EFFECTS OF HAY MATURITY ON THE ENTERIC METHANE EMISSIONS, FORAGE INTAKE, ENERGY METABOLISM AND DIGESTION BY BEEF HEIFERS

By

EMALEE JEAN FRIEND

Bachelor of Science in Plant and Soil Science

Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

Dr. S.A. Gunter

Thesis Adviser

Dr. P.A. Beck

Dr. A.P. Foote

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Abstract: Ruminants are a primary source of food security and play an essential part in sustainable agriculture. Ruminants consume fibrous feedstuffs and convert them to highly nutritional food and consumable products. While doing this, ruminants produce greenhouse gases that contribute to climate change. Enteric methane is produced as a byproduct of the fermentation of ruminants. Enteric methane production is also an energy loss to the ruminant system. Forage quality has an impact on the amount of enteric methane that is produced. As forages mature, nutritive value declines with declining crude protein and increasing fiber content. Enteric methane production tends to increase as the quality of forages decline. Due to the impact enteric methane production has on the environment and to the producer, it is important to research ways to mitigate enteric methane production. Globally, wheat is grown on more than 240 million hectares. Along with grain it provides pasture, hay, silage, and straw as feed for ruminants. Therefore, our objective of this experiment was to investigate the effects that feeding heifers long-stemmed wheat hay cut at three different maturities had on intake, digestion, metabolism, and enteric methane emission by beef heifers. Twelve heifers were used in a 34-day feeding experiment fed over three 7-day periods. Heifers were randomly assigned to 1 of 3 maturities including wheat hay cut at the stem elongation stage, wheat hay cut at the booting stage, and a mature wheat hay cut at the milk grain stage. Hay was fed at ad libitum plus a daily supplement of pellets were offered via an automated head-chamber system that measures carbon dioxide and methane emissions, and oxygen consumption while supplement was consumed. The results of our experiment showed that dry matter intake decreased linearly as the wheat matured. Hay maturity had a significant negative linear effect on metabolizable energy intake and digestible energy intake. As the hay matured, heat production decreased linearly. Hay maturity had a decreasing linear effect on total enteric methane emissions but did not have an effect on methane yield as expressed as g of methane/kg of DMI. However, carbon dioxide emissions and oxygen consumption decreased linearly as wheat matured. Digestibilities of the immature, intermediate, and mature hay were 76.22%, 68.01%, and 58.57%, respectively. These results indicate that as forage matures it has a negative effect on dry matter intake, carbon dioxide emissions, oxygen consumption, heat production, metabolizable energy intake and digestible energy intake, but did not have an effect on methane yield.

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ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
AHCS	Automated head chamber system
AIA	Acid insoluble ash
BMP	Best management practices
BW	Body weight
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
EBG	Empty body weight gain
EQSBW	Body weight equivalent to medium framed steer
EWB	Empty body weight
FA	Fatty acids
FSBW	Final shrunk body weight
GE	Gross energy
GQS	Gas quantification system
H_2	Hydrogen
HE	Heat energy
HI	Heat increment
HP	Heat production
Kcal	Kilocalorie
Kg	Kilogram
Mcal	Megacalorie
ME	Metabolizable energy
N	Nitrogen
N_2O	Nitrous Oxide
NDF	Neutral detergent fiber
NEm	Net energy for maintenance
NEr	Retained net energy
NFC	Non-fiber carbohydrate
N_{intake}	Nitrogen intake
NPN	Non-protein nitrogen
N_{urine}	Urine nitrogen excretion rate
O_2	Oxygen
OM	Organic matter
PR	Passage rate
RC	Respiration chamber
RE	Retained energy
RQ	Respiratory quotient
RRT	Ruminal retention time
RUP	Ruminal undegradable protein
SBW	Shrunk body weight
SDG	Sustainable Development goal
SF_6	Sulfur hexafluoride tracers
SRW	Standard reference body weight for the expected final body fat
SWG	Shrunk weight gain
TDN	Total digestive nutrients
VFA	Volatile fatty acids

CHAPTER I

INTRODUCTION

In recent decades, global atmospheric concentrations of carbon dioxide (**CO_2**), methane (**CH_4**), and nitrous oxide (**N_2O**) have risen due to human activities (IPCC, 2013). Fossil fuel use is the largest source of CO_2 in the United States, while methane sources include leaks from natural gas systems, raising of livestock, and natural sources such as natural wetlands (E.P.A., 2022). Twenty-five percent of the United States methane emissions are from enteric fermentation (E.P.A., 2022). Sources of N_2O include agriculture, fuel combustion, wastewater management, and industrial processes (E.P.A., 2022). One of the goals of the 2015 Paris Agreement of the United Nations Framework Convention on Climate Change is to limit global warming to 2° C above pre-industrial levels. The United Nations has also agreed to Sustainable Development Goals (**SDG**) to achieve sustainable development by 2030 (Assembly, 2015). Some of the goals are no poverty (SDG 1), no hunger (SDG 2), and climate action (SDG 13). Methane will need to be reduced by 24 - 47% by 2050 relative to 2010 to meet the 2.0° target (Arndt et al., 2021).

Increases in income and human population will result in an estimated gross increase of 70 to 80% in meat and milk demand compared to current levels (Drewnowski and Popkin, 2009; Hu, 2011), this includes a 69% increase in beef consumption (Godfray et al., 2018).The global meat production was 318 million tons in 2016 and the Food and Agriculture Organization (FAO) estimates an increase to 455 million tons by the year 2050 (FAO, 2022). As the supply of meat

and dairy products increases, so does agriculture's environmental footprint (Lee et al., 2017). Hence, developing means for mitigating enteric emissions from ruminants are essential to meeting the SDS goals set by the United Nations.

Ruminants are significant sources of enteric CH_4 (Gill et al., 2009). Microbial fermentation in the rumino-reticulum allows ruminants to digest fibrous feedstuffs not usable to humans and monogastric livestock (Newbold and Ramos-Morales, 2020). During microbial fiber digestion, enteric CH_4 is produced and emitted to the environment through eructation (NASEM, 2016). During this process, 2 to 12% of gross energy in feed is converted to CH_4 gas in the rumen (Johnson and Johnson, 1995). Variation in CH_4 production are related to dry matter intake (**DMI**) and the composition of the diet (Johnson and Johnson, 1995). There are many factors that affect CH_4 emissions through these two mechanisms. Some of the factors are level of intake, type of carbohydrate, the addition of lipids, and the addition of ionophores to the diet (Johnson and Johnson, 1995). Typically, as the ruminally digestible carbohydrate intake increases, so does the CH_4 emissions (Beauchemin et al., 2007).

