control treatment. This response to RDP supplementation is based on an increase in forage utilization in the rumen. Lardy et al. (1999) found that summer calving cows grazing native range during the breeding season lost less weight and maintained condition score when supplemented with RDP over cows that received no supplement or an energy supplement. Also performance did not differ from cows receiving a RDP plus RUP supplement. Performance of calves was greatest for the RDP and RDP plus RUP supplemented cows as well.

Differing supplement delivery is another way to alter performance and economics. Beaty et al. (1994) found that supplementing pregnant cows daily or 3 times per week had little to no effect on performance, but reduced input costs of the system. Cattle that are supplemented daily show signs of reduced grazing as they anticipate the delivery of supplement, this behavior is not observed when supplement is delivered less frequently (Melton and Riggs, 1964). Musgrave et al. (2012) showed a reduction in performance of cattle supplemented on the ground compared to those supplemented in bunks. This reduction in response is believed to be due to 36-41% of the DDGS offered

being wasted. Wasted supplement can become a concern, especially when a producer is supplementing large numbers of cattle.

Passage Rate

Animals that are consuming primarily forage based diets have a unique rumen environment. This environment is comprised of three distinct phases which are a liquid phase, a solid floating mat phase, and smaller solid particles that are found in the liquid phase (Vieira et al., 2008). Liquid and solid phases pass at different rates, so research focused on digesta movement attempts to account for each phase independently (Uden et al., 1980). Although passage rate is difficult to measure it is extremely important for several reasons, one of which is because it influences voluntary feed intake to a large degree, especially when forage comprises the majority of an animal's diet and gut fill limits intake (Bartocci et al., 1997). Passage rate is influenced by intake, forage:concentrate ratio, particle size and density, and environmental temperature (Bartocci et al., 1997). Passage rate is increased as intake increases and an assumption that increased passage rate leads to a reduction in digestibility has been generally accepted within the cattle world (Balch, 1950; Colucci et al., 1982). Depressions in forage digestibility at faster passage rates are due to a reduction in mastication and a shorter rumen fermentation period (Colucci et al., 1982). Diets containing different levels of forage, concentrate, and liquid have different rates of passage throughout the tract. Forage, in general, has a longer retention time than concentrate (Colucci et al., 1982). Temperature is a complex factor that has been shown to effect passage rate. As cattle are exposed to increased ambient temperatures their bodies attempt to maintain thermal neutral conditions by limiting intake, especially fiber, thereby reducing heat of fermentation. This reduction in intake reduces passage rate in an attempt to increase

digestibility and secure needed nutrients (Bernabucci et al., 1999). Passage rate undoubtedly has an effect on protein degradation, cell wall digestion, and microbial efficiency (Huhtanen and Kukkonen, 1995). The effects passage rate has on how protein is broken down in the ruminant animal can lead to changes in total MP supplied to the host. Passage rate affects the amount of protein that is available for microbial use, which leads to differences in the amount of protein available for host digestion that is not MCP. (Orskov and McDonald, 1970). When protein that is available in the rumen flows out prior to microbial fermentation the host animal is able to break it down and absorb it through the small intestine. This path of uptake changes the amount of MP supplied to the animal depending on the amino acid (AA) profile of the protein. Essential amino acids (EAA) are required to be consumed or produced outside of the host animal (Merchen and Titgemeyer, 1992). Microbes supply ruminants with some EAA, but this leads to other limiting AA that can reduce growth of animals. When only MCP is supplied methionine, lysine, and threonine are the first three limiting AA, in that order (Merchen and Titgemeyer, 1992). Protein sources that supply these AA are high quality protein because they meet the EAA requirement and help increase growth. However, microbes can utilize both good and bad quality protein to produce more MCP, which is why AA entering the small intestine vary in quantity and quality when compared to AA intake (Merchen and Titgemeyer, 1992). Good quality proteins are more beneficial to the animal when they bypass microbial use and are absorbed into the small intestine as AA (Menke et al., 1979). Understanding exactly how the animal is utilizing protein within the digestive tract is crucial to understand total MP supplied, which is what dictates the necessity of supplementation.

Field Peas

Field pea production in the United States has increased dramatically in recent years (Fendrick et al., 2005). In 2011 around 146,000 hectares were planted and in 2016 just over 513,000 hectares were allocated to field pea production (NASS, 2016). Field peas are primarily grown for human consumption; however, grain that fails to meet specifications for human consumption can be used for livestock feed (Birkelo et al., 2000; Loe et al., 2003; Lardy et al., 2009; Fendrick et al., 2005). Entry into the human consumption market is limited by slow processing equipment and also a high standard that the grain must meet. Recent increases in production of field peas are largely due to the benefits for subsequent harvest of crops in a rotational system. Rotational systems are becoming more popular in order to reduce the amount of engineered inputs by selecting ecological processes that target desired results (Liebman and Dyck, 1993). Generally peas are grown in a rotational system with other crops such as wheat or barley. One benefit is that peas help minimize the reliance on purchased fertilizers by fixing nitrogen in the soil (Anderson et al., 2007; Jenkins et al., 2011). Also rotational systems help reduce the prevalence of weeds while reducing the need for herbicides, which leads to an increase in crop production (Liebman and Dyck, 1993). Field peas also offer benefits like pest control due to the fact that they are not hosts to some pests that can destroy fields of barley or wheat. Studies have shown that planting non-host crops can greatly reduce the incidence of these pests in following years (Allen et al., 1970). Overall utilizing peas in a rotational system helps increase yields of other crops and decreases inputs compared to planting monocultures every year.

Field Peas in Cattle Diets

Field peas are a highly digestible legume and are rapidly fermented in the rumen similar to other cereal grains (Reed et al., 2004). Field peas contain 20-28% CP and around 44% starch, compared to dry rolled corn which contains around 72% starch (Fendrick et al., 2005; NASEM, 2016). Starch fermentation rate is similar to corn, but is slower than both barley and wheat (Anderson et al., 2007). Finishing diets show no difference in performance of cattle fed whole field peas or dry rolled field peas, which suggests that processing is an unnecessary step when utilizing peas (Fendrick et al., 2005). The protein fraction in peas is highly degradable (Mustafa et al., 1998) and RDP can be as high as 73.0% of total CP when using in situ bags to determine protein degradation (Aufreere et al., 1994). Estimates for RDP range from 65.0-73.0% of CP, making RUP anywhere from 27.0-35.0% (Anderson et al., 2007; Greenwell et al., 2018). Greenwell et al. (2018) used in situ bags to determine RUP content of field peas and also RUP digestibility. This study showed field peas contain 30-35% RUP as a percent of CP and 97.4-98.9% RUP digestibility. The nutrient profile of peas is comparable to other cereal grains making them a viable option in cattle diets.

Field peas have been studied in several performance studies ranging from creep feeding to finishing diets and comparing field peas to more traditional protein sources or cereal grains. Utilizing field peas in creep feed replacing wheat middlings did not result in any differences in ADG; however, unless intake was restricted by salt greater inclusions of field peas led to an increase in dry matter intake (DMI; Gelvin et al., 2004). Other studies showed that ADG was not different when peas were included at 33% or 67% of the creep feed with the remainder being wheat middlings, and that intake did not differ between treatments (Anderson, 1999; Landblom et al., 2000). In receiving diets

when field peas replaced 12% corn and 9% canola oil ending BW was greater, DMI was greater, but there were no differences in ADG or gain to feed (G:F) compared to the control diet (Gilbery et al., 2007). In another study replacing DDGS, wheat middlings, and barley malt with 20% field peas in a receiving diet resulted in no difference in receiving performance or finishing performance, but net returns were increased from \$8.90 to \$13.23 per head in favor of the field pea receiving ration (Landblom et al., 2007). In a third growing study no differences in DMI were observed, but an increase in VFA concentration and a lower mean pH were observed when peas replaced corn at increasing levels up to 50% of the total diet. Diets that contained 50% dry rolled corn and no field peas had a mean pH of 6.68 and when corn was completely replaced with field peas the mean pH dropped to 6.62. The change in mean pH coupled with the increase in VFA concentration indicates that peas were rapidly fermented and utilized by the microbes and were a suitable replacement for corn (Reed et al., 2004). A study looking at performance differences when a 70.8% corn, 24% condensed distillers solubles, and 5.2% urea supplement versus field peas were supplemented at 0.5% of BW to growing cattle grazing crested wheat grass showed a reduction in ADG from 0.99 to 0.87 kg/d and a lower ending BW for the field pea treatment. However, performance of the same cattle during the finishing phase resulted in a tendency ($P=0.07$) for field pea supplemented cattle to have a greater ADG. A tendency ($P=0.07$) for lower hot carcass weight (HCW) was also reported for the field pea supplemented cattle when compared to the soluble supplemented cattle with 394 kg and 403 kg HCW, respectively. However, replacing 20% DRC in the finishing diet with field peas resulted in no difference in ADG or HCW (Greenwell et al., 2018). Replacing dry rolled corn (DRC) with 15%, 30%, or 45%

inclusion of field peas resulted in no difference in performance for lambs fed finishing diets. In another study, DRC was replaced with field peas at 10% of the diet and there were no differences in performance of finishing cattle or carcass characteristics at the plant. In this study, whole field peas were also compared to rolled field peas and there were no differences in performance based on processing method. This study concluded that peas are a suitable replacement for DRC (up to 10% of diet DM) and there is no benefit from processing the field peas (Birkelo et al., 2000). Another study showed replacing DRC with field peas at 10%, 20%, and 30% of the diet resulted in no performance or carcass differences, however greater inclusion of peas increased overall desirability and flavor of the beef (Jenkins et al., 2011.) Overall field peas seem to be a viable option for replacing other cereal grains in creep feeds, receiving diets, and also in finishing diets without having detrimental effects on performance, and in some cases may improve economics depending on price procurement of peas.

