The Effect of Maturity at Harvest of *Eragrostis tef* (“Moxie”) on Nutritive Value as a Forage for Beef Cattle

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Authorization to Submit Thesis

This thesis of James R. Vinyard, submitted for the degree of Master of Science with a Major in Animal and Veterinary Sciences and titled "The Effect of Maturity at Harvest of *Eragrostis tef* ("Moxie") on Nutritive Value as a Forage for Beef Cattle," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Teff **(***Eragrostis tef* ; 'Moxie'), a warm-season annual grass, could be an excellent forage for beef cattle. However, there is limited information on its nutritive value to cattle when harvested at different stages of maturity. Thus, the objective of this research was to determine the effect of feeding teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity on nutrient intake, ruminal fermentation characteristics, omasal nutrient flow, and N utilization in beef cattle. Six ruminally cannulated beef heifers were used in a replicated 3 × 3 Latin square design with 28-d periods (18 d for adaptation and 10 d for measurements). Dietary treatments were BT, EH, or LH teff hay. Dry matter intake was measured daily. Indwelling pH loggers were used to measure ruminal pH from d 21 to 28. Ruminal fluid and omasal digesta were collected from d 26 to 28 to determine fermentation characteristics and omasal nutrient flow. Fecal and urine samples to quantify N excretion were also collected (d 26 to 28). Blood samples were collected 3 h post-feeding on d 28. There were no changes ($P > 0.28$) in the ADF or NDF content of teff with advancing maturity but iNDF increased ($P < 0.01$) with increasing maturity. Maturity had no effect ($P \geq 0.14$) on DMI, and ruminal total short-chain fatty acid (SCFA) concentration, pH, digestibility and outflow of DM, OM, NDF, ADF, and CP. However, the CP content of BT hay was greater ($P < 0.01$) than for EH and LH hay (18.1, 14.1, and 11.5%, respectively, DM basis), and this resulted in the higher CP intake ($P < 0.01$) for heifers fed the BT than the EH and LH hay. Consequently, ruminal ammonia-N (NH_3-N) concentration was greater ($P < 0.01$) for heifers fed BT than EH and LH hay, thereby possibly explaining the tendency for a decrease ($P = 0.08$) in PUN concentration, and a decrease ($P < 0.01$) in the excretion of total N, urine N and urea-N (UUN) with advancing maturity. However, fecal N excretion (g/d) did not differ ($P = 0.76$). In conclusion, despite a decrease in CP intake and ruminal

NH_3 -N concentration, feeding beef heifers EH and LH compared to BT teff hay did not compromise ruminal digestion and outflow of DM, OM, NDF, ADF, and CP, and microbial protein synthesis. Advancing maturity in teff hay also resulted in a decrease in the excretion of total N and urine N and UUN when fed to cattle. Therefore, beef producers could wait until the LH stage to harvest/graze ‘Moxie’ teff grass to maximize forage yield without severely compromising its nutrient value.

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Dedication

This thesis is dedicated to my parents, Ronald and Susan, and my brothers, Michael, Matthew, and Joseph, as well as my dog, Sully, who have been present through constant love and support throughout my time at the University of Idaho.

Table of Contents

List of Abbreviations

AA – amino acid

ADF – acid detergent fiber

AN – arcuate nucleus

BLM – Bureau of Land Management

BT – boot

BW – body weight

CART – cocaine- and amphetamine-regulated transcript

CCK – cholecystokinin

CHO - carbohydrate

CP – crude protein

CRH – corticotropin releasing hormone

DM – dry matter

DMI – dry matter intake

DN – dorsomedial nucleus

EH – early-heading

GABA - γ-aminobutyric acid

H_2SO_4 – sulfuric acid

iNDF – indigestible neutral detergent fiber

IVNDFD – *in vitro* neutral detergent fiber digesibility

LCFA – long-chain fatty acids

LH – late-heading

LHY – lateral hypothalamus

NE_m – net energy at maintenance

MP – metabolizable protein

N – nitrogen

NDF – neutral detergent fiber

NH_3-N – ammonia nitrogen

NPY – neuropeptide Y

OM – organic matter

PN – paraventricular nucleus

PNW – pacific northwest

PUN – plasma urea nitrogen

RDP – rumen degradable protein

RUP – rumen undegradable protein

SCFA – short-chain fatty acid

UUN – urine urea nitrogen

VN – ventromedial nucleus

Yb - ytterbium

List of Tables

List of Figures

Introduction

Ruminants have evolved to be unique in their ability to utilize cellulose as a source of energy (Van Soest, 1982). Ruminal fermentation of forages results in the production of short chain fatty acids (SCFA), microbial protein, carbon dioxide, and methane. Short chain fatty acids are then absorbed across the rumen wall and are used as an energy source by the animal. Microbial protein is digested in the small intestine to release peptides and amino acids that are then absorbed and used to meet the requirements for maintenance, growth and production purposes. The distinct advantage provided to ruminants by their rumen microbial communities is the ability to utilize cellulose, the most abundant biopolymer on the planet. Microbial digestion of cellulose enables cattle to utilize a source of feed that humans or other monogastric livestock cannot use (Van Soest, 1982).

In Idaho, over 60% of the land is public, as it belongs to either the state or federal government (Bureau of Land Management, 2016). These public lands are primarily rangeland that could be grazed by cattle. Because grazing fees charged by the land management agencies are very low, this is a very cost-effective way to feed beef cattle (Bureau of Land Management, 2016). The goal of rangeland cattle production is to produce cattle in a profitable manner while maintaining or improving the quality of the rangeland allotment being grazed. Rangeland cattle producers have to ensure proper grazing management and cooperate with the land management agencies to maintain ecosystem health.

Providing rangeland cattle a constant supply of nutritionally adequate forage can be challenging because of numerous factors, including weather patterns and environmental disturbances that alter forage quality and quantity (Adams et al., 1996). The three major environmental disturbances that are currently affecting rangeland forage production in the U.S. are the increasing frequency and severity of wildfire (Dennison et al., 2014), persistent drought conditions (Borsa et al., 2014), and the increasing domination of invasive annuals (Boyte et al., 2014). Drought and invasive species decrease the quality and quantity of forage available on range by preventing native plants from thriving. Once native plants start disappearing, they are replaced by undesirable grass species such as cheatgrass (*Bromus tectorum*), medusahead (*Taeniatherum caput-medusae*), and ventenata (*Ventenata dubia;* Bansal et al., 2014). Because their quality is low as a result of anti-quality factors such as high silica concentrations (Montes-Sánchez and Villaba, 2017), seeds that make eating them painful (Young et al., 1987), or declining nutritive value, invasive species are often left ungrazed. Coupled with drought conditions, this accumulation of biomass increases the potential for large and frequent fires (Davies et al., 2015). When fires occur, grazing may not take place for several years because of rest periods and grazing bans that are implemented by land management agency as a strategy to restore ecosystem health (Roselle et al., 2010). Thus, research examining alternatives to rangeland grazing in years when forage quality and quantity are lacking as a result of environmental disturbances such as fire, is highly needed.

CHAPTER 1

Literature Review

The beef cattle industry in the Pacific Northwest region

The variability of the terrain across the Pacific Northwest allows it to play host to all aspects of beef cattle production, including the cow-calf, backgrounding, and finishing phases. The vast expanses of rangeland allows for the grazing of cow-calf pairs on native pasture across parts of Montana, Idaho, Washington, and Oregon throughout much of the year. Rangeland cattle production allows for the utilization of widely available forage in a cost-effective manner. In Idaho, public rangeland comprises over 60% of the entire state with over 5 million hectares of public land available for cattle grazing from spring until fall (Bureau of Land Management, 2016).

Cattle are turned out onto range in spring, typically between March and May, when forage at lower elevations is beginning to grow after the snow has melted (Vavra and Raleigh, 1976). At this point in time, the cows have been re-bred, but they are still nursing their calves that were born just a few months prior. As the grazing season progresses, forage quality and availability declines in low elevation areas. Therefore, cattle are then moved to high elevation areas, which allows them to utilize forage of higher quality, with greater protein content and digestibility, as it is in earlier stages of growth and maintains its high quality for a longer period than in areas at lower elevation (Adams et al., 1996). Cattle will be brought back off the range in late fall to early winter, when forage quality and quantity are low and plants have gone dormant (Vavra and Raleigh, 1976). This will allow for dry-lot feeding, where cattle are supplied with feed in a bunk or feeder, or the grazing of crop residues after harvest until the next range-grazing season.

In winter, depending on availability of forage and snowfall, cattle may still graze, or they are supplemented with forage or energy or protein sources in order to meet their nutrient requirements and prevent the loss of body condition. Many producers will bring cattle in off of range in winter, so that they are able to ensure adequate nutrition especially during the last trimester of pregnancy, a period when two-thirds of fetal growth occurs (Prior and Laster, 1979). Approximately 54% of beef cattle operations in the United States have a distinct calving season, with up to 60% of calving occurring in spring (McBride and Mathews, 2011). Once calving occurs, cows will be re-bred before spring turnout with their calf to graze fresh forage.

Approximately six to nine months after calving, calves (180 to 320 kg BW) are weaned (McBride and Mathews, 2011). Heifers may be kept as replacements and be bred the following spring and bulls may be kept for their genetics. Weaned calves that are not used for breeding will be fed for harvest or backgrounded on the forages available until they reach a sufficient weight to be transported to a feedlot. These forages may be from the grazing of winter wheat pasture or hay from previous harvests. Depending on the availability of cost-effective forages and the price of cattle, a producer may decide to keep calves and feed them longer such that there is a heavier calf to sell if the price per pound of calf is greater than the price per pound of feed (NASS, 2016). Thus, the producer may also keep the calf to background in order to increase frame size and the amount of muscle on the calf before the transition to a feedlot. In a typical backgrounding setting, a calf will be fed to achieve a rate of gain of 0.35 to 1.15 kg/d until reaching 300 to 400 kg at 12 to 15 months of age (NRC, 2016). Backgrounding diets will generally be high in forage, but may also contain concentrates in order to increase the rate of gain (NRC, 2016).

A calf may also be sent directly to the feedlot after weaning. Once in the feedlot, calves will transition to a high concentrate diet (80 to 90% of DM) in order to reach slaughter weight (average of 581 kg live weight) in the shortest amount of time (NRC, 2016). On a high-energy feedlot ration, it will take approximately 7 kg of feed for the animal to accumulate 1 kg of lean tissue with an ADG of 1.2 to 1.8 kg/d (NRC, 2016). Cattle will spend anywhere between 120 to 240 days in a feedlot, depending upon arrival weight, age, the rate of gain, and desired finishing characteristics (NRC, 2016; NASS, 2016).

Rangeland cattle production

Rangeland cattle production is the method of raising cattle with naturally available forage. The forage available for cattle on rangeland varies from year to year, but ultimately is an excellent source of nutrients for pregnant and growing cattle. The goal of producing cattle on rangeland is to ensure that a cow has a calf every year for the smallest amount of monetary input while still maintaining the ecological integrity of the range on which the cattle are grazing. Grazing cattle on rangeland, however, does not come without challenges. The availability of forage on rangeland is widely variable due to numerous factors including the presence of invasive species, wildfire, drought, and seasonal changes. In addition, the nutrient requirements of the cow changes with physiological state. In order to be profitable, a producer must be able to match the nutrient requirements of the animal to the available nutrients on rangeland.

The biological cycle of the beef cow

A key production target for cow-calf operation is for cows to have a calf every year. In order to meet that goal, producers must provide cattle with a proper plane of nutrition.

The largest factor decreasing the number of calves sold annually for producers is the failure of a cow to conceive (Wiltbank et al., 1961). For every cow that does not have a calf, the producer is losing approximately $1,154 per year; $600 for the calf that could have been sold and $554 to feed an open cow for an entire year (Speer, 2016). These values are based upon feeding an open cow 15 kg of hay for 365 days and are most likely much greater than most production settings, however, it is imperative for the producer to provide adequate nutrients to their cows to prevent the loss of profit. The nutrient requirements, however, change throughout the year based on the physiological state of the animal. Therefore, matching nutrient supply to the requirements of the animal is key to meeting production targets and maintaining a profitable operation.

At approximately one year of age and 60% of their mature body weight (BW), heifers will be bred for the first time (Short et al., 1990). After conception, a heifer’s nutrient requirements will increase as the first trimester begins in order to support not only maintenance and growth, but now the growth of a fetus. During the first trimester, adequate nutrition is imperative as the development of the placenta and the organs of the fetus occur during the first 60 days of gestation (Funston et al., 2010). During the first trimester, a heifer requires 8.1 to 9.0 Mcal/d of net energy at maintenance (NE_m), 427 to 453 g/d of metabolizable protein (MP), 19.3 to 20.2 g/d Ca, and 11.5 to 12.2 g/d P (NRC, 2016). During the second trimester, nutrient requirements are only slightly greater than the first trimester to account for fetal and heifer growth. The NRC (2016) recommendations are 9.4 to 10.7 Mcal/d NE_m, 466 to 515 g/d MP, 20.4 to 21.0 g/d Ca, and 12.5 to 13 g/d P. For a multiparous cow, the nutrient requirements are lower during the second than first trimester as they are no longer growing, and can be met by feeding low quality forages (Rayburn, 2013). Weaning also occurs after the first trimester and nutrient requirements decrease, as the cow is no longer lactating (Rayburn, 2013). Cattle producers can take advantage of this period of low nutrient requirements and decrease their overall feed costs.

The point in the biological cycle at which the nutrient requirements are the highest is from the beginning of the third trimester of gestation until peak lactation. During the third trimester, up to 75% of fetal growth occurs (Robinson et al., 1977), increasing the dam’s nutrient requirements. During this period of growth, however, the dam’s intake is decreasing because of the increasing space needs of the fetus (Forbes, 1986), requiring the dam to consume feed of greater quality or smaller meals with greater frequency. During late gestation, the fetus’s metabolic requirement is almost double that of the dams, relative to body size (Reynolds et al., 1986) and the majority of its energy requirement is met via the oxidation of glucose and lactate (Hay et al., 1983). Approximately 10 to 15% of energy requirements is met by the uptake of acetate from the dam (Comline and Silver, 1976) with 30 to 40% from the oxidation of amino acids (Faichney and White, 1987). Thus, the requirements set by NRC (2016) are 11.9 to 16 Mcal NE_m, 561 to 741 g/d MP, 33.6 to 34.5 g/d Ca, and 18.2 to 18.8 g/d P, with the greatest requirements required just prior to parturition.

If provided adequate nutrition throughout gestation, the heifer will calve at approximately 85% of mature BW (Short et al., 1990) and enter lactation. This period continues until the dam reaches peak lactation, which is approximately 60 days after calving (Butler et al., 1981). During this period, the primiparous cow’s nutrient requirements are very high because of growth, lactation, and uterine involution requiring 15.3 to 16.2 Mcal NE_m, 780 to 850 g/d MP, 33.3 to 36.6 g/d Ca, and 22.3 to 24.1 g/d P (NRC, 2016).

However, during early lactation, DMI increases and lactating cows will spend more time grazing than non-lactating cows (Vanzant et al., 1991). After calving, there is a period of 83 days during which the dam must be rebred in order to have another calf the following year (Short et al., 1990). The nutrition of the dam is important, both before and after calving, and improper nutrition could be detrimental to reproductive performance (Wiltbank et al., 1962). Energy partitioning for maintenance, lactation, and growth takes priority over the energy needed for reproduction (Yates and Schoonover, 1982), meaning that a primiparous cow must have a high plain of nutrition in order to have adequate energy available for reproductive success. Undernutrition during the post-partum phase when a cow needs to be re-bred can prevent conception and reduce productivity (Diskin et al., 2003).

The use of public lands for beef cattle production

The availability of public lands for cattle production in the western United States enables producers to have low feed costs. Currently, the monthly cost of grazing is $1.41 per animal unit (Bureau of Land Management, 2018), with approximately 12 million animal unit months grazed annually (Bureau of Land Management, 2016). The compensation received by the land management agencies for grazing permits is then used to maintain rangeland integrity by funding programs focused on weed management, range monitoring, and restoration efforts (Bureau of Land Management, 2016). Although the grazing of public lands promotes inexpensive cattle production, the general public view rangeland livestock production negatively as around 300 million ha of public and private rangeland is used for both grazing and recreation (Havstad et al., 2007). The overlap in use by cattle producers and recreationists can cause conflicts on best use of available land (Wolf et al., 2016). Proper grazing, however, is also beneficial in maintaining and restoring ecological health of rangeland. Some of the benefits associated with proper rangeland grazing include the prevention of wildfires and spread of invasive species (Taylor, 2006), maintaining the integrity of riparian areas (Kauffman and Krueger, 1984), and improved or maintained wildlife habitat (Holechek et al., 1982).

