MEASUREMENT OF GREENHOUSE GAS EMISSIONS FROM BEEF CATTLE USING THE GREENFEED SYSTEM

by

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ABSTRACT

Production of air pollutants by beef cattle operations has incurred increased scrutiny in recent years. Two experiments quantified gaseous emissions of beef cattle in a feedlot and while grazing wheat pasture. In experiment 1, the effects of supplementing a finishing diet (14.4% CP; 1.47 Mcal/kg NE_g) for beef steers with a commercially-available condensed tannin extract (CT) at three levels (0, 0.5, and 1.0 % of diet, DM basis) were evaluated. Angus-cross steers (n = 27; initial BW = 350 $\pm$ 32 kg) were fed individually via Calan gates for 126 d. Ruminal methane (CH_4) and metabolic carbon dioxide (CO_2) fluxes were measured using a GreenFeed system (GF, C-Lock Inc., Rapid City, SD) for two sampling periods, that coincided with fecal and urine sampling.. Oxygen consumption was estimated from CO_2 production assuming a respiratory quotient of 1.05. Average daily gain (2.08, 2.14, and 2.08 kg/d for 0, 0.5, and 1.0% CT, respectively) and G:F did not differ (P = 0.88) among treatments. Apparent total tract starch digestibility during phase 1 decreased (P = 0.01) with inclusion of 1% CT. Fecal N excretion was greater (P = 0.05) for 1.0% CT during phase 1. Urinary N excretion was not different ($P \geq 0.39$) among treatments during both phases, but urinary N as a proportion of total N excretion decreased (P = 0.01) when CT was included in the diet during phase 1. No differences ($P \geq 0.23$) were observed for percentage of GE intake lost as CH_4 (phase 1: 2.99, 3.12, 3.09%; phase 2: 3.54, 3.55, and 4.35%) for 0, 0.5, and 1.0%

CT, respectively. In experiment 2, effects of concentrate supplementation of steers grazing wheat forage were evaluated. Thirteen Angus-cross steers (initial BW = 436 $\pm$ 24 kg) were used in a crossover design to evaluate the effects of corn supplementation on gas emissions, performance, and energetic losses of steers grazing wheat pasture. Treatments included either 0.2 kg of pelleted wheat middlings (CON), or a dry-rolled corn supplement fed at 0.5% of BW plus 0.2 kg of pelleted wheat middlings (SUPP). Forage intake was calculated using the determined fecal output and estimated forage digestibility. Ruminal CH_4 and CO_2 fluxes were measured using a GreenFeed system. Urine energy loss was assumed to be 1.4% of GE intake. Oxygen consumption was estimated from CO_2 production, assuming a respiratory quotient of 1.05. Fecal output was estimated using TiO_2 as an external marker. Forage intake as percent of BW did not differ (P = 0.15) between CON (3.22%) and SUPP (3.61%). Corn supplementation decreased (P = 0.02) CH_4 g/kg of DMI by 20.5%. Methane as percent of GE intake was decreased (P = 0.02) by 21.6% when steers consumed SUPP. Supplementation of condensed tannins in a feedlot or concentrate grazing production setting appears to alter emissions of air pollutants without affecting performance. However, more research is warranted to evaluate the varying levels of supplementation and interactions with various diets.

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CHAPTER I

Review of Literature

Effects of Condensed Tannins in Ruminant Diets

Tannin Structure and Sources

Hydrolyzable tannins (HT) and proanthocyanidins (PA) are the two main subclasses of tannins. Proanthocyanidins are also known as condensed tannins (CT). Hydrolyzable tannins are polymers of gallic acid linked together by a central D-glucose molecule (Fig. 1.1; Reed, 1995). Hydrolyzable tannins are more susceptible to enzymatic attack and are more water-soluble relative to CT. Gallic acid polymers of HT are metabolized ruminally and causes kidney disease, liver necrosis, and gastroenteritis (Reed, 1995).

Condensed tannins are polymers of flavanols (Fig. 1.2) and are linked by carbon-carbon bonds that cannot be degraded by anaerobic enzymatic degradation (Waghorn, 2008). Variations within CT structure include location and number of hydroxyl groups (Waghorn, 2008).

Tannins and Protein

Precipitation of dietary CP is the primary biological activity of CT (Naumann et al., 2013). Structural chemistry determines CT affinity for dietary CP (Naumann et al.,

2013). Binding capacity of CT is affected by structural chemistry, sources of CT, molecular weight of CT, and composition of dietary CP. Binding of CT and dietary CP consistently causes a decrease of ruminal ammonia (NH_3) production and total tract CP digestion (Waghorn, 2008).

Kumar and Singh (1984) reported four different potential bonds of CT and protein: hydrogen-bond formation, ionic bonds, covalent bonds, and interaction between aromatic ring and hydrophobic portions of protein. Hydrogen bonding is the most common CT-protein interaction (McAllister et al., 2005). Kumar and Singh (1984) reported specific bonding is dependent on type of tannin and composition of protein. Proline-rich-protein (PRP) has greatest affinity for CT because of increased access of phenol groups of CT to carboxyl groups of PRP (Naumann et al., 2013). Regardless of bond type, CT bound to protein effectively disrupts proteolysis, because proteolytic enzymes are unable to access peptide bonds with CT bound to protein (Reed, 1995). Binding affinity of CT is greatest when pH is near the isoelectric point of proteins, and degradation of CT protein complexes is greatest at pH below 3.0 (Barry and Manley; 1984; Naumann et al., 2013). Theoretically, pH of the abomasum should effectively disrupt CT-protein complexes, which has led to research using CT to create rumen undegradable protein (RUP) from dietary protein (Nishimuta et al., 1974; Waghorn et al., 1987; Perez-Maldanodo et al., 1996).

Researchers have postulated a greater amount of dietary CP flows to the small intestine if said dietary CP is bound to CT (McSweeney et al., 2001). Waghorn et al. (1987) fed lambs CT containing forage to lambs ruminally infused with polyethylene glycol (PEG). Lambs not given PEG infusions increased nitrogen (N) flow to the small

intestine by 39%, but total tract N digestion decreased. Perez-Maldanodo et al. (1996) fed one of three tropical forages to lambs with two of the three forages containing greater than 0.95% CT (DM basis). They reported a 49% increase in N flowing to the abomasum with increased CT concentration of forage. However, apparent total tract N digestibility decreased when lambs consumed forage-containing CT. Dietary CP flow to the small intestine increases with inclusion of CT in the diet, but total tract N digestion typically decreases. Theoretically, the acidic environment of the rumen dissociates the CT-protein interaction (Frutos et al., 2004). Due to sodium bicarbonate secretions in the duodenum, the pH of the first meter of the duodenum increases to greater than 5.0, which may cause a re-association of CT and protein within the small intestine (Waghorn, 2008). The re-association occurs because of the pH increasing greater than the isoelectric point of the protein. Furthermore, any CT not bound to protein entering the duodenum may bind to digestive enzymes and effectively mitigate enzymatic activity (Waghorn, 2008).

Tannins and Carbohydrates

Condensed tannins can also decrease carbohydrate digestion, though this is secondary to its activity on protein (Frutos et al., 2004). Affected carbohydrates include starch, cellulose, hemicellulose, and pectin. Decrease in digestion of carbohydrates is poorly understood, but the most accepted modes of action causing decreased digestion is binding of CT to feedstuffs, enzyme inhibition, and microbe inhibition (Frutos et al., 2004). Barry and McNabb (1999) reported CT binds to dietary CP first, and once CT outnumbers available dietary CP, CT inactivates microbial enzymes second.

Barry and Manley (1984) offered 7 kg/d of high-tannin birdsfoot trefoil (10% CT of DM) and 7.75 kg/d low-tannin birdsfoot trefoil (4% CT of DM) to ruminally and duodenally cannulated sheep (IBW = 37 kg), and measured flow and digestibility of

cellulose, hemicellulose, and readily fermentable carbohydrates (RFC) at each site. Amount of cellulose and hemicellulose increased in feces with sheep consuming high-tannin forage. Furthermore, apparent total tract digestibility of cellulose and hemicellulose decreased by 19% and 23%, respectively, with sheep consuming high-tannin forage. Post-ruminal digestion of cellulose did not differ among sheep consuming high-tannin forage. No difference in post-ruminal digestion may indicate the abomasum effectively disrupted the CT-protein interaction, which did not decrease hindgut fermentation. However, post-ruminal digestion is confounded by amount of substrate flowing to the hindgut between treatments. Digestion of RFC was unaffected by high-tannin forage, which may indicate CT having lower affinity for RFC.

In a similar experiment, Barry et al. (1986) offered 6 kg/d of high-tannin birdsfoot trefoil (9.5% CT of DM) to ruminally and duodenally cannulated sheep. Three treatments were provided: forage without supplement (CON), forage treated with 5.7 g of PEG/kg of forage (low-PEG), and 23.1 g of PEG/kg of forage (high-PEG). Flow and digestibility of cellulose, hemicellulose, and lignin were measured at each site. Apparent total tract digestibility of cellulose and hemicellulose were unaffected by treatment of PEG on forage. However, apparent total tract DM digestion increased with inclusion of PEG in the diet. Ruminal degradation of cellulose, hemicellulose, and lignin were greater with inclusion of PEG. Post-ruminal digestion of cellulose, hemicellulose, and lignin were greater with sheep consuming CON compared to high-PEG treatments. Condensed tannins having a greater affinity for PEG and not binding to feedstuffs or digestive enzymes may have caused greater ruminal degradation of carbohydrates.

The exact relationship between CT and fiber digestion is unclear, but decreased fiber digestion is a secondary effect of CT consumption. However, amount of fiber affected by CT is dependent of structure and source of CT.

Tannins and Lipids

Condensed tannins reportedly affect ruminal biohydrogenation (BH) of fatty acids, by suppressing microbes in the rumen (Khiaosa-Ard et al., 2009). *Butyrivibrio fibrisolvens* is a major ruminal bacterium known to contribute to BH, and CT inhibits *B. fibrisolvens* (Jones et al., 1994). Few experiments have studied effects of CT and dietary fat. However when studied, CT was fed to attempt to create a greater amount of conjugated fatty acids produced in lean muscle as they are implicated in greater human health (Khiaosa-Ard et al., 2009; Vasta et al., 2009a; Vasta et al., 2009b).

Khiaosa-Ard et al. (2009) determined effects of CT on BH via in vitro analysis of grass-clover hay incubated with or without addition of CT extract. Forage incubated with CT had smaller post-fermentation stearic acid content by 47%. A smaller amount of stearic acid indicates less BH, as stearic acid is a product of BH of unsaturated fatty acids. Furthermore, Khiaosa-Ard et al. (2009) reported a greater concentration of unsaturated fatty acids with inclusion of CT, which further supports lower BH activity.

Vasta et al. (2009a) fed sheep at *ad libitum* intake of a forage-based or concentrate diet with or without quebracho CT added to diets. Quebracho CT was added 4.0% of diet DM. Harvesting of sheep took place after 60 days of feeding and total ruminal contents were collected. Condensed tannins had minimal effects on BH of sheep consuming forage-based diet, most likely due to the small amount of fat in the diet. Sheep consuming concentrated diet with CT had decreased total saturated fatty acid content and increased monounsaturated fatty acid content in the rumen compared to sheep consuming

concentrate diet without CT. Change in fatty acid content of rumen contents further supports effects of CT on ruminal BH of fatty acids.

Both Khiaosa-Ard et al. (2009) and Vasta et al. (2009a) reported an increase in *iso* branched chain fatty acids, an intermediary of BH, when CT was included in the diet. According to Vlaeminck et al. (2006), cellulolytic bacteria are responsible for production of iso branched chain fatty acids, and production is an indication of good microbial function. Indication of activity by ruminal microbes gives further testimony to CT affecting ruminal microbes directly and not digestive enzymes, as reported with other macronutrients.

Negative Effects of Tannins

Aside from interactions of CT with above-mentioned macronutrients, CT also poses other negative nutritional effects such as decreased intakes, toxicity to ruminal microbes, and toxicity to the animal.

Voluntary feed intake decreases when CT concentration of diet is in excess of 5% (Barry and Manley, 1984; Frutos et al., 2004). Barry and McNabb (1999) reported decreased intake of *Lotus pedunculatus* (greater than 5% CT of forage DM) compared to *Lotus corniculatus* (less than 4.5% CT of forage DM) indicating that concentration greater than 5% CT of diet DM is a threshold for decreased intake.

Frutos et al. (2004) suggested three modes of action in reduction of intake by ruminants consuming high-CT diets: reduction in palatability, slowing of digestion of nutrients, and conditioned taste aversion. Reduction in palatability is most plausible as astringent flavor of CT interacts with taste buds (McLeod, 1974). Intake is not decreased in most undomesticated herbivore species, reportedly because they possess proline-rich saliva (Kumar and Singh, 1984; Frutos et al., 2004). Condensed tannins have greater

affinity for proline than other amino acids (Kumar and Singh, 1984). There is no indication of proline-rich saliva in domesticated cattle (Naumann et al., 2013).