Globally, ruminants contribute around 11.6% and cattle contribute 9.4% of all the anthropomorphic greenhouse gas emissions (FAOSTAT, 2022), while playing an essential part in sustainable agriculture by consuming fibrous feedstuffs and converting them to highly nutritional food (milk and meat) and consumable products (wool) for humans. Eighty-six percent of feed intake by livestock is made of materials that are not consumed by humans. Grass and leaves represent more than 57% of the ruminants intake (FAOSTAT, 2022).

Ruminants are one of the few sources of CH_4 production we can manipulate (Johnson and Johnson, 1995). They are also a primary source of food security (Smith et al., 2013) and represent the largest land-use system on Earth (Herrero et al., 2015). The combined economic benefits to the livestock industry and environmental benefits to society by decreasing ruminant CH_4

emissions makes research of the factors impacting CH_4 production important (Johnson and Johnson, 1995).

Winter wheat (*Triticum aestivum* L.) grown in Southern Great Plains of the United States can be used for grain or forage only, or for the dual purpose of both forage and grain (Redmon et al., 1995). Dual purpose wheat is grown on about 8 million hectares in southern Kansas, Oklahoma, and Texas (Lollato et al., 2017). The practice of grazing wheat is also common in Argentina, Australia, Morocco, Pakistan, Syria, and Uruguay (Epplin et al., 2000). Wheat pasture is high in protein, energy, and low in fiber, making it a source of high-quality forage (Hossain et al., 2003). It is also comparable to alfalfa (*Medicago sativa* L.) in terms of crude protein and digestibility (Hossain et al., 2003), which globally, is the main hay used in livestock production (Ronga et al., 2020). In the fall, lightweight calves from the Southeast, Midwest, and West are imported to the Southern Plains to graze winter wheat pastures (Epplin et al., 2000) to heavier weights before entering feedlots for finishing. In the southern plains, wheat is grazed from late November until early March, which is typically when other forage sources are low in quantity and quality (Hossain et al., 2003). If grain prices are low and cattle prices are high, producers will graze cattle on the wheat until June, or when wheat is no longer suitable for grazing, forgoing a grain crop (Stewart et al., 1981).

Globally, wheat is grown on more than 240 million hectares, which is larger than any other crop (Curtis, 2022). In the United States, wheat ranks third among field crops in planted acreage, production, and gross farm receipts (USDA-ERS, 2022). Around the globe in places lacking rainfall and winter-spring temperatures up to 25°C, wheat is the main forage used as hay, silage, or straw to provide feed to ruminants (Shaani et al., 2017). Wheat is tolerant to abiotic stresses, making it an important resource for livestock feed (Ronga et al., 2020). Harvesting wheat at different growth stages can have an impact on both yield and quality (Buxton, 1996). As the plant matures, yield increases but the nutritive quality decreases (Minson, 1990). Protein

concentrations declines while fiber content increases (Jung and Allen, 1995). Voluntary intake generally declines as forages advance in maturity, this is due to gut fill in the animal from the increase in neutral detergent fiber **(NDF)** concentration (Buxton, 1996). Declining crude protein and increasing NDF and acid detergent fiber **(ADF)** is thought to lead to increasing greenhouse gas (**GHG**) emissions while decreasing digestibility and livestock productivity.

The purpose of our study was to investigate the effects that feeding beef heifers long-stem wheat hay harvested at three different maturities had on forage intake, forage digestibility, energy metabolism and enteric CH_4 emissions when offered on an ad libitum basis.

CHAPTER II

REVIEW OF LITERATURE

Forage Maturity and Quality

Measures of Forage Quality

Ruminants have the unique ability to utilize high-fibrous plant material by fermentation through microbial actions to obtain greater energy from the plants when compared with monogastrics. Over 80% of the total feed consumed in the beef production system is forages (McGeough et al., 2012). Van Soest (1987) stated that forage quality is the most crucial factor influencing ruminant productivity. Forage quality can be defined as the relative performance of animals when consuming herbage on an ad libitum basis (Buxton et al., 1996). The quality of forage is the product of nutrient concentration, potential intake, digestibility, and metabolism within the animal (Buxton et al., 1996). The most important aspects of forage nutritional value are digestibility and crude protein (**CP**) concentration (Guyader et al., 2016) because energy and protein are the major nutritional constituents required by the ruminant for maintenance and growth. As forage digestibility increases, passage rate (**PR**) increases, leading to increasing forage intake (Deramus et al., 2003). As a plant matures, the cell wall concentration within the stems and leaves increase and cell solubles decrease, and as a result, digestibility of the forage declines (Buxton, 1996). Forage nutritive quality encompasses the quantity of plant cell walls, optimal digestibility and rate of digestion (Van Soest, 1987).

Influence of Plant Maturity on Nutritive Quality

A major factor affecting forage quality is the stage of development or maturity. As forages grow, the proportion of stems and cell wall constituents increase as the proportion of leaves decreases (Jung and Allen, 1995). Typically the leaves are of higher quality than the stems (Van Soest, 1987). However, this statement is based on the function of the leaves and stems. In alfalfa, for example, the stems are structural organs, and the leaves are metabolic organs; therefore, leaves maintain their quality as the plant ages (Van Soest, 1987). As most plants mature, they produce more stems in relation to leaves. Stems are lower in digestibility than leaves, and digestibility declines more rapidly with increased plant maturity than that of leaves (Jung and Allen, 1995); thus fibrous cell wall constituents increase. Also, with increasing maturity, fibrous cell wall constituents become more lignified. Lignification causes a decrease in structural carbohydrate degradability in the rumen (Himmelsbach, 1993). There is a strong correlation between the decline of forage quality and the proportion of lignified structural tissue (Van Soest, 1987). As the amount of lignin increases so does ADF, since ADF is composed mainly of cellulose and lignin. Lignin and ADF are negatively related to digestibility of forages (Jung and Allen, 1995). As grasses advance in stage of maturity, NDF increases while nutrient levels decline (Horrocks and Vallentine, 1999). The intake potential of a forage is inversely related to NDF (Buxton, 1996). Intake is limited by the filling effect of a forage. The filling effect is linked to its cell-wall concentration and rate of disappearance of cell walls from the rumen by digestion and passage (Buxton, 1996). Fiber ferments and passes from the reticulorumen more slowly than the non-fiber constituents of feeds (Jung and Allen, 1995), therefore, with an increase in NDF, there is a decrease in intake due to the high amount of fiber being consumed and slowing down digestion. Harvesting forage at an earlier stage of maturity increases fiber digestibility, CP, and voluntary intake (Van Soest, 1965).