Using livestock grazing to maintain or improve the rangeland ecosystem is an intricate task that requires attentive management by producers with an extensive knowledge of grazing behavior, forage production, and rangeland ecosystems. Improper grazing in sensitive ecosystems, similar to the sagebrush steppe in the Pacific Northwest, can damage native plants and pave the way for the dominance of invasive annuals (Daubenmire, 1940; Mack and Thompson, 1982; Reisner et al., 2013). Rangeland producers must use management tools to control their livestock to maintain the integrity of their allotment. This can be done by placing sought after feedstuffs, including energy supplements and salt licks, in areas where grazing is preferred (Bailey and Welling, 1999). Doing so allows for the utilization of areas of pasture that might not be highly desired by cattle, but still needs to be grazed in order to maintain ecological health. When grazing cattle on public lands, the distance to water is of utmost importance. Although they can graze further from it, cattle prefer to graze within 2 km of a water source (Ganskopp, 2011), particularly when temperatures increase. This decreases the use of forage further away (Roever et al., 2015). Cattle will readily graze pastures that are further from water early in the season when temperatures are lower and forage quality is greater (DelCurto et al., 2005). As the season advances, however, cattle will be drawn more towards riparian areas where forage is more palatable and water is readily available, which risks overgrazing of stream banks and soil compaction (DelCurto et al., 2005). Thus, the placement of man-made water sources is key in altering dispersion and forage utilization by cattle.

Although it is a cost-effective strategy to feed cattle, there are several challenges associated with the use of rangeland for grazing. The land management agency that controls a given allotment dictates the activity on that land. This includes when the rancher can start grazing and when they stop, as well as stocking rate and density. Land management agencies function to maintain or improve the ecological health of rangeland and the measures taken to do so may result in challenges for producers in meeting the nutrient requirements of cattle. In order to maintain rangeland health, the BLM has fundamental aspects that they monitor in order to make their decisions. Those aspects are watershed functionality, the cycling of nutrients and energy in the ecosystem, water quality, habitat for endangered and potentially endangered species, and habitat quality for native flora and fauna (Veblen, et al., 2014). Thus, if one or more of these fundamental aspects is compromised in an allotment, the BLM will act in order to restore ecological health. One example would be grazing restrictions after a fire. Following a fire, the ecosystem is highly vulnerable and the loss of native plants opens up the potential for weed and invasive species invasion as well as erosion of exposed soil (D’Antonio and Vitousek, 1992). Therefore, land management agencies can implement grazing bans that allows for the recovery of vegetation because cattle grazing immediately after a fire, when herbage is just beginning to recover, could potentially continue to damage the ecosystem. Overall, the decision to reintroduce grazing to an ecosystem should be done on a site-by-site basis and be based upon the pre-fire ecology of a given allotment (Roselle et al., 2010). Livestock grazing can, however, be used to improve rangeland ecosystems in many ways and producers and land management agencies must cooperate to ensure ecological health as well as forage availability to ensure economic viability for ranchers.

Meeting the nutrient requirements of rangeland cattle

As with any livestock production system, it is imperative that the nutrient requirements of cattle are met while they are on range. It is relatively more difficult, however, to monitor forage chemical composition, dry matter intake (DMI), BW, and body condition score of rangeland cattle when compared with cattle raised in a more intensive management system, such as feedlot production. In addition to the changes in the physiological state of cattle over the grazing season that can alter nutrient requirements, forage yield and quality of rangeland plants also changes with their phenological stage of growth. Therefore, this can result in challenges in meeting the protein, energy, mineral, and vitamin requirements of rangeland cattle over the grazing season.

Protein

Metabolizable protein (MP) supplies the AA required for maintenance, growth and production purposes in cattle. The MP requirements will change depending on the physiological state, be it growth, lactation, or gestation. To meet maintenance requirements, a beef cow requires approximately 3.8 g of MP per kg of metabolic BW ($BW^{0.75}$); therefore, a 454 kg cow needs 374 g of MP per day (NRC, 2016). The requirements for MP increase when cattle are growing, pregnant, or lactating. Growing cattle will require 305 g of MP per kg of live weight gain in addition to their maintenance requirements (Wilkerson et al., 1993). For a mature cow (550 kg, shrunk BW) that produced 8 kg of milk per day at peak milk yield and an average of 5 kg/day with 3.4% milk CP the requirement for MP is 780 g/d at parturition, which increases to 850 g/d until peak lactation and then decreases to 536 g/d after weaning. In an instance in which a cow produces more milk or milk with a greater concentration of protein, the MP requirements would increase (NRC, 2016). Once pregnant, in order to nourish the fetus, the MP required in addition to lactation and maintenance ranges from 1 to 3 g/d in the first trimester to 88 to 251 g/d in the third trimester (NRC, 2016). Thus, an appropriate amount of MP has to be provided throughout the various physiological states.

The three fractions that comprise MP are microbial protein, bypass or escape protein, and endogenous protein. Of these three fractions, microbial protein is the most important because it can provide up to all of the MP requirement in cattle, it is highly digestible, has a diverse AA profile, and is an efficient AA source (Owens and Bergen, 1983). For a high producing animal, such as a rapidly growing beef steer or a high producing dairy cow, bypass protein is also important because microbial protein might not be adequate (Firkins and Reynolds, 2005). From a quantitative standpoint, the endogenous fraction is not of great significance (Van Bruchem et al., 1997), although its contribution could be high when feeding high forage diets (Sandek et al., 2001).

Crude protein, which is comprised of rumen degradable (RDP) and rumen undegradable protein (RUP), is commonly used to determine nutritional adequacy of diets for cattle. RDP is sequentially broken down by the rumen microbes into small peptides, individual AA, and then NH_3-N, which can all be used for microbial protein synthesis. The source preferred by microbes is dependent upon their rate of growth. Rapidly-growing, starch fermenting (amylolytic) microbes such as *Bacteroides amylophilus*, *Selenomonas ruminantium*, and *Succimonas amylolytica* (Van Soest, 1982), have a preference for preformed peptides and amino acids (Russell et al., 1992). On the other hand, slow-growing microbes such as *Ruminococcus albus*, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens* (Van Soest, 1982), have a preference for NH_3-N (Russell et al., 1992). Although other nutrients including N and minerals such as, Sulphur are needed, energy is the major determinant of microbial protein synthesis (Bach et al., 2005; Russell et al., 1992). When energy is available, there is a greater incorporation of peptides, AA and NH_3-N into microbial protein. When a there is a greater utilization of peptides and AA into microbial protein, less NH_3-N is produced as less AA are deaminated (Russell et al., 1992).

Ammonia-N can supply up to 80% of the N required for microbial protein synthesis (Hristov and Jouany, 2005). When energy is limited, less sequestration of peptides and AA into microbial protein occurs because microbes cannot store excess peptides and AA, they hydrolyze them to NH_3-N, and process that leads to the production of ATP, CO_2, and SCFA. In addition, a limited supply of energy also reduces capture and use of NH_3-N, which increases its concentration in the rumen. Rumen NH_3-N can range from 0 to 130 mg/dL (Tillman and Sidhu, 1969). Pisulewski (1981), however, reported that 5 mg of NH_3-N/dL is required to optimize microbial protein synthesis. Miller (1973) also reported that a concentration of 29 mg of NH_3-N/dL was required for optimal microbial protein synthesis. This wide range reported in literature could possibly be related to differences in fermentable energy supply in those studies. For example, the requirement was 5 mg/dL when feeding a diet containing 35% (DM basis) barley grain , 25% corn starch, and 15% glucose (Pisulewski, 1981), whereas it was 2.2 mg/dL when feeding diets containing 45.4% cracked corn, 11.3% alfalfa, 11.3% Timothy, 6.8% molasses, and 24.4% dextrose (Slyter et al., 1979).

Bypass protein or RUP is of significance to meeting the AA requirements of the cow by allowing for metabolic access to the entire digestible AA profile (NRC, 2016). In addition, in high producing animals, including calves growing at a fast rate, microbial protein might not be adequate, which increases the importance of RUP (Firkins and Reynolds, 2005). The undegraded protein that flows into the abomasum is hydrolyzed into oligopeptides, dipeptides, and individual AA by gastric pepsin. Once released into the duodenum, proteases like trypsin, chymotrypsin, and carboxypeptidase breakdown the remaining oligopeptides into dipeptides and AA that can be absorbed via the brush border of the intestinal epithelium (NRC, 2016). The RUP fraction of a feed is largely variable and dependent upon the passage rate of the protein source, rumen pH, and microbial activity (Broderick et al., 1991), thus making RUP a difficult fraction to measure (Schwab et al., 2003).

Endogenous protein is any protein that is synthesized by the animal but is digested and recycled as amino acids that are absorbed in the small intestine. This includes sloughed off ruminal and intestinal epithelial cells and enzymes used for digestion (Lapierre et al., 2008). The diet has an impact on the contribution of endogenous protein to MP. The introduction of cellulose into the abomasum of sheep increased endogenous N flow by 17% (Zebrowska and Kowalczyk, 1991). Ouellet et al. (2010) reported increased endogenous N flow in cattle fed hay (46 g of endogenous N per day) over silage (35 to 40 g of endogenous N per day), which is related to a greater abrasive texture in dried forage increasing the sloughing of digestive epithelium (Sandek et al., 2001). The reabsorption of endogenous N is important in the reduction of N excretion and waste in livestock production systems (Van Bruchem et al., 1997).

For cattle on rangeland, the plant species and stage of growth have a significant impact on protein supply (Buxton, 1994). In general, legumes contain a greater concentration of protein than grasses, but are less digestible (Hoffman et al., 1998). Also, as a plant matures, its leaf:stem ratio decreases. Because the leaves generally contain more protein than the stems, protein content decreases with advancing maturity (Buxton, 1994).

Low quality forage (< 7% CP as a % of DM) may not provide an adequate amount of protein to a pregnant beef cow, even if quantity is not limiting (DelCurto et al., 2000). Villalobos (1993) and Hollingsworth-Jenkins and associates (1996) determined that the supply of RDP from a forage source must be 4 to 5% of the OM intake for a gestating beef cow or between 340 to 430 g/d. Thus, a cattle producer may have to provide a supplemental protein source to cattle in order to guarantee an adequate supply of RDP or RUP. Protein supplementation can be provided in a few different forms, the most common being oilseed byproducts such as soybean meal, canola meal, or dried distillers grains (DelCurto et al, 2000). Mid- to high-quality forage provided as hay or stockpiled for grazing also make excellent supplements when the quality of rangeland forages is low (DelCurto et al., 2000). Villalobos et al. (1997) supplemented cows grazing dormant Nebraska sandhills range (4.3 to 6.4% CP; as a % of DM) with 2.2 kg/day of meadow hay (15.1 to 15.5% CP) or 0.9 kg/day of 70% soybean meal, 30% wheat grain mixture (36% CP) over a period of 112 days and found that body condition score and BW increased or remained the same, whereas unsupplemented cattle decreased in both body condition score and BW. Hunt et al. (1988) reported that supplementing steers consuming low quality meadow fescue hay (6.6% CP) with cottonseed meal increased intake, but there were no differences in intake related to the frequency of supplementation (twice/day, once/day, every other day).

Energy

Carbohydrates, protein and fat can all be sources of energy for cattle. Carbohydrates, which can be structural or non-structural, are the most important energy source for grazing cattle and for microbial protein synthesis in the rumen. The structural carbohydrates are hemicellulose and cellulose; which along with lignin make up neutral detergent fiber (NDF). The NDF fraction of plants indicates the amount of fiber as a whole that is available to the animal and is an excellent predictor of forage intake (Waldo, 1986). It does not, however, indicate the digestibility of the fiber. The acid detergent fiber (ADF) concentration of a forage, which is comprised of cellulose and lignin, is a good predictor of digestibility (Van Soest and Mertens, 1977). The concentration of lignin, which accumulates as plants mature and is indigestible, aids in the determination of quality as it reduces digestibility (Jung and Vogel, 1986). Forage with a high concentration of lignin are of a low quality and usually at a later stage of maturity than a forage with a low lignin concentration (Griffin and Jung, 1983). When forage quality is low (less than 55% digestible DM and less than 10% starch and soluble sugars; NRC, 2016), supplementation of energy may be necessary to prevent a decrease in animal performance (Swanson, 1993). In times of low forage quality, cattle can be supplemented with either high quality forage (Vanzant and Cochran, 1994), cereal grains (Bowman et al., 2004), or other energy sources such as beet pulp (Swanson, 1993).

Nonstructural carbohydrates are comprised of organic acids, simple sugars, fructans, and starch. The major nonstructural carbohydrates found in forages are starch and sugars (glucose, fructose, and sucrose; Moore and Hatfield, 1994). Forages generally contain sugars at 2 to 15% of DM (Holt and Hilst, 1969) and starch from 1 to 11% of DM (Wilson and Mannetje, 1978; Bailey, 1958), depending on species and phenological maturity.

Nonstructural carbohydrate digestion occurs primarily in the rumen as starch and sugars are fermented rapidly and provide readily available energy to microbes, but can decrease rumen pH (Monroe and Perkins, 1939). The addition of rapidly fermentable carbohydrates in the diet of cattle consuming forage can enhance performance but if fed in excess of 30% of diet DM this can reduce intake and digestibility of forages because of a decrease in rumen pH (Ørskov, 1986).

Besides providing ATP for microbial protein synthesis, fermentation of carbohydrates in the rumen results in the production of SCFA that contribute up to 80% of caloric requirements of cattle (Bergman, 1990). The major SCFA are acetate, propionate, and butyrate. In animals consuming a high forage diet, like range cattle, acetate is proportionally, the main SCFA produced (Calsamiglia et al., 2008). In cattle fed diets containing a high proportion of concentrates, like feedlot cattle, propionate is produced at increased molar proportions (Ørskov, 1986). Greater concentrations of butyrate are observed when cattle are fed high amounts of disaccharides (DeFrain et al., 2004; Gao and Oba, 2016). Once producd, the bulk of SCFA are absorbed into portal circulation via the rumen epithelium. Approximately 90% of butyrate and 30% of acetate absorbed in the rumen are used by the epithelial cells of the rumen as a source of energy, and the end product of metabolism is β-hydroxybutyrate (Bergman, 1990; Forbes and Barrio, 1992). Approximately 50% of the propionate absorbed is metabolized by the epithelium of the rumen to yield lactate and CO_2 in cattle (Bergman, 1990). The SCFA not metabolized during absorption are transported in the portal vein to the liver. Butyrate is used for ketogenesis in the liver (Bergman, 1990) whereas propionate is largely utilized for gluconeogenesis and can provide over 60% of the glucose synthesized in lactating cattle (Wiltrout and Satter, 1972). The small amounts of butyrate and propionate not metabolized in the liver are metabolized in peripheral tissues, including the mammary gland (Bergman and Wolff, 1971). Only a small amount of acetate is metabolized in the liver (Bergman and Wolff, 1971) with the bulk metabolized in peripheral tissue including skeletal muscle, adipose tissue and the mammary gland (Bergman, 1990).

Regulation of feed intake

To be productive, animals must consume an adequate amount of protein, energy, vitamins, and minerals for nourishment. Therefore, feed intake is important in determining nutrient supply. A combination of physiological and dietary factors, including the physical capacity of their digestive tract, and nervous and endocrine signals produced in the brain and digestive tract that signal for the animal to start and stop eating, and the physical form of the diet all play a key role in regulating feed intake.

The regulation of feed intake can be broken down into two categories: short- and long-term. The mechanisms of regulation must function together in order to maintain intake at a level necessary to fuel maintenance, growth, lactation, or reproduction. Short-term regulation includes the chop length of forages and their subsequent rate of passage from the rumen, the actions of mechano- and chemoreceptors in the reticulorumen, as well as hormones secreted in the abomasum (ghrelin) and small intestine (CCK), and the hepatic oxidation of metabolic fuels (Allen, 2000; Havel, 2001). Short-term regulation allows for continued intake once the mechanism of satiation is alleviated, as digesta flowing into the omasum, thus relieving the distension of the rumen. The mechanisms of long-term regulation include the neuropeptides produced in the hypothalamus, as well as leptin (Havel, 2001). Rather than altering intake by causing meal cessation, long-term regulation of intake works over a period of days or weeks to adjust the level of intake (Van Itallie et al., 1977).