A reduction in DMI is plausible if CT were to slow digestion and emptying of digestion tract (Frutos et al., 2004). Hervás et al. (2003) ruminally dosed sheep quebracho CT and reported no reduction in DMI with CT dosage at 0.5g/kg or 1.5 g/kg of live weight (LW). However, when dosed at 3.0g/kg of LW sheep became anorexic, weakened, and depressed. Sheep were subsequently euthanized to prevent suffering. Even though DMI decreased with sheep consuming 3.0g/kg of LW, it is more likely sheep succumbed to toxicity of tannic acid, and it does not represent the second mode of action. Absence of a decreased DMI when sheep were ruminally dosed indicates that potential slowing of digestion ruminally does not affect DMI.

Aversions for feedstuffs are influenced by experiences early in life and aversions are created from sensory input while a feedstuff is consumed (Provenza, 1996). Provenza (1995) reported that within one hour of consumption the emetic system is activated, which causes a conditioned aversion. The astringent flavor of CT causes a negative sensory input. Provenza et al. (1990) offered pelleted blackbrush with or without CT to goats. Goats initially ate both pellets, but after one day of consumption, goats avoided CT containing pellets thereafter.

Microbial toxicity of CT has been well-documented (Blytt et al., 1988; Scalbert, 1991; McSweeney et al., 2001; Smith et al., 2005). However, interactions between CT and ruminal microbes are poorly understood (McSweeney et al., 2001). Scalbert (1991) reported CT disintegrates microbial cell walls, and interacts with protein and carbohydrates within microbial cell membranes. Scalbert (1991) also reported substrate

deprivation and metal ion deprivation are indirect modes of action of tannin toxicity. Binding of CT to substrate or metal ions inhibits ruminal microbes from reproducing because the metal ions are sources of energy for transportation in and out of the cell. McSweeney et al. (2001) reported complexing ability of CT means CT are reactive with microbial cell wall and their enzymes, and interaction most likely inhibits transportation of nutrients into cells. Smith et al. (2005) reported CT to bind to cell walls of *Butyrivibrio fibrisolvens*, *Streptococcus bovis*, *Prevotella ruminicola*, and *Ruminobacter amylophilus*. Substrate deprivation and binding of CT to metal ions are main factors in ruminal microbial inhibition, but CT permeates and damages cell walls of certain strands of bacteria.

Positive Effects of Tannins

Condensed tannins possess beneficial effects, especially in ruminants (Rochfort et al., 2008; Waghorn, 2008; Powell et al., 2009). Positive effects reported include bloat mitigation, methane (CH_4) mitigation, ammonia (NH_3) mitigation, and anti-parasitic properties.

The effect of CT on bloat in ruminants has been reviewed extensively (Aerts et al., 1999; Barry and McNabb, 1999; Min et al., 2003). Frothy bloat is common in cattle grazing highly digestible pastures, and interactions between ruminal microbes and soluble protein causes froth or foam to form (McMahon et al., 2000). Legumes such as birdsfoot trefoil, have not indicated or have less frequency of bloat due to CT in the plant precipitating protein and slowing or decreasing digestibility of dietary CP, which prevents formation of froth or foam (Frutos et al., 2004). Min et al. (2006) dosed quebracho at 1% and 2% of DMI directly into the rumen of steers grazing wheat forage and scored severity of bloat. Bloat was scored on scale of 0 to 3, 0 being no evidence of

bloat and 3 being severe distension. Steers dosed with the greatest amount of CT had the least incidence of bloat compared to steers receiving no CT and steers receiving 1% CT of DMI.

Internal parasites are problematic in livestock production operations (Waghorn, 2008). With CT affecting ruminal microbes, researchers have studied CT effects on internal parasites. Naumann et al. (2013) reported that effects of CT on parasites vary due to source of CT and type of parasites. Animals have been drenched with CT, supplemented with CT, and given forages with CT to observe effects of CT on parasites (Naumann et al., 2013). Villalba et al. (2010) reported that sheep infested with internal parasites preferred forage with a greater amount of CT (10% CT inclusion of diet DM), presumably to help mitigate affects of parasites. Molan et al. (2000) reported that sheep drenched with commercial anthelmintic and sheep not given anthelmintic but grazed CT containing forage (5% CT of diet DM) had similar performance and fecal egg counts. Sheep drenched with commercial anthelmintic or offered CT had a smaller fecal egg count than sheep receiving no form of anthelmintic. Molan et al. (2000) also reported that CT extracted from forages has similar affects as animals grazing CT containing forages. Butter et al. (2000) top-dressed diets fed to lambs with quebracho CT (4% of diet DM) while sheep were consuming high or low protein diets. Diets were formulated to be isocaloric, and fishmeal was added at 17% to create a high-protein diet. After sheep were confirmed to have nematode eggs present in feces, CT was dosed to sheep and nematode egg counts taken daily. Sheep consuming CT had least amount of eggs present in feces. Although interactions of CT and parasites are not completely understood, multiple studies have reported an ability of CT to have some parasitic function. With growing concern of

antibiotic use in animals, CT may be a good alternative to commercially available antibiotics.

Condensed tannins have been cited for decreasing greenhouse gas emissions in vitro and in vivo (Woodward et al., 2004; Min et al., 2005; Min et al., 2006; Beauchemin et al., 2007; Rochfort et al., 2008; Powell et al., 2009). Rochfort et al. (2008) suggested CT directly inhibit methanogens as a primary mode of action for reduction in CH_4 emissions observed. Woodward et al. (2004) measured CH_4 emissions of dairy cows grazing ryegrass (0% CT of DM) and *Lotus* grass (2.5% CT of DM). Total CH_4 excreted was not different among dairy cows grazing ryegrass or *Lotus*, but intake increased for cows grazing *Lotus.* Corrected for DMI, dairy cows grazing *Lotus* excreted 17.6% less CH_4. Methane data is confounded by differences in NDF values of forage used. Ryegrass and *Lotus* were 41.10% NDF and 28.43% NDF, respectively. Lower NDF content caused increased in DMI, lower NDF forages produce less CH_4, and increased DMI correlates to decreased CH_4 production (Johnson and Johnson, 1995).

In an in vitro analysis, Pellikaan et al. (2011) reported a decrease in CH_4 production when CT was added to mixed hay (*Medicago sativa L.*). Addition of CT to mixed hay decreased CH_4 production by 24%. Beauchemin et al. (2007) added quebracho CT extract at 1% and 2% of DMI to a forage-based diet for heifers and steers, and reported no differences in CH_4 emission corrected for DMI. Potential for CT to decrease CH_4 emissions is evident, but further investigation needs to study interactions of CT with various forages and interaction of sources of CT and with diets in vivo.

Aside from CH_4 emissions, CT decreases NH_3 emissions in dairy excreta (Powell et al., 2009). When dietary CP consumption is greater than an animal's requirement, NH_3

from the excess dietary CP consumed is converted to urea via the ornithine cycle in the liver. The CP converts to urea, because ammonia from the dietary CP is toxic to the animal. The primary excretion site of urea is in the urine (Krober et al., 2000). Nitrogen excreted in urine produces greater emissions than N excreted in feces because of volatilization and mineralization that changes urea to NH_3 (Oenema et al., 2001). Ammonia is a pollutant and respiratory tract irritant (Cole et al., 2008). Powell et al. (2009) fed dairy cows a high-tannin forage (1.66% CT of DM) or low-tannin forage (0.66% CT of DM), and reported a shift in N excretion from urine and a greater proportion N excretion occurring in feces. Greater proportion of N content of feces is most likely due to binding of dietary CP, and consequently dietary CP becoming unavailable to ruminal microbes. Without availability of dietary CP to ruminal microbes, dietary CP passed through the GI tract relatively undigested. Nitrogen in feces is bound to organic matter, thus unavailable for volatilization and mineralization to NH_3 relative to N excreted in urine (Webb, 2001).

Summary of Condensed Tannins

Condensed tannins have varying effects in GI tracts of ruminants. Condensed tannins affect the digestibility of protein, carbohydrates, and fat at throughout the GI tract. The affect of CT on protein is the most researched CT/nutrient relationship. The astringent flavor of CT reduces intake and conditions animals to avoid consuming forages with CT. Nonetheless, when CT are fed in moderation benefits are observed (i.e., bloat mitigation).

Ammonia is considered a respiratory pollutant (Cole et al., 2008), and feeding CT in the feedyard may potentially decrease NH_3 emissions due to a larger amount of NH_3

contained in the feces. In addition, it appears that a greater proportion of N is excreted in feces with inclusion of N in the diet (Powell et al., 2009). With a greater amount of N in feces, less N is volatilized or mineralized to ammonia because the N is bound to organic matter (Oenema et al., 2001).

Creatinine

Creatinine Synthesis

Creatinine (CRN) is a product of protein turnover in the body (Heymsfield et al., 1983). Creatine (CR) is a nitrogenous compound found primarily in muscle, and participates in energy transfer for muscle contractions (Heymsfield et al., 1983). Synthesis of CR initiates in the kidneys (Fig. 1.3) with production of guandinoacetate from glycine and arginine (Borsook and Dubnoff, 1947). Transamidinase regulates CR production in kidneys (Heymsfield et al., 1983). Creatine intake also influences production of CR in kidneys, and CR production reaches full activation with consumption of vegetarian diet (Folin, 1905; Chanutin, 1926; Crim et al., 1975). Intake of CR decreases endogenous production of CR. Endogenous CR or dietary CR releases into blood from the liver, and active transport against a concentration gradient transports CR into muscle (Heymsfield et al., 1983). Uptake of CR replaces the 2% turnover of CR already in muscle (Fitch et al., 1968). Once in muscle CR undergoes phosphorylation with creatine kinase, and phosphocreatine is a product used to transfer energy to myosin and actin (Salway, 2004). Dehydration of CR or phosphocreatine converts both the former and latter to CRN (Heymsfield et al., 1983). Creatinine diffuses out of cells into blood and expelled by kidneys in urine at a constant rate and consistent with lean muscle mass (Heymsfield et al., 1983).

Creatinine and Muscle Mass

Correlation of CRN to human lean body mass is reported in several experiments (Folin, 1905; Hobson, 1939; Van Niekerk et al., 1963; Chinn, 1967). Folin (1905) first described CRN excretion is constant and proportional to body weight when subjects consumed a CR free diet. Consumption of CR in diet varied excretion of CRN by 2-3% (Folin, 1905). However, Folin (1905) noted that subjects with a greater amount of fat affected total excretion of CRN per kg of LW. Myers and Fine (1913), using methods of Folin (1905) to determine CRN in urine, were first to determine direct correlation between CRN and total CR in the body. Thus, muscle mass in the body may be determined. To determine muscle mass in the human body four assumptions are accepted: 1.) All CR in the body is located within skeletal and smooth muscle; 2.) CR-free diet does not affect total CR pool; 3.) CR converts irreversibly to CRN; and 4.) excretion of CRN is constant (Heymsfield et al., 1983). Lofgreen and Garrett (1954) reported a correlation ($r = 0.67$) of creatinine excreted and percentage of separable lean of steers after slaughter. Van Niekerk et al. (1963) reported a correlation ($r = 0.97$) between CRN excretion and total empty body protein after measuring CRN excretion in sheep and directly measuring total body protein after slaughter. Kumar et al. (1959) reported a coefficient of variation of less than 2% when predicting fat-free mass using CRN excretion and when directly measuring fat-free mass after slaughter of beef calves. In a meta-analysis, Chinn (1966) reported a strong correlation ($r = 0.96$) in rats, between total body protein directly measured and total body protein predicted using CRN. Thus, CRN excretion is useful in predicting lean body composition of animals (Van Niekerk et al., 1963).

Creatinine and Prediction of Urine Volume

Total urine collection in nutrition research is important in order to quantify nitrogen balance or energetic balance. Collecting total urine in livestock experiments is difficult without utilizing invasive procedures (i.e., catheters) or using metabolism crates, which often negatively affect intake and performance. Therefore, spot sampling urine and extrapolating to determine total urine volume would be highly useful. Folin (1905) stated the amount of CRN eliminated in urine is constant regardless of urine volume, N eliminated in urine, and intake of dietary CP. Several experiments further support a constant CRN excretion rate (Rose, 1933; Hobson, 1939; Crim et al., 1975). Thus, knowing total urine output would be determined by using total CRN excretion as an internal marker.

Total CRN excretion can be determined by determining fat-free body mass, but determining fat-free body mass is impossible without harvest of animals. Empty BW is highly correlated to fat-free body mass, thus, correlation of BW and CRN production is strong (Folin, 1905). Therefore, a secondary option for determination of total urine excreted is to determine CRN excreted per kg of live shrunk BW, which determines total CRN excretion (Folin, 1905; Rose, 1933; Brody et al., 1934; Hobson 1939; Cryer and Sode, 1970; Crim et al., 1975). Folin (1905) first suggested that CRN excretion is constant per kg of BW, but also suggested that CRN excreted per kg of BW decreases as percent of fat increases in body composition. Narayanan and Appleton (1980) concluded that CRN excreted per kg of BW is inaccurate because fat contributes to body weight, which can underestimate CRN output. Van Niekerk et al. (1963) countered Narayanan and Appleton (1980) by reporting a maximum percentage of body fat before fat affects CRN. Van Niekerk et al. (1963) concluded sheep above 28% had decreased excretion of

CRN per kg of shrunk BW, compared to sheep below 28% empty body fat. According to Van Niekerk et al. (1963), CRN excretion is not affected by fat when body fat is below 28% in sheep. The correlation of CRN and fat-free body mass (r = 0.67) reported by Lofgreen and Garrett (1954), CRN is expressed per kg of BW.