Conclusion

In conclusion, protein work in grazing cattle is challenging due to the fact that cattle feeders are essentially supplying two creatures protein that each use at different efficiencies. The metabolizable protein system does a fairly adequate job of allowing producers to maximize protein utilization by understanding RDP and RUP requirements. However, the need for research in protein is still great. Values for both RDP and RUP are difficult to calculate because many factors affect actual MP supply. Passage rate, nitrogen recycling, and energy availability are all key components that impact the amount of RDP being converted into MCP. Values for the amount of RUP that can be absorbed as MP depend heavily on RUP digestibility and passage rate.

A greater supply of field peas than current markets can support has led to interest in using field peas as cattle feed. Field peas have been shown to be beneficial in both growing and finishing cattle diets. The nutrient profile of field peas has led to interest in using them as a supplement for growing cattle. The objective of my first study was to compare DDGS and field peas as grazing supplements for growing cattle. This study is designed to look at performance and economics of field peas compared to a well-known product that has an established price and market.

The lack of research and difficulty calculating RDP and RUP values has led to my second study, which will look at passage rate, protein utilization, and digestibility. The objective is to determine passage rate of different forages, which will increase accuracy in RDP and RUP values. A better understanding of where in the digestive tract forages are broken down will allow producers to supplement only what cattle actually need in order to meet a desired production level. This exact supplementation will ultimately lead to less environmental impact and also an increased economic savings for producers.

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Chapter II. Economics of Field Pea Supplementation for Cattle Grazing Crested Wheatgrass

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Abstract

Field peas are widely grown in the panhandle of Nebraska, however, human consumption markets quickly become saturated. A two year experiment was conducted comparing field peas to dry distillers grains with solubles (DDGS) as a protein supplement for grazing cattle. The objective was to establish a price producers could pay for field peas relative to DDGS based on performance differences. In yr. 1, 112 heifers (294 kg; SD=34.6) grazed crested wheat grass pasture (n = 12) using a 2×2 factorial treatment design. In yr. 2, 114 spayed heifers (306 kg; SD=16.3) were utilized. Treatments included peas or DDGS supplemented at either 0.4% or 0.8% of BW. Pasture was the experimental unit and cattle were blocked by BW. Two day weights were collected and averaged to calculate beginning and ending BW. Interim BW was also collected to adjust the amount of supplement offered. There was no interaction between type and level of supplement ($P = 0.23$). Also, level of supplement was not significant ($P = 0.15$), cattle fed field peas at 0.4 or 0.8% of BW gained 0.97 and 0.98 kg/d, respectively, compared to 1.02 and 1.15 for the low and high DDGS supplemented heifers. There was a significant difference in ADG due to type of supplement ($P = 0.02$). Field pea supplemented heifers had 10% lower ADG compared to DDGS supplemented heifers. Economically, this means if corn is priced at \$124.58/ 909 kg DM, and DDGS is priced similar to corn, a producer should pay \$112.13/ 909 kg DM, or \$2.89/ 27 kg, for field peas.

key words: beef cattle, DDGs, field peas, protein supplementation

Introduction

Calf-fed and yearling systems are the two most common production systems in the US. One study has shown that low input grazing systems may be more economical than high input drylot systems when animals are retained through the finishing phase (Mathis et al., 2008). Supplementing cattle during the grazing season has been used to increase average daily gain (ADG). Dry distillers grains plus solubles (DDGS) is one of the most common supplements that has been used in parts of Nebraska.

The lack of DDGS availability in western Nebraska has led to a search for a competitive alternative, such as field peas (*Pisum sativum*), a highly digestible legume (Reed et al 2004). Field peas are grown in western Nebraska, and production in the US has increased due to dryland wheat farmers' interest in rotational cropping systems (Fendrick et al., 2005; Titlow et al., 2014). Peas, like other legumes, offer nitrogen fixation into the soil (Anderson et al., 2007) and help reduce weeds and pests that destroy subsequent crops (Liebman and Dyck, 1993; Allen et al., 1970). Also peas provide the option to either graze or harvest them prior to planting subsequent crops (Titlow et al., 2014). Field peas are grown primarily for human consumption, but this market becomes quickly saturated causing a surplus that is available for the livestock industry to use (Birkelo et al., 2000; Loe et al., 2003; Fendrick et al., 2005; Lardy et al., 2009; Greenwell et al., 2018a). Operations with limited storage availability or enterprises that grow crops and cattle can see economic benefits from feeding field peas instead of retaining them until markets reopen (Pesta et al., 2012). Peas are high in crude protein (CP; 20-28%) and are around 44% starch (Fendrick et al., 2005; NASEM, 2016). Unlike DDGS, which are 63% rumen undegradable protein (RUP; Castillo-Lopez et al., 2013), field peas

contain 27-35% RUP both as a percent of CP (Anderson et al., 2007; Greenwell et al., 2018b).

The objectives of this study were to determine the value of field peas as a protein supplement for grazing cattle, and to determine a pricing mechanism relative to DDGS.

Materials and Methods

Two hundred and twenty-six yearling heifers were used in this trial. The study was conducted over a two year period at the High Plains Ag Lab (HPAL) near Sidney, NE. Prior to arrival at HPAL, heifers were vaccinated against respiratory and clostridial viruses and administered a growth implant. All animals involved in this trial were managed in accordance with the protocols approved by the Animal Care and Use Committee at the University of Nebraska.

Animal Management

In Yr 1, 112 intact predominately British and British cross heifers (294 kg; SD=34.6) were utilized during the summer of 2016. Grazing was initiated May 20 and heifers were removed from grass on September 19. In Yr 2 114 spayed British and British cross heifers (306 kg; SD=16.3) were utilized during summer of 2017. Grazing was initiated May 23 and ended September 7. Both years heifers were individually weighed on the morning of day 0 and 1. Heifers were weighed off of one large pasture. These weights were averaged to determine initial BW of the heifers. Cattle were blocked by body weight into a light, medium, and heavy body weight and then assigned randomly to pastures. Treatments were assigned randomly to each pasture. Treatments were arranged in a 2x2 factorial design with whole field peas or DDGS supplemented daily at either 0.4% or 0.8% of body weight (BW). Supplement amount was based on the initial BW at the initiation of the trial, but was prorated to be delivered only six days per week.

Supplement was weighed into buckets and hand delivered Monday through Saturday into bunks near each pasture's water source. Interim weights were collected on July 13 and July 12 during Yr 1 and Yr 2, respectively. This weight was used to adjust the supplement amount for the duration of the trial. No supplement refusals were ever present throughout the trial. The trial ended on day 124 and day 108 for Yr 1 and Yr 2, respectively. Two day individual weights were taken on the morning of d 124 and 125 for Yr 1 and d 108 and 109 for Yr 2. Heifers were weighed off of pastures and grazed a large pasture as a group in between weigh days. These weights were averaged to calculate ending BW. Average daily gain (ADG) was calculated using initial and ending BW.

Pasture Management

Treatment groups were assigned randomly to one of twelve pastures to start the trial each year. Pastures were stocked such that 4.25 ha were allotted per animal and cattle were rotated through the pastures every two weeks to minimize pasture effect on treatment differences. Nine pastures were larger and were stocked with 10 hd and 3 pastures were smaller and were stocked with 8 hd. Animals in the larger groups rotated through all nine pastures over the course of the trial and animals on the smaller pastures rotated through only the smaller pastures.

Statistical & Economical Analysis

This trial was set up in a 2x2 factorial arrangement with supplement type and level being the two factors. Cattle were blocked by initial BW into light, medium, and heavy blocks both years. Each block had one replication per treatment per year, which allowed for a total of six replications (3/yr) per treatment over the two year study. Groups of cattle were used as the experimental unit and block and year were treated as fixed effects. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc, Cary,

NC) and probabilities were considered significant if $P \leq 0.05$. The economic analysis was calculated by using percent change in performance (ADG) of the DDGS minus the ADG of the field peas divided by the ADG of the DDGS. Corn price per bushel as-is was used to determine DDGS price per 909 kg. Field pea price was calculated based on the performance difference when compared to DDGS.

Results

Cattle Performance

Initial BW did not differ among treatments ($P \geq 0.60$; Table 1). There was no interaction between level and type of supplement ($P = 0.23$). Level of supplement was not statistically significant ($P = 0.15$), cattle fed field peas at 0.4 or 0.8% BW gained 0.97 and 0.98 kg/d respectively. Heifers supplemented with DDGS at 0.4 or 0.8% gained 1.03 and 1.14 kg/d respectively. Griffen et al. (2009) pooled 35 pasture studies and reported a 13.7% increase in ADG when DDGS supplementation increased from 0.4 to 0.8% BW. Similarly, this study showed a 10.7% increase in ADG at the differing levels, although it was not statistically different. Type of supplement was statistically different ($P \leq 0.02$). Field pea supplemented heifers had 10% lower ADG compared to DDGS supplemented heifers at 0.98 and 1.09 kg/d respectively. Ending BW was also greater for DDGS supplemented heifers ($P \leq 0.04$) than field pea supplemented heifers. Supplementation level was not different ($P \geq 0.46$). Greenwell et al. (2018a) reported an increased ADG when growing cattle grazing crested wheatgrass pastures were supplemented with a dry rolled corn (70.8%), condensed distillers solubles (24.0%), and urea (5.2%) supplement compared to either field peas or no supplement. Supplementation occurred at 0.5% of BW on a dry matter basis. Field pea supplemented cattle gained 0.87 kg/d whereas the control cattle and the corn blend supplemented cattle gained 0.69 and 0.99 kg/d,

respectively. Performance of cattle in this study was consistent with the current study's results. Conversely, Landblom et al. (2007) reported no difference in ADG when field peas replace DDGS, wheat middlings, and barley sprouts in a growing diet. Gelvin et al. (2004) also reported no difference in calf ADG when field peas replaced soybean meal in a creep feed.