The central location for the integration of short- and long-term regulation of intake is the hypothalamus (Forbes, 1996). Branches of the vagus and splanchnic nerves that form the enteric nervous plexus innervate the ruminant gastrointestinal tract and liver (Harding and Leek, 1972). These nerves relay signals to and from the hypothalamus in order to regulate feed intake and rumen motility. The hypothalamus is divided into several sections that play different roles in the production and secretion of, and the response to orexigenic (e.g., neuropeptide Y [NPY], opioid peptides, and certain AA) and anorexigenic compounds (e.g., corticotropin releasing hormone [CRH], melanocortin, and cocaine- and amphetamine-regulated transcript [CART]). These sections are the arcuate nucleus (AN), the ventromedial nucleus (VN), dorsomedial nucleus (DN), paraventricular nucleus (PN), and the lateral hypothalamus (LHY; Kalra et al., 1999). The AN is the site for the synthesis of orexigenic compounds including NPY, opioid peptides, γ-aminobutyric acid (GABA), and glutamate. The VN is also known as a satiety center and disruption of the VN results in hyperphagia and obesity (Brobeck, 1946). When the hypothalamus receives satiation signals from the peripheral nervous system, the frequency of neuronal discharge increases in the VN, but decreases in the LHY (Forbes, 1996). This is because the LHY is known as the feeding center and produces several orexigenic compounds (Brobeck, 1946). The DN is thought to be the site of interaction between NPY and leptin and an area that helps regulate the release of orexigenic and anorexigenic signals from the hypothalamus (Yokosuka et al., 1998). The PN contains receptor sites for most, if not all, of the compounds that regulate feed intake and is one the most important sites regarding the regulation of synthesis and release of compounds controlling feed intake (Li et al., 1994).

Short-term regulation of feed intake controls the commencement and cessation of meals but does not play a role in overall body energy status (Havel, 2001). It is controlled via hormonal (Geary, 2004), mechanical (Leek and Harding, 1975), and chemical (Baile and Mayer, 1969) stimulation of receptors in the reticulo-rumen, small intestine, and liver (Allen et al., 2009). These signals are sent to the hypothalamus via the vagus nerve from receptors throughout the nerve plexus of the gastrointestinal tract and regulate both the duration and frequency of meals to control feed intake.

The commencement of a meal is signaled by the hormone ghrelin. Ghrelin, a 28-amino acid peptide hormone, is synthesized in the abomasum and increases appetite in cattle (Gil-Campos et al., 2006). Concentrations of ghrelin will increase before a meal and decrease shortly thereafter, thus aiding to increase feed intake and reduce the utilization of fat for energy. Ghrelin receptors are present in the brainstem (Geary, 2004) and signal the AN to synthesize NPY to stimulate intake (Kamegai et al., 2001) and block the suppressing action of leptin (Nakazato et al., 2001). Ghrelin is also synthesized in the AN and may trigger intake via the hypothalamus (Geary, 2004). The intravenous infusion of ghrelin will increase intake by increasing meal size and frequency (Geary, 2004).

The cessation of a meal in ruminants is regulated in part by the contents of the reticulorumen. In a study conducted by Campling and Balch (1961), removal of rumen contents during feeding resulted in a 70 to 85% increase in hay intake. Bladders (11.3, 22.7, 34.0, or 45.4 kg) filled with warm water (35°C) were also placed into the rumen to simulate an increase in rumen volume; there was a 0.5 kg decrease in DMI for each additional 4.5 kg of water added to the rumen. Due to the rumen's physical capacity, the movement, tension, and distension caused by digesta within the reticulorumen decreases feed intake. Distension receptors are located within the muscular layer of the rumen wall and are stimulated by the stretching of the rumen epithelium as gut fill increases (Harding and Leek, 1972). The movement of digesta within the reticulo-rumen stimulates mechanoreceptors that are located primarily in the epithelium of the reticulum and the cranial rumen (Leek and Harding, 1975).

Forage NDF is considered an excellent chemical predictor of intake in ruminants because of its bulkiness (Waldo, 1986). Forage intake will decrease as the NDF content increases due to increased retention time of digesta in the reticulorumen (Mertens, 1980; Allen and Mertens, 1988). Reticuloruminal retention time is a function of the rate of degradation and passage of digesta into the omasum. Differences in the rate of degradation are related to concentrations of lignin and indigestible NDF (iNDF) that vary with the maturity, growing conditions (temperature, altitude, precipitation, etc.), and species of forage. A smaller amount of forages containing high concentrations of lignin and iNDF will be consumed by cattle than forages of similar NDF concentrations but with lower indigestible fractions (Oba and Allen, 1999). The size of forage particles can also negatively impact intake. Welch and Smith (1978) fed cattle polypropylene ribbon (with a consistency similar to grass hay) at lengths of 0.5, 1.0, 1.5, and 2.0 cm and collected feces to determine the amount of ribbon excreted intact. The majority of one half cm particles passed without signs of mechanical degradation. However, as particle length increased, the proportion of particles that had been mechanically digested increased. Thus, particles beyond 0.5 cm must be degraded to an extent prior to passage into the omasum. Forage type also has an impact on intake. Grasses tend to be more digestible than legumes as they contain lower concentrations of lignin and iNDF, but legumes have a faster rate of passage from the rumen due to the fragility of particles (Allen, 2000; Waghorn et al., 1989).

Besides mechanical regulation, there is evidence (Baile and Mayer, 1969; Oba and Allen, 2003; Forbes et al., 1992) that feed intake is suppressed by chemical stimulation within the reticulo-rumen. Receptors in the epithelium are sensitive to the size and acidity of molecules either present in feed (lactate and acetate) or produced as a result of fermentation (lactate, acetate, propionate, and butyrate) as well as the osmolality of the rumen environment (Forbes et al, 1992). Baile and Mayer (1969) ruminally injected goats with sodium acetate, propionate, butyrate, or a combination of all three each time they consumed feed. Feed intake on days they were not infused with SCFA solutions served as a control; Baile and Mayer (1969) reported depression of feed intake when goats were ruminally injected with solutions of SCFA compared to the control. Similarly, intraruminal infusion of increasing amounts of propionate (a 1.56 M SCFA solution containing 0, 20, 40, 60, 80, or 100% propionate) resulted in a decrease in feed intake in cows during early and mid-lactation (Oba and Allen, 2003). Forbes et al. (1992) infused increasing amounts of either sodium acetate or NaCl into the rumen of cattle offered grass silage (ad libitum) and reported that the increasing osmolality depressed silage intake.

The hepatic oxidation theory suggests that the oxidation of metabolic fuels in the liver results in satiety signals being sent from hepatocytes to the brain to reduce feed intake (Allen et al., 2009). The main metabolic fuels that have an impact on feed intake are propionate and non-esterified fatty acids (Allen, 2000) and their oxidation and subsequent production of ATP causes hyperpolarization of hepatocyte membranes. This hyperpolarization decreases the firing of the afferent nerves, decreasing the signaling to the hypothalamus. The decreased signaling to the feed centers of the hypothalamus, discussed previously, decrease the synthesis of orexigenic stimulants. The infusion of propionate directly into the portal vein decreased intake to a greater extent than either acetate or butyrate (Anil and Forbes, 1980), most likely because of the greater extent of oxidation of propionate compared to acetate and butyrate in the liver (Allen et al., 2009). Long chain fatty acids (LCFA) also elicit hypophagic responses related to hepatic β-oxidation. The inclusion of LCFA decreased DMI in dairy cows, with unsaturated fatty acids having a greater impact on intake than saturated (Harvatine and Allen, 2006). This is most likely related to the accelerated oxidation of unsaturated compared to saturated fatty acids (Allen et al., 2009). Although it elicits hypophagic signaling in nonruminants, infusion of glucose in adult ruminants fails to reduce feed intake because it is not excessively oxidized by the liver (Allen et al., 2009).

Meal cessation is also stimulated by the release of CKK within the small intestine. Cholecystokinin is a peptide hormone secreted by the epithelial cells of the duodenum and jejunum (Buffa et al., 1976). The secretion of CCK is stimulated by the presence of digesta in the duodenum, primarily the amino acid and fatty acid components of a meal (Rehfeld, 1998). The secretion of intestinal CCK prompts the release of pancreatic enzymes (trypsin, chymotrypsin, amylase, and lipase) and bile (Rehfeld, 2004). The synthesis and secretion of CCK also occurs within the brain. When CCK antibodies were injected into the cerebrospinal fluid of sheep to inhibit the action of CCK, feed intake was increased by approximately 100% (Della-Fera et al., 1981). Injection of CCK will reduce meal size by ending the meal, but not frequency or number of meals, and does not always reduce total intake because of potential increased meal frequency (Geary, 2004). Thus, the action of CCK is to inhibit feed intake by signaling the end of a meal as well as to aid in digestion by signaling enzyme and bile secretion.

The signaling responsible for the long-term regulation of feed intake is caused by the synthesis and secretion of neurotransmitters (orexigenic and anorexigenic) and hormones that act upon receptors within or acting upon the hypothalamus. Leptin is an anorexigenic hormone that is synthesized in adipose tissue and acts upon the hypothalamus (Kalra et al., 1999). These orexigenic and anorexigenic signals are responsible for the long-term regulation of feed intake because of their ability to alter the body's energy status by altering adiposity (Geary, 2004).

Neuropeptide Y synthesis and secretion is increased in times of food deprivation and its chronically high concentrations lead to obesity (Kalra, 1997). Neuropeptide Y is a 36-amino acid peptide that is considered to be the most potent of the orexigenic compounds/appetite stimulants (Miner, 1992). Injections of NPY into the hypothalamus will increase feed intake, regardless of the animal's current state of satiety (Clark et al., 1984) Opioid peptides, such as dynorphin and β-endorphin, are also appetite stimulants produced in the hypothalamus, although they are much weaker than NPY (Finley et al., 1981). When injected, opioid peptides stimulate eating, but only for a short period of time and not to a great extent (Finley et al., 1981). Glutamate and GABA, which are AA that act as orexigenic compounds, are the most abundant neurotransmitters in the hypothalamus. Glutamate receptor stimulation results in immediate, but brief episodes of feed intake (van den Pol et al., 1990). On the other hand, GABA receptor stimulation results in longer periods of feeding and GABA is co-expressed with NPY in the AN (Decavel and van den Pol, 1990).

All of these orexigenic compounds work in the hypothalamus in order to maintain energy homeostasis by upregulating feed intake, however, they must be coupled with anorexigenic compounds in order to prevent overeating and obesity in animals.

The hunger center of the hypothalamus is continuously active (Cone, 2005). Thus, to prevent constant consumption of food, there must be signals that will reduce appetite and increase the amount of fat mobilization (Pullina et al., 2013). Leptin, a hormone synthesized by adipocytes, has receptors spread throughout the body, however, the receptors that regulate feed intake are found in the AN of the hypothalamus (Geary, 2004). The dosing of leptin in animals causes a decrease in intake, body weight and fat stores while energy expenditure increases (Pelleymounter et al., 1995; Levin et al., 1996). Leptin does not decrease the number of meals, but does decrease meal size (Vasselli et al., 1997). Leptin acts on the hypothalamus, which inhibits the synthesis of NPY (Cusin et al., 1996). Blood leptin concentration increases at the beginning, remains elevated during, and decreases at the end of a meal because of the feedback cycle through the hypothalamus to terminate eating (Kalra et al., 1999). There are many functions of CRH; with regards to feed intake, it acts upon the PN, which inhibits feed intake (Morely, 1987). Melanocortin also functions to reduce intake and mobilize body fat. When melanocortin receptors are stimulated, energy expenditure is increased but intake is reduced, which can cause a reduction in body weight (Balthasar et al., 2005). The injection of CART into the AN of the hypothalamus decreases feed intake but does not affect the levels of NPY being produced (Stanley et al., 2001). Thus, CART most likely does not affect meal frequency, but only meal size.

Consideration of the regulation of feed intake, both short- and long-term, is imperative when feeding and investigating forages and goes hand-in-hand with forage quality. Low quality forages must be consumed in greater quantities by cattle in order to meet their nutrient requirements, however, even if forage is offered *ad libitum*, consumption is limited by the animal. Thus, it is necessary to understand how bulk fill of the rumen and intake regulation signaling occurs in order to more adequately provide feed for cattle, whether it is by reducing particle size or providing feed at different times throughout the day.

The challenges associated with feeding cattle on rangeland

Although the use of rangeland for grazing is one of the cheapest options to feed cattle in the United States, ranchers face numerous challenges in ensuring an adequate supply of nutrients to match requirements. Besides the need to account for the changes in the nutrient requirements of cattle as dictated by the physiological state (e.g., pregnant and lactating vs. pregnant and not lactating), ranchers also have to deal with the changes in nutrient composition of rangeland plants with advancing maturity. In addition, in recent years, the domination of invasive annuals, persistent drought conditions, and severe rangeland fires have also resulted in challenges in the provision of nutritionally adequate forage for cattle during the grazing season.

Changes in forage quality and quantity with advancing maturity

The growth stage of a plant is the major determinant of forage quality (Buxton, 1994). In most plants, leaves tend to have a greater concentration of protein and a lower concentration of fiber, whereas stems tend to have the opposite. The CP content of switchgrass and big bluestem leaves ranges from 8.3 to 11.4% of DM, whereas stems range from 3.6 to 7.3% of DM. Stem tissue also typically contains a greater concentration of NDF and lignin than leaf tissue does. Switchgrass and big bluestem leaves also contain 63.4 to 67.1% NDF and 3.8 to 5.4% lignin, whereas stems contain 64.1 to 78.1% NDF and 4.4 to 8.6% lignin (Griffin and Jung, 1983). As plants mature, the leaf:stem ratio decreases. For instance, the percentage of leaf tissue of switchgrass and big bluestem decreased from 68% of the plant to just 30% from late June to early August (Griffin and Jung, 1983). With the increase in overall stem tissue of the plant, the nutrient composition changes as well. These changes occured in the stems of big bluestem and switchgrass, whereas the leaves maintained a relatively constant CP and NDF concentrations (Griffin and Jung, 1983). Stem CP decreased from 7.3 to 5.2% of DM and 6.6 to 4.8% of DM for big bluestem and switchgrass, respectively, from late June to early August. Additionally, stem NDF concentration increased from 64.1 to 78.7% of DM and 69.7 to 78.1% of DM for big bluestem and switchgrass, respectively (Griffin and Jung, 1983). Similar changes occur in timothy hay whereby CP content decreases (10 to 5.9% of DM) and crude fiber content (27.8 to 33.2% of DM) and lignin (5.3 to 10.1% of DM) increase when maturity advanced from early bloom to post bloom (Lloyd et al., 1961). Griffin and Jung (1983) also measured the change in phosphorus concentration with advancing maturity for big bluestem and switchgrass and reported that P content only decreased slightly (0.22 to 0.20% of DM) for leaves but decreased by 0.10% for stems (0.24 to 0.14% of DM).

The phenological maturation of a plant not only alters its nutrient profile, but also the nutrient availability as digestibility decreases with advancing maturity. Griffin and Jung (1983) reported *in vitro* DM digestibility ranges from 57.7 to 66.1% of DM for leaves and 39.7 to 61.4% of DM for stems of big bluestem and switchgrass. Thus, as the leaf:stem ratio decrease, digestibility decreases as well. Digestibility of stem tissue from big bluestem and switchgrass decreased from 61.4 to 39.7% and 58.5 to 42.7%, respectively, from late June to early August. Digestibility of leaf tissue from big bluestem and switchgrass decreased from 61.7 to 60.8% and 66.1 to 57.7%, respectively, in the same time period (Griffin and Jung, 1983). Dry matter digestibility for timothy hay decreased from 65 to 48% with advancing maturity (early bloom to post bloom; Lloyd et al., 1961).

Digestibility is not only affected by phenological maturity, but also by species. For example, Buxton and Marten (1989) reported that Reed canarygrass, orchardgrass, and smooth bromegrass were more digestible than tall fescue because of their greater leaf:stem ratios. The type of forage (warm-season vs. cool-season grasses vs. legumes) also affects the digestibility. Warm-season grasses generally have a greater concentration of NDF than cool-season grasses, leading to decreased digestibility (Reid et al., 1988). Reid et al. (1988) compared the digestibility of thirty six cool-season grasses, sixty legumes, and fifty seven warm-season grasses in beef cattle and found the DM digestibility to be 66.9, 62.8, and 59.8%, respectively.

The nutritive value of a forage is determined by the combination of its nutrient composition and the digestibility of those nutrients. A low quality, forage has a CP concentration less than 8% (DM basis), DM digestibility of less than 55%, and soluble sugars and starch content of less than 10% of DM (Leng, 1990). The concentrations of ADF, lignin, and iNDF generally increase with advancing maturity, which reduces digestibility of a forage (Buxton, 1994). Animal performance, when grazing, is influenced by the nutritive value of forage; the greater the nutritive value, then the greater the potential for growth, reproduction, and lactation performance.

Forage selection can influence the quality of feed that animals consume when grazing. The leaf:stem ratio is one of the key factors that has an impact on forage selection by cattle. Cattle prefer plants that have a greater leaf:stem ratio and also prefer plants that are more green than brown, dormant tissue (Hardison et al., 1954). Therefore, this can influence nutrient supply in cattle grazing on range. Ganskopp and Bohnert (2009) reported that cattle preferred sections of large pastures that had forages that were more digestible, had greater concentrations of CP, and lower concentrations of NDF.