Beck et al. (2009) examined the impacts of cutting wheat forage at different maturity levels on animal performance. Dry matter (**DM**) yield, chemical composition, digestibility, passage kinetics, and performance of growing calves were evaluated when fed mixed diets containing 20 or 40% wheat forage cut at the boot and dough stages. The forage that was cut at the dough stage yielded 125% more dry matter than the forage cut at the boot stage, with 6.3 percent units less CP. They found that detergent fiber concentrations were not affected by maturity, but the non-fiber carbohydrates (**NFC**) were greater in the forage cut at the dough stage. When included in the diet at 20% of DM, maturity of the forage did not have an effect on PR, ruminal retention time (**RRT**), or fecal output. When the diet consisted of 40% wheat hay, they saw greater total tract DM digestibility as well as a greater total tract NDF digestibility with boot stage hay compared with the dough stage. They found no difference in body weight (**BW**), BW gain or average daily gain (**ADG**) when growing calves were fed a total mixed diet containing 20% or 40% wheat forage. The authors stated that even though ADG was not different in the experiment, increased maturity of forages can cause differences in DMI, total tract DM, and NDF digestibility of growing calves at the higher inclusion rates.

Cell Wall and Plant Cell Contents

Minson (1990) developed a model to link plant anatomy to chemical composition as the foundation for the variations of the potential digestibility of the various nutrient fractions. The model is based on the idea that forages are made up of two major components: cell contents and cell wall. The cell contents contain most of the organic acid, soluble carbohydrates, CP, fats, and soluble ash (Van Soest, 1987). The cell wall constituents include hemicellulose, cellulose, pectin, lignin, cutin, silica, and minerals that resist the normal digestive processes (Hatfield, 1989; Van Soest, 1965). The cell-wall portion of the forage impacts the intake by ruminants (Waldo, 1986), makes up 40-80% of the forage, and is the less digestible part of the plant cell (Horrocks and Vallentine, 1999). The microbial degradation of cellulose rarely is complete; much of the

cellulose is unchanged, especially if the cellulose is lignified, and excreted in the feces (Blaxter and Czerkawski, 1966). The proteins, non-protein nitrogen, lipids, and other solubles are highly digestible, but the sugars, starch, pectin, and other soluble carbohydrates are essentially completely digestible (Horrocks and Vallentine, 1999).

Protein

Protein is essential for muscle, milk, wool, hair production and maintenance. After energy, protein is the nutrient that most commonly limits animal production (Minson, 1990). Since nitrogen is the building block for amino acids that forms protein, the amount of nitrogen in the forage is used to determine CP in laboratory analysis. Nitrogen can be divided into true protein and non-protein nitrogen (**NPN**). Non-protein nitrogen includes nucleic acid, free amino acids, amides, and nitrates (Buxton et al., 1996). In the rumen, NPN is converted to ammonia by ruminal microbes and then is incorporated into microbial protein. The limitation of microbial protein synthesis is ruminally availability carbohydrates to provide energy for microbial growth (Buxton et al., 1996). True protein consists of cytoplasm and chloroplasts and makes up 60-80% of the herbage nitrogen (Van Soest, 1987). Buxton and Marten (1989) found that the concentration of CP declined linearly with increasing maturity in all species due to the decrease in CP concentrations of both the leaves and stems and because the stems made up a large portion of total herbage as the reproductive tillers matured. Ruminal undegradable protein (**RUP**) (dietary protein not hydrolyzed in the rumen) as a percentage of CP increases as plants mature (NASEM, 2016).

Carbohydrates

Carbohydrates are the primary energy source for maintenance and the production processes associated with growth, pregnancy, and lactation (Armstrong, 1965). Carbohydrates provide the physical fiber needed to stimulate rumination and reticuloruminal motility and supply

digestible energy to both the ruminal microorganisms and the animal itself (NASEM, 2016). Carbohydrates have both digestive and physiological roles and have the broadest range in digestibility of any nutrient. Digestibility of carbohydrates ranges from 100% for sugars to 0% for indigestible fiber and, combined with lignin, makeup 70 to 80% of the portion of the diet (Hall and Mertens, 2017).

Both the chemical and physical characteristics of carbohydrates affect rumen function, the ruminal fermentation pattern, metabolism and production (Hall and Mertens, 2017). The carbohydrate digestibility has an immense impact on digestible nutrients and net energy of forages (Hall and Mertens, 2017). Carbohydrates can be classified into two categories based on their chemistry and functionality; structural and nonstructural (NASEM, 2016).

Nonstructural Carbohydrates. Nonstructural carbohydrates are the cell contents and contain simple sugar, starch, and hemicellulose, which are easily digestible. Starch is the main storage of carbohydrates in plants and is found abundantly in cereal grains. Starch is the main energy component in ruminant diets due to its availability (Gómez et al., 2016). The bacteria fermenting starch produce a higher proportion of propionic acid than the bacteria that ferments cellulose or hemicellulose (Orskov, 1986). Particle size and gelatinization of starch play a prominent role in its digestion and utilization. (Hall and Mertens, 2017). In cereal grains, starches are found in the endosperm. Starch granules contain amylopectin, amylose, and noncrystalline amylopectin (Svihus et al., 2005). Starches from cereal grains are typically composed of 16 to 35% amylose and 65 to 84% amylopectin (Svihus et al., 2005). If the grain does not get processed sufficiently, the ratio of amylose:amylopectin is usually negatively correlated with starch digestion (Foley et al., 2006). Grain processing reduces particle size and exposes the starch to microbial digestion in the rumen (NASEM, 2016).

Water-soluble carbohydrates consist of monosaccharides and oligosaccharides. Monosaccharides includes glucose, fructose, galactose, arabinose, and xylose (Rooke and

Hatfield, 2003). Oligosaccharides include disaccharides, raffinose, and stachyose (Rooke and Hatfield, 2003). Water-soluble carbohydrates provide a rapidly available energy source for ruminal microorganisms (NASEM, 2016). The content of water-soluble carbohydrates varies due to environmental conditions; high light intensity and photosynthesis increase the content, and high temperatures decrease the content (Van Soest, 1987). Studies have shown that increasing the water-soluble carbohydrate content has been shown to improve animal performance (Brito et al., 2009; Fisher et al., 1999), dry matter intake (Brito et al., 2009), in vitro digestibility (Fisher et al., 1999), and nitrogen use efficiency (Brito et al., 2009).

Structural Carbohydrates. Structural carbohydrates are the cell wall material that contains hemicellulose, cellulose and lignin. Structural carbohydrates have slower fermentation rates and produce more CH_4 per unit of substrate fermented than nonstructural carbohydrates (Holter and Young, 1992; Moe and Tyrrell, 1979) due to its impact on pH of the rumen and the microbial population (Johnson and Johnson, 1995).

The dietary fiber concentration primarily impacts the digestibility and thus the quality of forage (Van Soest, 1987). Nutritionally, fiber can be defined as fractions of feeds that are slowly digested or not digestible and that occupy space in the gastrointestinal tract of animals (Mertens, 1997). The primary standard of the chemical evaluation of forages is the detergent method (Minson, 1990) that Dr. Peter Van Soest developed. The procedure was developed to rapidly determine the insoluble cell wall matrix and estimate its major subcomponents, hemicellulose, cellulose, and lignin (Van Soest, 1987).