Economic Analysis

Economically, the reduction in heifer performance means that field peas should be worth 10% less than DDGS. Corn price is often used as the basis for pricing DDGS. Assuming DDGS is priced equal to corn, which is priced around \$124.58/ 909 kg dry matter (DM), a producer could afford to pay \$112.13/ 909 kg DM, or \$2.89/ 27 kg, for field peas. Field pea price is calculated based on the DDGS price \$124.58 minus [$124.58(0.10 \text{ reduction in ADG})$]. As corn price fluctuates (Table 2), the price a producer can afford to pay for field peas will vary as well. Currently field peas are entering the human consumption market at \$6.50/ 27 kg, however this market is quickly saturated. Pricing of supplement calculated in the table does not include trucking costs. Field peas are primarily grown in the western panhandle of Nebraska, which is an area with few ethanol plants and limited access to DDGS. Assuming DDGS is hauled at \$3/loaded 1.6 km and local peas require minimal trucking, for producers in that part of the state, the economics of supplementing field peas becomes much more relevant. One example would be a producer located in Sidney, NE that is purchasing DDGS from an ethanol plant in Hastings, NE. This product would be hauled 418 km and would increase the cost of DDGS by \$34.67/ 909 kg assuming a 22,700 kg load capacity per truck, 90% DM DDGS product, and \$3/loaded 1.6 km. If this producer decides to purchase DDGS from a plant in Kearney, NE they would need to haul the product only 351 km. This

would reduce trucking costs to \$29.07/909 kg. The reason DDGS is more appealing than using WDGS is because the DM of DDGS is 90%, whereas the DM of WDGS is around 30%. This means that a producer is hauling less DM on each truck resulting in a higher increase in DM cost per 909 kg.

Alternatively, using the assumption that the response from DDGS is linear, animals receiving no supplement would have an ADG of 0.92 kg/d. This means that the additional response in gain from feeding field peas is worth 0.06 kg/d compared to 0.17 kg/d when supplementing DDGS. This results in field peas having only 35% the response above the control compared to DDGS when supplemented at 0.4% or 0.8% of BW.

Implications

Utilizing field peas in the panhandle of Nebraska is a viable option for producers in need of a protein supplement for cattle. Understanding the 10% reduction in ADG compared to DDGS is an important concept to account for when determining price procurement of the field peas. Also factoring in trucking costs of the DDGS is key due to the large distance from most available sources. Integrated producers may recognize benefits on both the agronomic side and cattle performance side from growing and feeding field peas within their current systems.

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Table 1. Performance of heifers grazing crested wheatgrass pastures supplemented with whole field peas or distillers grains plus solubles

	<u>Treatments³</u>				SEM	Type	<u>P-value</u>	
	0.4 % D ⁴	0.8% D	0.4% P ⁵	0.8% P			Level	Interaction
Initial BW ¹ , kg	301	300	301	298	9.6	0.88	0.60	0.82
Ending BW ¹ , kg	419	431	412	410	13.6	0.04	0.46	0.26
ADG ² , kg	1.03	1.14	0.97	0.98	0.09	0.02	0.15	0.23

¹BW=body weight

²ADG=average daily gain

³Treatments=amount of supplement fed based on % of body weight

⁴D=distillers grains plus solubles

⁵P=whole field peas

Table 2. Economic value of field peas compared to DDGS

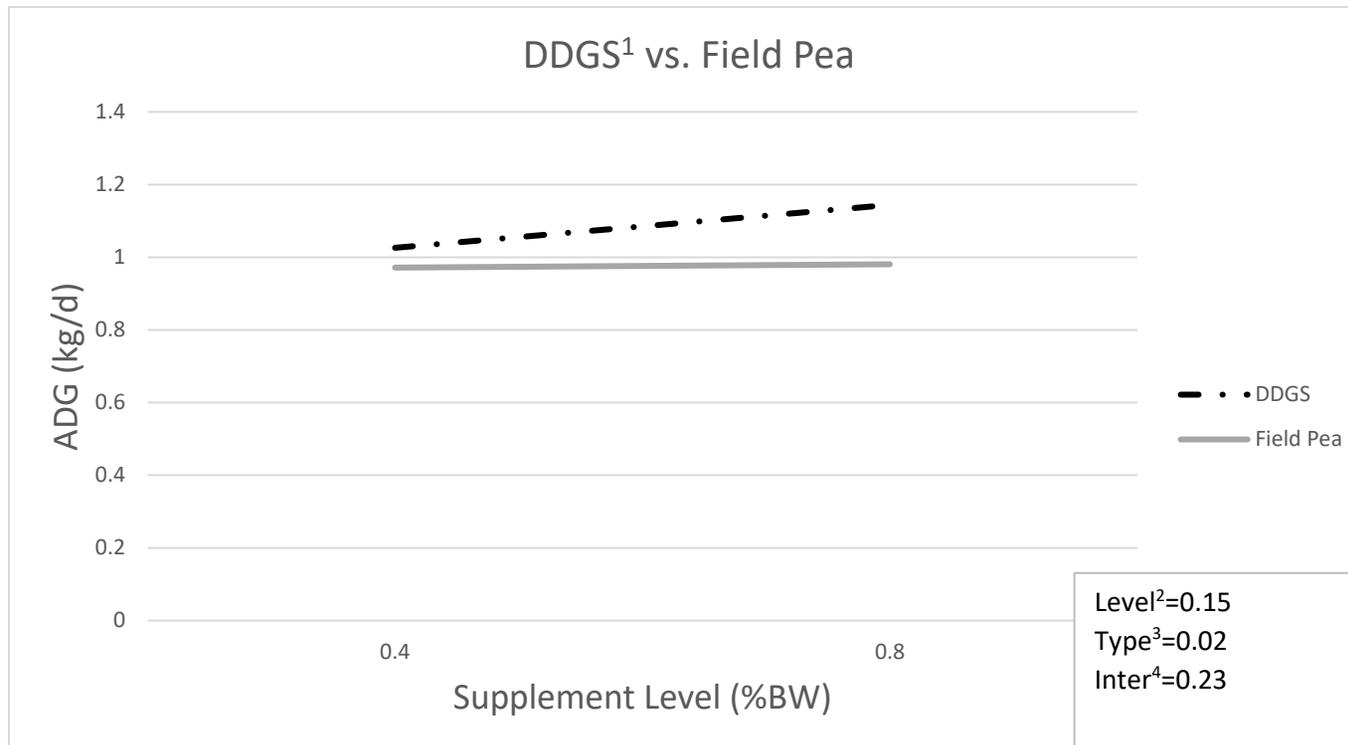
Corn \$/25.5 kg	DDGS ¹ \$/909 kg DM	Field Pea \$/27 kg ²
3.00	124.58	2.89
3.50	145.35	3.38
4.00	166.11	3.86
4.50	186.88	4.34
5.00	207.64	4.82

¹DDGS = dry distillers grains plus solubles; prices shown are equivalent to corn price

²Equivalent price for field peas given a 10% reduction in ADG compared to DDGS

$$-[\text{DDGS} - (\text{DDGS} \times 0.10)] = \text{Field Pea}/909\text{kg}$$

Figure 1. Performance of heifers supplemented with field peas or dry distillers grains plus solubles while grazing crested wheatgrass pastures



¹DDGS=dry distillers grains plus solubles

²Level=p-value related to level of supplement

³Type=p-value related to type of supplement

⁴Inter=p-value related to the interaction between level and type of supplement

Chapter III. Utilization of Forage Crude Protein and the Need for Rumen Undegradable Protein Supplementation

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Abstract

Variable sources of grazed forages are used in cattle backgrounding systems across the US. Often, metabolizable protein (MP) is limiting in these backgrounding systems and rumen undegradable protein (RUP) supplements are fed to increase ADG and G:F. The objective of this study was twofold, the first objective was to determine if RUP is limiting when forage is immature and provides large amounts of protein to growing cattle. The second objective was to determine if forages that have rapid passage rates and are highly digestible allow some rumen degradable protein (RDP) to bypass the rumen prior to utilization by the microbes and become available for absorption in the small intestine, similar to RUP. A pooled analysis was conducted looking at RUP supplementation of growing animals grazing a variety of forages. Average daily gain (ADG) increased with increased RUP supplement, with the exception of animals grazing forages that were 17% crude protein (CP) or greater. This led to a metabolism study utilizing 4 cannulated animals ad libitum fed a low quality brome (LQB), high quality brome (HQB), alfalfa (ALF), or an oat/alfalfa (OAT) blend (70% DM and 30% DM respectively). Animals were fed twice daily and adapted to new diets over a period of 10 days. Results showed that animals fed LQB and HQB diets had lower intakes and lower digestibility values compared to ALF and OAT treatments. Several internal markers were used along with titanium dioxide as an external marker to evaluate markers in forage based diets.

key words: RUP supplementation, digestibility markers, forage digestibility

Introduction

Forages are the predominant source of feed used in beef production (NASEM, 2016). However, differences in digestibility, CP, and palatability are caused by changing plant species, maturity, and management (Bohnert et al., 2011). Forages are composed of cell wall components and cell solubles. Cell solubles are nearly 100% digestible, so differences in forage digestibility can be attributed to cell wall differences (Deinum, 1981). Forage quality and yield are also variable throughout a grazing season, but are closely related to plant maturity, although environmental factors and grazing management result in changes as well (Buxton, 1996). Determining what factors are impacting the quality and yield of the forage is difficult when there are so many interacting factors (Deinum, 1981).