Although a decrease in forage quality and digestibility is observed throughout the growing season, forage yield increases allowing for more herbage available for consumption. As plants mature, the accumulation of carbohydrates, particularly cellulose and hemicellulose, increases. This increase in carbohydrates, mostly in stem tissue, greatly increases the overall forage yield of a plant. The increase in biomass yield could provide greater amounts of forage on rangeland for cattle. There is a compromise, however, between the quantity and the quality of biomass. In general, although herbage yield increases with advancing maturity, quality decreases and could potentially limit nutrient availability for cattle. Thus, the key for producers is to ensure that they graze or harvest the greatest amount of biomass that still provides an adequate amount of nutrients for cattle.

Rangeland forages in Idaho

The grasses available for cattle grazing can be classified as either cool or warm season grasses. The rangeland grasses of Idaho are predominantly cool season. Bluebunch wheatgrass (Pseudoroegneria spicata), Idaho fescue (*Festuca idahoensis*) and Sandberg bluegrass (*Poa secunda*) are common cool season grasses found on Idaho rangeland. These grasses follow the C3 pathway of respiration and tend to have greater protein content and digestibilities than warm season grasses (Barbehenn et al., 2004). Cool season grasses begin to grow when soil temperatures reach 4 to 7°C, thus, they grow well in spring and fall when temperatures are low (Forage Information System, 2018). These species will have two distinct periods when their growth rates are high; cool temperature in both spring and fall. This growth pattern is beneficial to Idaho cattle producers as it avails high quality forage for cattle during the spring calving season when nutrient requirements are because of the high demand to support lactation, growth (primiparous cows) and reproduction functions. Cool season grasses, however, enter a period of dormancy in the summer because of high temperatures and low precipitation. This results in “summer slump” whereby forage quality and quantity is low (Forage Information System, 2018).

One method to combat the “summer slump” is to utilize warm season grasses, they follow the C4 pathway of respiration and tend to have greater yields and fiber concentrations than cool season grasses (Barbehenn et al., 2004). Warm season grasses begin to grow when soil temperatures reach 15 to 18°, thus they grow well in the summer when temperatures are high (Forage Information System, 2018). The period of high forage quality for warm season grasses occurs during the “summer slump” period for cool season grasses, thus they are ideal to bridge the gap in quantity and quality of forage required. The presence of warm season grasses in Idaho, however, is very limited and would not be present in large enough quantities to provide much relief for the “summer slump”. Warm season grasses, however, could be grown and harvested for use as supplements during the winter months when grazing might not be possible.

Domination of invasive species

Invasive species are able to dominate landscapes because of several factors, including the ability to outcompete native species for nutrients in the soil (Vasquez et al., 2008; Young and Mangold, 2008), greater growth rates than native species (Young and Mangold, 2008), unique seed dispersal methods (Monaco et al., 2005; Young et al., 1987), and anti-quality factors that prevent consumption (Hulbert, 1955; Bovey et al., 1961; Montes-Sánchez and Villaba, 2017). Invasive plants cost the United States billions of dollars every year in losses and damages to native species as well the effort to control alien species (Pimentel et al., 2005). There are three major invasive species in the Pacific Northwest that inhibit the production of cattle on rangeland: cheatgrass (*Bromus tectorum*), medusahead (*Taeniatherum caput-medusae*), and ventenata (*Ventenata dubia*; Sheley et al., 2014). Each of these three species can dominate landscapes that were predominately native forbs and perennial grasses and have been present in the Pacific Northwest for decades (Hironaka, 1961). Each species comes with its own method for domination within a given ecosystem, but all three effectively decrease the profits of producers trying to work on infested land. The presence of invasive species can severely decrease the value of hay harvested, especially if it is to be exported and they decrease the nutritive value of a pasture or allotment for a livestock producer (Hironaka, 1961).

Cheatgrass

Cheatgrass, also known as downy brome or bronco grass, is a winter annual that first appeared in Idaho in the late 1890's (Klemmedson and Smith, 1964). Since then, it has become increasingly invasive and dominates the landscape in certain parts of the state (Vallentine, 1989). Growth of cheatgrass begins in early fall, slows down once snow begins to fall, before increasing at a fast rate in spring as snow thaws. Because cheatgrass has a good nutritive profile during spring when it is growing rapidly, it can be a good forage source for cattle (Cook and Harris, 1952; Murray et al., 1978; Murray and Klemmedson, 1968). Its nutritional quality, however, declines as it matures, becoming unpalatable to cattle (Hull and Pechanec, 1947; Klemmedson and Smith, 1964; Vallentine, 1989). Once mature, its sharp seeds may also increase the incidence of lumpy-jaw or eye injuries in cattle (Young et al., 1987). Cheatgrass is a very difficult grass to control, as it will return in stronger stands even after it has been burned (Boyte et al., 2014). Because animals will consume it, dormant season grazing can be used to reduce cheatgrass invasion of native landscapes (Davies et al., 2015).

Medusahead

Medusahead is one of the worst range weeds present in Idaho with the potential to damage millions of acres of rangeland (Davies and Johnson, 2008). It is a winter annual grass native to Europe (Abrams, 1955) that was first documented in the United States in 1887 (Furbush, 1953). Its seeds spread across landscapes via adhesion with awns and barbs to people, animals, and vehicles (Monaco et al., 2005). The dispersal of seeds from medusahead occurs over a long period (typically July to October), aiding in its ability to be very invasive (Davies, 2008). Thus, livestock should not graze areas infested with medusahead during the period of seed disarticulation because of their potential as dispersion vectors (Davies, 2008). Domination of medusahead limits both the amount and quality of forage available for livestock production. Davies and Svejcar (2008) reported an 87% decrease in the biomass yield of desirable native plants following the invasion of medusahead. The grazing of medusahead is also largely not a feasible option. Although reported to contain 5.3 to 12.4% CP, 56.2 to 67.4% NDF, and 31.5 to 46.7% ADF (DM basis; Montes-Sánchez and Villaba, 2017), it is unpalatable to livestock because of its high silica content (up to 11% of DM; Bovey et al., 1961; Montes-Sánchez and Villaba, 2017). Another problematic characteristic of medusahead is its slow rate of decomposition. Because it is not grazed and decomposes slowly, its domination increases the fuel load that increases the incidences and severity of rangeland fires (Torell et al., 1961).

Ventenata

Ventenata, also known as wire grass or North Africa grass, is a cool-season annual that is native to Northern Africa and Southern Europe. Ventenata infestations in the Pacific Northwest have been documented since the 1950s, and this has reduced the number of native forbs and perennial grasses and also led to increased erosion because it has a shallow root system relative to native plants (Wallace et al., 2015). Ventenata can also cause economic losses by infesting hay fields and decreasing crop yield (Fountain, 2011). In addition, it poses a risk to native grasses and crops as it can be a host to disease. For instance, barley yellow dwarf virus, a disease known to compromise the production of many crops and native rangeland plants, has been detected in ventenata populations throughout the PNW (Ingwell and Bosque-Pérez, 2014). As with medusahead, ventenata also contains a high silica concentration (up to 3% of DM), decreasing its palatability for grazing animals (Pavek et al., 2011).

Drought conditions

Throughout the past three decades, most of the western United States has been experiencing a decrease in annual precipitation, leading to widespread drought (Easterling et al., 2017). During drought, water stress can limit or inhibit plant growth, which can reduce both the quantity and quality of forage available for grazing. In addition, drought conditions also increase the incidences of fires occurring on rangeland, with the dryness in an area influencing ignition, propagation and intensity (Littell et al., 2016). As global temperatures continue to rise, the frequency and severity of droughts will continue to increase (Cook et al., 2014). Therefore, this will result in challenges for producers in meeting the nutrient requirements of cattle. Depending on the severity of the drought conditions, practical strategies that producers could implement include decreasing the stocking rate on rangeland and the use of supplemental feeds.

Use of alternative forages as a potential solution to feed availability

Because of the increasing prevalence of exotic annual grasses, persistent drought conditions, and the greater frequency and severity of rangeland fire, the provision of nutritionally adequate forage to rangeland cattle is becoming increasingly difficult. Therefore, alternative feeding and grazing strategies are urgently needed by cattle producers in order to supply cattle with a year-round source of high quality forage to ensure an adequate nutrient supply. One such strategy is the identification and growth of alternative forage species that would be high quality, suitable replacements for rangeland forage for producers to graze or feed as a supplement.

When selecting the ideal alternative forage, quick growth and high yield are imperative in Idaho, as well as high forage quality (greater than 55% digestible DM and 8% CP; DelCurto et al., 2000). In the western United States, an excellent alternative forage must also be drought resistant because of frequent periods with little to no water. The west has a very wide range of terrain and climates, thus any forage grown must be hardy and quick growing in order to provide cattle valuable nutrients when range forage is low in quality and/or quantity. Based on these criteria, *Eragrostis tef* is one such grass that has potential to be fed as an alternative forage to rangeland beef cattle (Miller, 2011).

***Eragrostis tef* as a potential forage source for beef cattle in Idaho**

Eragrostis tef, or teff grass, is a warm-season, annual grass that is native to Ethiopia where it is used as a grain crop rather than a forage crop. Teff is a drought tolerant crop (Miller, 2011). It has a rapid rate of growth and yield; the first cutting can be as early as 40 days after planting, with rapid regrowth allowing for up to three cuttings in one season (Miller, 2011; Saylor 2017). Norberg and Felix (2014) reported yields of Tiffany teff between 4.19 to 7.23 t/ha when grown in Ontario, OR (44.0°N, 117.0°W; Owyhee silt loam soil; 10 cm of water from furrow-irrigation weekly).

Teff is also highly palatable, with anecdotal evidence (Miller, 2011) suggesting that it is comparable to timothy in terms of its nutritive value. Therefore, this has resulted in interest in its use as horse feed (Staniar et al., 2010; McCown et al., 2012). McCown and colleagues (2012) harvested teff at the early-heading and late-heading stages of maturity and timothy and alfalfa at similar stages of maturity. The palatability of teff at both stages of maturity was then compared to alfalfa and timothy hay in the one study, and then with just timothy hay in another study. In the first study, horses preferred alfalfa and timothy over either stage of maturity of teff but preferred early-heading teff compared to late-heading teff. In the second study, DMI was similar in mares fed either timothy or teff hay (1.85 vs. 1.65 kg DM/100 kg BW, respectively). Staniar and colleagues (2010) harvested teff at the boot, early-, and late-heading stages of maturity to determine the effect of maturity at harvest on DMI and apparent digestibility in mature mares. Mares consumed a greater amount of boot (9.7 kg/d) and early-heading (9.2 kg/d) stage hay than late-heading (8.1 kg/d) stage of maturity. Dry matter, CP, and NDF digestibility decreased by 5.3, 5.3, and 8.6%, respectively, from the boot stage to the early-heading stage, and an additional 3.8, 7.6, and 7.8% from the early-heading stage to the late-heading stage. Because DMI was comparable, and nutrient digestibility did not decrease in a drastic manner, Staniar et al. (2010) concluded that boot stage and early-heading stage maturities of teff were suitable for horses (Staniar et al., 2010). In spite of the availability of this information for horses, little is known about the nutritive value of teff as cattle feed.

The nutritive value of a feed is determined by its chemical composition and digestibility. Several studies have been conducted to determine the chemical composition of teff. McCown and others (2012) harvested teff of two varieties at the early-heading (“Tiffany”) and late-heading (“Horse Candy”) stages of maturity and reported 89.7% DM, 66.0% NDF, 38.0% ADF, and 8.9% CP for the “Tiffany” harvest and 90.3 % DM, 71.7% NDF, 44.6% ADF, and 10.1% CP for the “Horse Candy” harvest. McCown and associates (2012) did not report the agronomic conditions nor the harvest dates of the teff fed in their study. The “Tiffany” teff fed by Staniar et al. (2010) was harvested at the boot, early-heading, and late-heading stages of maturity and was fertilized with N, once, 28 days after planting (67 kg/ha) and after each harvest (45 kg/ha). Staniar and colleagues (2010) reported values of 92.0% DM, 68.1% NDF, 35.7% ADF, and 16.4% CP for the boot stage harvest, 92.1% DM, 71.1% NDF, 40.2% ADF, and 10.8% CP for the early-heading stage harvest, and 92.5% DM, 70.8% NDF, 41.5% ADF, and 7.5% CP for the late-heading stage harvest. In a greenhouse study by Saylor (2017), “Corvallis”, “Dessie”, “Moxie”, and “Tiffany” teff varieties were harvested at 40, 45, 50, 55, and 60 days after planting. The DM yield of teff increased by 1.1 g per day from days 40 to 60 (4.1 to 26.1 g of DM per pot). Ash free NDF content increased at a rate 0.6% of DM per day from days 40 to 60 (51.7 to 63.5% of DM), whereas CP content decreased at a rate of 0.9% of DM per day (28.7 to 11.2% of DM). The digestibility of teff has been analyzed *in vitro* (Sugg, 2016; Saylor, 2017) and *in vivo* using lactating dairy cows (Saylor et al., 2018). Sugg (2016) collected biweekly samples of “Tiffany” teff grown for 107 days in the Southern High Plains and reported that advancing maturity of both the whole plant and the canopy structure did not affect *in vitro* digestibility. Sampling by Sugg (2016) began at 51 days after planting and they did not report values for digestibility. Saylor (2017) reported that seed variety of teff (“Tiffany”, “Moxie”, “Corvallis”, and “Dessie”) had no effect on the *in vitro* NDF digestibility (49.7 to 51.9%). *In vitro* NDF digestibility, decreased from 60.8 to 41.2% when cutting age increased from 40 to 60 days after planting. The lack of differences reported by Sugg (2016) could be attributed to the delay of sampling until 51 days after planting, whereas Saylor (2017) began sampling at 40 days after planting. When harvested at 51 days post-planting (Sugg, 2016), the teff could have matured beyond the point when the concentrations of lignin and fiber change at a fast rate. There was no effect on the apparent total tract digestibility of DM (64.3 to 67.7%) and NDF (50.0 to 54.9%) when corn silage, alfalfa hay, and prairie hay (23.9, 19.4, and 1.6% of DM offered, respectively) were replaced with teff (29.6 and 27.3% of the DM offered) in two TMR fed to dairy cattle (Saylor et al., 2018). Saylor and associates (2018) speculated that the similar digestibilities could have been related to a longer retention time and greater extent of fermentation of teff compared to the alfalfa and a quicker passage rate and decreased digestibility of alfalfa compared to the teff. *In vivo* digestibility of teff, however, when fed as the sole feed has not been measured in either dairy or beef cattle. In addition, the ruminal fermentation characteristics, including the production of SCFA and the synthesis of microbial protein, which are crucial in the provision of energy and AA to cattle, have yet to be evaluated.

The ultimate measure of nutritive value of a feed is the animal performance. Young et al. (2014) fed growing beef steers a TMR containing either teff hay (44.0% teff hay [unspecified variety], 20.7% corn silage, 31.4% rolled barley; DM basis) or alfalfa hay (20.5% alfalfa hay, 43.0 % corn silage, and 32.6% rolled barley; DM basis). The inclusion of teff in the TMR increased DMI (7.41 to 8.04 kg/d) but did not affect total BW gain or average daily gain. Young and others (2014) also fed growing dairy heifers a TMR containing either teff hay (8.5% alfalfa hay, 42.0% teff hay, 11.3% corn silage, 13.7% steam-flaked corn, and 24.5% dried distiller's grains; DM basis) or alfalfa hay (54.1% alfalfa hay, 24.8% corn silage, and 21.2% steam-flaked corn; DM basis). The inclusion of teff in the diet of growing dairy heifers increased intake (5.95 to 6.77 kg/d), total BW gain (96.1 to 104.7 kg) and ADG (1.13 to 1.34 kg/d). Thus, Young and others (2014) concluded that teff hay was a palatable and low-cost alternative to alfalfa hay in both beef steer and dairy heifer diets.

Saylor and associates (2018) evaluated the use of teff as the sole forage source for lactating dairy cattle. Replacing corn silage, alfalfa hay, and prairie hay with teff hay had no effect on DMI, apparent total tract digestibility, or the percent and yield of milk fat and lactose. The percent of milk protein, was greater (3.14 and 3.18%) for the teff-containing diets than the control diet (3.07%) even though the CP content of teff-containing diets was only 0.3% greater than the control (17.1 versus 16.8% CP). The concentration of milk urea N (12.08 and 11.63 vs. 11.53 mg/dL) and yield of milk protein (1.27 and 1.30 vs. 1.23 kg/d) also tended to be greater for teff-containing diets than for the control diet. The greater yield of milk protein was potentially related to a greater fermentability of teff hay when compared to alfalfa hay because legumes have a faster rate of passage than grasses due to the fracturing of leaves within the rumen, which results in a decrease in digestibility (Moseley and Jones, 1979).