The cell contents are removed by digesting the forages in a solution of sodium lauryl sulfate and ethylenediaminetetraacetic acid (**EDTA**) that has a neutral pH of 7. The cell contents, or the neutral detergent solubles, include sugars, starch, pectin, lipids, soluble carbohydrates, protein, non-protein nitrogen, and water-soluble vitamins and minerals (Horrocks and Vallentine, 1999). The part of the forage that is insoluble in neutral detergent is called neutral detergent fiber

and contains hemicellulose, cellulose, and lignin. Neutral detergent fiber values are related to digestibility, feed intake, and rate of digestion (Mertens, 1997). The NDF component is digestible in the rumen, but resistant to microbial degradation. This leads to a slower digestion rate, slower PR, increase in ruminal residence time, and thus lowers intake (Van Soest, 1987). A ruminant consuming an all-forage diet will have a higher DMI if the NDF is lower. (Lee et al., 2017). Waldo (1986) states that the NDF of forage diets is the single best chemical predictor of forage intake.

To determine the amount of lignin and cellulose in the forage, the NDF can get further digested with an acid detergent solution. The acid detergent is a strong acid solution of quaternary detergents (Van Soest, 1987). The lignin and cellulose are termed ADF. As the value of ADF increases, the forage digestibility decreases (Horrocks and Vallentine, 1999). Thus, ADF is used to predict the digestibility and therefore the energy content of feeds.

Lignin is not a carbohydrate, but is included in the NDF and ADF carbohydrate fraction (NASEM, 2016). Lignin is resistant to digestion and negatively affects the digestibility of NDF and ADF (NASEM, 2016). Immature plant tissues have a primary cell wall. As the plant matures and cell elongation ceases, plants develop a secondary cell wall inside the cell (Jung and Allen, 1995). This new cell wall is thicker, giving the plant cell tensile strength. As the forage gets more mature, lignin starts to form in the space between the plant cells called the lamella and the primary wall region, then forms in the secondary wall. Most of the lignin is found in the middle lamella/primary wall region (Jung and Allen, 1995). Lignin benefits the plant by providing structural integrity by giving additional strength and rigidity to the plant, but decreases the cell wall digestibility (Van Soest, 1993). Lignin is the single fiber component most limiting nutrient availability (Van Soest, 1987).

Zadok's Decimal Code for the Growth Stages of Cereals

The effects of the environment and age of the herbage impact growth and development and are seen in the phenological development and forage quality. Therefore, a growth-stage classification system can be useful in comparing it with forage quality. The Zadok's decimal code scale describes the growth stages of cereal grains. It was developed by Zadoks et al. (1974) and is an expansion of the system developed by Feekes (1941) . The decimal code is based on 10 principal growth stages: germination, seedling growth, tillering, stem elongation, booting, inflorescence emergence, anthesis, milk development, dough development, and ripening. Each of the ten growth stages is then divided into secondary growth stages. A one-digit code designates the principal growth stages, and the secondary stages are designated by a two-digit code (Zadoks et al., 1974).

Table 2.1. Zadok's decimal code scale with descriptions of the principal and secondary growth stages

	2-digit code	General Description
0	Germination	
	00	Dry Seed
	01	Start of imbitition
	02	-
	03	Imbibition complete
	04	-
	05	Radicle (root) emerged from caryopsis
	06	-
	07	Coleoptile emerged from caryopsis
	08	-
	09	Leaf just at coleoptile tip
1	Seedling growth	
	10	First leaf through coleoptile
	11	First leaf unfolded
	12	2 leaves unfolded
	13	3 leaves unfolded
	14	4 leaves unfolded
	15	5 leaves unfolded
	16	6 leaves unfolded
	17	7 leaves unfolded
	18	8 leaves unfolded
	19	9 or more leaves unfolded
5	Inflorescence (ear/panicle) emergence	
	50	-
	51	First spikelet of inflorescence just visible
	52	-
	53	1/4 of inflorescence emerged
	54	-
	55	1/2 of inflorescence emerged
	56	-
	57	3/4 of inflorescence emerged
	58	-
	59	Emergence of inflorescence completed
6	Anthesis	
	60	-
	61	Beginning of anthesis
	62	-
	63	-
	64	-
	65	Anthesis half-way
	66	-
	67	-
	68	-
	69	Anthesis complete

2	Tillering	
	20	Main shoot only
	21	Main shoot and 1 tiller
	22	Main shoot and 2 tillers
	23	Main shoot and 3 tillers
	24	Main shoot and 4 tillers
	25	Main shoot and 5 tillers
	26	Main shoot and 6 tillers
	27	Main shoot and 7 tillers
	28	Main shoot and 8 tillers
	29	Main shoot and 9 or more tillers
3	Stem elongation	
	30	Ear at 1 cm
	31	1st node detectable
	32	2nd node detectable
	33	3rd node detectable
	34	4th node detectable
	35	5th node detectable
	36	6th node detectable
	37	Flag leaf just visible
	38	-
	39	Flag leaf ligule just visible
4	Booting	
	40	-
	41	Flag leaf sheath extending
	42	-
	43	Boots just visibly swollen
	44	-
	45	Boots swollen
	46	-
	47	Flag leaf sheath opening
	48	-
	49	First awns visible
7	Milk development	
	70	-
	71	Caryopsis water ripe
	72	-
	73	Early milk
	74	-
	75	Medium milk
	76	-
	77	Late milk
	78	-
	79	-
8	Dough development	
	80	-
	81	-
	82	-
	83	Early dough
	84	-
	85	Soft dough
	86	-
	87	Hard dough
	88	-
	89	-
9	Ripening	
	90	-
	91	Caryopsis hard (difficult to divide)
	92	Caryopsis hard (not dented by thumbnail)
	93	Caryopsis loosening in daytime
	94	Over-ripe, straw dead and collapsing
	95	Seed dormant
	96	Viable seed giving 50% germination
	97	Seed not dormant
	98	Secondary dormancy induced
	99	Secondary dormancy lost

Energy Metabolism

The feed digestibility largely determines its energy content (NASEM, 2016). All animals require energy, as it is essential for basal bodily functions maintaining body temperature (Minson, 1990), breathing, excretion, digestion, and reproductive processes. The animal's energy requirements mainly depend on body size, sex, age, physiological state, and the environment in which they live. The carbohydrates and fats found in forages in the feed are primarily used as energy sources. The unit energy is most commonly measured is calories. A calorie is defined as the amount of heat needed to raise the temperature of one gram of water by one degree Celsius.