Whittet al. (2004) determined CRN excretion is constant of growing heifers ranging from 0.026 to 0.028 g/kg of shrunk BW. Chizzotti et al (2008) further agreed with Whittet et al. (2004) after evaluating CRN of growing heifers. Lofgreen and Garrett (1954) found similar CRN excretion values for steers. Therefore, sex does not affect CRN excretion. Multiplying CRN excretion per kg of shrunk BW by shrunk BW of animals will determine total CRN excretion per d. Mathematically, urine output per d can be determined after CRN is determined from a spot sample of urine using:

$$\text{Urine output/d} = \frac{0.027\text{g/kg of shrunk BW}}{\text{Total CRN excreted/d}}$$

Physiological stages can affect CRN excretion within animals. Both Whittet et al. (2004) and Chizzotti et al. (2008) reported lesser CRN excretion than 0.025 g/kg of shrunk BW at older ages of maturity. Values for growing heifers are inappropriate for use in older cattle. Chizzotti et al. (2008) noted that as an animal ages, proportion of fat increases, and decreases CRN excretion per kg of BW. Older cattle would need a lesser CRN constant to determine urine volume.

Indirect Calorimetry

Indirect Calorimetry vs. Direct Calorimetry

Measuring heat production is the most practical option for measuring the oxidation of nutrients within the body (Blaxter, 1962). Heat production is how caloric values are

assigned to nutrients, and heat production is considered the energy expenditure of an animal (Blaxter, 1962). Fasting heat production is the equivalent of basal metabolism or maintenance requirements of the animal (Ferrell and Oltjen, 2008). Direct calorimetry is the measure of heat produced by the animal. Direct measuring calorimeters consisted of placing an animal in an insulated chamber equipped with an inner and outer wall (Blaxter, 1962). The medium between the two walls is typically water, and heat production of the animal was determined by measuring the temperature change of the medium (Ferrell and Oltjen, 2008).

Indirect calorimetry is possible by an assumption of the first law of thermodynamics or Hess's Law (Blaxter, 1962). Indirect calorimetry measures gas exchange, oxygen consumption and CO_2 excretion, to estimate heat production (Ferrannini, 1988). Nitrogen-containing compounds present another layer to estimating HP, as N is not fully oxidized in the body, but excreted in urine (Blaxter, 1962). Lastly, incomplete oxidation of carbohydrates or bacterial digestion of carbohydrates produces CH_4, which must be corrected for in the indirect method (Blaxter, 1962). The equation of Brouwer (1965) sums all the aforementioned losses and consumption of oxygen into one equation. The equation of Brouwer (1965) estimates heat production and presents coefficients or correction factors for the consumption of oxygen and excretion of CO_2, urinary N, and CH_4. With the collection of those four variables, heat production can be estimated.

Whole-Body Calorimetry Chambers

Methods to measure gaseous emissions, indirectly, of ruminants traditionally use whole-body calorimetry chambers. Within chambers, airflow is constant and measurement of carbon dioxide, methane, and oxygen of air, estimates consumption or

excretion by animal (McLean and Tobin, 1987). With the use of respiratory quotients, one can estimate oxygen consumed from CO_2 excreted. Nonetheless, use of whole-body calorimetry chambers is costly due to gas analyzing equipment, maintenance of chambers, maintenance of buildings housing chambers, and finally chambers require intensive labor to conduct experiments. Moreover, chambers do not estimate environmental factors surrounding the animal on gaseous emissions and chambers typically decrease feed consumption (Ferrell and Oltjen, 2008). Benefits of chambers include capturing hindgut fermentation CH_4 emissions and chambers accounting for diurnal variation in emissions.

Portable Technology to Measure Gaseous Emissions

With increasing concern of greenhouse gas (GHG) emissions, a greater need to measure CH_4 emissions of cattle in a natural environment is warranted. For this reason, development of equipment other than whole-body chambers has occurred. Development of sulfur hexafluoride (SF_6) tracer gas technique is an example. Sulfur hexafluoride tracer gas technique has been discussed in several reviews, and considered a relatively invasive procedure (Johnson et al., 1994; Johnson and Johnson, 1995; Hammond et al., 2013).

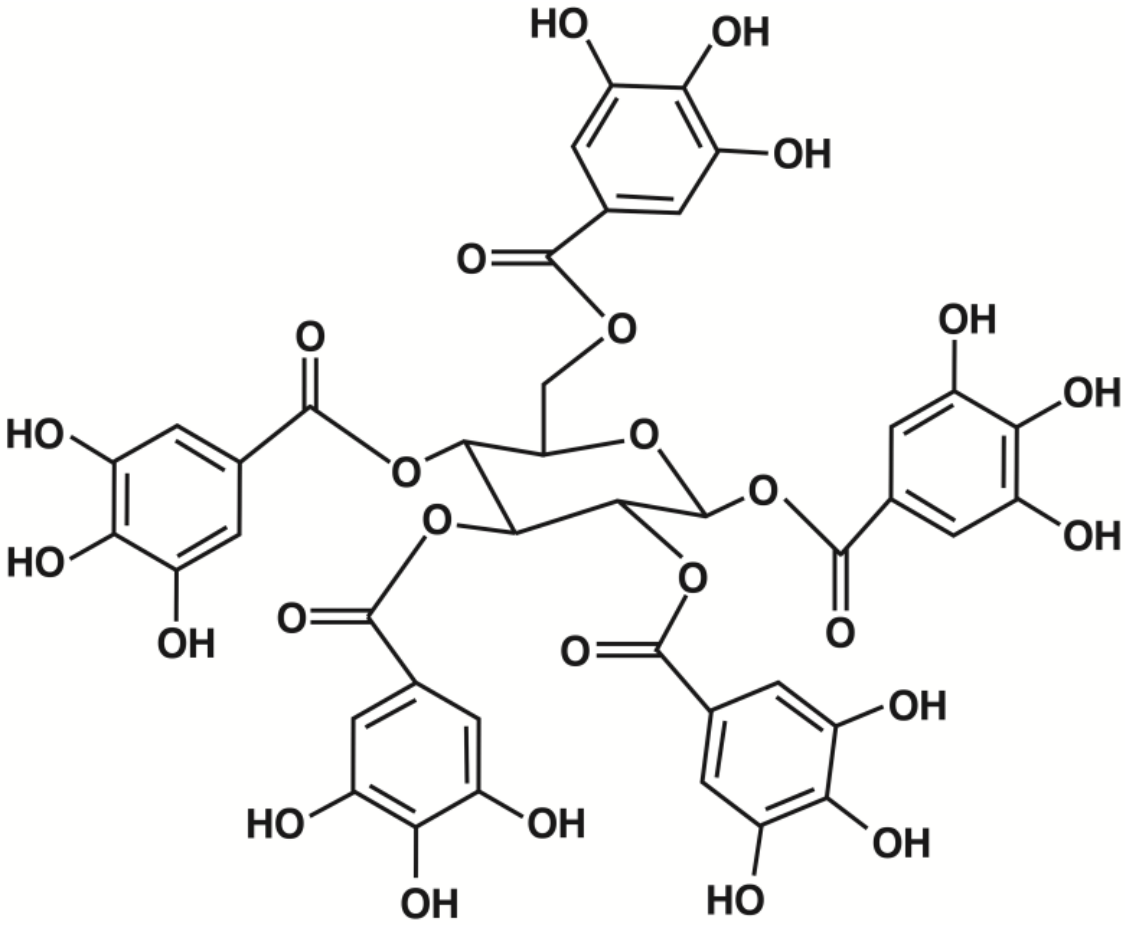
Johnson and Johnson (1995) describe the use of head boxes that fit entirely around animal's head. Head boxes are typically enclosing an animal's head for 24 hours or less. While the head box is attached, air excreted by the animal is captured using a pump system within the head box. Gas chromatography or other methods analyze samples for gas concentrations. Head boxes do decrease intake and do not capture hindgut fermentation (Johnson and Johnson, 1995). Not capturing CH_4 from the hindgut is a negative impact of the head box system.

The GreenFeed system (GF; C-Lock Inc., Rapid City, S.D.) is a commercially available head box that analyzes spot samples of breaths excreted by animals. Constant flow of air via a fan pulls air into system under negative pressure, and GF analyzes excreted air for CO_2 and CH_4 with on-board nondispersive infrared sensors (Fig 1.4). GreenFeed is stationary and does not require attachment to the animal. GreenFeed uses feed to entice animals to insert their head into an enclosed feeding area for a minimum three-minute feeding. Benefits to using a GF include constant access of the unit by animals, spot samples during a 24 h period can account of diurnal variation, and intake of main diet is unaffected. Negative attributes include an inability to capture hindgut CH_4 and feed used to entice need be accounted for in intake estimates. Hammond et al. (2013) found similar gaseous emissions comparing GF to or accepted methods (i.e., whole-body chambers, head boxes, and SF_6). Hristov et al. (2015) and Huhtanen et al. (2013), agreed with Hammond et al. (2013), and found no differences emissions between methods and similar repeatability of GF relative to or methods. Therefore, GF is a viable option to measure gaseous emissions.

The ability of the head boxes to measure CO_2 and CH_4 presents a novel research model that may potentially estimate environmental impacts on net energy requirements of ruminants. A head box system will collect the CO_2 and CH_4 emissions of cattle grazing or in a pen setting and the use of a respiratory quotient will estimate oxygen consumption. The last input to the equation is urinary N. To estimate urinary N, total urine collection is required. Using a catheter or metabolism crate to collect urine would contradict the novel research method aforementioned. However, spot-sampling urine and the use of CRN will estimate total urine output. Thus, all variables for use in the equation of Brouwer (1965)

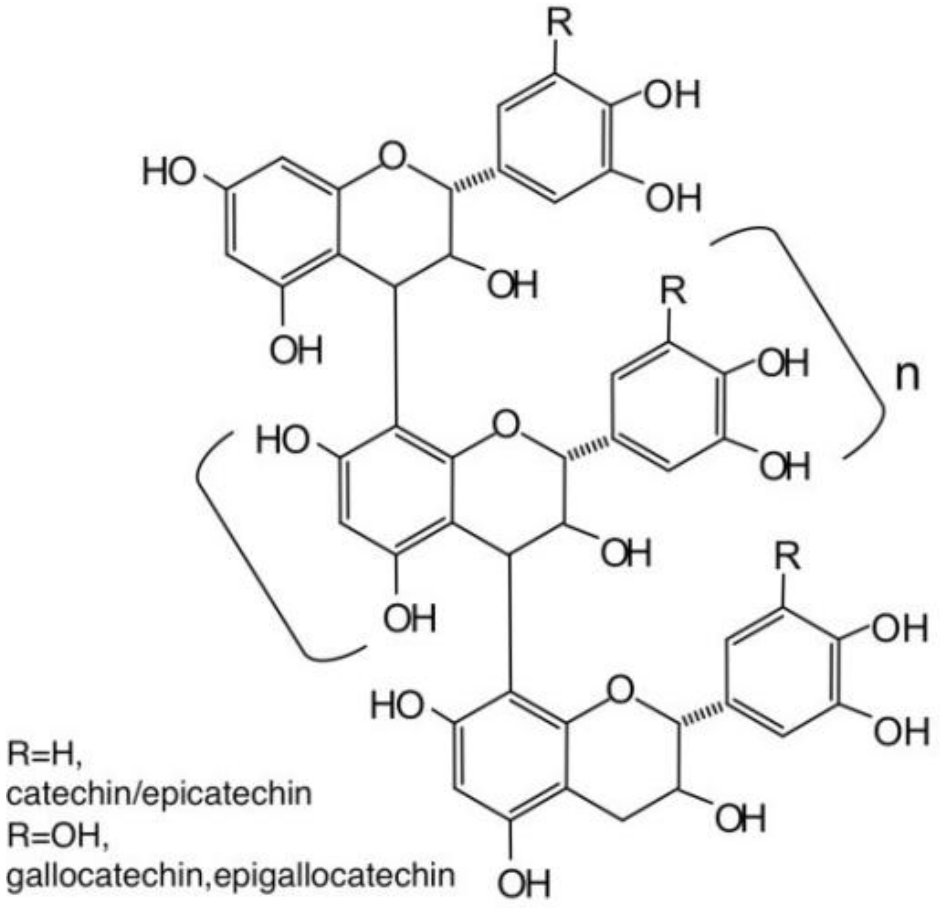
are determined, and can be used to estimate heat production or increment. This novel method may be an answer to the negative impacts of whole-body calorimeters.