Although several studies have determined the nutrient composition of teff as well as the impact of its inclusion in TMR for cattle on production performance, little is known about its degradation in the rumen. The effects of feeding teff on the ruminal fermentation characteristics is yet to be evaluated, and is critical to the understanding of its nutritive value as cattle feed. Similarly, N utilization in cattle fed teff has also not been reported. Thus, research on the effects of feeding teff as the sole forage in beef cattle diets on ruminal fermentation characteristics and N utilization is needed. This will ensure its judicious use as an alternative forage source for rangeland cattle production.

Hypothesis

The nutritive value of ‘Moxie’ teff as a preserved forage source for beef cattle changes relative to its stage of maturity.

Objective

Our objective was to determine the effect of feeding ‘Moxie’ teff hay harvested at either the boot, early- or late-heading stage of maturity on nutrient intake, ruminal fermentation characteristics, omasal nutrient flow, and nitrogen utilization in beef cattle.

CHAPTER 2

Effects of maturity at harvest on the nutritive value and ruminal digestion of *Eragrostis tef* (cv. Moxie) when fed to beef cattle[1]

MATERIALS AND METHODS

All procedures in this study were approved by the Institutional Animal Care and Use Committee at the University of Idaho (Protocol # 2016-35).

Agronomic Practices

Teff used in this study was seeded on June 20th, 2016 (soil temperature of 18°C) at the University of Idaho Nancy M. Cummings Research, Extension, and Education Center in Carmen, Idaho (latitude 45°14′ N, longitude 113°53′ W, altitude 1,166 m). Environmental data for the growing and harvest phases (Table 1) was collected from National Centers for Environmental Information at the Salmon Idaho Weather Station in Salmon, ID (22.5 km south of growth site). Prior to seeding (Melroe Planter; Melroe Manufacturing Company, Gwinner, ND), the field was grazed to remove any remaining biomass, disked three times to remove the remaining stem and root tissue from the soil surface, roller-harrowed, and rolled to ensure a firm seed bed. Seed was broadcast and then rolled to ensure seed-to-soil contact.

The soil was tested just prior to planting and the recommendation was for application of 56 kg/ha N. However, inorganic N fertilizer was not applied primarily because of nitrate

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[1] A version of this chapter has been published: Vinyard, J. R., J. B. Hall, J. E. Sprinkle, G. E. Chibisa. 2018. Effects of maturity at harvest on the nutritive value and ruminal digestion of Eragrostis tef (cv. Moxie) when fed to beef cattle. J. Anim. Sci. 96:3420-3432. doi:10.1093/jas/sky202

toxicity concerns. In addition, the planted area is used for winter-feeding, with hay spread out across the field, allowing for the use of manure as fertilizer. The seeding rate was 11 kg/ha. Fields were watered using a center-pivot irrigation system to ensure a 15-cm soil moisture depth prior to emergence, and a 20-cm soil moisture depth after emergence. Prior to emergence, fields were watered daily with approximately 0.65 cm of water applied each time. After emergence, watering occurred one to three times each week, as needed, with 1.25 to 2.00 cm applied each time. Weeds were controlled using a commercially available mixture of dicamba (3,6-dichloro-2-methoxybenzoic acid) and 2,4-dichlorophenoxy-acetic acid (Latigo; Helena Chemical Company, Collierville, TN) according to label instructions. Spraying was carried out once, after emergence, with a tractor-mounted boom-sprayer. Teff grass was harvested (Discbine disc mower; New Holland Agriculture, Turin, Italy) at the BT, EH, and LH stages of maturity (Moore et al., 1991) and left in a windrow for five days. Following field curing, hay was baled (Hesston 4655 Square Baler, AGCO) and moisture content was measured using a hay probe. Yield was estimated for each stage of maturity by weighing 3 groups of 10 bales to obtain an average weight and multiplying it by the total number of bales harvested. Hay was transported to Moscow, ID for the feeding trial, which began on November 11, 2016.

Animals, Experimental Design, and Treatments

Six non-pregnant ruminally-cannulated (10 cm diameter, Bar Diamond, Inc., Parma, ID) British crossbred beef heifers (initial BW ± SD; 476 ± 32.6 kg) were used in a replicated 3 × 3 Latin square design with 28-d experimental periods. Heifers were housed in individual tie-stalls at the University of Idaho Dairy Center and were fed twice daily at 0630 and 1830 h for ad libitum intake. Dietary adaptation took place in the first 18 d of each period, which was then followed by 10 d of sample collection. The 3 dietary treatments were teff hay that was harvested at the BT, EH, or LH stages of maturity. To provide a uniform particle size (5 to 10 cm), hay was coarsely ground through a tub grinder. Animals were also offered a complete mineral supplement (J.R. Simplot Company, Caldwell, ID) daily to either meet or exceed NRC (2016) requirements. The supplement was fed free-choice in a separate feeder and it contained 6.0% Ca, 6.0% P, 5.0% NaCl, 5.0% Mg, 0.3% K, 2,008 ppm Cu, 2,014 ppm Zn, 37.8 ppm Se, and 99,792 IU/lb Vitamin A. At the beginning of the trial, cattle were vaccinated against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) types 1 and 2, parainfluenza3 (PI3), and bovine respiratory syncytial virus (BRSV) (2 mL intramuscular; Bovi-Shield Gold 5; Zoetis, Parsippany, NJ) and *Clostridium* (5 mL subcutaneous; Ultrabac 8; Zoetis, Parsippany, NJ). Cattle were also treated for the control of internal and external parasites (10 mL subcutaneous; Dectomax injectable; Zoetis, Parsippany, NJ), and given a subcutaneous injection of trace minerals (6.5 mL subcutaneous; MultiMin 90; MultiMin USA, Fort Collins, CO).

Measurements

All heifers were weighed prior to morning feeding on two consecutive days at the beginning of each experimental period and at the end of the study. To determine DMI, hay offered and orts were recorded daily. Hay samples were collected from hay offered on three consecutive days each week and composited by experimental diet. Orts were collected daily and were composited by animal and week. The collected hay and orts samples were dried at 55°C for 72 h and then sequentially ground through a 4- and 2-mm screen (Retsch Cutting Mill SM 200, Retsch, Haan, Germany).

To estimate nutrient digestion and flow out of the rumen, $YbCl_3$ was used as a digesta marker. Briefly, $YbCl_3$ was infused continuously into the rumen at a rate of 3.35 g of Yb/d (Siddons et al., 1985) using a peristaltic pump (323S; Watson & Marlow, Wilmington, MA) from d 22 to 28 of each period. A 50-mL subsample of each batch of solution made for each cow for every period was collected prior to infusion. Omasal digesta was then collected on d 26 at 0900, 1500, 2100 h and d 27 at 0300, 1200, and 1800 h and d 28 at 0000 and 0600 h using the sampling technique described by Huhtanen and associates (1997). Approximately 600 mL of omasal digesta was collected at each sampling time point and composited by animal and period.

To measure fermentation characteristics, approximately 1 L of ruminal digesta from the cranial ventral, caudal ventral, central, and cranial dorsal regions of the rumen was collected at the same time as omasal digesta. Digesta samples were strained through four layers of cheesecloth and two 5 mL aliquots were collected for the measurement of short-chain fatty acids (SCFA) and NH_3-N concentrations. The first aliquot (5 mL) was mixed with 1 mL of metaphosphoric acid (H_2PO_4; 25% wt/vol) for later analysis of SCFA and the second 5-mL aliquot was mixed with 1 mL of 1% H_2SO_4 for later analysis of NH_3-N. Ruminal digesta samples were collected in the same manner on d 19 at 0630, 0700, 0800, 0900, 1000, 1200, 1500, 1800, and 2100 h, and on d 20 at 0000 and 0300 h to determine the diurnal changes in ruminal NH_3-N concentration. Ruminal pH was measured continuously from d 22 to 28 of each period using indwelling pH loggers (LRCpH; DASCOR, Inc., Escondido, CA) as described by Penner and colleagues (2006). Each logger was programmed to record rumen pH every minute.

To determine apparent total tract nutrient digestibility and nutrient excretion, grab fecal and spot urine samples were collected at the same time as omasal digesta sampling. The collected fecal samples were immediately frozen (-20ºC). A 50-mL subsample of the collected urine was immediately acidified with 3 mL of 2*M* H_2SO_4 to a pH < 2.5 and placed on ice to prevent the loss of NH_3-N. Thereafter, 1 mL of the acidified urine was diluted by addition of 9 mL of distilled H_2O, composited by animal and period, and frozen (-20ºC) for later analysis of total N, urea-N, creatinine, and purine derivatives.

On the last day of each period (d 28) blood samples were collected via coccygeal venipuncture 3 h post-feeding using evacuated sodium heparin tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were centrifuged (645 × *g* for 25 minutes at 4°C) immediately after which plasma was harvested and frozen (-20ºC) for later PUN analysis.

Sample Analyses

Fecal samples were thawed overnight at room temperature, composited by period, dried at 55 ºC for 72 h and sequentially ground through a 4- and 2-mm screen (Retsch Cutting Mill SM200, Retsch, Haan, Germany). The ground hay, orts, and fecal samples were then analyzed for DM (AOAC, 1990; method 930.15), OM (AOAC, 1990; method 942.05), NDF and ADF (Van Soest et al., 1991), lignin (Goering and Van Soest, 1970), and CP using the Kjeldahl procedure (Foss Analytics; Hillerød, Denmark; AOAC, 1990; method 976.05). Sodium sulfite and alpha-amylase were used for NDF determination. The indigestible NDF (iNDF) content of hay samples was also determined as described by Valente and associates (2011). Briefly, samples (0.6 g) were weighed into nylon bags (F57, Ankom Technology, Macedon, NY) that were then incubated for 288 h in the rumen of 2 cows. After incubation, the residues were analyzed for NDF as previously described. Hay samples were composited by treatment for the entire study and sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for the analysis of starch, simple sugars, mineral, and nitrate content. Starch was extracted from samples by incubating at 40ºC and filtered prior to thermal solubilizing, after which samples were incubated with glucoamylase to produce dextrose. Dextrose was then analyzed using a YSI Analyzer (YSI 2700 SELECT Biochemistry Analyzer, YSI Incorporated Life Sciences, Yellow Springs, OH). Simple sugars were analyzed as described by Hall and colleagues (1999). Samples for mineral composition analysis were digested using a Microwave Accelerated Reaction System (MARS6 CEM, Matthews, NC) and analyzed using inductively couple plasma (Thermo Fisher Scientific, Waltham, MA) as described by Wolf and colleagues (2003). Chloride content was analyzed using the method described by Cantliffe and colleagues (1970). Nitrate content was analyzed using an RQflex reflectometer (MilliporeSigma; Burlington, MA).

Ruminal fluid samples preserved with H_2SO_4 were centrifuged (10,800 × *g* for 20 minutes at 4°C) and the supernatant was collected and analyzed for NH_3-N using a phenol-hypochlorite assay (Broderick and Kang, 1980). To determine SCFA concentration in ruminal fluid samples, samples were thawed at room temperature, mixed thoroughly and centrifuged (12,000 × *g* for 10 minutes at 4°C). The supernatant was collected and centrifuged again (16,000 × *g* for 10 minutes at 4°C). The resultant supernatant was filtered through a 0.2 µm Nylon filter and diluted 1:1 with distilled water. The concentration of SCFA was determined using a gas chromatograph with a flame-ionization detector (GC-FID; 6890 Series, Hewlett-Packard; Palo Alto, CA) as described by Coats and associates (2011).

The composite omasal digesta samples were thawed at room temperature overnight in preparation for oven drying (60°C for 72 h). Dried samples were ground with a mortar and pestle. The omasal digesta and fecal samples were then digested (AOAC, 2005; method 2006.03) in preparation for Yb analysis using Microwave Plasma-Atomic Emission Spectrometry (MP-AES; Agilent Technologies, Santa Clara, CA). The infused $YbCl_3$ solution samples were analyzed for Yb.

Acidified urine composites were thawed and analyzed for total N using the Kjeldahl procedure (Foss Analytics; Hillerød, Denmark; AOAC, 1990; method 976.05). Commercial kits (Arbor Assays; Ann Arbor, MI) were used for the analysis of urine creatinine and urine urea-N (UUN), and PUN. Urine allantoin and uric acid concentrations were determined using a method adapted from Stentoft and others (2014). Briefly, quantification was carried out using a HPLC/MS (Waters Corporation, Milford, MA) fitted with a reversed-phase column (C18, 5µm particle size, 2 mm × 250 mm; Phenomenex, Torrance, CA) using a 5% methanol mobile phase.

Calculations

The flow of DM to the omasum and as feces was calculated by dividing the amount of Yb infused (g/d) by the amount of Yb (g/kg of DM) in the whole omasal digesta or feces. The ruminal or fecal nutrient outputs were determined by multiplying the omasal or fecal OM, NDF, ADF, and CP concentrations (DM basis) by the DM flow. Apparent nutrient digestibilities were calculated using the following equation:

$$\text{Digestibility, \%} = ([\text{Nutrient intake, g} - \text{Nutrient output, g}]/\text{Nutrient intake, g}) \times 100$$

Urine output was estimated using the concentration of creatinine measured in urine, and BW and creatinine constant of 29 mg/kg BW per day (Valadares et al., 1999) according to the following equation:

$$\text{Urine output, kg/d} = (29 \times \text{BW}^{0.75}) \div \text{creatinine concentration, mg/L}$$

Apparent nitrogen balance was calculated as the difference between N intake and excretion (fecal + urine).

The excretion of allantoin and uric acid was used to estimate the total absorption of purine derivatives (PD) as described by Chen and Gomes (1992) according to the following equation:

$$\text{PD}_{\text{excreted}}\text{, mmol/d} = 0.85(\text{PD}_{\text{absorbed}}) + (0.385\ \text{BW}^{0.75}),$$

where 0.85 is the recovery of absorbed purines as PD and 0.385 $\text{BW}^{0.75}$ is representative of purine excretion from endogenous sources. The flow of microbial N was calculated using the following equation:

$$\text{Microbial N, g/d} = 70(\text{PD}_{\text{absorbed}}) \div (0.116 \times 0.83 \times 1000),$$

where 70 represents the N content of purines, 0.83 is the digestibility of purines, and the ratio of purine-N:total N in rumen microbes is 11.6:100

Statistical Analysis

All data on nutrient intake, ruminal fermentation characteristics, nutrient digestibility and omasal flow, and nutrient excretion were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC) for a replicated 3 × 3 Latin square. The model included the following independent variables: cow, period, square, and stage of maturity of teff hay

(BT, EH and LH). Period, square, and stage of maturity were considered fixed whereas cow within square was considered as random. Temporal ruminal NH_3-N data was analyzed accounting for repeated measures through the inclusion of additional terms for time (hour) and stage of maturity × time interaction in the model described previously. The variance–covariance structure of the repeated measures was modeled separately with an appropriate structure fitted using the lowest values of the fit statistics based on the Bayesian information criteria. Data are presented as least square means. Significance was declared at $P < 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Feed Composition

Crude protein content decreased ($P < 0.01$) with advancing maturity (Table 2). Indigestible NDF content was greater ($P = 0.01$) for EH compared to BT hay, whereas hemicellulose content tended ($P = 0.098$) to decrease with advancing maturity. However, NDF, ADF, acid detergent lignin, and cellulose content were similar ($P \geq 0.288$) across the three stages of maturity. Although statistical analysis was not conducted, estimated forage yield was numerically higher for EH and LH compared to BT hay. There were minor differences in starch content across the three stages of maturity. However, the simple sugars content of EH and LH hay was numerically higher than for BT hay.

Rumen Fermentation

There was no treatment effect ($P \geq 0.58$) on mean, minimum, or maximum pH (Table 3). Similarly, the duration for pH < 6.2 or 5.8, and the area of the curve for pH < 6.2 or 5.8 also did not differ ($P \geq 0.24$) across treatments. Although total SCFA concentration was similar (*P* = 0.30) across treatments, the molar proportion of acetate, was greater (*P* < 0.01) for heifers fed BT and EH hay compared to LH hay. The molar proportion of butyrate increased (*P* < 0.01), whereas the molar proportions of isobutyrate and isovalerate decreased (*P* < 0.01) with advancing maturity. The acetate:propionate ratio tended to be greater (*P* = 0.05) for heifers fed BT and EH hay than the heifers fed LH hay.

Nutrient Intake, Omasal Flow, and Ruminal and Total Tract Digestibility

The stage of maturity at harvest had no effect (*P* ≥ 0.70) on the intake of DM, OM, NDF, or ADF (Table 4). However, heifers fed BT hay consumed a greater (*P* < 0.01) amount of CP than heifers fed EH and LH hay. The stage of maturity at harvest also had no effect (*P* ≥ 0.14) on apparent ruminal digestibility and omasal flow of DM, OM, NDF, ADF, and CP. Similarly, stage of maturity had no effect on apparent total tract digestibility of DM, OM, NDF, and ADF. However, apparent total tract CP digestibility decreased (*P* < 0.01) with advancing maturity of teff grass.