Backgrounding systems often use grazed forages to produce yearling cattle and target higher total sale prices at the sale barn (Gillespie-Lewis et al., 2016). However, metabolizable protein (MP) requirements are often not met when growing cattle are grazing forages alone (Creighton et al., 2003). Metabolizable protein deficiency results from forage protein being primarily rumen degradable protein (RDP), which is used by the microbes in the rumen to produce microbial crude protein (MCP). Rumen degradable protein is often the first limiting nutrient that grazing calves need to increase body weight gain on grass (Creighton et al., 2003). Small amounts of RDP do escape the rumen due to rate of passage and poor microbe utilization when high quality forages are consumed (Volden et al., 2002). However, the amount of bypass soluble protein and rumen undegradable protein (RUP) vary among plants (Klopfenstein et al., 2001).

The first objective of this study was to determine if cattle grazing immature forage sources that supply large amounts of protein are limited in RUP. The second objective was to determine if forages that have rapid passage rate and are highly digestible allow some rumen degradable protein (RDP) to bypass the rumen prior to utilization by the microbes and become available for absorption in the small intestine, similar to RUP.

Materials and Methods

Experiment 1

Data were collected from 10 previously conducted studies published in the Nebraska Beef Cattle Reports. Studies were conducted from 1987 to 1991 and included 458 steers and 210 heifers grazing a variety of pastures. All data in this analysis are from published data and no live animals were used to conduct this study. Titles and authors are listed in Table 1. Studies selected were all designed with growing animals grazing pastures and individually receiving varying levels of RUP supplement. Type of RUP supplement varied and animals received anywhere from 0 to 0.255 kg per animal daily. Additionally, each RUP treatment was formulated to provide equivalent energy as the control to ensure any average daily gain (ADG) response was due to RUP supplement alone. In order to compare response across trials, ADG was regressed above the ADG of the control treatment. Crude protein (CP) values of the grazed forage varied from 10.4% to 21.7% and were measured by collecting forage samples via clipping or diet sampling over the grazing period from cannulated steers.

Anderson et al. (1988) conducted two trials. Trial 1 utilized 59 steers (277 kg) grazing smooth bromegrass from April to July. Treatments included an energy control (corn starch and 30% molasses) and 0.08, 0.17, and 0.26 kg/animal/d of RUP

supplemented. This supplement consisted of corn gluten meal, bloodmeal, and 15% molasses. Animals were supplemented individually using a Calan gate system. In trial 2 60 steers (244 kg) grazed smooth brome grass pastures from August to November. Treatments mirrored trial 1, but 0.23 kg/animal/d of soyhulls was added to all treatments and molasses in the RUP treatments increased to 30% due to palatability issues. Forage samples were collected at 2 week intervals via hand clipping approximately 2.5 cm above ground. Forage sampled during trial 1 contained 10.4% CP and 60.1% in-vitro dry matter digestibility (IVDMD). Forage sampled during trial 2 contained 13.4% CP and 56.4% IVDMD.

Drouillard and Klopfenstien (1989) conducted a trial utilizing 60 steers (263 kg) grazing smooth brome grass pastures from May to July. Treatments consisted of two levels of RUP supplementation and three levels of Tetronasin (ionophore) in a 2 x 3 factorial arrangement. Treatments included either control or 0.17 kg/animal/d of RUP supplement. The RUP supplement contained corn gluten meal, bloodmeal, and 30% molasses. Control animals received corn starch and 30% molasses in order to supply equal amounts of energy as the RUP supplemented treatment. Tetronasin was fed at 0, 30, or 90 mg/animal/d. Forage samples showed that smooth brome grass was 16.3% CP.

Goedeken et al. (1988) conducted a trial using 60 steers (270 kg) grazing smooth brome grass from April to July. Animals were supplemented cornstarch to provide energy at either 0 or 1.36 kg/animal/d. Also animals were allocated to receive a blend of corn gluten meal and blood meal which provided RUP at 0, 0.06, or 0.12 kg/animal/d. Treatments were set up in a 2 x 3 factorial arrangement with energy level and RUP level

as the two factors. Molasses was added at 30% to help reduce any palatability issues. Pastures in this study provided 16.3% CP.

Hafley et al. (1993) conducted two trials looking at RUP response. Trial 1 utilized 72 steers (286 kg) grazing pastures consisting of big bluestem, switchgrass, and Indian grass from June to August. Animals were assigned to one of three treatments. Treatments included an energy control (cornstarch and molasses) and energy control plus 0.08 or 0.15 kg/animal/d of RUP. The supplement consisted of corn gluten meal and blood meal. Energy was equal across all treatments to ensure that gain above the control was a protein response. Trial 2 utilized 90 heifers (240 kg) grazing the same pastures as trial 1, but receiving one of five possible treatments. Animals grazed from June to August. Treatments were negative control (no supplement), energy control, energy control plus 0.14 kg/animal/d RUP, energy control plus 0.22 kg/animal/d RDP, or an energy control plus 0.36 kg/animal/d blend of RUP and RDP. Non-enzymatically browned soybean meal plus feather meal provided the RUP supplement and corn steep liquor plus urea provided the RDP supplement. Blending the two supplements resulted in the final treatment that provided both RUP and RDP. For both studies pastures were sampled three times during the grazing period using esophageally fistulated animals. Values reported for trial 1 averaged 48.57% in vitro DMD and 11.5% CP as a percent of DM. Pastures in trial 2 had a 49.2% in vitro DMD and 10.4% CP as a percent of DM on average.

Hollingsworth et al. (1993) also conducted two trials. Trial 1 utilized 120 heifers (240 kg) grazing either piper sudangrass or pearl millet from July to September. Treatments included an energy control (cornstarch and molasses) or 0.09, 0.14, or 0.19 kg/animal/d RUP supplement (corn gluten meal and bloodmeal). Treatments were

arranged in a 2 x 4 factorial arrangement with forage source and supplement amount being the two factors. Forage was sampled using fistulated animals and also by collecting hand clipped samples. Sudangrass had 65.6% IVOMD and provided 18.0% CP on average. Pearl millet had a 63.3% IVOMD and provided 15.0% CP on average. Trial 2 utilized 60 steers and 60 heifers (272 kg) grazing piper sudangrass from June to August. Treatments were a negative control (molasses), an energy control (corn starch and molasses), 0.09 kg/animal/d RUP (molasses, corn gluten meal, and bloodmeal), 0.09 kg/animal/d RUP plus energy (molasses, corn gluten meal, bloodmeal, and corn starch), or 0.17 kg/animal/d RUP (molasses, corn gluten meal, and bloodmeal). Treatments 2, 4, and 5 were all isoenergetic. Ruminally fistulated steers were used to collect forage samples along with hand clipped samples. Piper sudangrass had 70.0% IVOMD and provided 21.7% CP on average.

Irlbeck et al. (1990) utilized 100 steers divided into two groups. Group one grazed 127 d (274 kg) and group two grazed for 70 d (330 kg) both on smooth bromegrass pastures. Treatments were an energy control (corn starch and molasses) or 0.04, 0.09, 0.13, or 0.18 kg/animal/d of RUP supplemented. This supplement contained molasses, corn gluten meal, and bloodmeal. Forage samples were collected weekly with esophageally fistulated steers. Analysis on forage samples resulted in 15.0% CP during the 127 d grazing period and 16.3% CP during the 70 d grazing period.

Karges et al. (1992) utilized 40 steers (326 kg) grazing summer native range from June to September. Treatments included in the analysis were an energy control (corn starch and molasses) and 0.07, 0.14, or 0.21 kg/animal/d of RUP from molasses, soybean meal, and feather meal. Forage samples were collected at the beginning of each month

from esophageally fistulated steers. Forage analysis resulted in 9.4% CP as a percent of DM on average.

Studies were divided into several groups to look at correlations between ADG and initial body weight (BW), type of forage, or CP of the forage. The pooled analysis included three studies with initial BW under 250 kg, five studies with initial BW between 250 to 300 kg, and two studies with initial BW over 300 kg (in one study one-half of the cattle were over 300 kg and half were between 250 to 300 kg and these were subdivided). The Hollingsworth et al. (1993) trial was removed from the BW response if CP of forage grazed was above 17.0% due to lack of response to supplement. Studies were also divided into three types of forage being grazed. Within this analysis five studies evaluated cattle grazing brome grass, three studies evaluated cattle grazing warm season grasses, and two studies evaluated cattle grazing summer annuals. Once again the goal was to see if type of forage had an effect on ADG with increasing RUP supplementation. The last division in this analysis was based on CP of the forages. This analysis had five studies with cattle grazing forages with less than 13.5% CP, three studies with forage CP between 13.5 to 17%, and two studies in which the forage had over 17% CP.