Figure 1.1 Structure of Condensed Tannin



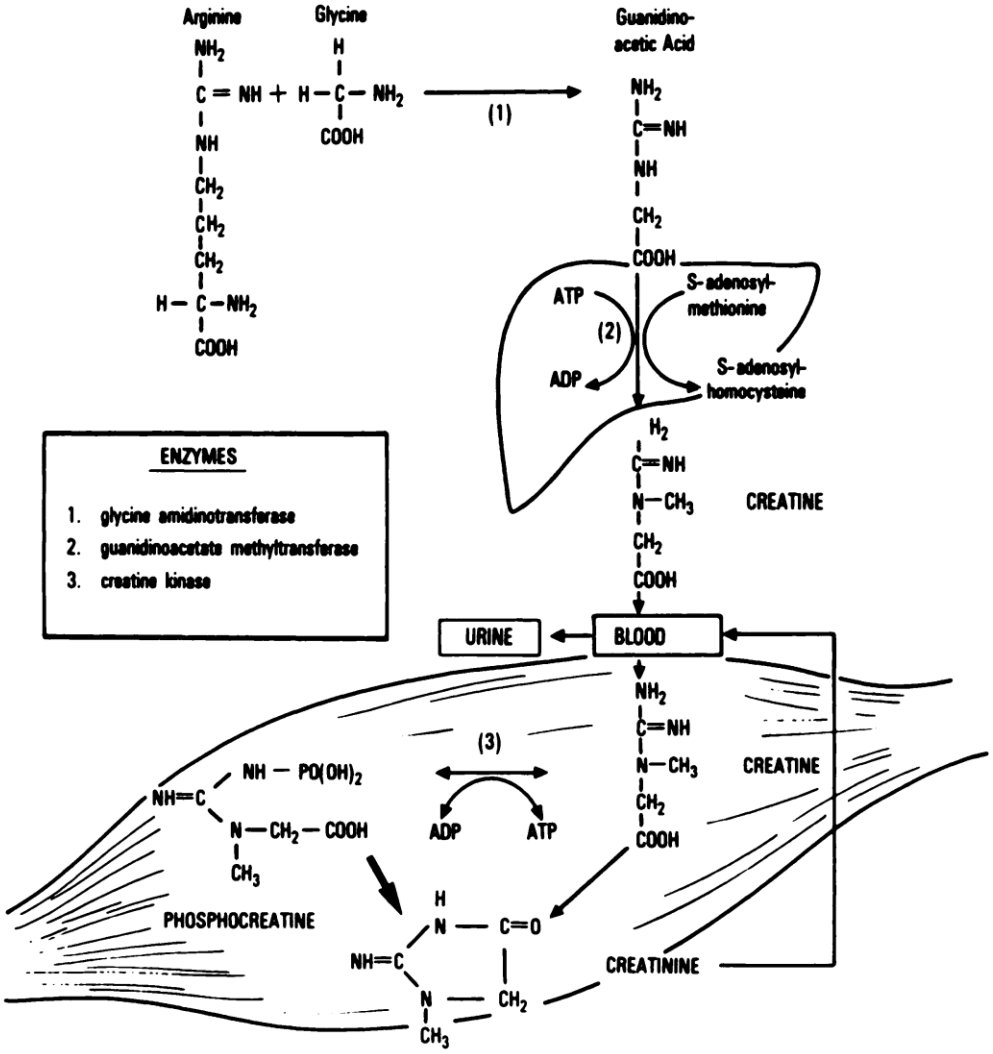
Adapted from Naumann et al. (2013)

Figure 1.2 Structure of Hydrolyzable Tannin



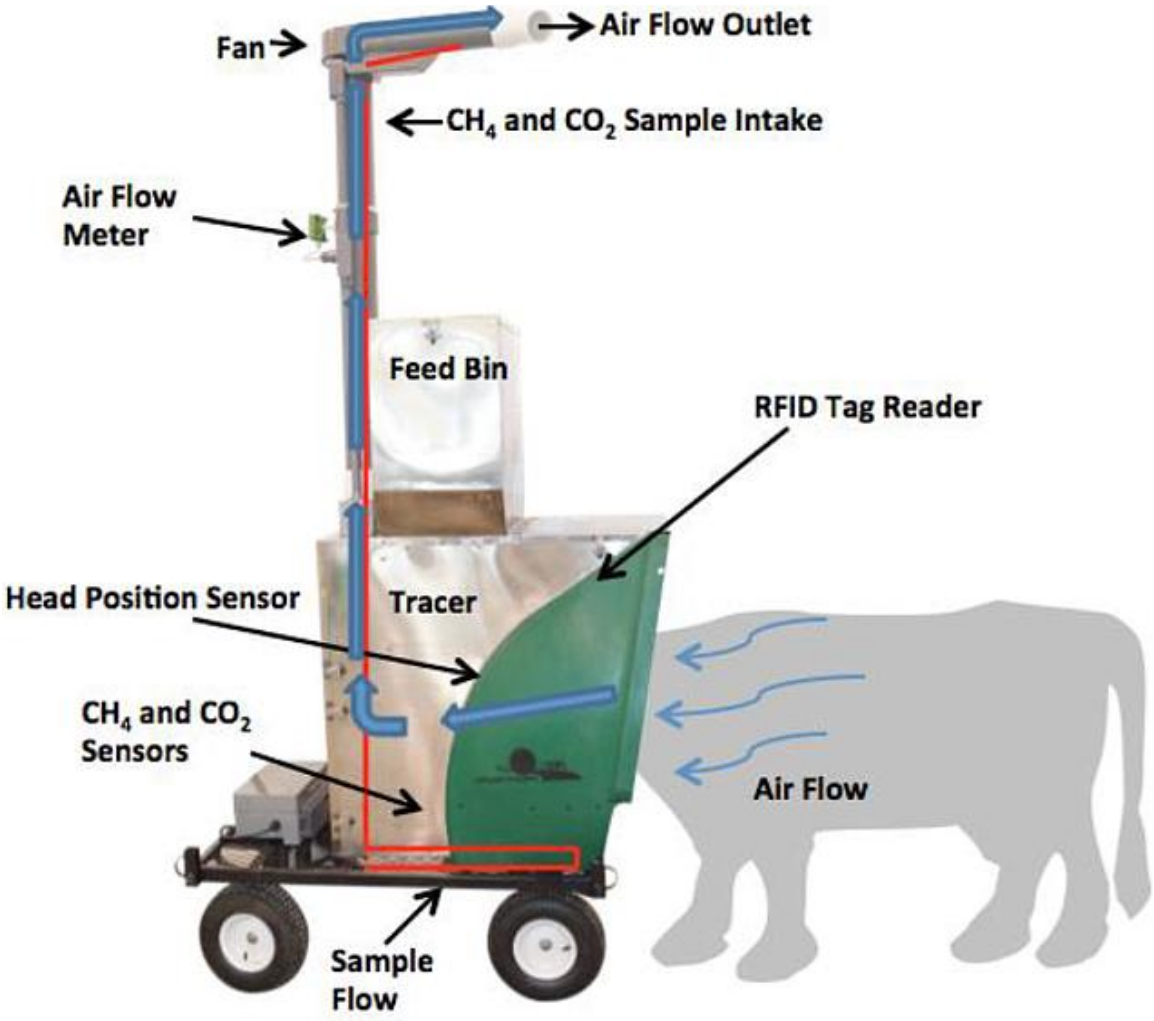
Adapted from Nascimento et al. (2013)

Figure 1.3 Metabolic Pathway of Creatinine Metabolism



Adapted from Heymsfield et al. (1983)

Figure 1.4 Greenfeed Schematic



Adapted from Hristov at al. (2015)

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CHAPTER II

Effect of condensed tannin extract supplementation on performance, nitrogen balance, gas emissions, and energetic losses of beef steers

Introduction

Decreasing nitrogen (**N**) loss from fed cattle is of interest to feeders, regulators, and the general public. Nitrogen excreted in urine is a greater concern than N excreted in feces because much of the N in urine is excreted as urea and hydrolyzed to ammonia (NH_3) and nitrous oxide (Krober et al., 2000; Oenema et al., 2001; Vasconcelos et al., 2007; Waldrip et al., 2015). Nitrogen in feces is considered to be less volatile as it is in organic form (Bierman et al., 1999, Webb, 2001; Luebbe et al., 2012). Emissions from concentrated animal feeding operations (**CAFO**) have been a growing concern of regulatory bodies and agencies in recent years. Examples include The Emergency Planning and Community Right-to-Know Act (U.S. EPA, 2007) and air quality regulations on fine particulates as $PM_{2.5}$ (U.S. EPA, 2006). Factors affecting N losses largely stem from the diet, namely protein degradability and quantity (Cole et al., 2008). Waldrip et al. (2013, 2015) reported a correlation between greater dietary CP and greater urinary N excretion. Inclusion of by-products in feedlot diets, as well as erroring on the margin of safety to practically guard against inherent variation in cattle type

(Vasconcelos et al., 2007), can increase diet CP above the steer's requirements, which are thought to range from 12.5 to 13.5% (Gleghorn et al., 2004).

Condensed tannins (**CT**) are a class of plant polyphenols that form complexes with dietary protein, which disrupts proteolysis (Reed, 1995). Forages containing CT have been studied for the effects on performance, methane emissions, and lessening diet costs (Beauchemin et al., 2007; Stewart et al., 2015). Extracts of CT have also been studied for the aforementioned reasons (Min et al., 2005; Barajas et al., 2013). Therefore, CT binding with excess dietary CP may alter N excretion, increase fecal N excretion, and decreased ammonia volatilization. Furthermore, energetic maintenance cost may be decreased due to less CP being metabolized.

Materials and Methods

All procedures involving the use of animals were approved by the West Texas A&M University/CREET Institutional Animal Care and Use Committee (approval number 102014).

Twenty-seven Angus-cross steers (initial BW = 350 $\pm$ 32 kg) were used in a completely randomized design to determine the effects of CT supplementation during the finishing period on performance, carcass characteristics, N balance, and gas emissions. Steers were weighed two consecutive days (d0 and d1) to minimize differences in gut fill and obtain an accurate initial BW, and steers were fed the finishing ration at 1.0% of BW on d0 and d1. Steers were stratified by BW and assigned randomly to one of three treatments; CT added at 0% of diet DM (**CON**), 0.5% of diet DM (**0.5% CT**), or 1.0% of diet DM (**1% CT**). On d 1 of the experiment, steers were implanted with Revalor-S (120 mg trenbolone acetate, 24 mg estradiol; Merck Animal Health, De Soto, KS). All steers were fed using Calan gates (American Calan, Northwood N.H.) to monitor individual

feed intake, and bunks were managed such that steers had *ad libitum* access to feed. Steers were contained in 3 pens with nine Calan gates/pen. However, gates in the fence line between pens were removed to allow a larger pen environment. The finishing diet fed was formulated to be consistent with typical industry concentrations of by-product use and CP concentrations (Vasconcelos and Galyean, 2007). Therefore, the diet was formulated to contain 14.0% CP, 1.50 NEg Mcal/d, 8.5% roughage, 60% steam-flaked corn, 15% wet distiller's grains with solubles, 10% corn distiller's solubles and 6% fat (Table 2.1). Steers were trained to use the Calan gates before the experiment. Grain adaptation consisted of offering the basal diet initially at 1.0% of BW, and increasing feed calls by no more than 0.23 kg per d for the first 21 d. All steers were fed the basal diet for 126 d. The basal diet was mixed in a stationary mixer then deposited into feed carts, and weighed to the nearest tenth of a kg and delivered by hand. Condensed tannins were not fed during the grain adaptation in an attempt to match dietary supplied protein to animal requirements. Protein requirements as a percent of DMI decline over the feeding period (NRC, 1996), thus we delayed CT supplementation to ensure that protein supply did not impair performance. Condensed tannins were added to the ration after the 21 d basal diet adaptation.

Condensed tannins (By-Pro: quebracho extract, 95% tannic acid, Silvateam, Ontario, CA) were mixed daily with the basal diet to form a premix (10% CT and 90% basal diet), using a 90 kg capacity, electric mixer (L200DA, Leland Double Action Mixer, Detroit, MI). Premix was fed as a top dress at either half or one percent of the diet DM. Weekly DM adjustments were made after weekly ingredient samples were taken and weekly complete diet samples were taken prior to mixing and feeding, respectively,

and composited for each period to determine DM content. Dry matter of the composited samples was determined using a forced-air oven at 55° C for 48 hours.

Fecal and urine samples were collected during two periods, each 6 d in length, to determine digestibility and N balance. The first period took place after 34 days on feed (**phase 1**) and the second period took place after 95 days on feed (**phase 2**). Feces and urine were collected twice daily with a two hour advancement every 24 hours. Each sampling, cattle were taken to the processing chute and palpated for a fecal sample. At the same time, funnels with threaded bottoms were temporarily adhered to the sheaths of the steers and a plastic bottle was screwed to the bottom of the funnel. A 300 mL sub-sample from each steer was collected and composited by each collection period. Urine was immediately placed in a -20º C freezer to prevent N loss.

Titanium dioxide (**TiO_2**) was used as an external marker to estimate fecal output (Titgemeyer et al., 2001). Titanium dioxide was dosed at 15 g/head daily for the 8 days leading to each collection as well as the 6 days during the collection. Titanium dioxide was weighed each day and top-dressed on the diet to ensure consumption. Orts were collected and analyzed for residual TiO_2.

Urine output was determined by using creatinine as an internal marker (Whittet et al., 2004; Chizzotti et al., 2006). Creatinine is a product of muscle metabolism, is excreted in the urine at a consistent proportion to BW, and is largely independent of diet type, age, and physiological stage (McCarthy et al., 1983; Whittet et al., 2004; Ceconi et al., 2015). Therefore, urine volume was determined from urine creatinine concentration, BW, and the excretion rate value of 0.028 g/kg of BW suggested by Whittet et al. (2004)

for estimation of total creatinine excreted per d. Weights were taken three consecutive days for an accurate BW (shrunk 4%) to use in creatinine calculations.