Nitrogen Utilization

Heifers fed BT hay consumed a greater (*P* < 0.01) amount of N and had a greater concentration of NH_3-N in the rumen than heifers fed EH or LH hay (Table 6). There were temporal changes in rumen NH_3-N concentration; it increased post-feeding on all diets, with feeding BT hay resulting in greater changes than EH and LH hay (Figure 1). Plasma urea-N concentration in heifers fed BT hay tended (*P* = 0.08) to be greater than in heifers fed EH and LH hay. Total urinary output and the urinary excretion of N and urea-N were greater (*P* ≤ 0.01) for heifers fed the BT than for EH or LH hay. However, the stage of maturity at harvest had no effect (*P* ≥ 0.11) on total urine N excretion as a percentage of N intake, urinary excretion of allantoin, uric acid, total purine derivatives, as well as estimated microbial N flow. Fecal N excretion did not differ (*P* = 0.76) across treatments. However, fecal N excretion as a percentage of N intake was lower (*P* < 0.01) for heifers fed the BT than the EH or LH hay. Although total N excretion decreased (*P* < 0.01) with increasing maturity, there was no treatment effect (*P* = 0.75) on total N excretion as a percentage of N intake. Apparent N balance tended (*P* = 0.07) to be greater for heifers fed the BT than the EH and LH hay.

DISCUSSION

Because of persistent drought conditions, widespread domination of invasive grass species and increased incidences and severity of fire on rangeland, there is great interest in the use of alternative forages, such as 'Moxie' teff, as cattle feed. However, the judicious use of such forages is dependent on the adequate characterization of their nutritive value as influenced by the numerous biotic and abiotic factors. The stage of maturity at harvest is the major determinant of forage quality, and climatic and agronomic conditions modulate its impact (Buxton, 1996). Since there is still limited information on the nutritive value of teff grass the objective was to determine the effects of feeding 'Moxie' teff harvested at the BT, EH or LH stages of maturity on nutrient intake, ruminal fermentation characteristics, omasal nutrient flow, and N utilization in beef cattle.

With grasses, leaves typically contain a higher amount of CP and a lower amount of fiber than stems; therefore, the decrease in the leaf:stem ratio with advancing maturity results in a decrease in CP and an increase in fiber content (Van Soest, 1982; Buxton, 1996). These changes greatly influence nutrient intake and digestibility, and, thus nutrient supply and productive performance. As expected, the CP content was lower for LH than BT hay

(11.5 vs. 18.1% CP on a DM basis; harvested 66 vs. 53-d post-seeding) in the current study. Staniar and others (2010) also reported a decrease in the CP content with advancing maturity for field-grown first cut 'Tiffany teff' (16.4, 10.8, and 7.5% CP on a DM basis for BT, EH and LH hay harvested 47, 61 and 75 d post-seeding, respectively), with all values at least 1.7% lower compared to the current study. Saylor (2017) did not observe differences in the CP content of greenhouse-grown 'Moxie', 'Tiffany', 'Corvalis' and 'Dessie' teff harvested 40, 45, 50, 55 or 60 d after seeding. However, across variety, CP content decreased at a rate of 0.88%/d between d 40 (average of 28.7% CP on DM basis) and 60 post-seeding (11.2%). This was a greater change in CP content than reported by Staniar and colleagues (2010) and in the current study, and this could be explained by several factors including differences in soil fertility, climatic and growing conditions, and harvest ages. It was anticipated that advancing maturity in 'Moxie' teff would also result in the accumulation of cellulose and lignin, and, thus, result in an increase in the ADF and NDF content (Jung, 2012). There were no changes, however, in the cellulose, lignin, ADF and NDF content across the three stages of maturity. This is in contrast to Staniar and associates (2010) who noted a 2.7 and 5.8% increase in NDF and ADF content, respectively, with advancing maturity. Staniar and others (2010), however, harvested BT hay 6 d earlier (d 47 vs. 53) and LH teff 9 d later (d 75 vs. 66) than in the current study. Given the rapid growth rate of teff grass (Miller, 2011), the longer duration between harvest dates (29 vs. 13 d) could possibly explain the greater increase in NDF and ADF content in the study by Staniar and colleagues (2010) compared to the current study. Saylor (2017) also observed a 11.8% increase in ash free NDF content of 'Moxie', 'Tiffany', 'Corvalis' and 'Dessie' teff with advancing age. However, this was a quadratic increase; the rate of accumulation was rapid between d 40 and 55, and it plateaued thereafter (d 55 to 60). Saylor (2017), however, did not report the physiological stages corresponding to the harvest dates. Based on age, the BT stage hay in the current study was harvested 13 d later than Saylor (2017). Similarly, the harvest date for BT hay was also 7 d later in the study by Staniar and associates (2010). Therefore, the marginal changes in fiber content reported by Staniar and others (2010), and in this study relative to Saylor (2017) could possibly be due to the harvest dates for BT hay (d 47 to 54) corresponding with the time when the rate of fiber accumulation starts to slow down. It is also probable that minor changes occur in fiber accumulation beyond d 55; however, this still needs to be determined in field-grown teff grass.

Most often, there is greater correlation between forage quality and physiological maturity than with age; however, physiological maturity and age can be confounded by climatic/growing and agronomic conditions (Buxton, 1996). Compared to the current study, teff grass was greenhouse-grown by Saylor (2017) and the average temperature was maintained at 24.6ºC throughout the growth period. This was 3.8ºC higher than in this study (20.8ºC) and could have resulted in a higher rate of lignin and cellulose accumulation (Moore and Jung, 2001; Jung, 2012), thus, explaining the increase in the fiber content. In addition, minimum air temperature, which also affects the rates of plant respiration and photosynthesis, has a great impact on growth in annual forages (Hatfield and Prueger, 2015). In this study, ‘Moxie’ teff was grown at a high elevation (Carmen, ID; elevation = 1,166 m). Therefore, the cool (average of 13.7 ºC) night temperature during the growing season could have also slowed down the rate of fiber accumulation. However, other factors including soil moisture, light and soil fertility can also influence forage growth patterns (Moore and Jung, 2001). Therefore, further studies are needed to determine the effects of growing the various forage teff varieties under different climatic, elevational, and agronomic conditions, as well as using harvest dates that are based on physiological maturation and age, on nutrient composition. Dry matter intake was similar across diets in the current study (Table 4). A key factor that regulates DMI in forage-fed cattle is bulk fill of the rumen. Typically, DMI decreases as dietary NDF content increases due to greater fill effects (Jung and Allen, 1995; Allen, 2000). However, substantial changes in NDF content between the BT and LH stages of maturity were not observed (Table 2). However, iNDF content was greater in the EH hay than in the BT hay. Therefore, it is possible that digesta retention time was lower in heifers fed BT hay than those fed EH hay because NDF contents and digestibility were similar across treatments even though the indigestible fraction varied across treatments, resulting in the lack of a diet effect on DMI (Allen, 2000). Since DMI did not change, stages of maturity also did not result in differences in the intakes of OM, NDF and ADF as OM, NDF and ADF content of teff hay was similar across diets. However, as expected, heifers fed BT hay, which had a higher CP content, consumed a greater amount of CP than the heifers fed EH and LH hay.

A concern when harvesting a forage at an advanced stage of maturity is the increase in lignification that can reduce ruminal digestion, thus, limiting nutrient supply (Buxton, 1996; Moore and Jung, 2001). Initially, it was anticipated that there would be an increase in the degree of lignification with advancing maturity; however, it was not observed (Table 2). It is possible that a lack of increase in acid detergent lignin with advancing maturity in the teff hay fed in this study resulted in similar ruminal fiber digestion. Similarly, *in vitro* true digestibility, an indicator of digestible NDF, did not change for ‘Tiffany’ teff harvested at bi-weekly intervals starting on d 51 post-seeding (Sugg, 2016). To the contrary, *in vitro*

NDF digestibility of ‘Moxie’, ‘Tiffany’, ‘Corvalis’ and ‘Dessie’ teff decreased at a rate of 0.95% per day when harvested between d 40 and 60 post-seeding (Saylor, 2017). Although Saylor (2017) did not measure lignin content, it is possible that lignin increased because Saylor (2017) also noted an increase in ash free NDF. Therefore, a greater lignification could account for decreased *in vitro* NDF disappearance. In the current experiment, differences in apparent ruminal digestion of OM and CP across diets were not observed (Table 4). Similarly, omasal flow of DM, OM, NDF, ADF and CP did not differ across diets, as advancing maturity did not reduce apparent ruminal digestion. Stage of maturity at harvest also had no effect on apparent total-tract digestion of DM, OM, NDF, and ADF (Table 4), which may be expected because lignin concentration was similar across all maturities of teff hay. However, apparent total-tract digestion of CP was higher for heifers fed BT compared to LH hay possibly reflecting the higher CP intake for BT than LH heifers (Table 4).

Ruminal fermentation of carbohydrates (CHO) in forages is crucial as it leads to the production of SCFA, which can contribute up to 80% of caloric requirements in beef cattle (Bergman, 1990). Additionally, ruminal fermentation also avails substrates for the synthesis of microbial protein, which can potentially supply all of the required metabolizable protein in cattle (Virtanen, 1966; Storm et al., 1983, Clark et al., 1992). Total ruminal SCFA concentration did not differ across diets (Table 3). The lack of substantial changes in the fiber and starch content of teff hay with advancing maturity makes it probable that ruminal fermentable CHO supply was comparable across diets, thereby, resulting in the lack of a diet effect on total SCFA concentration and all measures of ruminal pH. Acetate made up the bulk (≥ 73.5%) of ruminal SCFA across diets which was expected with forage based diets

(Calsamiglia et al., 2008). The molar proportion of acetate was greater for heifers fed BT and EH hay compared to LH hay (average of 75.0 vs. 73.5%). Feeding forage at later stages of maturity typically results in an increase in ruminal acetate concentration due to the accumulation of fiber (Van Soest, 1982; McCollum et al., 1985). The molar proportion of butyrate was greater in heifers fed the EH and LH hay compared to BT hay (Table 3). Since the simple sugar content of teff hay also increased by 4.7 percentage units with advancing maturity, it could account for the higher butyrate concentration in heifers fed EH and LH than BT hay (DeFrain et al., 2004). The molar proportions of isobutyrate and isovalerate, however, were lower for heifers fed EH and LH compared to BT hay (Table 3). It is probable that the decrease in CP intake with advancing maturity also reduced branched chain-AA supply, thus, limiting branched chain fatty acid synthesis (Johnson et al., 1994).

Peptides, AA and NH_3-N are the three forms of N utilized by the ruminal microbes to synthesize protein. Although peptide and AA concentrations were not quantified, ruminal NH_3-N, which supplies up to 80% of N required for microbial growth was measured (Hristov and Jouany, 2005). For optimal growth of microbes to occur, ruminal NH_3-N concentration should be at least 5.0 mg/dL (Satter and Slyter, 1974). In the current study, the decrease in CP intake with advancing maturity resulted (Table 5) in a decrease in average ruminal NH_3-N concentration (composite of samples collected from d 26 to 28 to represent a 24-h feeding cycle) such that it was close to the 5.0 mg/dL threshold for heifers fed LH teff. Since ruminal NH_3-N concentration changes relative to feeding, the changes in ruminal NH_3-N concentration post-feeding were measured (Figure 1). The ruminal NH_3-N concentration was below the 5.0 mg/dL threshold for a number of time points for heifers fed EH and LH teff hay. Although this suggests a deficiency, microbial N supply did not differ across diets. However, microbial N supply was 12.4 to 21.3% lower for heifers fed LH and EH compared to BT hay, and this possibly caused the tendency for a decrease in apparent N retention (Table 5) with delayed harvest, which could reduce production performance, depending on class of animal. Therefore, the effects of delaying harvest of teff grass on production performance in beef cattle, still need to be evaluated.

There was a tendency for a decrease in PUN and a decrease in the excretion of total N, urine N and urea-N with advancing maturity (Table 5). Others (Pendlum et al., 1980; Park et al., 1994; Cline et al., 2009) made similar observations, albeit, when feeding different grasses, including tall fescue and wheatgrass. Such changes are suggestive of an improvement in ruminal N utilization, possibly due to limited spillage of NH_3-N into blood and the increased recycling of endogenous urea-N to the rumen that reduces its irreversible loss in urine (Huntington et al, 2009). However, it is important to ensure that nutritional strategies to improve the efficiency of N use in the rumen should not restrict microbial growth and limit MP supply, thereby, compromising animal performance. Since, harvesting or grazing forages at later stages of maturity could limit MP supply, whereas the high CP content of forages at earlier stages of growth could reduce the efficiency of N utilization, there has been interest in the use of protein or energy supplements to improve the use of nutrients from teff grass by cattle. Although others (Fieser and Vanzant, 2004; Añez-Osuna et al, 2015) have reported supplementation to be beneficial in increasing both N use and productivity when feeding other forages, there is still no information for teff grass. Therefore, research on determining the ideal supplementation strategies to use when feeding teff hay is warranted. Supplemental by-pass protein with LH hay could increase MP supply, whereas energy supplementation when feeding BT hay could reduce N wastage and increase productivity.

Although forage quality is important, forage yield, which increases with advancing maturity, is another important factor to consider when deciding harvest or grazing time. Although not subjected to statistical analysis, estimated forage DM yield for the harvested area was 2.86 t/ha for the BT stage compared to 5.18 t/ha for the LH stage. Although others (Staniar et al., 2010; McCown et al., 2012) harvested teff at the same physiological stages of maturity as in the current study, they did not report forage yield. However, just as with quality, numerous factors, including variety, soil type, and climate conditions, can influence forage yield. Therefore, there is need for additional studies to determine forage yield for teff grass grown under different climatic and agronomic conditions.

CONCLUSION

In conclusion, advancing maturity did not result in appreciable changes in the degree of lignification of ‘Moxie’ teff hay, and this could have resulted in the lack of a diet effect on DMI, ruminal digestion and outflow of DM, OM, NDF, ADF, and CP, and apparent total tract digestion of DM, OM, NDF, and ADF. However, the decrease in CP content with advancing maturity in teff grass reduced CP intake. Subsequently, this resulted in a decrease in rumen NH_3-N, PUN, and UUN, suggesting an improvement in N utilization. However, there was a tendency for microbial N supply to be lower for heifers fed EH and LH hay compared to BT hay. Based on the results of the current study, delaying the harvesting of ‘Moxie’ teff from the BT to EH stage of maturity to potentially maximize forage yield does not severely compromise hay quality. However, future studies are needed to further evaluate effects of advancing maturity in teff grass on animal performance, and whether the provision of supplemental protein or energy improves N use and productivity.

Table 1. Environmental conditions[1] and harvest data of 'Moxie' teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity.

Maturity	Date	Temperature, °C			Precipitation, mm	Growing degree days[2]
		Minimum	Average	Maximum		
Cutting[3]						
BT	August 12, 2016	13.6	20.7	27.8	20.3	146
EH	August 19, 2016	13.8	20.9	28.1	20.3	179
LH	August 25, 2016	13.6	20.8	27.9	20.3	186
Baling[4]						
BT	August 16, 2016	15.0	22.6	30.1	0.0	22.8
EH	August 23, 2016	12.4	19.9	27.4	0.0	9.72
LH	August 29, 2016	12.7	19.9	27.1	0.0	9.44

[1] Data collected from the National Centers for Environmental Information (Salmon Idaho Weather Station, Salmon, ID; https://www.ncdc.noaa.gov/; National Oceanic and Atmospheric Administration, 2017).

[2] Calculated as (maximum temperature + minimum temperature)/2 – base, the base is 18°C.

[3] Data is calculated from the seeding date until the cutting date; days post planting for BT = 53; EH = 60; and LH = 66.