Correlations and slopes of each line were analyzed using PROC REG of SAS. Differences in slopes of lines within each category were analyzed by PROC GLM with $P \leq 0.05$ being considered significant and $P < 0.10$ being considered a tendency. Treatment within study was the experimental unit in this pooled analysis.

Experiment 2

Two ruminally and duodenally cannulated heifers and two ruminally cannulated steers were utilized in a digestion trial to better understand protein utilization of ruminants when fed different forages containing high, medium, or low amounts of protein. Steers were housed individually in 2.4 x 1.5 m² concrete slatted floor pens with ad-libitum access to water. Housing rooms were temperature controlled at 25°C. Treatments (n = 4) consisted of low quality brome (LQB), high quality brome (HQB), high quality alfalfa (ALF), and a 70% oat and 30% alfalfa blend (OAT) fed ad-libitum. Animals also had access to a pressed mineral block containing salt, manganese, iron, copper, zinc, iodine, and cobalt.

Animals were fed twice daily at 0830 and 1630 h, feed refusals were collected daily prior to feeding and weighed back on an as fed basis. Refusals were re-fed as part of the following days feeding on d 10 to 13, but on d 14 they were sampled and removed from the bunks to start the next diet. Refusal samples from d 14 were dried for 48 h in a 60°C forced air oven to determine DM and correct for DMI. Ingredient samples were collected on d 10 and 12, subsampled, composited by period and frozen. One subsample was dried for 48 h in a 60°C forced air oven to correct ingredient DM. After trial completion, ingredient samples were ground through a 1-mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Oat samples were freeze dried (Virtis Freezemobile 25ES) prior to grinding.

Periods consisted of 14 d with 10 d of adaptation and 4 d of collection. Animals were continuously dosed with 8 g of TiO₂ via rumen cannula, as an indigestible marker, twice daily at 0830 and 1630 h for a total of 16 g/d of TiO₂. Collections occurred from d

11 to d 14, as well as a collection the morning following d 14. During collection on d 11 to d 14 fecal samples were collected at 0830, 1230, 1630, and 2030 h. Duodenal samples were also collected d 12 to d 14 at 0830, 1230, 1630, and 2030 h. Three g of Cobalt EDTA (0.4 g Co) dissolved in 200 mL ddH₂O was dosed via rumen cannula at 0830 h on d 11 and rumen liquid samples were collected at 0830 prior to dosing, 1030, 1230, 1430, 1630, 1830, 2030, and 2230 h. A sample of rumen liquid was also taken at 0830 h on d 12 to ensure the marker had completely passed out of the rumen. All samples were immediately frozen at -20°C. After each period, fecal samples were composited by day for each animal, freeze dried, and ground through a 1-mm screen using a Wiley Mill (Thomas Scientific). Composites were formed by sub-sampling an equal weight from each time point into the daily composites. Following grinding, samples were composited by period for each animal. Whole rumen samples and omasal samples were collected following a complete rumen evacuation at 0630 the morning following d 14 prior to feeding the new diet. Samples were frozen immediately at -20°C. Following the trial, duodenal samples were freeze dried and ground through a Tecator cyclotec sample mill (American Instrument Exchange, Haverhill, MA). Then samples were composited into period samples. Omasal samples were freeze dried and then ground through a 1-mm screen using a Wiley Mill (Thomas Scientific).

Feed, orts, duodenal, omasal, and fecal samples, composited by period, were dried at 100°C for 24 h to determine DM and then burned in a cool muffle furnace at 600°C for 6 h to determine OM (organic matter). Crude protein of feed samples were determined for each period and averaged over the entire trial. Additionally, all samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) as described

by Van Soest et al. (1991). Sodium sulfite was added to all samples at 0.5 g. Following ADF analysis, dried filters (GE Healthcare Whatman 541) from the ADF analysis were placed in a cool muffle furnace at 600°C for 6 h to determine acid insoluble ash (AIA) content. The AIA content of each sample was used to calculate fecal output and digestibility.

An additional marker, indigestible ADF (IADF) was used to calculate digestibility values for each treatment. Ankom F58 bags containing 0.5 g of feed, orts, duodenal, and fecal samples were ruminally incubated in two heifers for 168 h with 2 bags / animal for each sample type. An additional 2 bags / animal for feed samples were incubated for 240 h to confirm complete digestion within the bags. Ninety bags were placed into each mesh bag and inserted into the ventral sac of the rumen. Weight was added to the mesh bags to keep samples in the ventral sac of the rumen. After 168 h or 240 h, bags were removed and analyzed for IADF using the Ankom Fiber Analyzer (Ankom Technology). Fecal output, duodenal flow, and digestibility were calculated using IADF as a second internal marker.

In vivo digestibility values were adjusted back to in vitro digestibility values using the Geisert et al. (2007) adjustment. These adjustments were determined by feeding eight animals five types of hay. Prairie hay and mature and immature alfalfa and brome were used. These in vivo digestibility values were calculated using total fecal collection and regressed against in vitro digestibility values on the same hay samples. Geisert et al. (2007) determined adjustment values for each standard that was included in this in vitro run, which allows a standard adjustment to be applied to all unknown forage samples and help predict in vivo digestibility for the unknown. Adjusted in vitro digestibility values

are presented in table 4.1 and 4.2 and were compared to in vivo digestibility values calculated using both internal and external markers.

Rumen liquid was analyzed by atomic absorption spectrophotometry to determine ruminal liquid dilution rate. Dilution rates were calculated from ruminal cobalt concentrations from 2 to 14 h after dosing. Lastly duodenal and omasal samples were analyzed for purine concentration to determine microbial flow using a modified Zinn and Owens (1986) procedure with a more dilute HClO_4 to hydrolyze material containing purines (as described by Crawford et al., 2008). Purine concentration was determined on a spectrophotometer (Spectra Max 250 Spectrometer, Molecular Devices) at 260 nm. Measuring purines, which are present in all DNA and RNA, and N in these samples, allows for the amount of MCP that is flowing through the system, to be calculated based on a common purine to N ratio found in the microbes. This study used a 0.25 purine to nitrogen ratio (Mass et al., 2001).

Results

Experiment 1

After breaking the studies into different categories some correlations were apparent. The first subdivision was with initial body weights to determine if cattle at lighter weights required more RUP supplement to facilitate their rapid growth and development (Figure 1). Within these trials smaller cattle had the lowest response to RUP supplement, 0.44 kg/d increase in ADG for each 1.0 kg increase in RUP supplement compared to 0.97 kg/d for larger cattle. Medium weight cattle had a 0.51 kg/d increase in ADG for each 1.0 kg increase in RUP supplement, which was not different than the response for the smallest cattle ($P = 0.94$), but both medium and small cattle had lower responses ($P < 0.02$) compared to heavy cattle. This may be due to the MP being more

efficiently converted into net protein (NP) in the smaller animals (NASEM, 2016). Growing cattle are using MP for maintenance and gain, larger cattle would require more MP for maintenance leaving them more deficient and resulting in a larger response from supplemental protein (Wilkerson et al., 1993). Correlations for ADG compared to BW were all very strong with cattle >300 kg having the lowest correlations ($r^2 = 0.70$). All the cattle analyzed in these trials were between 238 and 330 kg.

Looking into the correlation between ADG and amount of RUP supplement relative to type of forage was the next analysis (Figure 2). Warm season grasses had the strongest correlation ($r^2 = 0.81$) and showed an increase of 0.43 kg ADG for each 1.0 kg increase in RUP supplement provided. Brome grass showed a similar trend with 0.63 kg ADG increase with each additional 1.0 kg of RUP supplementation; however the correlation was lower ($r^2 = 0.66$). Summer annuals had no correlation ($r^2 = 0.00$) and did not show a response to the RUP supplementation. Response of these animals was not different from 0 ($P = 0.84$). The low correlation is due to the fact that animals grazing pearl millet (15.0% CP) had a positive response in ADG, but animals grazing sudangrass (18.0-22.0% CP) had a negative response. No statistical difference was detected between grass types ($P \geq 0.43$). The numerical differences observed due to type of forage may be due to forage quality, specifically CP content.