During 90 d of the experiment (coinciding with sampling periods) steers had access to a GreenFeed system (**GF**, C-Lock Inc., Rapid City, S.D.) to measure carbon dioxide (**CO_2**) and methane (**CH_4**) emissions (Hammond et al., 2013; Waghorn et al., 2013). Steers were trained prior to the experiment to use the GF system. For more comprehensive descriptions of the GreenFeed system, readers are referred to Hristov et al. (2015) and Huhtanen et al. (2015). Briefly, the GF system is a stationary headbox that uses feed to entice steers to use the system. As the steer inserts their head into the headbox, a radio-frequency identification (**RFID**) tag reader recognizes the steer and drops a controlled amount of pelleted feed. While the steer stands with their head in the headbox and consumes an allotment of feed, the GF system measures a three to five-minute spot sample of air expelled by the steer and analyzes it for CH_4 and CO_2. Exhaled gases are pulled through a tube under negative pressure where airflow mass is measured continuously. Measurement of CH_4 and CO_2 concentration are analyzed using on-board nondispersive infrared sensors and production estimates are then calculated and adjustments made to standard temperature and pressure. The GF was programmed to allow a total of 6 three to five-minute spot samples per day with a minimum four h gap between feeding events. Weekly gas calibrations and monthly CO_2 recovery tests were performed to ensure proper sensor function. Carbon dioxide recovery tests for the unit averaged 94.8 $\pm$ 0.64%. An equal parts blend of a transition pellet (14% CP; Cargill Animal Nutrition, Minneapolis, MN) and pelted cottonseed hulls (81% NDF; Livestock Nutrition Center, Altus, OK) were used as a treat reward to entice the steers to use the GF

in the current experiment. The transition pellets consisted of a mix of roughage and by-products and its intended purpose is to transition steers to a concentrate diet. Steers were allowed a total of 500 g of transition/cottonseed hull pellets per day over 6 different feeding events.

Steers were fed for 126 d to an average final BW of 601 $\pm$ 50 kg. Steers were harvested at a commercial abattoir (Tyson Fresh Meats, Amarillo, TX) at d 127. Carcass data was collected by trained personnel of the West Texas A&M University Beef Carcass Research Center (Canyon, TX).

Laboratory Analyses

Dry matter of feces, feed, and orts were analyzed by drying samples to a constant weight at 55° C in a forced-air oven for 48 hours. Samples were ground through 1mm screen using a Wiley Mill (Model 4, Thomas-Wiley, Philadelphia, PA). Lab corrected DM on fecal, ort, and diet samples were conducted with forced-air oven at 105° C for 24 hours. Ash content was determined using a muffle-furnace at 450^{o} C for 8 hours. Gross energy of feces and feed were determined using an automatic bomb calorimeter (Parr 6400 Calorimeter, Parr Instrument Company, Moline, IL). Total N of feed and feces were determined by combustion in a C/N analyzer (Elementar C/N Vario Max Cube, Elementar Americas Inc., Mt. Laurel, NJ). Starch content of feed and feces was determined using an enzyme kit (Megazyme International Ireland Ltd., Wicklow, Ireland; method 996.11; AOAC, 2003).

Concentration of TiO_2 in fecal and ort samples were determined using a spectrophotometer (Synergy 2, Biotek, Winooski, VT) using the method described by Myers et al. (2004). A blank fecal background was used to correct for any prior TiO_2 in fecal samples (Morgan et al., 2014). Urinary creatinine concentrations were determined

using a spectrophotometer, and using a creatinine determination kit (ADI-907-030A, Enzo Life Sciences, Farmingdale, NY).

Calculations

Digestibility was calculated by dividing fecal output, determined using the TiO_2 procedure, by DMI subtracted from 100 (Merchen, 1988). The creatinine constant used for urinary creatinine excretion was 0.028 g/kg of BW (McCarthy et al., 1983; Whittet et al., 2004; Ceconi et al., 2015). Creatinine excretions were determined for each steer by multiplying BW, taken from each collection period, by the creatinine constant. Urine output was equal to the creatinine excretion value divided by the creatinine concentration in each urine sample.

Using the CH_4 and CO_2 collected by the GF unit, the N determined from the urine samples, and a respiratory quotient of 1.05, heat production was calculated using the equation of Brouwer (1965). Recent reports, using whole-body calorimetry chambers (Hales et al., 2012) and head boxes (Thornton and Owens, 1981; Hales et al., 2015) suggest an RQ near or less than 1.0. The RQ value of 1.05 was chosen because by definition, an RQ of greater than 1.0 suggests the accretion of fat, which would clearly be the case for these steers fed for slaughter. Biological limits for RQ have been reported from 0.7 for fasting animals to greater than 1.2 for force-fed animals (Blaxter and Wainman, 1965). While the possibility exists for RQ to vary by treatment, other calorimetry research has not reported this (Hales et al., 2012, 2015). Urinary energy was calculated as the quotient of gross heat (2.3 kcal/g) of urea assuming all urinary N was urea (Street et al., 1964; Hales et al., 2013).

Statistical Analysis

Growth performance and carcass characteristics were analyzed as a linear mixed model with one-way treatment structure in a completely randomized design (PROC MIXED; SAS Institute Inc., Cary, NC), with animal serving as the experimental unit. The class statement included steer and treatment and the model statement included treatment.

Digestion, N balance, and gaseous emissions were analyzed as a linear mixed model with one-way treatment structure in a completely randomized design (PROC MIXED; SAS Institute Inc., Cary, NC), with animal serving as the experimental unit. The class statement included steer and treatment. The model statement included treatment, period, and treatment *x* period. No interactions between treatment and period were detected ($P > 0.10$); therefore main effects of treatments and periods are reported.

Means separation and *P*-values were determined using LSMEANS with PDIFF option. Orthogonal contrasts were used to separate linear and quadratic effects of CT inclusion in the diet. Treatment differences are discussed when $P \leq 0.05$; tendencies are discussed when $P > 0.05$ and < 0.10.

Results and Discussion

Performance and Carcass Characteristics

Dry matter intake did not differ ($P = 0.57$; Table 2.2) across treatments. Condensed tannins have been reported to affect palatability of a diet (Reed, 1995), however, no differences were observed in DMI among treatments. It can be assumed the treatments did not affect the palatability of the diet in the current experiment. No differences ($P \geq 0.86$) among treatments were observed in final BW, ADG, and G:F. These results are similar to findings by Krueger et al. (2010), who reported no difference among ADG, G:F, or DMI when CT was included at 1% of a finishing diet compared to 0% CT

inclusion. Hot carcass weight, USDA yield grade, dressing percent, KPH percent, and marbling were not different ($P \geq 0.26$). Fat thickness at the 12th rib and LM area were not different among treatments ($P \geq 0.12$). The carcass results are comparable to Barajas et al. (2010), which found no differences in LM area and marbling when CT was included at 0.30% of the diet against 0% CT inclusion. However, Barajas et al. (2010) reported a decrease in dorsal fat thickness with CT inclusion in the diet and this differed from the current experiment's reported fat thickness. It should be noted however, that while Barajas et al. (2010) reported dorsal fat thickness this may not be at the same location as 12th rib fat thickness reported in the current experiment. Krueger et al. (2010) reported a lower HCW when CT was included at 1% of the diet compared to CT at 0% of DMI. No difference was found in HCW in the current experiment and the difference that Krueger et al. (2010) reported may have simply been due to variation in a small sample size.

Intake and Digestion

No differences ($P \geq 0.13$; Table 2.3) were observed in OM intake, starch intake, and fecal OM among treatments during phase 1. Fecal starch excreted tended ($P = 0.06$) to be greater when CT was included at 1% of the diet. Condensed tannins have the ability to form hydrogen bonds with carbohydrates (Smith et al., 2005), which may explain the greater starch content in the feces. Fecal starch concentrations for 0% CT, 0.5% CT, and 1.0% CT were 4.4, 4.2, and 6.0%, respectively. Binding of CT to carbohydrates may explain the decrease ($P = 0.03$) in starch digestibility during phase 1 for the 1% CT treatment. Nevertheless, Smith et al. (2005) noted that decreased digestibility could be overcome by adaptation of the microbes to CT. Furthermore, Smith et al. (2005) suggested that quebracho CT had less inhibitory effect on microbes than other sources of

CT. Quebracho CT was the source of CT for the current experiment. Lastly, OM digestibility was not different ($P = 0.30$) across treatments during phase 1.

Organic matter and starch intakes during phase 2 were not different ($P \geq 0.42$) among treatments. Fecal OM and OM digestibility also did not differ ($P \geq 0.30$) during phase 2. Organic matter digestibility averaged 68.9 (CON), 68.2 (0.5% CT), and 64.4% (1% CT), which is less than in other metabolism trials (Arias et al., 2012; Luebbe et al. 2012). The low OM digestibility observed during the trial may be indicative of the slightly greater roughage concentration and roughage supplied NDF than industry average (Vasconcelos and Galyean, 2007), and also that gut fill may have regulated level of intake. Intake by cattle consuming high-concentrate diets is generally regarded as being controlled by caloric intake and various chemostatic mechanisms (Allen et al., 2009). Finally, fecal starch content and starch digestibility were not different ($P \geq 0.11$) across treatments during phase 2, which is different than observed during phase 1. The lack of difference during phase 2 in starch digestibility may be due to the acclimation of ruminal microbes (Smith et al., 2005). Had the sampling occurred later than phase 1, differences in starch digestibility may not have been observed, due to the ruminal microbes being acclimated. Fecal starch concentrations for 0% CT, 0.5% CT, and 1.0% CT were 6.9, 10.7, and 8.8%, respectively during phase 2. Zinn et al. (2002) suggested that assessing the quality of steam-flaked corn should be based on fecal starch concentration and optimal fecal starch concentration should be 2 to 3%. Aside from the quality of the steam-flaked corn, the large DMI observed across treatments may have led to greater fecal starch concentrations than the optimal fecal starch concentration reported by Zinn et al. (2002).

Nitrogen Balance

Nitrogen intake did not differ ($P = 0.16$; Table 2.4) among treatments during phase 1. Fecal N did linearly increase ($P = 0.01$) as CT inclusion in the diet increased. This increase may be due to the CT precipitating the dietary protein in the rumen and making it unavailable to the microbes and available to the small intestine for digestion and absorption. Urinary N and retained N were not different ($P \geq 0.39$) among treatments during phase 1. Powell et al. (2009) tested the effects of high-tannin birdsfoot trefoil compared to low-tannin birdsfoot trefoil, alfalfa, and red clover in dairy cattle diets and observed no difference in fecal N, but did report a decrease in urinary N when cattle were fed the high-tannin forage. This differs from the current experiment. Beauchemin et al. (2007) supplemented a 70% forage-based diet with 1 and 2% quebracho tannins and reported a decrease in CP digestibility when CT was included. Luebbe et al. (2012) speculated that the relatively small particle size of distiller's grains, which decreases retention time in the rumen and increases passage rate, thus increasing the passage of cellulose and hemicellulose (from the distiller's grain) to the hindgut for fermentation. As more substrate is available to the microbes in the hindgut, urea is recycled to the hindgut and used as the N source for the microbes. Therefore, a greater amount of N from the recycled urea may be excreted in feces, as the hindgut is inefficient in absorbing the MCP (Van Soest, 1994). Urinary N, as proportion of total N excretion linearly decreased ($P < 0.01$) with a greater inclusion of CT in the diet, and fecal N excretion, as a proportion of total N excretion, linearly increased ($P < 0.01$) with a greater inclusion of CT in the diet. This is due to an increase in fecal N and not a decrease in urinary N. Powell et al. (2009) found a similar change in urinary N excretion, as a proportion of total N excretion in

dairy cows fed CT. However, the decrease was accompanied by a decrease in total urinary N, not an increase in fecal N.

Nitrogen intake during phase 2 was not different ($P = 0.66$) among treatments. Unlike phase 1, fecal N did not differ ($P = 0.21$) among treatments during phase 2. There was no difference ($P \geq 0.27$) in urinary N or retained N. Urinary N was expected to increase in the current study as the CT was expected to precipitate excess dietary CP in the current diet. The current diet was greater than optimal dietary CP requirements (12.5 to 13.5%; Gleghorn et al., 2004). Furthermore, it was our hypothesis that if less N was excreted in urine, this could have led to lower energetic costs for the disposal of urea and this may have transferred into better performance. In addition, urinary N, as proportion of total N excretion, was not different ($P \geq 0.27$) during phase 2.

The hypothesis of the current experiment does not coincide with these results. A greater repartitioning of N excretion was assumed later in the feeding period because of lower protein requirements of steers closer to physiological maturity. According to Byers and Schelling (1988), as the ruminant animal ages the body composition changes to include a greater proportion of fat and a lesser proportion of protein; therefore, reducing the protein requirement and the growth of the animal. The dietary CP not needed by the animal theoretically would have been bound by the CT and unavailable for digestion. Possibly, feeding a greater dose of CT may have resulted in a shift in N excretion during phase 2. However, that may have rendered the steer protein deficient, therefore, affecting performance.