[4] Data is calculated from the cutting date until the baling and storage data

Table 2. Chemical composition of 'Moxie' teff hay harvested at the boot (BT), early heading (EH), or late-heading (LH) stages of maturity

	Maturity at harvest[1]				
Variable	BT	EH	LH	SEM	*P*-Value
Yield, t/ha	2.86	4.02	5.18	_	_
DM[2], %	91.3^{a}	89.1^{ab}	84.7^{b}	1.3	0.010
Nutrient content, % DM					
CP	18.7^{a}	14.7^{b}	11.9^{c}	3.9	< 0.001
ADF	28.0	28.7	27.6	0.6	0.39
NDF	62.9	62.6	61.3	0.7	0.29
iNDF	14.0^{b}	15.4^{a}	15.1^{ab}	0.3	0.01
Acid detergent lignin	2.25	2.23	2.08	0.11	0.55
Cellulose[3]	25.7	26.4	25.5	0.6	0.51
Hemicellulose[3]	34.9	34.0	33.7	0.4	0.10
Simple sugars[4], %	5.20	10.0	9.80	_	_
Starch[4], %	0.40	0.50	1.10	_	_
TDN[4, 5]	56.0	57.0	59.0	_	_
Nitrate[4], %	1.55	0.83	0.38	_	_
Ash[4], %	11.5	10.2	9.2	_	_
Calcium[4], %	0.48	0.51	0.47	_	_
Phosphorus[4], %	0.33	0.27	0.25	_	_
Magnesium[4], %	0.19	0.20	0.17	_	_
Potassium[4], %	2.67	2.38	2.13	_	_
Sodium[4], %	0.04	0.04	0.03	_	_
Sulfur[4], %	0.35	0.33	0.29	_	_
Chloride[4], %	0.72	0.82	0.69	_	_
Iron[4], mg/kg	403	230	191	_	_
Zinc[4], mg/kg	31	33	25	_	_
Copper[4], mg/kg	11	10	9	_	_
Manganese[4], mg/kg	38	33	34	_	_
Molybdenum[4], mg/kg	2.2	2.0	1.7	_	_

[1] Age of teff hay at harvest (post-planting): BT = 53 d; EH = 60 d; and LH = 66 d.

[2] DM content of hay post-field drying.

[3] Calculated using the values for NDF, ADF, and acid detergent lignin.

[4] Composite samples collected and sent to Dairy One for analysis.

[5] Summative calculation according to Weiss et al. (1992).

Table 3. Rumen fermentation characteristics for heifers fed 'Moxie' teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity

	Maturity at harvest[1]				
Variable	BT	EH	LH	SEM	*P*-Value
pH					
Mean pH	6.32	6.32	6.34	0.07	0.82
Min pH	5.89	5.83	5.83	0.09	0.74
Max pH	6.67	6.72	6.70	0.06	0.58
Duration pH<6.2 (min/d)	367	445	379	154	0.64
Duration pH<5.8 (min/d)	55.2	85.8	56.9	50.8	0.32
Area pH <6.2	73.2	104	80.0	48.3	0.40
Area pH <5.8	6.12	10.5	7.25	6.39	0.24
SCFA					
Total, *mM*	94.5	90.6	89.5	2.7	0.30
Acetate, mol/100 mol	75.3^{a}	74.7^{a}	73.5^{b}	0.3	0.003
Propionate, mol/100 mol	15.2	14.8	15.4	0.2	0.08
Butyrate, mol/100 mol	6.90^{c}	7.89^{b}	9.01^{a}	0.20	< 0.001
Isobutyrate, mol/100 mol	0.908^{a}	0.754^{b}	0.547^{c}	0.034	< 0.001
Valerate, mol/100 mol	0.840^{b}	0.993^{a}	0.946^{ab}	0.042	0.018
Isovalerate, mol/100 mol	0.929^{a}	0.792^{b}	0.563^{c}	0.044	< 0.001
Acetate:propionate	4.97	5.04	4.78	0.082	0.05

[a-c] Means with different superscripts differ ($P < 0.05$).

[1] Age of teff hay at harvest (post planting): BT = 53 d; EH = 60 d; and LH = 66 d.

Table 4. Nutrient intake, apparent ruminal and total tract nutrient digestibilities for heifers fed ‘Moxie’ teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity

	Maturity at harvest[1]				
Variable	BT	EH	LH	SEM	*P*-Value
Intake, kg/d					
DM	11.2	11.4	11.7	0.7	0.77
OM	11.0	11.3	11.5	0.7	0.77
NDF	7.03	7.17	7.16	0.40	0.94
ADF	3.13	3.28	3.23	0.18	0.70
CP	2.09^{a}	1.68^{b}	1.39^{b}	0.15	0.001
Ruminal outflow, kg/d					
DM	6.97	7.30	6.63	0.48	0.58
OM	4.91	5.14	4.79	0.35	0.76
NDF	1.75	1.89	1.99	0.21	0.88
ADF	0.839	0.890	0.935	0.105	0.57
CP	1.26	1.31	1.14	0.10	0.23
Ruminal digestibility, % of					
DM	41.8	36.1	43.5	3.2	0.14
OM	58.7	54.3	58.5	2.7	0.28
NDF	76.9	73.2	72.3	2.7	0.27
ADF	73.5	72.4	71.0	3.3	0.87
CP	74.4	72.4	71.0	3.2	0.35
Total tract digestibility, %					
DM	69.5	72.3	72.4	3.7	0.56
OM	78.4	78.8	79.4	1.3	0.77
NDF	82.8	82.5	81.2	1.1	0.45
ADF	80.8	81.5	79.8	1.3	0.58
CP	83.3^{a}	81.2^{ab}	78.3^{b}	1.9	0.008

[a,b]Means within a row with different subscripts differ ($P < 0.05$).

[1]Age of teff hay at harvest (post-planting): BT = 53 d; EH = 60 d; and LH = 66 d.

Table 5. Fecal N excretion, urine N and purine derivative excretion, microbial N supply and rumen ammonia and plasma urea-N concentrations for heifers fed 'Moxie' teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity

	Maturity at harvest[1]				
Variable	BT	EH	LH	SEM	*P*-Value
N intake, g/d	349^a	270^b	227^b	16	< 0.001
Fecal excretion					
DM, kg/d	3.99	4.12	4.32	0.23	0.47
N, g/d	89.4	85.3	85.2	5.0	0.76
N, % of N intake	25.6^a	31.8^b	38.0^c	1.4	< 0.001
Urinary Excretion					
Total output, kg/d	15.5^a	11.5^b	11.7^b	1.4	0.012
N, g/d	167^a	120^b	97.1^b	12.4	<0.001
Urea-N, g/d	106^a	74.6^b	53.8^b	7.6	<0.001
Urea-N, % of total urine	63.6	63.3	54.3	3.1	0.09
Total N, % of N intake	48.6	44.1	40.2	2.8	0.15
Allantoin, mmol/d	67.4	61.6	64.8	4.1	0.60
Uric acid, mmol/d	13.5	11.5	11.5	0.8	0.11
Total purine derivatives,	80.9	73.1	76.3	4.7	0.49
Microbial N flow, g/d	31.4	24.7	27.5	4.3	0.54
Total N excretion,					
g/d	257^a	205^{ab}	164^b	15	0.004
% of N intake	74.2	75.8	71.5	4.5	0.75
Apparent N retention, g/d	92.3	64.6	62.9	11.2	0.07
Rumen NH$_3$-N, mg/dL	14.0^a	8.82^b	5.01^c	0.63	<0.001
Plasma Urea-N, mg/dL	19.1	15.9	14.1	1.4	0.08

^{a,b}Means within a row with different subscripts differ ($P < 0.05$).

[1] Age of teff hay at harvest (post-planting): BT = 53 d; EH = 60 d; and LH = 66 d.

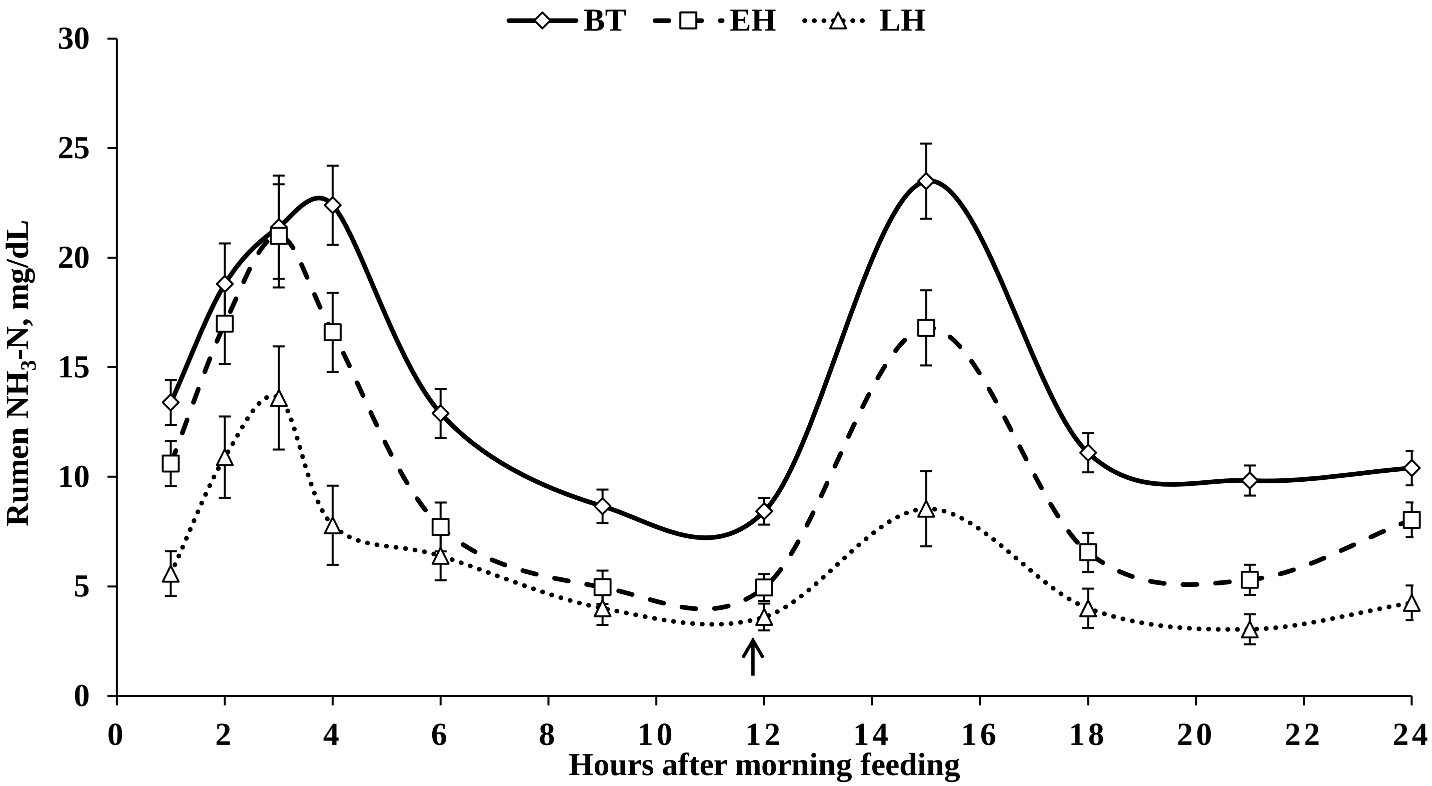

Figure 1. Rumen NH_3-N concentration for heifers fed ‘Moxie’ teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity. Heifers were fed twice daily at 0630 and 1830 h (indicated by the arrow). Diet, $P < 0.01$; Time, $P < 0.01$; Diet × Time interaction, $P = 0.01$. The error bars reflect the SEM associated with time.

CHAPTER 3

General Discussion

The hypothesis for this study is that the nutritive value of ‘Moxie’ teff, as a preserved forage source for beef cattle, changes relative to its stage of maturity. The results of this study do not support this hypothesis, though some aspects of this hypothesis are supported by our data. Given that most forage species will have an increase in fiber and lignin accumulation as they mature (Buxton, 1996), we expected to see, with advancing maturity, a decrease in digestibility due to an increase in lignin and fiber content in teff. However, the NDF (61.3 to 62.9%; DM basis) and ADF (27.6 to 28.7%; DM basis) content were similar across stages of maturity. The greatest difference in teff composition due to advancing maturity was the decrease in CP content from 18.7% of DM in boot stage hay to 14.7 and 11.9% of DM for early- and late-heading stages, respectively. The maturity at harvest did not impact the digestibility of DM, OM, NDF, or ADF of teff when fed to beef cattle, and this possibly was due to similar lignin and indigestible NDF across treatments. Thus, producers that are growing teff can delay harvest from the boot stage to the early-heading stage or later without compromising nutrient digestion. This will allow for the maximum yield of biomass for cattle to consume without compromising nutrient availability as a result of decreases in digestibility typically observed with advancing maturity.

Teff grass has many positive attributes, including high yield, quick growth rate, and drought tolerance. The low fiber and lignin contents of teff also allow for a highly digestible forage to provide nutrients to cattle. Although it is highly digestible, teff does not provide a lot of energy. The lack of energy in teff could potentially inhibit microbial growth in the rumen and limit productivity for the animal. To fully investigate the potential of a forage, animal performance must be evaluated. Currently, there has been a few studies evaluating growth performance in beef cattle fed teff grass in total mixed rations containing other forage sources. However, to the best of our knowledge, the impact of feeding teff grass as the sole forage source is yet to be evaluated. Thus, information on growth performance in beef cattle fed teff as the only forage source is needed. These trials will allow for the accurate determination of the feasibility of feeding teff to cattle in seasons in which rangeland quality is poor or winter forage is needed. In addition, the effect of feeding supplementatal energy or protein on the utilization of teff needs to be investigated. Feeding hay harvested at later stages of maturity or teff grown in soils with a low N content could potentially limit microbial protein synthesis and, thus, MP available to cattle. Thus, the supplementation of bypass protein could be beneficial in providing adequate amounts of MP to cattle. Teff with a high CP content could potentially reduce N utilization efficiency related to limited energy availability. To increase the efficiency of N utilization, energy could also be supplemented to cattle fed teff as a strategy to reduce N wastage.

In order to determine its viability as an alternative forage source, teff should also be compared to other forage sources. Saylor and colleagues (2018) compared teff to a mixture of alfalfa, prairie hay, and corn silage and found out that teff improved milk protein yield. Young and others (2014) compared teff with alfalfa hay in growing cattle and observed increased gain in animals fed teff over alfalfa. Although these comparisons are beneficial, teff should be compared with other alternative forages that are typically used on beef operations when primary sources are depleted or not available. Triticale has been used as a grain source to replace barley or corn; however, it can also be used as a forage source for grazing cattle. In one study (Ruiter et al., 2002), triticale reached the boot stage approximately 50 to 55 days after planting, which is comparable to teff. However, forage yield was lower for triticale than teff (2.4 vs 2.6 t/ha). Although triticale has been reported to be a high energy forage source for cattle (Tanaka et al., 2005) there is need for a direct comparison of its nutritive value to teff. There is anecdotal evidence suggesting comparable nutritional quality between teff and timothy grass (Miller, 2011). McCown and others (2012) compared teff and timothy hay as feed for mares and noted that mares preferred timothy. However, McCown and others (2012) did not evaluate whether there were differences in forage growth, digestibility, or nutrient composition. Therefore, a direct comparison of the nutritive value of teff to timothy grass as beef cattle feed when harvested at similar stages of maturity is also warranted.

Numerous factors including climatic conditions, influence forage quality. Because the teff used in the current study was grown in one location, this can make it challenging to extrapolate our observations to other locations in Idaho. As mentioned previously, the degree of lignification is greatly influenced by temperature. At higher elevations, such as Carmen, ID, the cool temperature may have resulted in the low degree of lignification we observed across stage of maturity. Had the teff used in this study been grown at a lower elevation, with higher day and night temperatures, we possibly could have noted a greater degree of lignification, which influences digestibility. Because of the differences in climatic conditions (e.g., rainfall and temperature) as influence by elevation across the state, forage production trials at various locations across Idaho are necessary to generate location-specific feeding recommendations. Very few publications include forage yield or agronomic data. This information, however, is important, particularly to producers, when using teff as a forage source. Our study is one of the few studies that reported forage yield; however, we did not conduct an actual experiment to investigate ideal soil conditions, insecticides, herbicides, or fertilizers. Nor did we replicate forage production to perform statistical analysis to report true yields of forage. Therefore, generation of this information would be useful for cattle producers.

At the onset of the study, our goal was to use the double marker technique to estimate ruminal nutrient digestion and outflow. Therefore, we infused both chromium EDTA and $YbCl_3$. However, following Cr and Yb analysis for the omasal digesta samples, the estimated ruminal outflow rates we obtained indicated marker dysfunction. Therefore, instead of the double marker system, we ended up using the single marker (Yb) method. Although the use of 2 markers for the solid (Yb) and liquid phases (Cr) is recommended as it gives good estimates, Yb has been used as a single marker with success in the past (Siddons et al., 1985). Teeter and associates (1984) observed that Yb fed mixed with ground corn readily associated with fiber sources within the rumen and provided accurate estimates of digestibility. The combination of Yb as a single digesta marker and omasal sampling to estimate ruminal degradation has also been used in another study with beef cattle (Górka et al., 2015). Therefore, although we could not use the double marker system, the estimates we generated are useful in advancing our knowledge on use of teff grass as cattle feed.