Since the Calorie is a small amount of energy; the kilocalorie (**kcal**) and the megacalorie (**Mcal**) is used in conjunction with animal feeding standards (NASEM, 2016). The kcal is equal to 1,000 calories and the Mcal is equal to 1,000 kilocalories. Several energy measures are commonly used to evaluate the nutrition of the feed. Energy values are expressed as gross energy (**GE**), digestible energy (**DE**), metabolizable energy (**ME**), heat energy (**HE**), net energy for maintenance (**NEm**), and retained net energy (**NEr**).

The equality of ME=RE+HE is accepted because of the first law of thermodynamics; that energy cannot be created nor destroyed but can be transformed from one form to another (NASEM, 2016). If two entities are measured, the third entity can be calculated by the difference. Metabolizable energy is defined as the energy consumed that is not excreted in feces, urine, or combustible gases. Retained energy (**RE**) is the energy deposited in animal tissues or products, and HE is simply the heat generated by the animal.

Energy Values

Gross Energy. Gross energy is the heat energy from completely oxidizing an organic substance to CO_2 and water. Gross energy does not provide information about the availability of energy to the animal (NASEM, 2016). The Nutrient Requirements of Beef Cattle (NASEM, 2016) defines DE as the "gross energy of the food minus the energy lost in the feces." Most of the energy lost from a feed is via feces. The amount of protein, fats, and minerals lost in the feces is directly proportional to the amount of forage dry matter and organic matter eaten (Minson, 1990). A large portion, around 60-75%, of DE is derived from rumen fermentation (NASEM, 2016). Digestible energy reflects diet digestibility; however, it fails to account for major energy losses in relation to the urination, digestion processes, and metabolism (NASEM, 2016).

Metabolizable Energy. Metabolizable energy is a term used to describe the amount of accessible energy a feed provides to the animal. Metabolizable energy is DE minus energy lost in the urine

and gasses produced, which is mainly CH_4 (NASEM, 2016). Most of the ME gets dissipated in the form of heat (Salles et al., 2018). Therefore, metabolizable energy and DE are closely related. Metabolizable energy is calculated as 82% of digestible energy (Van Soest, 1987) but can vary depending on intake, age of animal, and feed source (NASEM, 2016). The ME:DE ratio can range from 0.82 to 0.93 in growing cattle (Vermorel and Bickel, 1980).

Heat Production. Heat production (**HP**) is the by-product of metabolic processes caused by the digestion and assimilation of food. The magnitude of HP is determined by many variables such as genetic composition, sex, age, physiological condition, climate, and quality and quantity of feed (MacRae and Lobley, 1982). The increase in HP is known as heat increment (**HI**) or the specific dynamic effect. Heat increment can further be defined as the difference between ME and RE. The heat produced helps maintain body temperature when cold-stressed, but when not cold-stressed, the heat is considered an energy loss that is not accounted for by ME (NASEM, 2016). Diets high in fiber are linked to a high HI (Van Soest, 1987). Heat increment is the largest loss of digestible energy, which is around 20 to 30% in maintenance animals, 30% in lactating animals, and 42% in fattening animals (Van Soest, 1993).

Retained Energy. Retained energy is the fraction of dietary energy that is deposited in body protein, body fat, conceptus, milk, hair, etc. (Ferrell and Oltjen, 2008). Retained energy can be defined as the net energy for gain, which is the energy content of the tissue deposited. Retained energy is generally <20% of energy loss in beef cattle of intake energy (Ferrell and Oltjen, 2008). Intake of feed is utilized more efficiently for energy maintenance than for energy retention (NASEM, 2016). Retained energy can be calculated by the equation $RE = 0.0635 \times \text{empty body weight}^{0.75} \times \text{empty body weight gain}^{1.097}$ (NASEM, 2016). Energy is retained as either protein or fat, therefor the composition of the gain at different weights can be estimated from RE (NASEM, 2016). The relationships between RE, proportion of fat in gain, and the proportion of protein in gain are influenced by dietary ME concentration (NASEM, 2016).

Ruminants

Ruminants have a unique digestive tract that allows for the digestion of forages and other fibrous feeds via anaerobic fermentation. Anaerobic microorganisms such as ruminal bacteria, protozoa, and fungi that are found in the rumen ferment plant cell wall polysaccharides, starch, protein, and other materials, the byproducts of which provide energy and supports microbial growth, which then provides the animal with amino acids from microbial proteins. The oxygen (**O_2**)-intolerant environment in the digestive tract produces products that do not get completely oxidized. The end products produced in the rumen from anaerobic microorganisms are CO_2, CH_4, volatile fatty acids (**VFA**), and microbial cells. The VFAs are mainly acetate, propionate, and butyrate. Formate, isobutyrate, 2-methylbutyrate, isovalerate, valerate, and caproate are found in lesser amounts in the rumen (NASEM, 2016). Other products like ethanol, lactate, and succinate are produced, but concentrations are very low because they are used as substrates by other microorganisms (NASEM, 2016). Diet, protozoa, and status of the methanogen population in the rumen influences the proportions of acetic, propionic and butyric acid (Van Soest, 1987). The concentrations of VFAs in the rumen are regulated by a balance between production and absorption (Van Soest, 1987). Increased production rates can induce higher VFA concentrations (Giesecke, 1970)

Gas Composition

Microbial fermentation yields heat, CO_2, CH_4, and hydrogen (**H_2**) (Johnson et al., 2000). During feeding, the ruminant swallows air, which contains nitrogen and O_2. Shifts in the rumen ecology and fermentation balance cause the composition of gases to vary from animal to animal and day to day (Hales and Cole, 2017). The typical composition of rumen gases is 0.2% H_2, 0.5% O_2, 7.0% nitrogen, 20-30% CH_4, 45-75% CO_2, and minor % of N_2O and H_2 sulfate (Clarke and Reid, 1974; Min et al., 2006). The proportion of CO_2 is normally 2 or 3 times that of CH_4 (Van Soest, 1987). Nitrogen gets eructated, and the O_2 gets absorbed or used by facultative organisms

(Van Soest, 1987). Methane gets removed from the rumen by eructation or absorption across the rumen wall and exhalation via the lungs (Van Soest, 1987). Some CO_2 gets removed by eructation, but its fate is not as clear as CH_4 because of the pooling and recycling of metabolic carbon as urea and bicarbonates in saliva (Van Soest, 1987).