The idea to look at the relationship between CP of the forage and ADG related to increasing RUP supplementation (Figure 3) stemmed from the trends observed in the types of grass. Summer annuals made up the high CP subdivision, except for pearl millet, which had a CP of 15.0%. Studies in which cattle grazed forages with 13.5 to 17.0% CP showed the greatest response in ADG when supplemented higher levels of RUP, 0.70 kg

of additional gain for each 1.0 kg of RUP supplement with a strong correlation ($r^2 = 0.65$). Cattle grazing forages with less than 13.5% CP had a similar response ($P = 0.68$) with 0.47 kg increase in ADG for each 1.0 kg increase in RUP supplement with a strong correlation ($r^2 = 0.83$). Amount of RUP supplement provided only reached 0.20 kg/d for cattle on grass that had 13.5 to 17% CP, but ADG reached nearly the same level as it did in the cattle supplemented 0.25 kg/d on grass with 13.5% CP or less, which was 0.125 kg above their respective controls. The last group was cattle grazing forages with CP at 17% or greater. These cattle showed a negative response to RUP supplementation which tended to be different than low CP cattle ($P = 0.09$) and was significantly different than medium CP cattle ($P = 0.02$). Also the slope of this group's response was different than 0 ($P = 0.003$). Poppi and McLennan (1995) pooled experiments and saw a similar response with low quality forages (< 0.55 DM digestibility) responding linearly to increasing RUP supplementation, however high quality forages (> 0.60 DM digestibility) showed some response to RUP supplementation, but the effect was much less. When low quality and high quality forages were supplemented with 4 g of RUP per kg of BW per day animals grazing low quality forages gained 0.90 kg per day more than the control whereas animals grazing high quality forages only gained 0.25 kg per day more than the control. Rogers et al. (1996) supplemented RUP (corn and caromeal blend) to steers grazing warm season pasture averaging 20% CP. Compared to an energy control (corn) that supplied equal energy as the RUP supplements and 0.08 kg/d RUP there was no difference in ADG of the high or low (0.159 and 0.239 kg/hd/daily respectively) RUP supplemented pastures suggesting RUP was not the first limiting nutrient. Worrell et al. (1990) observed a similar response when 96 crossbred yearling steers grazed rye pasture.

This study utilized a control that received no supplement and a treatment that received 0.45 kg/d of cottonseed meal (CSM). Animals grazed a fall period from mid-December to the end of January and a spring period from mid-February to the end of March. During the fall period the rye ranged from 13.1 to 17.5% CP and during the spring regrowth rye ranged from 20.0 to 28.8% CP. The CSM used in this study was 42 to 48% RUP as a percent of CP and led to a response in ADG from 1.43 kg/d for the control to 1.72 kg/d for the CSM treatment during the fall period when forage CP was lower. However, during the spring period there was no difference in the control and CSM supplemented ADG at 1.13 kg/d and 1.16 kg/d, respectively.

Yearlings grazing wheat pasture is another example of high CP forages lack of response to supplemental CP. Vogel et al. (1989) used 256 growing animals across a three year study grazing wheat pasture. Treatments included no supplement, a corn based energy supplement, a meat meal based RUP source, or a cottonseed meal based RUP source. All supplements were provided on an equal energy basis. Wheat pasture averaged 23.0% CP as a percent of DM over the three year trial. Supplemented cattle had greater ADG than the animals that received no supplement, but additional protein supplement resulted in similar ADG to the energy control supplemented treatment ($P > 0.30$).

Responses shown here could be due to large intakes and rapid passage of forage through the rumen. This may allow undegraded RDP to pass from the rumen in the liquid contents and enter the omasum and eventually the small intestine. The undegraded RDP is utilized in the small intestine as RUP and increases the total metabolizable protein available for the animal. This extra “RUP” may be why the supplemental RUP did not improve ADG in these steers. Response during the grazing season will vary. A study in

2011 demonstrated a greater response to supplementation later in the grazing season, supplementing DDGS resulted in 0.15 kg ADG increase above the control early in the grazing season (first 60 days) and jumped to 0.34 kg ADG above the control for the remaining 96 days of the study (Watson et al., 2012). Buckner et al. (2013) collected samples at 5 time points across a grazing season on smooth bromegrass pastures. These samples were collected by ruminally fistulated steers. Samples were analyzed for in-vitro digestibility, CP, and RUP. Crude protein was 18.6% on a DM basis in May and IVDMD was 68.1%. Values for CP dropped to around 13.7% in June and July, but returned to around 15.5% in August and September due to fall regrowth. Values reported for RUP as a percent of CP ranged from 11.7% to 14.4%. This means that 85% to 90% of the protein being supplied from this pasture was RDP. Digested RUP as a percent of DM was reported to be 1.31% in May and declined to around 0.80% during the other sampling months. However, RUP values were measured using the mobile bag technique (Vanzant et al., 1998) with a calculated rumen incubation time equal to 75% of mean retention time based on in vitro dry matter digestibility (Klopfenstein et al., 2001). This technique may not correctly assess RUP content of highly digestible forages consumed ad libitum with rapid passage rate. In May, when the smooth bromegrass is highly digestible and provides a large amount of RDP in a diet that has a relatively fast passage rate, chances for bypass of soluble protein out of the rumen are at their peak. Bypass of soluble protein coupled with digested RUP values being at their peak explain why RUP supplementation is less effective early in the grazing season. As the forage matures and CP and IVDMD decrease MP again becomes limiting and responses increase.

Experiment 2

Dry matter intakes (DMI) and organic matter intake (OMI ; Table 2) followed the same trend, LQB and HQB treatments consumed the least, with ALF having the greatest intake and OAT being intermediate. Intakes observed on a neutral detergent fiber basis (Table 2) followed the opposite trend with LQB and HQB treatments having the highest neutral detergent fiber intake (NDFI) and ALF having the lowest NDFI with OAT again being intermediate. This trend is due to a low amount of NDF in both the ALF and OAT diets as a percent of DM when compared to LQB and HQB treatments. Stefanon et al. (1996) observed similar differences when looking at nutrient profiles of brome compared to alfalfa. Brome contained much higher amounts of NDF as a percent of DM. Also as both alfalfa and brome mature, NDF content increases. The LQB treatment was mature brome and the HQB treatment was harvested as fall regrowth, but contained mature stems as well. The ALF diet was immature, high quality alfalfa and the OAT diet contained 30% alfalfa and also very immature oat forage resulting in both diets being low in NDF. Crude protein of the forages (Table 3) on a DM basis were 8.23%, 11.95%, 17.48%, and 17.44% for the LQB, HQB, ALF, and OAT treatments, respectively. Both the LQB and HQB treatments would be expected to have very little bypass of soluble protein based on experiment 1, but the ALF and OAT treatments were both expected to have greater passage rates leading to bypass of some soluble protein prior to microbial utilization due to excess RDP available in the rumen.

Total tract digestibility of DM (Table 4.1) and OM (Table 4.2) were measured using titanium dioxide, AIA, and IADF and compared to in-vitro digestibility of the diets. Trends were similar across all markers with respect to total tract DM and OM digestibility. Each marker showed that OAT and ALF treatments had similar digestibility,

which were greater than both LQB and HQB treatments. However, using in-vitro digestibility as a standard measure, AIA consistently overestimated both DM and OM digestibility. Undersander et al. (1987) reported that AIA as a marker both overestimated and underestimated DM digestibility of alfalfa grown under differing levels of water stress. They concluded that AIA may be a useful marker in grasses but isn't useful for alfalfa. Sunvold and Cochran (1991) observed overestimated digestibility measures when using AIA as an internal marker on alfalfa hay, when compared to total fecal collection methods on the same feed. However, AIA was closely related to the brome diets within this trial. Diets containing less than 0.75% AIA show variable results as a marker and also contamination of dirt within feed ingredients can lead to skewed digestibility values by increasing the amount of AIA in the feed. In this study the average AIA in the diet across the trial was 0.72%. Due to the fact that alfalfa was fed as a complete diet and also comprised 30% of the OAT diet, the overestimation of digestibility follows the previous work. Both the HQB and LQB diets contained considerable amounts of small particles at the bottom of the bunks, which could lead to sampling error and effect digestibility values.

Using an external marker of titanium dioxide (TiO_2) underestimated DM and OM digestibility values compared to in-vitro values. However, OAT and ALF values calculated were much closer than HQB and LQB values when TiO_2 was used. Titgemeyer et al. (2001) observed numerically lower digestibility values when using TiO_2 as a marker in forage based diets fed ad-lib compared to total fecal collections, however marker method was not statistically different. Values for DM digestibility were

12% lower when calculated using TIO₂ in that study. This study showed similar differences and agreed with previous research.

When using IADF as a marker, both DM and OM digestibility values were very close to the values determined using in-vitro techniques. Incubating feed samples for 168h and 240h helped ensure that only indigestible material was remaining. Samples incubated for 168h and 240h had similar IADF values, which helped validate that only indigestible material was remaining. The HQB treatment resulted in a 5.0% greater digestibility, which was the largest deviation away from the in-vitro values for DM digestibility. Using IADF in this study resulted in the closest digestibility values of any marker used, when compared to in-vitro digestibility values. Conversely, Undersander et al. (1987) reported that using IADF as a marker had a low correlation to total fecal collection when digestibility values were calculated for alfalfa grown under differing levels of water stress. However, they did report that recovery of IADF averaged 101.1% and was not variable. Similarly, Sunvold and Cochran (1991) reported that IADF as a marker underestimated OM digestibility compared to total fecal collection when diets of alfalfa, brome, and prairie hay were limit fed to cattle housed in pens. Penning and Johnson (1983) reported that IADF was a suitable marker when used over a variety of forages fed to whether lambs. Using IADF as a marker had a higher correlation to in vivo methods than using in vitro methods to predict digestibility. Overall, using IADF as a marker in the current study resulted in values comparable to in-vitro digestibility values of the feeds. The method used to measure IADF is important to ensure no washout of particles from in situ bags as well as enough time for complete degradation of ADF within the rumen.