Gaseous Emissions

Carbon dioxide (kg/d) and CH_4 (g/d) emissions were not affected by CT inclusion for days on feed (DOF) 20 to 40 ($P \geq 0.37$; Table 2.5) or DOF 90 to 116 ($P \geq 0.74$; Table

2.6). Pellikaan et al. (2011) determined CT and specifically CT sourced from quebracho trees can mitigate CH_4 through an *in vitro* gas production using lucerne hay (*Medicago sativa L*.) as substrate. Puchala et al. (2005) compared feeding Sericea lespedeza (CT = 17.7% of DM) or crabgrass/tall fescue (CT = 0.5% of DM) to goats and reported that Sericea lespedeza reduced CH_4 emissions/kg of DMI by 50%. Beauchemin et al. (2007) supplemented a 70% forage-based diet with 1 and 2% quebracho tannins reported no differences in any expressions of CH_4 emissions, which is in contrast to Pellikaan et al. (2011). The difference in CH_4 emissions between Puchala et al. (2005) and Beauchemin et al. (2007) may be due to the source of CT. Different sources of CT have varying effects on the ruminant (Puchala et al., 2005). The source of CT (quebracho) in the current experiment was the same as Beauchemin et al. (2007) and the effects on CH_4 production were similar. In addition, in the experiment by Puchala et al. (2005), the NDF concentration of Sericea lespedeza was 40.1% and crabgrass/tall fescue was 55.7% , therefore, the forage quality may have confounded the CT effects of CH_4 emissions. Moreover, the lower NDF concentration (16.3%) in the current experiment may have prevented CH_4 from being decreased to a greater extent. Feeding a CT extract may also have different effects from administering CT via forage.

The lack of incremental CH_4 mitigation by CT in the current experiment may be due to the presence of the known methane inhibitors in the basal diet; steam-flaked corn grain, high fat corn grain by-products, fat, and an ionophore (Hristov et al. 2013). The combination of steam-flaked corn, wet distillers' grains and condensed distillers solubles in the basal diet should likely promote a low acetate:propionate fermentation which, coupled with rapid fermentability and low pH, favors inherently low methane production

(Wolin, 1960). Fat supplementation within the current experiment's diet (wet distiller's grains and yellow grease) is an indirect methanogensis inhibitor (Beauchemin and McGinn, 2005). Fat supplementation has a variety of methane inhibiting actions, the first being a hydrogen sink through biohyrdogenation, greater propionate production, and replacing less fermentable substrates that would increase methane production (Johnson and Johnson, 1995). In addition, monensin does promote lower acetate:propionate ratio within the rumen, but its effect on methane production has been inconsistent across various diet matrices (Thornton and Owens, 1981; McGinn et al,. 2004; Guan et al., 2006; Hristov et al., 2013).

Energetics

No differences ($P \geq 0.16$; Table 2.5) were observed in GE intake, DE intake, and ME intake across treatments during DOF 20 to 40. There were no differences ($P \geq 0.36$) in CH_4 energy, CH_4 energy as a percent of GE intake, and CH_4 energy as a percent of DE intake. In addition, urinary energy, urinary energy as a percent of GE intake, heat production, heat production as a percent of GE intake, and heat production per kg of metabolic BW were not different among treatments ($P \geq 0.39$). During DOF 90 to 116, GE intake, DE intake, and ME intake were not different across treatments ($P = 0.31$; Table 2.6). Moreover, CH_4 energy, CH_4 energy as a percent of GE intake, and CH_4 energy as a percent of DE intake did not different ($P \geq 0.23$) among treatments. In addition, urinary energy, urinary energy as a percent of GE intake, heat production, heat production as a percent of GE intake, and heat production per kg of metabolic BW were not different among treatments ($P \geq 0.49$). Lastly, the NRC (1996) assumes ME as a proportion of DE (ME:DE) is 82%. However, in the current experiment ME:DE was 14% greater than the 82% suggesting that the 82% conversion between DE and ME

overestimates the energetic losses. The results agree with Hales et al. (2012, 2013, and 2015). It should be noted that CH_4 emissions from hindgut fermentation were not taken into consideration.

Hales et al. (2013) fed varying levels of wet distiller's grain with solubles with a steam flaked corn diet at 2x maintenance in indirect calorimetry chambers. Hales et al. (2013) reported similar urinary energy (as percent of GE intake) and similar heat production (as a percent of GE intake). Johnson and Johnson (1995) noted a diet containing an excess of 90% concentrate had CH_4 losses ranging from 2 to 3% of GE intake, whereas Beauchemin and McGinn (2005) observed 2.8 to 4.0% loss of GE intake as CH_4; both of which are similar to the current experiment. Total urinary energy (Mcal/d) and heat production (Mcal/d) in our experiment differed from Hales et al. (2013) due to the fact the steers in the current experiment were fed at *ad libitum* intake, and Hales et al. (2013) were fed at 2x maintenance. In a different experiment, Hales et al. (2015) allowed steers *ad libitum* access to a dry-rolled corn based diet with 25% wet distiller's grains with solubles inclusion. Energy partitioning with O_2, CO_2, and CH_4 were measured by indirect calorimetry using portable head boxes. When feeding the dry rolled corn with 25% wet distiller's grains (CP = 18.31%), Hales et al. (2015) reported greater total urinary energy (Mcal/d) losses and greater urinary energy as a percent of GE intake, which would most likely be caused by the 27% increase in CP compared to the current experiment's diet. However, methane energy as a percent of GE intake and heat production as percent of GE intake were similar for both experiments. While CT sourced from quebracho trees had no effects on the energy partitioning in this trial, other sources of tannins may affect the rumen differently.

The California Net Energy System (**CNES**) was established using long-term comparative slaughter methods rather than the typical calorimetric methods used by Armsby, Kellner, Blaxter, etc. (Ferrell and Oltjen, 2008). The equation ME = RE + heat production (HP) is important in the CNES system. By knowing two of the three variables, one can calculate the third. Lofgreen and Garrett (1968) regressed ME intake on RE and calculated HP by difference. Whole-body calorimeters can be used to determine ME intake and HP, but these methods still require labor intensive digestion collections and gas-quantification equipment, which is costly and requires considerable expertise. Further, the invasiveness of whole-body calorimetry chambers can cause poor intake and requires considerable animal adaptation (Hales et al., 2015). In the current experiment, caloric intakes and energetic losses were determined by measuring DMI, fecal and urine collections, and measuring CO_2 and CH_4 emissions. By feeding steers with a Calan gate system it allowed steers to maintain pen dynamics and still measure individual feed intake. With validation of the GF system (Hammond et al., 2013; Waghorn et al., 2013; Huhtanen et al., 2013) CO_2 and CH_4 were able to be determined. Consequently, HP was determined using the equation of Brouwer (1965) assuming a respiratory quotient of 1.05. The current experiment differs from other metabolism trials on several accounts. Presumably, intake was not depressed because of metabolism stalls or calorimeter chambers. Cattle were still able to have pen interactions, and the current model theoretically accounted for the pen environment. In addition, the current model used a less invasive procedure such as the sulfur hexafluoride (SF6) procedure (Hammond et al., 2013) to measure CO_2 and CH_4. However, this model does come with limitations. Limitations include training animals to use the GF system, the GF uses spot samples to

extrapolate for an entire 24 h period, which may not take in diurnal variation into consideration. Furthermore, the GF system cannot measure hindgut fermentation. However, the ability of the current experimental model could have implications in determining true energy requirements and feed energy values in a pen or even a pasture setting because of the ability to capture samples in the steer's typical environment.

Implications

Under the conditions of this experiment, feeding condensed tannins altered route of nitrogen excretion but this impact was unrelated to performance, nitrogen balance, or energy partitioning of beef steers. It would appear that the differences in our experiment compared to others may be explained by the variability in tannin type, structure, and source. According to literature, the effects of tannins in the ruminant vary widely depending on the types, structures, and sources of tannins fed. Therefore, further research should evaluate how tannin type and source interacts with feedstuffs, amino acids, and the microbes of the rumen. Further, the methodology employed in the current experiment may serve as a less invasive method to measure beef cattle energy losses in the field.

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Table 2.1 Diet composition

Ingredient composition	DM, %
Steam flaked corn	60.6
Corn wet distiller's grains	15.0
Condensed distiller's solubles	10.0
Sorghum stalks	8.5
Trace mineral premix[1]	3.0
Limestone	1.15
Yellow grease	0.95
Urea	0.8
Salt	0.3
Analyzed nutrient composition[2]	
DM, %	58.75
CP, %	14.0
Starch, %	57.5
NDF, %	16.3
ADF, %	13.4
Ether extract, %	6.1
Ca, %	0.53
P, %	0.45
S, %	0.2
NEm, Mcal/kg[3]	2.16
NEg, Mcal/kg[3]	1.47

[1]Provided to diets (per kg of DM): 0.1 mg Co, 6.3 mg Cu, 0.6 mg I, 37 mg Fe, 27 mg Mn, 0.2 mg Se, 40 mg Zn, 1440 IU Vitamin. A, 148 IU Vitamin. D, 59 mg Vitamin E, 26 mg Monensin (Elanco Animal Health, Indianapolis, IN), and 5.9 mg Tylosin (Elanco Animal Health).

[2]Nutrient analysis conducted by commercial laboratory (Servi-Tech Laboratories, Amarillo, TX).

[3]Calculated from nutrient analyses according to NRC (1996).

Table 2.2 Effect of CT inclusion on performance and carcass characteristics during entire trial

	CT extract, % of DM[1]						
Item	0	0.5	1.0	SEM	*P*-value	Linear	Quad
No. of observations	9	9	9				
BW, kg							
Initial	337	337	335	10.8	0.99	0.89	0.94
Final	600	607	596	17.6	0.91	0.90	0.69
DMI, kg	11.3	11.8	11.2	0.43	0.57	0.80	0.31
ADG, kg/d	2.08	2.14	2.08	0.098	0.88	0.97	0.62
G:F	0.184	0.182	0.186	0.0057	0.86	0.78	0.64
Carcass characteristics							
Dressing, %	64.83	64.84	64.51	0.488	0.86	0.64	0.78
HCW, kg	389	393	385	11.4	0.89	0.83	0.67
Fat thickness, cm	1.76	1.38	1.28	0.167	0.12	0.54	0.50
Yield grade	3.06	2.96	2.86	0.312	0.90	0.65	0.99
LM area, cm^2	97.47	92.57	90.86	2.834	0.25	0.11	0.65
KPH, %	1.94	1.94	1.83	0.126	0.77	0.54	0.72
Marbling score[2]	46	50	43	2.8	0.26	0.40	0.16

[1]CT extract: Condensed tannins (By-Pro: quebracho extract, 95% tannic acid, Silvateam, Ontario, CA)

[2]30=slight, 40=small, 50=modest, ex. 42=Small 20

Table 2.3 Effect of CT on nutrient digestibility during phase 1 (after 34 DOF) and phase 2 (after 95 DOF)

	CT extract, % of DM[1]						
Item	0	0.5	1	SEM	*P*-value	Linear	Quad
Phase 1							
Intake, kg/d							
OM	9.17	10.50	10.05	4.715	0.15	0.20	0.13
Starch	4.97	5.68	5.43	2.570	0.16	0.22	0.14
Fecal output, kg/d							
OM	2.46	2.74	3.06	2.069	0.14	0.05	0.95
Starch	0.14	0.15	0.22	0.266	0.06	0.03	0.31
App. total tract dig., %							
OM	73.3	73.4	68.7	2.41	0.30	0.19	0.42
Starch	97.2	97.4	95.7	0.49	0.03	0.04	0.15
Phase 2							
Intake, kg/d							
OM	12.44	12.48	11.78	7.078	0.74	0.52	0.67
Starch	7.47	7.47	6.80	4.089	0.42	0.25	0.51
Fecal output, kg/d							
OM	3.75	3.96	4.09	2.271	0.56	0.29	0.89
Starch	0.33	0.50	0.44	0.570	0.11	0.17	0.10
App. total tract dig. %							
OM	68.9	68.2	64.4	2.16	0.30	0.16	0.56
Starch	95.5	93.2	93.4	0.84	0.13	0.10	0.24

[1]CT extract: Condensed tannins (By-Pro: quebracho extract, 95% tannic acid, Silvateam, Ontario, CA)

Table 2.4 Effect of CT on nitrogen intake, excretion, and retention during phase 1 (after 34 DOF) and phase 2 (after 95 DOF)

	CT extract, % of DM[1]						
Item	0	0.5	1	SEM	*P*-value	Linear	Quad
Phase 1							
Nitrogen, g/d							
Intake	221	252	240	11.1	0.16	0.23	0.13
Fecal	84	102	111	7.2	0.03	0.01	0.61
Urine	82	86	74	6.0	0.39	0.39	0.29
Retained	55	64	54	12.4	0.84	0.96	0.55
Urine volume, L/d	30	39	27	6.2	0.38	0.68	0.19
Fractional N excretion							
Urine, % of total[2]	49.8	45.8	39.7	2.000	0.01	<0.01	0.070
Fecal, % of total[2]	50.2	54.2	60.3	2.000	0.01	<0.01	0.070
Phase 2							
Nitrogen, g/d							
Intake	322	321	301	18.0	0.66	0.42	0.68
Fecal	126	140	146	7.9	0.21	0.09	0.68
Urine	139	150	136	10.1	0.59	0.85	0.32
Retained	57	31	19	16.5	0.27	0.12	0.71
Urine volume, L/d	41	47	33	7.5	0.44	0.44	0.31
Fractional N excretion							
Urine, % of total[2]	52.3	51.4	48.1	2.000	0.30	0.14	0.62
Fecal, % of total[2]	47.7	48.6	51.9	2.000	0.30	0.14	0.62