Gas Composition Changes with Forage Maturity and Changing Forage Quality

Understanding the impacts that forage quality has on the gas compositions of the rumen is important, not only in terms of energy loss from microbial digestion but also in terms of environmental impacts. As forages mature, nutritive value declines with declining crude protein and increasing fiber content (NASEM, 2016). Methane production is thought to increase as the fiber content of feed increases, and decrease as the protein content of feed increase (Johnson and Johnson, 1995). When ruminants are fed diets containing higher levels of nonstructural carbohydrates, such as in early-harvested higher quality forages, CH_4 production in reduced (Johnson and Johnson, 1995). Chagunda et al. (2010) studied the effects of forage nutritive quality on enteric CH_4 production by dairy cows. The cattle were fed grass silages that were divided into 3 groups and classified as high, medium, and low quality based on ME. Forage was fed ad libitum, and the concentrate portion of the rations was formulated to meet the targeted milk production and maintenance of the cow. Their study showed that the CH_4 intensity (CH_4/kg milk) from the cows that received the low-quality based forage was 33% more than the cows that received the high-quality forages.

Cole et al. (2020) studied the effects of dietary nutritive quality on CH_4 emissions of beef steers. Treatments included old world bluestem hay that was cut in October, in the flower/dead stage (designated as low-quality, 2.60 CP%, 72.4 NDF%, 41.1 ADF%), Old World bluestem hay, that was cut in June and July in the mid-vegetative/early elongation to late boot stage (designated as medium-quality, 8.4 CP%, 65.7 NDF%, 34.8 ADF%), and alfalfa hay that was obtained commercially (designated as high-quality, 18.12 CP%, 54.3 NDF%, 47.9 ADF%). Steers were

fed at 90% of their ad libitum intake. The average total DMI for the steers on the low-quality diet was 5.40 kg/d, medium quality diet was 6.20 kg/d and the high-quality diet was 6.38 kg/d. The NDF and ADF concentrations decreased as diet quality increased. Their study concluded that nitrous oxide emissions averaged 18.5 mg/day for steers fed the low-quality diet, 21.2 mg/day for steers fed the medium-quality diet, and 21.3 mg/day for steers fed the high-quality diet. When looking at the N_2O emissions on a mg/kg of DMI basis, the steers fed the low-quality diet produced 3.43, steers fed the medium-quality diet produced 3.41, and the steers fed the high-quality diet produced 3.30. A large portion of the nitrous oxide emissions were enteric; but potentially included some emissions from feces, urine, feed, and equipment. Forage quality did not affect O_2 consumption per kilogram of DMI and CO_2 production. The medium and high-quality hays had a greater respiratory quotient (**RQ**) than the low-quality hay. The RQ is the ratio of CO_2 production to O_2 consumption, which allows assessment of carbohydrates and fat utilization (Van Soest, 1987). Diet quality did not affect daily CH_4 production, but CH_4 production per kg of digestible organic matter (**OM**) and NDF decreased as the forage quality increased. However, it is important to point out that this study used a legume for their high-quality hay and a grass for the low and medium-quality hays. Ominski et al. (2006) study on enteric CH_4 emissions from backgrounded cattle showed that an increase in emissions may be associated with lower forage nutritive quality. When the forage quality was low and dry matter availability was limited (738 kg/ha) was when they saw the highest CH_4 emissions. Boadi and Wittenberg (2002) reported emissions were lowest for early summer grazing when forage nutritive quality was high.

Methane

Methane is a colorless and odorless gas that is a potent greenhouse gas. Methane is about 28 times more potent than carbon dioxide at warming the Earth on a 100-year timescale (NASA, 2020). Natural sources of CH_4 include wetlands, volcanoes, vents on the ocean floor, and termites (NASA, 2020). Anthropogenic sources of CH_4 are the production and combustion of natural gas and coal, biomass burning, livestock farming, and waste management (E.P.A., 2022). In 2021, the atmospheric methane levels averaged 1,895.7 ppb , which is around 162% greater than pre-industrial levels (Stein, 2022).

Globally, CH_4 makes up 17.62% of all GHG emissions (FAO, 2022). Anthropogenic CH_4 accounts for 10.57% of the total GHG emissions and enteric fermentation accounts for 3.17% of the total GHG emissions (UN, 2022). In the United States, methane accounts for about 11.5% of all U.S. GHG (E.P.A., 2022). Enteric fermentation from ruminants account for 2.9% of the total emissions (E.P.A., 2022)

Figure 2.2 Global GHG emissions and sources of methane

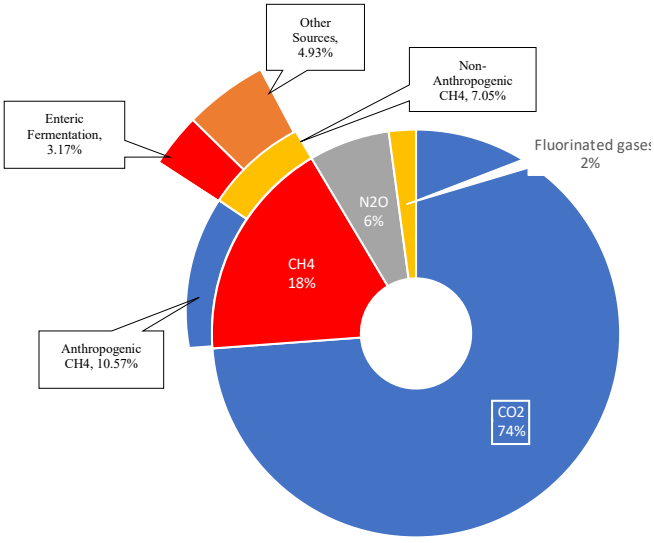
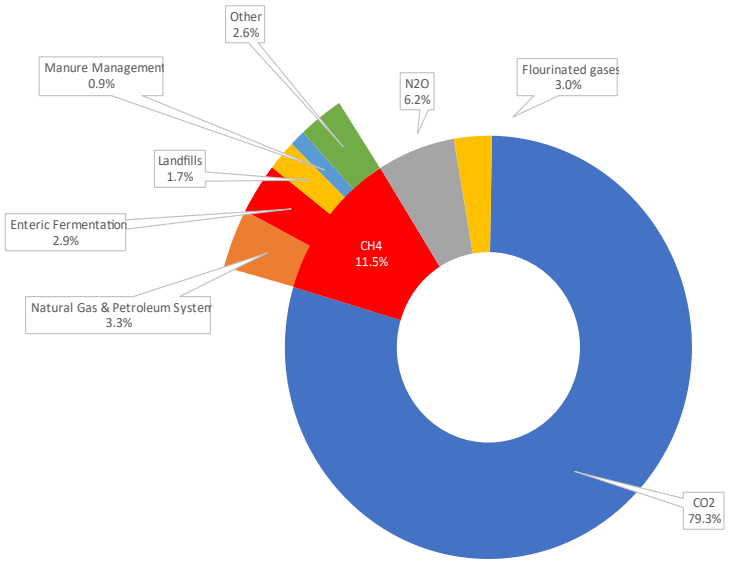