Trends observed for NDF digestibility values were similar to DM and OM digestibility values for all diets except ALF. The ALF treatment had the lowest NDF digestibility in relation to all other treatments. Buxton and Brasche (1991) reported similar findings during a 2 year study conducted using alfalfa, birdsfoot trefoil, brome grass, and orchardgrass. Forages were grown and harvested in order to determine NDF content, lignin content, and NDF digestibility using lab procedures. Alfalfa had the greatest lignin content at around 12.6% of DM compared to brome at 5.6% of DM. Lignin, which is considered indigestible, reduces digestibility by chemically binding to hemicellulose. Hemicellulose is a large portion of the NDF content of a plant, which led to lower NDF digestibility of alfalfa (50.1%) compared to brome (62.0%). However, alfalfa also had a lower NDF content on a dry matter basis compared to brome at 28.0% and 47.4%, respectively. The current study showed similar results with alfalfa having a lower NDF content and also a lower NDF digestibility compared to all other forages.

Measuring protein site of degradation in this digestion study was compromised due to issues in duodenal sampling. T-style cannulas were utilized as duodenal cannulas, which led to variable samples that may be inconsistent with actual duodenal flow. Inconsistent sampling led to large amounts of variation and resulted in inconclusive findings.

Passage rate was calculated by determining the concentration of cobalt in the rumen at each given time point and using the natural log to determine percent passing per hour. All treatments were statistically different ($P < 0.01$). Alfalfa had the highest liquid passage rate at 13.18%/h with the LQB treatment having the lowest liquid passage rate at 6.98%/h. Passage rates of 9.29%/h and 10.84%/h were calculated for the HQB and OAT

treatments, respectively. These liquid passage rates align with the lack of need for supplementation in the high CP and highly digestible forages in experiment 1. With an increase in liquid passage rate in these highly digestible treatments like OAT and ALF the chance for a bypass of soluble protein increases. These diets have high CP values and soluble protein in the form of peptides and amino acids can leave the rumen in the liquid fraction and be absorbed in the small intestine similarly to RUP.

Bypass RDP out of the rumen prior to microbe utilization was calculated based on omasal samples that were collected. Using total CP of the omasal samples and subtracting microbial protein, ammonia, and NDFN isolated the endogenous and bypass RDP fractions. Calculations resulted in 10.01%, 8.19%, 4.22%, and 4.44% omasal CP of these fractions, for ALF, OAT, HQB, and LQB, respectively. An assumption that no bypass RDP is present in the HQB and LQB diets means that around 4.3% CP is from endogenous sources. If this assumption is true, then animals fed the ALF and OAT treatments have around 5.7% and 3.9% of DM bypass RDP within the omasum. Using IVDMD to calculate fecal output and assuming 1.25 times fecal output to calculate omasal flow allowed for calculations of total bypass RDP on a DM basis. Bypass RDP as a percent of total forage CP was calculated to be 13.2% and 8.8% for the ALF and OAT treatments, respectively. These values suggest some bypass of RDP occurred, however, there are many assumptions in the calculations and the values are represented as relative and not absolute values. For example, the microbial crude protein values based on purine analysis appear to be low for the ALF and OAT diets.

Using omasal flows previously described multiplied by omasal CP values and then divided by the total feed CP on a DM basis allowed for calculations of protein

recovery in the omasal samples. Calculations resulted in 51.0%, 39.4%, 81.7%, and 100.8% recovery for ALF, OAT, HQB, and LQB treatments, respectively. The lack of recovery in the higher quality diets is due to ammonia absorption in the rumen and the complete recovery of the LQB treatment helps validate calculations. Overall, results of this trial show that some bypass RDP is present in the ALF and OAT diets and would result in a lower MP deficiency in these diets, which would align with the results from experiment 1.

Overall, utilizing markers to calculate digestibility of feedstuffs creates challenges. Marker recovery is variable, dosing of external markers creates challenges, and feed contamination from outside sources presents issues with internal markers. This is one area that needs further research to help improve metabolism and digestion research within our field.

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Table 1. Pooled analysis papers, authors, and years.			
Trial	Authors	Title	Year
1/2	Anderson, S. J., T. J. Klopfenstein, and V. A. Wilkerson	Escape Protein Supplementation of Yearling Steers Grazing Smooth Brome Grass Pastures	1988
3	Drouillard, J. and Klopfenstein, T.	Protein Sparing Effects of Tetronasin in Grazing Steers	1989
4	Goedeken, F., T. Klopfenstein, and R. Stock	Protein and Energy Supplementation of Calves Grazing Smooth Brome	1988
5/6	Hafley, J. L., B. E. Anderson, and T. J. Klopfenstein	Supplementation of Growing Cattle Grazing Warm-Season Grass with Proteins of Various Ruminal Degradabilities	1993
7/8	Hollingsworth, K. J., T. J. Klopfenstein, and M. H. Sindt	Metabolizable Protein of Summer Annuals for Growing Calves	1993
9	Irlbeck, N., T. Klopfenstein, J. Drouillard, and D. Blasi	Corn Gluten Meal Versus Corn Gluten Meal:Blood Meal	1990
10	Karges, K. K., T. J. Klopfenstein, V. A. Wilkerson, and D. C. Clanton	Effects of Ruminally Degradable and Escape Protein Supplements on Steers Grazing Summer Native Range	1992

Figure 1. Pooled response in ADG above the control calves to RUP supplement in 10 studies subdivided by initial BW. The additional ADG above the control supplement is graphed relative to amount of RUP supplement calves received.
 Solid line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals with an initial BW of 300 kg or over (Hi).
 Dashed line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals with an initial BW of 250 to 300 kg (Med).
 Dotted line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals with an initial BW of 250 kg or below (Lo).

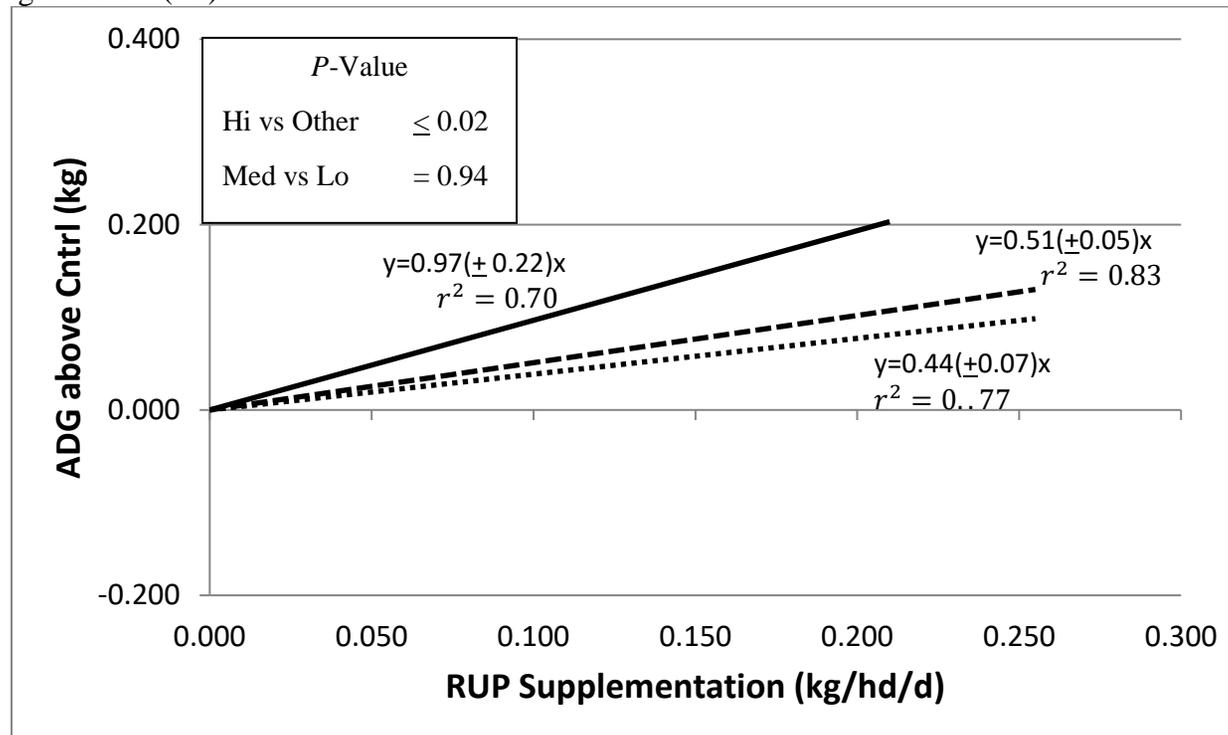


Figure 2. Pooled response in ADG above the control calves to RUP supplement in 10 studies subdivided by grazed forage type. The additional ADG above the control supplement is graphed relative to amount of RUP supplement calves received.

Solid line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals grazing summer annual forages.

Dashed line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals grazing brome grass.

Dotted line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals grazing warm season grass.