[1]CT extract: Condensed tannins (By-Pro: quebracho extract, 95% tannic acid, Silvateam, Ontario, CA)

[2]Sum of urine and fecal excretion

Table 2.5 Effect of CT inclusion on gas production and estimated energetics of beef steers during DOF 20 to 40

	CT extract, % of DM[1]						
Item	0	0.5	1	SEM	*P*-Value	Linear	Quad
GE intake, Mcal/d	51.1	57.1	54.2	2.15	0.16	0.31	0.10
DE intake, Mcal/d	38.3	42.9	38.6	2.22	0.28	0.93	0.12
% of GE intake	75.17	74.95	70.17	2.112	0.19	0.11	0.39
ME intake, Mcal/d	36.3	40.6	36.5	2.16	0.30	0.95	0.13
% of GE intake	71.33	71.05	66.35	2.154	0.21	0.11	0.41
ME:DE	0.95	0.95	0.94	0.003	0.69	0.46	0.67
CO_2, kg/d[2]	8.6	9.1	9.0	0.38	0.58	0.41	0.54
CH_4, g/d[2]	116	136	126	9.5	0.37	0.46	0.23
CH_4, g/kg of DMI	4.0	4.2	4.5	0.31	0.53	0.27	0.91
CH_4 energy, Mcal/d	1.53	1.79	1.66	0.126	0.36	0.47	0.22
% of GE intake	2.99	3.12	3.09	0.203	0.90	0.74	0.75
% of DE intake	10.67	11.1	11.0	0.72	0.90	0.74	0.75
Urine energy, Mcal/d[3]	0.43	0.45	0.39	0.031	0.39	0.38	0.29
% of GE intake	0.84	0.78	0.73	0.059	0.42	0.19	0.94
Heat production, Mcal/d[4]	22.9	24.3	24.1	1.01	0.59	0.41	0.54
% of GE intake	44.9	42.5	45.0	1.50	0.42	0.98	0.20
per kg $BW^{0.75}$	0.23	0.24	0.24	0.008	0.40	0.26	0.45

[1] CT extract: Condensed tannins (By-Pro: quebracho extract, 95% tannic acid, Silvateam, Ontario, CA)

[2] Collected using a GreenFeed system (C-Lock, Inc.; Rapid City, SD) from d 20 to 40 (Phase 1) and 90 to 116 (Phase 2) were used in calculations

[3] Calculated as the quotient of gross heat of urea (2.3 kcal/g) and urine N concentration, assuming urea was the primary source of energy in urine (Street et al., 1964).

[4] Calculated using the Brouwer (1965) equation, assuming an RQ of 1.05.

Table 2.6 Effect of CT inclusion of gas production and estimated energetics of beef steers during DOF 90 to 116

	CT extract, % of DM[1]						
Item	0	0.5	1	SEM	*P*-value	Linear	Quad
GE intake, Mcal/d	65.4	64.4	60.0	3.58	0.31	0.15	0.58
DE intake, Mcal/d	47.1	44.7	40.8	3.13	0.35	0.15	0.85
% of GE intake	71.35	69.41	66.70	1.991	0.24	0.09	0.87
ME intake, Mcal/d	44.1	41.6	37.7	3.02	0.31	0.13	0.85
% of GE intake	66.69	64.63	61.64	2.025	0.20	0.08	0.85
ME:DE	0.93	0.93	0.92	0.005	0.28	0.12	0.67
CO_2, kg/d[2]	12.0	11.9	12.1	0.70	0.99	0.93	0.93
CH_4, g/d[2]	173	174	190	17.8	0.74	0.47	0.75
CH_4, g/kg of DMI	5.0	5.1	6.0	0.49	0.33	0.16	0.55
CH_4 energy, Mcal/d	2.28	2.30	2.50	0.234	0.73	0.47	0.75
% of GE intake	3.54	3.55	4.35	0.391	0.23	0.13	0.41
% of DE intake	13.5	13.3	13.4	1.94	0.98	0.92	0.91
Urine energy, Mcal/d[3]	0.73	0.79	0.71	0.053	0.59	0.85	0.32
% of GE intake	1.12	1.24	1.20	0.093	0.64	0.53	0.49
Heat production, Mcal/d[4]	31.9	31.7	31.7	1.86	0.99	0.92	0.92
% of GE intake	49.6	49.8	55.8	4.44	0.49	0.28	0.59
per kg $BW^{0.75}$	0.32	0.31	0.32	0.015	0.94	0.92	0.75

[1] CT extract: Condensed tannins (By-Pro: quebracho extract, 95% tannic acid, Silvateam, Ontario, CA)

[2] Collected using a GreenFeed system (C-Lock, Inc.; Rapid City, SD) from d 20 to 40 (Phase 1) and 90 to 116 (Phase 2) were used in calculations

[3] Calculated as the quotient of gross heat of urea (2.3 kcal/g) and urine N concentration, assuming urea was the primary source of energy in urine (Street et al., 1964).

[4] Calculated using the Brouwer (1965) equation, assuming an RQ of 1.05.

CHAPTER III

Effect of corn-based supplementation on gas emissions, performance, and energetic losses of steers grazing wheat pasture

Introduction

Enteric methane production represents an energetic loss of fermentation in the rumen and lower gut of cattle. Cattle consuming forages compared to cattle consuming high concentrate diets produce greater amounts of CH_4 g/kg of DMI (Johnson and Johnson, 1995). Wheat is a valuable dual purpose crop that provides high-quality forage during winter and spring before grain harvest in early summer. Traditionally, whole-body calorimeters are used to measure gaseous emissions of ruminants. Nevertheless, whole-body calorimeters do not account for environmental factors cattle encounter in production settings. Sulfur hexafluoride (**SF_6**) is a method of measuring CH_4 emissions in a production setting, but SF_6 is a laborious and invasive procedure (Hammond et al., 2013). The GreenFeed (**GF**, C-Lock Inc., Rapid City, S.D.) system is an automated headbox that measures carbon dioxide (**CO_2**) and methane emissions (**CH_4**). Feed automatically dispenses to entice cattle to use the unit. It is considered a less invasive system relative to whole-body calorimeters and SF_6 systems (Hammond et al., 2013).

Few experiments have studied the effects of corn supplementation to cattle grazing wheat pasture and the CH_4 mitigation effects concentrates impose.

Supplementation of grain to cattle may decrease acetate:propionate and change the microbial population of the rumen, reducing CH_4 production (Hristov et al., 2013). The purpose of the current experiment was to determine gas emissions of cattle grazing wheat pasture using a noninvasive automated headbox, and to determine effects of energy supplementation on CH_4 emissions of cattle grazing wheat pasture.

Materials and Methods

All procedures involving the use of animals were approved by the West Texas A&M University/CREET Institutional Animal Care and Use Committee (approval number 41014).

Thirteen Angus-cross steers (initial BW = 417 $\pm$ 13 kg) were used in a crossover design to determine the effects of concentrate supplementation on greenhouse gas emissions of steers grazing wheat pasture from March 8 to May 7, 2015. Steers were weighed two consecutive days (d0 and d1) to minimize effects of gut fill. Steers were randomly assigned to 1 of 2 treatments; wheat pasture plus 0.2 kg of pelleted wheat middlings (**CON**) or wheat pasture plus 0.2 kg pelleted wheat middlings and a corn-based supplement fed at 0.5% of BW (**SUPP**; Table 3.1). The CON treatment was provided to dose steers with external marker and to establish equal daily handling of steers. The experiment consisted of two 30 day periods (Period 1: March 8 to April 7; Period 2: April 8 to May 7, 2015). After the first 30 day period, steers were alternated to the opposite treatment. Steers were allowed *ad libitum* access to a single 8.3 ha of wheat (*Triticum aestiuum*; Table 3.2*)* for 23 h/d with the remaining hour used for treatment supplementation. Steers were given access to an additional 2.7 ha in the second period to ensure *ad libitum* access of wheat forage because of concerns with lack of precipitation decreasing forage availability. Forage availability, determined using a clipping square,

before the experiment equaled 1,344 kg/ha, and after first period forage availability equaled 1,130 kg/ha. After addition of the 2.7 ha, forage availability increased to 1,376 kg/ ha. Steers were gathered each day at 1100, and sorted into 13 individual pens. Steers were allotted one hour to consume supplement and orts were collected. Treatments were readily consumed and only one steer left a measurable amount of orts during the collection period. All steers had access to a free choice mineral with magnesium oxide (10% magnesium oxide; Hi-Pro Feeds, Friona, TX) and access to a mineral block containing polaxalene (Sweetlix Livestock Supplement System, Mankato, MN) to mitigate frothy bloat. The mineral block was formulated to administer 15 g of polaxalene/d.

Titanium dioxide (**TiO_2**) was used as an external marker to estimate fecal output (Titgemeyer et al., 2001). Titanium dioxide was mixed with the pelleted wheat middlings, and provided 15 g of TiO_2 per head/d for 14 days leading up to each collection and the six days during each collection. Orts were collected and analyzed for residual TiO_2.

Fecal collections were taken twice daily for six consecutive days starting on d 24 of each period to determine digestibility and energetic losses. Time was advanced two hours every 24 hours to minimize effects of diurnal variation. Steers were gathered, walked to a working chute on site, and rectally palpated for a fecal sample. Fecal samples were composited at end of collection period.

During the experiment, steers had access to a GreenFeed (**GF**, C-Lock Inc., Rapid City, S.D.) unit, which measures CO_2 and CH_4 emissions (Hammond et al. 2013; Waghorn et al. 2013). For more comprehensive descriptions of the GreenFeed system, see Hristov et al. (2015) and Huhtanen et al. (2015). Briefly, the GF system is a stationary

headbox that uses feed to entice steers to use the system. As the steer enters the headbox, a a radio-frequency identification (**RFID**) tag reader recognizes the steer and drops a controlled amount of pelleted feed. While the steer stands with their head in the headbox and consumes an allotment of feed, the GF system measures a 3 to 5 minute spot sample of CH_4 and CO_2 the steer exhales. Exhaled gases are pulled through a tube under negative pressure where airflow mass is measured continuously. Measurement of CH_4 and CO_2 concentration are analyzed using on-board nondispersive infrared sensors, production estimates are then calculated, and adjustments made to standard temperature and pressure. The GF is also equipped with a proximity sensor and any time a steer removed their head or did not consume the allotment of feed for the full 3 to 5 minute spot sample the data collected was discarded. The GF was programmed to allow six 3 to 5 minute spot samples per day with a four hour gap between feeding events. Weekly gas calibrations and monthly CO_2 recovery tests were performed to ensure proper sensor function. Carbon dioxide recovery tests were greater than 95% recovery. Commercially produced pellets (14% CP; Cargill Animal Nutrition, Minneapolis, MN) were used as a treat reward to entice the steers to use the GF. The transition pellets consisted of a mix of roughage and by-products and were intended to transition steers to a concentrate diet. Steers were allowed a total of 500 g of pellets per day over six different feeding events. Two steers in the first period and one steer in the second period did not utilize the GF unit. Their data were not used to determine means for energetic losses or gaseous emissions.

Laboratory Analyses

Dry matter of feces, feed, and orts were analyzed by drying samples to a constant weight at 55° C in a forced-air oven for 48 hours. Wheat forage samples were taken daily

during each collection period. Samples were then ground through a 1mm screen using a Wiley Mill (Model 4, Thomas-Wiley, Philadelphia, PA). Lab corrected DM on fecal, ort, wheat, and supplement samples were conducted with forced-air oven at 105° C for 24 hours. Ash content was determined by combusting fecal, ort, and supplement samples in a muffle-furnace at 450^{o} C for 8 hours. Gross energy of feces and feed were determined using an automatic bomb calorimeter (Parr 6400 Calorimeter, Parr Instrument Company, Moline, IL). Total N of feed and feces were determined by combustion in a C/N analyzer (Elementar C/N Vario Max Cube, Elementar Americas Inc., Mt. Laurel, NJ). Starch content of feed and feces was determined using an enzyme kit (Megazyme International Ireland Ltd., Wicklow, Ireland; method 996.11; AOAC, 2003).

Concentration of TiO_2 in fecal and ort samples were determined with a spectrophotometer (Synergy 2, Biotek, Winooski, VT) using the method described by Myers et al. (2004). Blank fecal background was used to correct for baseline TiO_2 in fecal samples (Morgan et al., 2014). A fecal background sample was collected from steers prior to TiO_2 dosing.

To determine wheat forage digestibility, wheat forage was ground to 1mm and 1g samples were weighed into F57 filter bags (Ankom Technology, Macedon, NY), and incubated in 250 ml bottles for 48 h at 39° C using an in vitro gas production system (Ankom Technology, Macedon, NY). Wheat samples were analyzed in triplicate with triplicate blanks as a correction. Ruminal fluid was used from two steers consuming a ration in excess of 50% forage. Ruminal fluid was strained through 4 layers of cheese cloth after collection. A 2:1 McDougall's buffer:ruminal fluid was used for the

incubation. A subsequent NDF analysis on the filter bags was used to determine true IVDMD.