Figure 2.3 U.S. methane emissions, by source



Ruminant Methane Production

Methanogenic bacteria, or methanogens, are single-celled microorganisms in the phylum Euryarchaeota and the domain Archae. They are located in the rumen and hindgut of the animal (Hook et al., 2010). Volatile fatty acids are the main products of the fermentation of the fermentation of the carbohydrates and proteins. The main VFAs produced are acetate, propionate, and butyrate (NASEM, 2016). During the production of VFAs, H_2 and CO_2 are produced (Hook et al., 2010). During the production of acetate and butyrate, H_2 is liberated, and propionate serves as a net H_2 sink (Beauchemin et al., 2009). Methanogens use H_2 to reduce the CO_2 in the rumen to prevent the accumulation of reducing equivalents. This process prevents the impediment of ruminal fermentation but also produces CH_4 (Beauchemin et al., 2009). Enteric CH_4 production is closely associated with the levels of VFAs present in the rumen (Johnson and Johnson, 1995). Therefore, if all the carbohydrates is fermented to acetic acid and no propionic acid is produced,

energy loss as CH_4 would be 33% (Chagunda et al., 2010). Eighty-seven percent of ruminant CH_4 is produced in the rumen, and the other 13% is produced in the large intestines (Murray et al., 1976). The diet composition and the amount of feed consumed are the main factors affecting the amount of CH_4 emitted (Johnson and Johnson, 1995). Methane production is influenced by forage quality. As forage digestibility decreases, forage intake decreases and the acetate: propionate ratio increases, which favors increased CH_4 production per unit of forage consumed (McAllister et al., 1996).

Passage Rate

According to Van Soest (1987), passage is defined as the flow of undigested residues through the digestive tract. Rumen outflow includes bacteria, potentially digestible feed resides, and unavailable lignified fiber, with more digestion of bacteria and feed matter occurring at succeeding stages. The fecal material is mainly made up of bacterial and plant cell walls and some endogenous matter (Van Soest, 1987).

Ingested feed gets removed from the digestive tract through two paths: digestion or passage. The processes of digestion and passage compete for the same material. The extent of digestion depends partly on the relative digestion rate and PR due to different feed components having inherently different digestion rates (Van Soest, 1987).

Non-cell wall components of feeds are digested 3 to 10 times faster than the PR (Mertens, 1993). Cell wall rate of digestion is commonly of the same extent as the rate of passage; however, cell wall digestion varies and depends on plant species and the maturity of the forage (Mertens, 1993). Retention of undigested fiber in the rumen increases ruminal fiber digestion, but feed intake is decreased (Allen, 1996). Conversely, an increase in the rate of passage will increase feed intake (Balch and Campling, 1961), which increases energy intake but decreases total tract digestibility (Firkins et al., 1998). Several factors affect the PR, such as particle size of feed, feed

additives, feeding method, breed of animal, ambient temperature, level of feed intake, and animal variability (Owens and Goetsch, 1988). A faster PR is associated with more digestible forages; this increases intake resulting in more fermentable substrates in the rumen and therefor higher CH_4 production (Van Soest, 1987).

Indigestible Markers

Fecal output has been estimated using indigestible markers for many years. Fecal output data are used to calculate digestibility and feed intake (Prigge et al., 1981). The ideal marker must not affect or be affected by the digestion process, must be nonabsorbable, and be physically similar to the material it is to mark (Fahey and Jung, 1983). They are categorized into internal markers and external markers. Internal markers are plant constituents including items such as: silica, lignin, and ADF (Fahey and Jung, 1983; Huhtanen et al., 1994; NASEM, 2016). External markers are substances added to the diet, including chromium, rare earth elements (such ytterbium chloride, yttrium chloride, and alisprosium) titanium, and cobalt (Krysl et al., 1985; Udén et al., 1980).

Internal markers are used in pasture studies and other studies where it is essential to know an estimate of digestibility (Mayes et al., 1986) and where direct measurements of intake is difficult (Van Soest, 1987). Digestion is the product of different dynamic processes. These processes include reduction of the size of feed particles, fermentation, and digesta flow. The ingested feed stuffs, the rumen's microbial population and the type of animal impacts these processes (Bernard and Doreau, 2000). According to Van Soest (1987), to estimate digestibility an equation is obtained by plotting digestibility versus fecal content. This process is applicable to any indigestible fecal constituent. The direct association between digestibility and fecal concentration is curvilinear (Van Soest, 1987).

Internal markers can also be used to determine PR. Passage rates are determined from turnover measurements based on gut contents obtained by emptying the rumen (Van Soest, 1987). The turnover rate can be calculated by determining the rumen volume and dividing it by intake (Paloheimo et al., 1959). Passage rates can also be determined from the duodenal flow of the marker and the ruminal pool size of the marker estimated by the evacuation of ruminal contents (Voelker and Allen, 2003).

External markers can be used to determine PRs, rumen volume, and yield of rumen fecal output fermentation products. In addition, they save on labor by helping to avoid total fecal collections (Van Soest, 1987). External markers can be given to the animal at a constant level for digestibility studies or at a pulse dose to determine the PR by administration of a pulse dose of a marker followed by numerous collections over a period of days (Van Soest, 1987). Administering the marker can be given by mouth or fistula. The collections can occur at the fistulated sites, in feces, or both (Van Soest, 1987).

Methane Mitigation

It is estimated that growing cattle could gain an additional 75 g/d of body weight if their CH_4 production was reduced by 20 percent (Beauchemin and McGinn, 2020). Mitigating enteric CH_4 emissions has been investigated over the last several decades. This is due to economic benefits of the livestock industry and the benefit to the environment (Johnson and Johnson, 1995). These studies have mainly focused on animal nutrition, genetics, and management, but other efforts looked at vaccines (Wright et al., 2004), defaunation of the rumen (Van Nevel and Demeyer, 1996; Whitelaw et al., 1984), and chemical additives (Dong et al., 1997; Van Nevel and Demeyer, 1995), to name a few. However, manipulating the diet is the most direct and effective means of lowering CH_4 emissions in most production systems (Beauchemin et al., 2009).