Measuring microbial protein synthesis was important in determining the nutritive value of teff as it can provide potentially all of the amino acids required by beef cattle. The initial plan to estimate microbial protein was to isolate bacterial pellets from rumen contents and measure the concentration of purines therein. This is a good method for the estimation of microbial protein synthesis. Bacterial pellets must be lyophilized prior to analysis; during this step, our freeze drier malfunctioned and our samples were compromised. Thus, we employed the use of purine derivatives excreted in urine as a marker to estimate microbial protein synthesis. This is a widely used method and it allows for decent estimates; however, it is not as accurate as microbial purines or ^{15}N.

Although this experiment provided great insight into the nutritive value of teff, there is still a long way to go in terms of the research that needs to be conducted. Future studies investigating supplementation strategies, grazing and feeding growing animals, as well as forage production are all necessary in order to truly understand the potential of *Eragrostis tef* as an alternative forage source for beef cattle.

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APPENDIX A: ANIMAL CARE AND USE PROTOCOL APPROVAL

University of Idaho
Institutional Animal Care and Use Committee

Date: Thursday, June 16, 2016

To: Gwinyai Chibisa

From: University of Idaho
Institutional Animal Care and Use Committee

Re: Protocol 2016-35
Investigating the use of Eragrostis tef ('Tiffany teff') as an Alternative Forage Source for Beef Cattle

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on Thursday, June 16, 2016.
This protocol was originally submitted for review on: Friday, June 3, 2016
The original approval date for this protocol is: Thursday, June 16, 2016
This approval will remain in effect until: Friday, June 16, 2017
The protocol may be continued by annual updates until: Sunday, June 16, 2019
Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Barrie Robison, IACUC Chair

APPENDIX B: ABSTRACTS PRESENTED

Effects of feeding *Eragrostis tef* hay harvested at different stages of maturity on nutrient intake, ruminal fermentation, and nitrogen utilization in beef cattle

J. R. Vinyard, J. B. Hall, J. E. Sprinkle, G. E. Chibisa

Journal of Animal Science, 95, Supp 4:137

Presented at: American Society of Animal Sciences Annual Meeting, July 8-12, 2017, Baltimore, MD

Eragrostis tef (‘Tiffany’ teff), a warm-season annual grass, could be an excellent forage for beef cattle. However, there is limited information on its nutritive value at different stages of maturity. Therefore, our objective was to determine the effect of feeding teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity on nutrient intake, ruminal fermentation, and N utilization in beef cattle. Six ruminally cannulated beef heifers (476 kg [SD 32.6] initial BW) were used in a replicated 3 × 3 Latin square design with 28-d periods (18 d for adaptation and 10 d for measurements). Dietary treatments were BT, EH, or LH teff hay. Dry matter intake was measured daily, and indwelling pH loggers were used to continuously measure ruminal pH from d 21 to 28. Ruminal fluid for ammonia determination and spot urine samples for measurement of urine output, N, and urea N excretion were collected from d 26 to 28. Blood samples for plasma urea N (PUN) determination were collected 3 h after feeding on d 28. The NDF and ADF content were similar across diets. However, CP content was greater for the BT hay than for EH and LH hay (18.1, 14.1, and 11.5%, respectively, DM basis). Dry matter intake was higher ($P = 0.03$) for heifers fed the LH hay (11.6 kg/d) compared with heifers fed the BT hay (10.9 kg/d), whereas CP intake increased ($P = 0.03$) for heifers fed the EH hay (1.76 kg/d) compared with heifers fed the LH hay (1.65 kg/d). There was no diet effect ($P > 0.05$) on mean, minimum, and maximum pH and the duration and area pH < 6.2 and 5.8, but ruminal ammonia concentration was greater ($P < 0.05$) for heifers fed BT hay (14.04 mg/dL) than for heifers fed EH (8.82 mg/dL) and LH hay (5.01 mg/dL). Plasma urea N concentration also tended to be higher ($P = 0.08$) for BT hay compared with EH and LH hay (19.1, 15.9, and 14.1 mg/dL, respectively). Urine output increased ($P = 0.01$) in heifers fed the BT hay (15.2 kg/d) than in heifers fed the EH (11.5 kg/d) and LH hay (11.7 kg/d). Similarly, urine N and urea N excretion increased ($P < 0.01$) for heifers fed the BT hay compared with heifers fed the EH and LH hay. In conclusion, although there was no diet effect on ruminal pH, the decrease in CP content with advancing maturity resulted in a decrease in the ruminal ammonia and PUN concentrations and urine N and urea N excretion when teff hay was fed to beef cattle.

Effects of maturity at harvest on the nutritive value of *Eragrostis tef* (Moxie) when fed to beef cattle

J. R. Vinyard, J. B. Hall, J. E. Sprinkle, G. E. Chibisa

Presented at: Pacific Northwest Animal Nutrition Conference, January 16-17, 2018, Boise, ID

The provision of nutritionally adequate forage for rangeland cattle is increasingly difficult in the US due to persistent drought conditions, the domination of invasive species, and an increase in the frequency and severity of wildfires. Therefore, feeding strategies that enable producers to have a reliable forage source all-year-round are urgently needed. A potential strategy is the identification and increased use of alternative forages as cattle feed. *Eragrostis tef* ('Moxie' teff), a warm-season annual grass, could be an excellent forage for beef cattle. However, there is limited information on its nutritive value as cattle feed when harvested at different stages of maturity. Thus, our objective was to determine the effect of feeding teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity on nutrient intake, ruminal fermentation characteristics, omasal nutrient flow, and N utilization in beef cattle. Six ruminally cannulated beef heifers (mean initial BW ± SD, 476 ± 32.6) were used in a replicated 3 × 3 Latin square design with 28-d periods (18 d for adaptation and 10 d for measurements). Dietary treatments were BT, EH, or LH teff hay. Dry matter intake was measured daily. Indwelling pH loggers were used to measure ruminal pH from d 21 to 28. Ruminal fluid and omasal digesta for the determination of fermentation characteristics and nutrient flow to the omasum, respectively, were collected from d 26 to 28. Fecal and urine samples for the measurement of N excretion were also collected (d 26 to 28). Blood samples for plasma urea-N (PUN) determination were collected 3 h after feeding on d 28. There were marginal changes in the fiber fractions of teff with advancing maturity. Diet had no effect ($P > 0.05$) on DMI, and ruminal total short chain fatty acid concentration, pH, digestibility and outflow of DM, OM, NDF, ADF, and CP. However, the CP content of BT hay was greater than for EH and LH hay (18.1, 14.1, and 11.5%, respectively, DM basis), and this resulted in the higher CP intake for heifers fed the BT than the EH and LH hay. Consequently, ruminal ammonia-N (NH_3-N) concentration was greater ($P < 0.05$) for heifers fed BT than EH and LH hay, thereby explaining the tendency for a decrease ($P = 0.08$) in PUN concentration, and a decrease ($P < 0.01$) in the excretion of total N, urine N and urea-N (UUN) with advancing maturity. However, fecal N excretion did not differ ($P > 0.05$). In conclusion, despite a decrease in CP intake and ruminal NH_3-N concentration, feeding beef heifers EH and LH compared to BT teff hay did not compromise ruminal digestion and outflow of DM, OM, NDF, ADF, and CP, and microbial protein synthesis. Advancing maturity in teff hay also resulted in a decrease in the excretion of total N and urine N and UUN when fed to cattle. Therefore, beef producers could wait until the LH stage to harvest/graze 'Moxie' teff grass to maximize forage yield without severely compromising its nutrient value.

Effects of maturity at harvest of *Eragrostis tef* (Moxie) on nutrient intake, digestibility and omasal flow in beef cattle

J. R. Vinyard, J. B. Hall, J. E. Sprinkle, G. E. Chibisa

Presented at: American Society of Animal Science and Canadian Society of Animal Science Annual Meeting, July 8-12, 2018, Vancouver, BC, Canada

Eragrostis tef ('Moxie' teff) could be a viable alternative forage for beef cattle. However, there is limited information on ruminal and total tract nutrient digestibility when teff hay harvested at different stages of maturity is fed to cattle. Therefore, our objective was to determine the effects of feeding teff hay harvested at the boot (BT), early-heading (EH), or late-heading (LH) stages of maturity on nutrient intake, ruminal digestion, omasal flow, and apparent total tract digestion in beef cattle. Six ruminally cannulated beef heifers (mean initial BW ± SD, 476 ± 32.6) were used in a replicated 3 × 3 Latin square with 28-d periods (18 d for adaptation). Dietary treatments were BT, EH, or LH teff hay, and DMI was measured daily. Omasal digesta was collected (d 26 to 28) for measurement of ruminal digestion and omasal nutrient flow, as were fecal samples for apparent total tract nutrient digestibility determination. Data was analyzed using PROC MIXED in SAS. The NDF and ADF content of teff did not change ($P \geq 0.29$) with advancing maturity. Diet also had no effect ($P \geq 0.14$) on intake, ruminal digestion, omasal flow and apparent total tract digestion of DM, NDF, and ADF. Crude protein content decreased ($P < 0.01$) with advancing maturity (18.7, 14.7, and 11.9%, respectively). Crude protein intake was lower ($P < 0.01$) for heifers fed EH and LH than BT hay. Although there was no diet effect ($P > 0.23$) on ruminal digestion and omasal flow, total tract digestion of CP was ($P < 0.01$) lower for heifers fed LH than BT hay (83.3 vs. 78.3%). In summary, delaying harvest of teff hay had no effect on NDF and ADF intake, digestibility and omasal flow. However, delaying harvest resulted in a decreased in CP content, intake and apparent total tract digestion.

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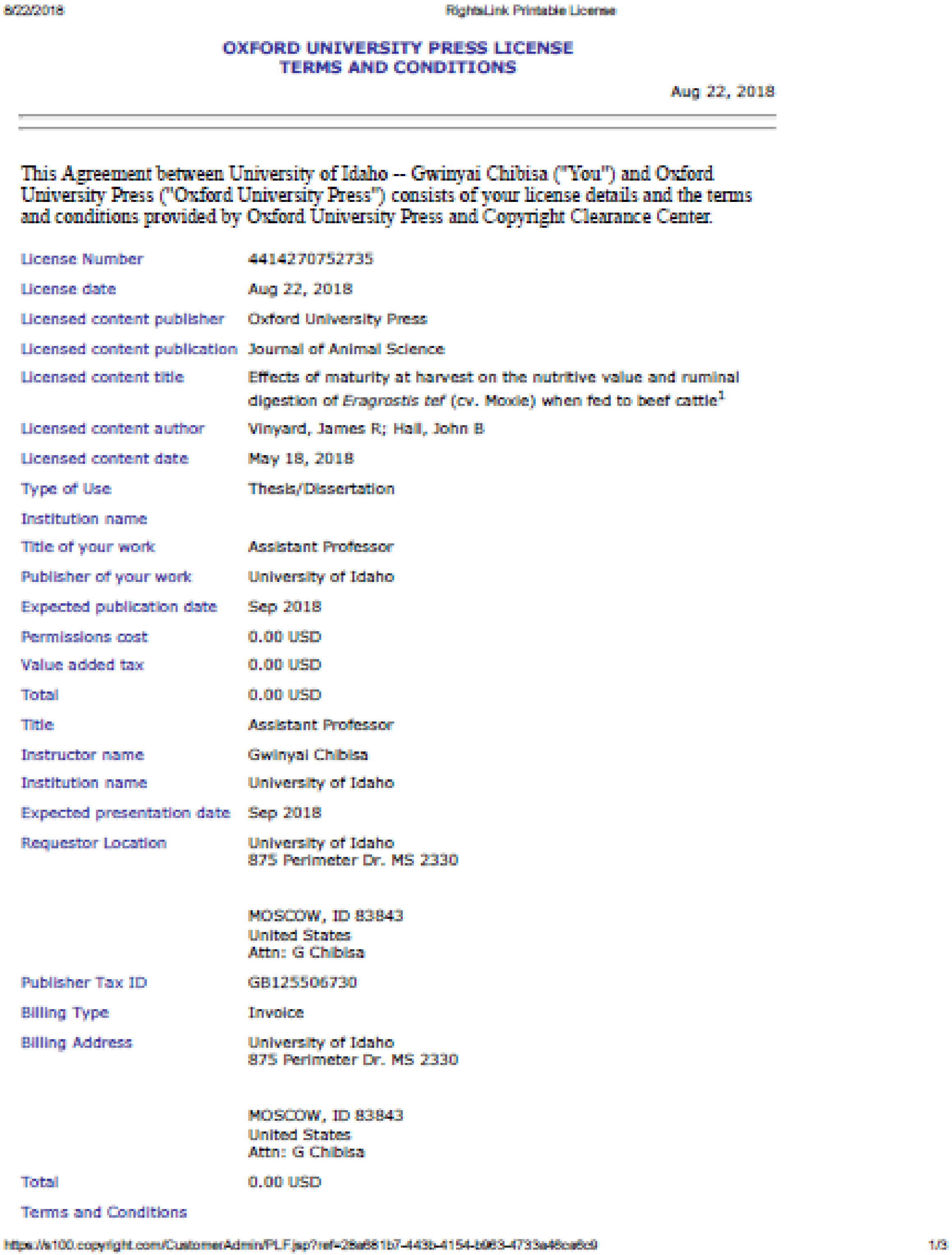

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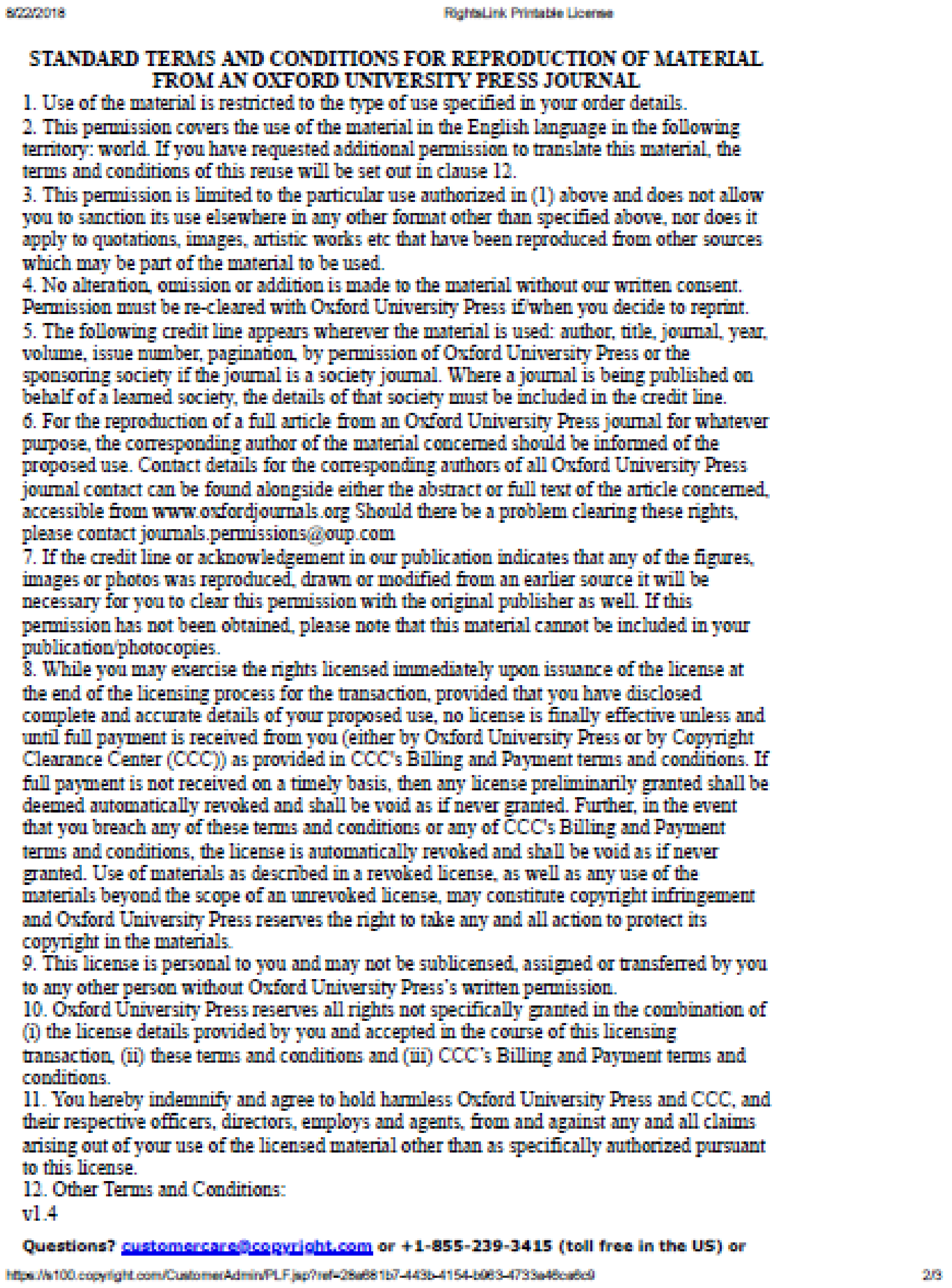

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