Calculations

Dry matter intake of wheat forage was calculated by dividing fecal output by in vitro DMD and then subtracting the known intakes of the supplements (Merchen, 1988)

Using CH_4 and CO_2 estimated from the GF unit, and assuming urine energy was 1.4% of GE intake (unpublished data), and a respiratory quotient of 1.05, heat production was calculated using the equation of Brouwer (1965). Recent reports, using whole-body calorimetry chambers (Hales et al., 2012) and head boxes (Thornton and Owens, 1981; Hales et al., 2015) suggest an RQ near or less than 1.0. The RQ value of 1.05 was chosen because by definition, an RQ of greater than 1.0 suggests the accretion of fat, and given the physiological stage of the steers, fat would be deposited greater than protein. Biological limits for RQ have been reported to be from 0.7 for fasting animals to greater than 1.2 for force-fed animals (Blaxter and Wainman, 1965). While the possibility exists for RQ to vary by treatment, other calorimetry research has not reported this (Hales et al., 2012, 2015).

Statistical Analysis

Growth performance and gaseous emissions were analyzed as a completely randomized crossover design (PROC MIXED; SAS Institute Inc., Cary, NC) as a mixed model with one-way treatment structure, with animal serving as the experimental unit. The class statement included steer and period. The model statement included treatment, period, and treatment *x* period. No interactions between treatment and period were detected ($P > 0.10$); therefore main effects of treatment are reported. Means separation

and *P*-values were determined using LSMEANS. Treatment differences were discussed when $P \leq 0.05$; tendencies were discussed when $P > 0.05$ and < 0.10.

Results and Discussion

Intake and Digestibility

Steers provided the corn-based supplement had greater ($P < 0.01$; Table 3.3) DMI (forage and supplement) than non-supplemented steers. Forage intake was not influenced ($P = 0.13$) by SUPP, and forage intake as a percent of BW was not influenced ($P = 0.15$) by SUPP. Intake estimates in the current experiment agree with forage intake estimations from other experiments (Andersen and Horn, 1987; Branine and Galyean, 1990). Branine and Galyean (1990) supplemented cattle grazing wheat pasture (> 20% CP) with 0.5 kg of a steam-flaked milo and observed no differences in intake between cattle consuming the supplement and cattle consuming no supplement. Branine and Galyean (1990) offered one third of the grain relative to the grain offered in the current experiment. Judkins et al. (1997) supplemented steers, grazing fescue pasture (15% CP; Table 3.2), ground corn 0.4% of BW, and did not observe a reduction in forage intake compared to steers not supplemented. Concentrate supplementation to ruminants consuming low-quality forage is reported to cause decreased forage intake and decreased forage digestibility (Mould et al., 1983; Horn and McCollum, 1987; Moore et al., 1999). However, the negative associative effect of concentrate supplementation and grazed forage is typically observed with consumption of low-quality forage. Forage with concentrations of CP above 15% may eliminate the negative association effects of concentrate supplementation of steers consuming forage. Moore et al. (1999) describes a TDN:CP of the diet above 7 as the threshold for decreased forage intake. The CP content of the wheat (15% or greater) in the current experiment and other experiments may cause a sufficient "surplus" of CP that

supplemented concentrate did not increase the TDN:CP above seven. Olson et al. (1999) ruminally dosed increasing levels of starch concurrently with increasing levels of rumen degradable protein (**RDP**) to steers consuming low-quality forage. Starch decreased forage intake as expected, but increasing levels of supplemental RDP mitigated the intake effects of the starch.

Organic matter digestibility and NDF digestibility were not affected ($P \geq 0.47$) by SUPP. Forage digestibility is a possible concern when supplementing forage diets with a concentrate, as the reduction in pH from rapid fermentation of concentrates produced in the rumen is harmful for cellulolytic species (Horn and McCollum, 1987). Hess et al. (1996) supplemented steam-flaked corn at 0.34% of BW to ruminally-cannulated steers grazing endophyte-free fescue and reported no differences in NDF digestibility or OM digestibility after a 96 h in situ digestion. Judkins et al. (1997) reported no differences in NDF and OM digestibility when supplementing steers grazing fescue with ground corn at 0.4% of BW. Elizalde et al. (1998) supplemented dry-rolled corn (1.4 kg/d) to steers grazing native tall fescue (>20% CP) and reported no differences in NDF and OM digestibility between steers receiving corn supplement and steers not receiving supplement. The lack of differences in the current experiment of NDF and OM digestibility may be explained by the "surplus" of dietary CP provided by forage CP as explained above. Olson et al. (1999) reported tendencies for CP to mitigate starch effects on digestibility, similar to the intake effects of starch.

Klevesahl et al. (2003) ruminally dosed increasing levels of starch concurrently with increasing levels of RDP to steers consuming low-quality forage, and reported decreased NDF digestibility with increasing starch. However, rumen pH of steers was not

less than 6.3 among treatments, suggesting no effect of pH on fiber digestion. Olson et al. (1999) reported differences in fiber digestion among treatments, but pH was not less than 6.25. Reductions in digestibility may be due to the "carbohydrate effect" (Mould et al., 1983; Arroquy et al., 2005). According to Arroquy et al. (2005), the "carbohydrate effect" decreases digestion because ruminal microbes have a greater affinity for more readily digestible nutrients, and the decreased cellulose digestion is not entirely caused by a reduced pH. Wheat is more readily digestible than low-quality forages, which may also explain the lack of differences observed in the current experiment.

Performance

Average daily gain among treatments was not different ($P = 0.54$; Table 3.3), but G:F was less ($P < 0.01$) for steers consuming the SUPP. The decrease in the G:F is due to an increase in DMI without a concurrent increase in ADG. Horn et al. (1995) supplemented steers grazing wheat pasture with a 1.22 kg/d of ground corn, and reported a greater ADG for steers consuming the ground corn supplement compared to steers without supplementation. Hess et al. (1996) supplemented steam-flaked corn at 0.34% of BW while ruminally-cannulated steers grazed endophyte-free fescue and reported increased ADG of steers consuming the supplement compared to steers not supplemented. The wheat forage had similar energy content to SUPP may explain the lack of performance differences between treatments. Concentrate supplementation typically increases performance of cattle grazing forages (Horn and McCollum, 1987), but SUPP in the current experiment was 69.3% concentrate. Had SUPP been 100% concentrate, a performance advantage may have been observed. Also, the period length may not have been sufficient to observe performance differences.

Gaseous Emissions and Energetic Losses

Whole-body calorimetry is the standard method for measuring gaseous emissions and energy metabolism of cattle (Ferrell and Oltjen, 2008). However, whole-body calorimeters cannot measure gaseous emissions of grazing animals. The GF system is portable, and more than one animal can utilize the system. Thus, GF can accompany animals in a pasture setting, and potentially account for environmental factors not accounted for in the controlled environment of whole-body calorimetry. With the use of respiratory quotients and the heat production equation of Brouwer (1965), one can estimate net energy.

Carbon dioxide exhaled (kg/d) tended ($P = 0.08$; Table 3.4) to increase with SUPP. Methane emissions (g/d) were not influenced ($P = 0.43$) by treatment. However, CH_4 excreted per kg of DMI was decreased ($P = 0.02$) by 20.5% for steers consuming SUPP. The SUPP did not affect ($P = 0.11$) CH_4 emissions when expressed as g/kg of NDF intake. There were no differences ($P = 0.43$) in Mcal of CH_4 produced between treatments, but CH_4 as a percent of GE intake was decreased ($P = 0.02$) 21.6% when steers consumed the SUPP. Lastly, Mcal of heat production (**HP**) produced was not different ($P = 0.11$) among treatments, but HP as a percent of GE intake was 20.6% lower for steers consuming the SUPP.

Methane lost as a percent of GE intake in the current experiment agrees with reported CH_4 emissions (Johnson and Johnson, 1995). Methane lost as a percent of GE intake was predicted to be 6 to 8% by Johnson and Johnson (1995) and the numbers observed from the GF system in the present experiment (CON: 8.21%; SUPP: 6.44%) aligns with those predictions. The reduction CH_4 as a percent of GE intake in the current trial between treatments may be attributed to a greater propionate production at the

expense of acetate production (Hristov et al., 2013). A greater amount of propionate produced in the rumen, generally causes a reduction in available hydrogen for CH_4 production. Olson et al. (1999) and Klevesahl et al. (2003) reported increased ruminal propionate production with starch supplemented at a minimum of 0.15% of BW. Another possible reason for the reduction in CH_4 as a percent of GE intake is due to the increased total DMI by steers consuming the SUPP. Johnson and Johnson (1995) reported an increased level of intake decreased CH_4 by 1.6% with each level.

McGeough et al. (2010) fed whole crop wheat silages (**WCW**) with varying grain to forage ratios, (range from 11% to 47% grain to steers), and reported a 14% decrease in grams of CH_4 excreted per kg of DMI as grain increased. The reported amounts of CH_4 per kg of DMI were 25% greater than the grams of CH_4 per kg of DMI reported in the current experiment, which could be explained by the lower digestibility of the forage in WCW. Furthermore, McGeough et al. (2010) reported a similar loss of CH_4 as a percent GE intake, and reported a 23% decrease in CH_4 as percent of GE intake when steers consumed the WCW with the greater concentration of grain. Zhao et al. (2015) measured CH_4 emissions of sheep using whole-body calorimeter chambers after feeding fresh-cut forage at *ad libitum* intake, and supplementing 0.5 kg of a concentrate ration containing 33% barley grain, 25% beet pulp, and 25% soybean meal. Zhao et al. (2015) reported no differences among treatments in CH_4 emissions when corrected for DMI and when expressed as a percent of GE intake among sheep. Even though the supplement was fed at 1% of BW, the supplement may not have contained sufficient starch to observe the same results as the current experiment, which had 65% dry-rolled corn in the supplement.

Implications

Under the conditions of this experiment corn supplementation to cattle grazing wheat pasture altered CH_4 emissions, most likely from increased DMI. Corn supplementation may be a potential opportunity to mitigate CH_4 emissions of livestock grazing forage and help relieve concerns over livestock air pollution.

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Table 3.1 Composition of supplements

Ingredient, % DMB	CON[1]	SUPP[1]
Pelleted wheat middlings	87.1	30.7
Dry-rolled corn	0.0	65.5
Molasses	4.6	3.8
TiO_2	8.3	0.0
Analyzed nutrient composition, % DMB		
Starch,	18.6	51.3
CP,	17.0	11.6

[1]CON = 0.2 kg of pelleted wheat middlings; SUPP = supplemented 0.5% BW of corn-based supplement plus 0.2 kg of pelleted wheat middlings

Table 3.2 Composition of wheat forage

Item, % DMB	Period 1[1]	Period 2[1]
DM	39.7	36.6
CP	20.9	15.1
NDF	37.3	40.5
ADF	22.0	25.4
IVDMD	84.4	78.4

[1]Period 1 (March 8 – April 7) Period 2 (April 8 – May 7)

Table 3.3 Performance and nutrient digestibility of steers grazing wheat pasture and consuming a corn-based supplement

	Treatment[1]			
Item	CON	SUPP	SEM	*P*-value
No. of observations	13	13		
Initial BW, kg	417	417	5.6	0.95
Final BW, kg	497	497	6.7	0.96
DMI, kg	14.4	18.1	0.75	<0.01
Forage intake, kg[2]	14.0	15.8	0.75	0.13
Forage intake, % of BW[2]	3.22	3.61	0.181	0.15
ADG, kg	1.36	1.30	0.07	0.54
G:F	0.098	0.073	0.0054	<0.01
App. total tract digestibility, %				
OM	84.9	84.6	0.47	0.71
NDF	82.5	83.1	0.84	0.63

[1]CON = 0.2 kg of pelleted wheat middlings; SUPP = supplemented 0.5% BW of corn-based supplement plus 0.2 kg of pelleted wheat middlings.
[2]Determined using IVDMD of wheat forage and TiO_2 as a marker for fecal output

Table 3.4 Gas emissions of steers grazing wheat pasture consuming a corn-based supplement

	Treatment[1]			
Item	CON	SUPP	SEM	*P*-value
No. of observations	12	11		
CO_2, kg/d[1]	9.8	10.5	0.30	0.08
CH_4, g/d[1]	334	351	15.0	0.43
CH_4, g/kg of DMI	24.4	19.4	1.48	0.02
CH_4, g/kg of NDF intake	63.7	53.8	0.42	0.11
CH_4 energy, Mcal/d	4.4	4.6	0.20	0.43
CH_4, % of GE intake	8.21	6.44	0.499	0.02
Heat production, Mcal/d[1]	25.3	27.2	0.81	0.11
% of GE intake	47.86	38.01	3.147	0.03

[1]CON = 0.2 kg of pelleted wheat middlings; SUPP = supplemented 0.5% BW of corn-based supplement plus 0.2 kg of pelleted wheat middlings.

[2] Collected using a GreenFeed device (C-Lock, Inc.; Rapid City, SD)

[3] Calculated using the Brouwer (1965) equation, assuming an RQ of 1.05, and assuming urine energy is 1.4% of GEI.