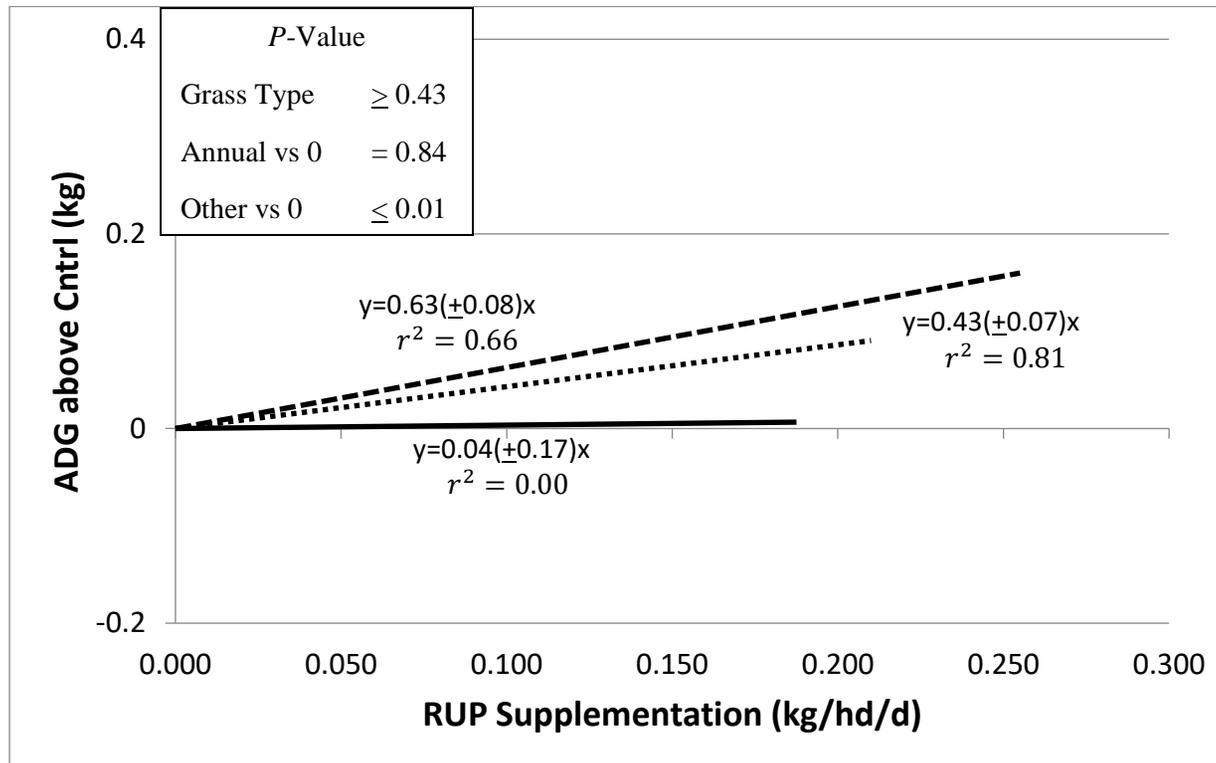


Figure 3. Pooled response in ADG above the control calves to RUP supplement in 10 studies subdivided by CP of grazed forage. The additional ADG above the control supplement is graphed relative to amount of RUP supplement calves received.

Solid line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals grazing forages containing 13.5% CP or less (Lo).

Dashed line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals grazing forages containing 13.5 to 17.0% CP (Med).

Dotted line: Relationship between amount of RUP supplement received and the ADG above the control animals for animals grazing forages containing 17.0% CP or more (Hi).

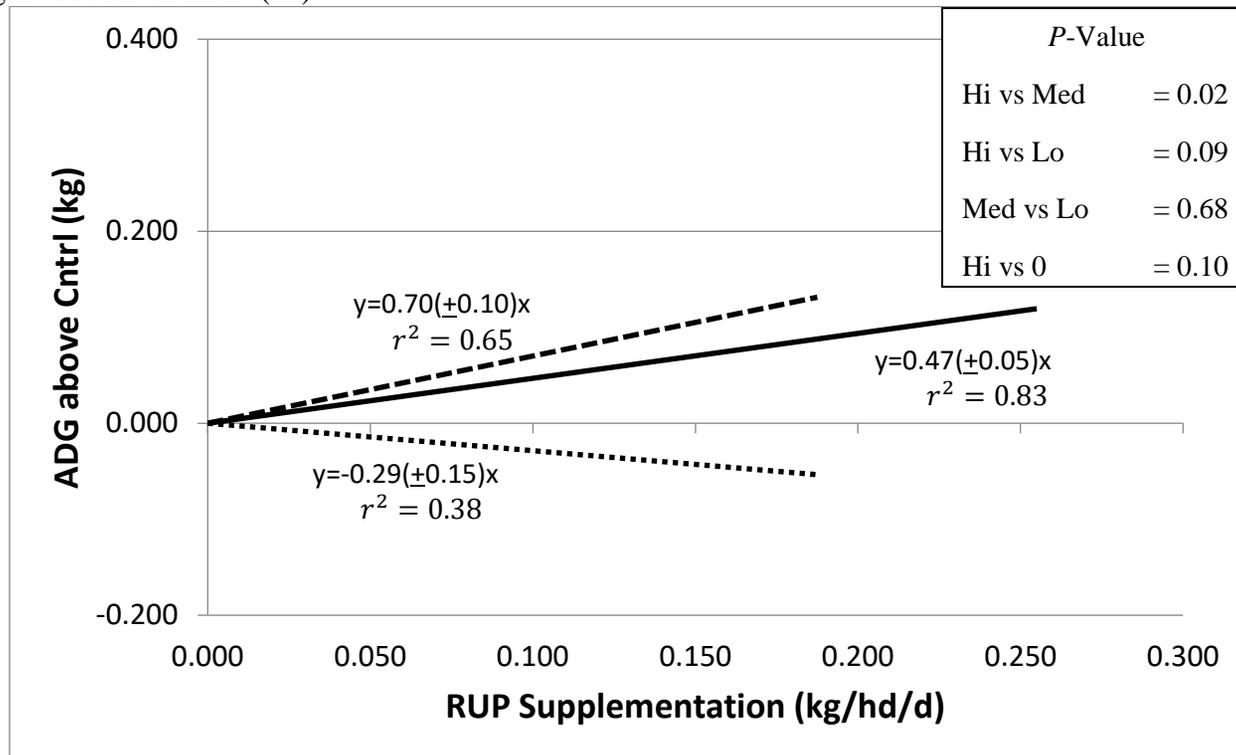


Table 2. Average dry matter, organic matter, and neutral detergent fiber intake of treatments

Intake (g/d)	Treatment			
	OATS ⁴	ALF ⁵	HQB ⁶	LQB ⁷
DMI ¹	8362	9199	6966	6475
OMI ²	7489	8496	6899	6308
NDFI ³	3810	3336	4684	4689

¹DMI- dry matter intake

²OMI- organic matter intake

³NDFI- neutral detergent fiber intake

⁴OATS- 70% oat and 30% alfalfa treatment

⁵ALF- alfalfa treatment

⁶HQB- high quality brome treatment

⁷LQB- low quality brome treatment

Table 3 Feed nutrient analysis including NDF, ADF, and CP

	Treatment			
	OATS ⁴	ALF ⁵	HQB ⁶	LQB ⁷
NDF ¹	53.24%	36.75%	61.51%	67.97%
ADF ²	31.37%	29.80%	40.50%	49.34%
CP ³	17.44%	17.48%	11.95%	8.23%

¹NDF – neutral detergent fiber on a dry matter basis

²ADF- acid detergent fiber on a dry matter basis

³CP- crude protein on a dry matter basis

⁴OATS- 70% oat and 30% alfalfa treatment

⁵ALF- alfalfa treatment

⁶HQB- high quality brome treatment

⁷LQB- low quality brome treatment

Table 4.1 Dry matter digestibility values using in-vitro, AIA, TiO₂, and IADF as markers

Marker	Treatment			
	OATS ⁵	ALF ⁶	HQB ⁷	LQB ⁸
In-Vitro ¹	66.62%	67.39%	49.20%	44.00%
AIA ²	75.14%	79.76%	59.65%	60.50%
TiO ₂ ³	62.77%	57.52%	36.69%	37.18%
IADF ⁴	66.94%	66.85%	54.49%	45.51%

¹In-Vitro- dry matter digestibility calculated using in-vitro measures

²AIA- acid insoluble ash

³TiO₂- titanium dioxide

⁴IADF- indigestible acid detergent fiber

⁵OATS- 70% oat and 30% alfalfa treatment

⁶ALF- alfalfa treatment

⁷HQB- high quality brome treatment

⁸LQB- low quality brome treatment

Table 4.2 Organic matter digestibility values using in-vitro, AIA, TiO₂, and IADF as markers

Marker	Treatment			
	OATS ⁵	ALF ⁶	HQB ⁷	LQB ⁸
In-Vitro ¹	68.48%	67.50%	52.60%	46.30%
AIA ²	77.97%	80.92%	63.02%	64.04%
TiO ₂ ³	66.99%	59.97%	42.43%	42.27%
IADF ⁴	69.42%	65.74%	55.13%	44.74%

¹In-Vitro- dry matter digestibility calculated using in-vitro measures

²AIA- acid insoluble ash

³TiO₂- titanium dioxide

⁴IADF- indigestible acid detergent fiber

⁵OATS- 70% oat and 30% alfalfa treatment

⁶ALF- alfalfa treatment

⁷HQB- high quality brome treatment

⁸LQB- low quality brome treatment

Table 4.3 Neutral detergent fiber digestibility values using AIA, TiO₂, and IADF as markers

Marker	Treatment			
	OATS ⁴	ALF ⁵	HQB ⁶	LQB ⁷
AIA ¹	74.08%	67.60%	65.52%	62.44%
TiO ₂ ²	61.25%	34.59%	40.31%	44.63%
IADF ³	64.08%	42.75%	57.71%	47.59%

¹AIA- acid insoluble ash

²TiO₂- titanium dioxide

³IADF- indigestible acid detergent fiber

⁴OATS- 70% oat and 30% alfalfa treatment

⁵ALF- alfalfa treatment

⁶HQB- high quality brome treatment

⁷LQB- low quality brome treatment