Lipids. Several studies have established that supplementing diets with lipids reduces enteric CH_4 emissions (Beauchemin et al., 2008; Boadi et al., 2004; Dohme et al., 2000). Dietary lipid supplementation works to reduce CH_4 emissions by reducing ruminal organic matter fermentation activity of methanogenic bacteria and decreasing the number of ruminal protozoa (Johnson and Johnson, 1995). Medium-chain fatty acids ($C_8 – C_{16}$) and long-chain fatty acids ($>C_{18}$) reduce CH_4 production (Blaxter and Czerkawski, 1966), but long-chain fatty acids also reduce fiber digestion (Broudiscou et al., 1990) and are less effective in reducing CH_4 production than medium-chain fatty acids (Cottle et al., 2011). Dohme et al. (2000) concluded that with the addition of 53 g/ kg DM of canola oil, coconut oil, or palm kernel oil, CH_4 production decreased by 20, 21, or 34%, respectively. This study was carried out using the rumen simulation technique (RUSITEC). Beauchemin et al. (2007) conducted a study to determine the impact of 3 lipid sources (tallow, sunflower oil, and sunflower seeds) on CH_4 emissions from growing cattle at 3% of diet. The basal diet consisted mainly of whole-crop barley silage (650 g/kg of DM) supplemented with steam-rolled barley and sources of protein (soybean meal and canola meal) minerals, and vitamins. The 3 lipid sources reduced enteric CH_4 emissions by 15% when the differences were accounted for in DE intake. However, tallow and sunflower seeds also reduced digestible energy intake, which could impact animal performance. In addition to lowering CH_4 production, sunflower oil increased digestible energy intake, increased the rate of gain, and had minimal effects on fiber digestibility.

Ionophores. Ionophores are antimicrobials that are used in the beef and dairy cattle industries as well as the poultry industry. Ionophores improve feed efficiency, increase weight gain, and reduces morbidity and mortality (McGuffey et al., 2001). The most commonly used ionophore is rumensin (Boadi et al., 2004). Ionophores decrease the acetate-to-propionate ratio of the VFAs (Beauchemin et al., 2008). When glucose gets converted to acetate from acetogenic bacteria, two net carbons are lost, usually as CH_4 (Van Soest, 1987). When glucose gets converted to

propionate, there is no net carbon loss (Van Soest, 1987). Ionophores work by inhibiting acetogenic bacteria, which gives an advantage to the propionate producing bacteria. In short-term studies, rumensin decreased enteric CH_4 emissions by 10 to 25% in finishing cattle (McGinn et al., 2004; Tedeschi et al., 2011; Tedeschi et al., 2003). However, due to the development of resistance to the antibiotic (Boadi et al., 2004), CH_4 production does not stay suppressed with prolonged or repeated use of ionophores (Guan et al., 2006; Johnson and Johnson, 1995; Sauer et al., 1998; Van Nevel and Demeyer, 1995). Guan et al. (2006) reported that CH_4 emissions, expressed as liters per kilogram of DMI or as percentage of GE intake, can be decreased by 27% within 2 weeks of ionophore supplementation in animals consuming high-concentrate diets, and by 30% in 4 weeks in animals consuming a high-forage diet. However, CH_4 production levels were restored to original levels by the third week in animals consuming a high-concentrate diet with ionophore supplementation and by the sixth week in animals consuming a high-forage diet with ionophore supplementation.

Genetics. Selectively breeding livestock with a higher feed efficiency or that produces less CH_4 per unit of DMI is another way to mitigate CH_4 production. Methane production and DMI are often highly correlated, but the relationship between the two varies highly among animals (Cottle et al., 2011). For example, a study by Nkrumah et al. (2006) conducted on fattening cattle shows that steers with high feed efficiency produced ~20% less CH_4 than the low feed efficiency steers (Nkrumah et al., 2006). In another study, Canadian steers with a high net feed efficiency also produced ~21% lower annual CH_4 than the low NFE steers (Okine et al., 2002).

Ruminal PR and digesta mean retention time can affect CH_4 emissions (Huhtanen et al., 2016). Animals with a faster PR of feed from the rumen produce less CH_4 emissions per unit of food ingested (Boadi et al., 2004). Huhtanen et al. (2016) studied ruminal digesta mean retention time using the mechanistic Nordic dairy cow model Karoline. Their data indicated that with a 1-hour increase in mean retention time, CH_4 emissions increased by 0.37 g/kg DMI in dairy cattle

and 0.33 g/kg DMI in sheep. Predicted CH_4 yield was 22.3 and 27.0 g/kg DM for dairy cows and sheep, respectively. The increase in CH_4 emissions could be due to the digesta PR indirectly controlling the methanogenesis pathway genes (Shi et al., 2014). According to a model, based on microbial growth kinetics and fermentation thermodynamics, the differences in PRs through the rumen would affect ruminal H_2 production (Janssen, 2010). According to the model, an increase in particle PR is associated with higher rumen H_2 concentration, which results is less H_2 formation during fermentation, which results in less CH_4 production (Janssen, 2010). With a lower ruminal H_2 concentration, the methanogens have to increase expression of methanogenesis pathway genes to maintain the H_2 turnover rate (Shi et al., 2014). In addition to the study conducted by Huhtanen et al. (2016), other studies (Goopy et al., 2014; Pinares-Patino et al., 2011) have concluded that sheep that had increased ruminal digesta PR had reduced CH_4 emissions. Mean retention time has been shown to be a heritable (Smuts et al., 1995) and repeatable trait (Orskov et al., 1988). A faster PR not only reduces CH_4 emissions per unit of food ingested, but also affects propionate and microbial yield, which would positively impact production benefits (Boadi et al., 2004).

Pasture Management and Forage Quality. In beef production, the cow-calf sector contributes around 60-84% of total GHG emissions (Grainger and Beauchemin, 2011), while the finishing cattle sector makes up most of the rest. The difference in CH_4 production between the two sectors can be attributed to the fact that the majority of cow herds graze pastures, and the fermentation of fiber produces more CH_4 than the starch being fermented in the grain-based finishing diets (Montes et al., 2013). Also, the cattle being fed in the market sector are fed for a relatively short time before slaughter while cows are commonly on high forage diets year-round. Therefore, according to Beauchemin et al. (2010), the most effective mitigation strategies are those aimed at reducing enteric CH_4 emissions from the cow-calf sector.

Improving pasture quality is viewed as a way to reduce CH_4 emissions, due to the increase in animal productivity and reduced acetate formation due to less fiber content in the sward, which results in less dietary energy lost as CH_4 (Beauchemin et al., 2008). A three-year study was conducted by Deramus et al. (2003) on beef cows and heifers in Louisiana. They compared CH_4 emissions from cattle grazing unimproved continuously grazed pastures to cattle grazing management-intensive, best management practices (**BMP**) pasture system. They concluded that when forage quality was high, emissions were lower. However, when forage quality declined, the emissions were greater during the summer and the fall. When seasonal variations were accounted for, the cattle grazing the BMP systems emitted 22% less CH_4 than those grazing the unimproved pastures.

Legumes have a greater digestibility and PR than grasses (McCaughey et al., 1999). McCaughey et al. (1999) studied the impacts of pasture type on CH_4 production in beef cattle. In a 69-d trial, cattle grazed pastures with 78% alfalfa (spp) and 22% meadow bromegrass (spp) or 100% meadow bromegrass. They concluded that the cattle grazing the alfalfa-grass pasture emitted less CH_4 than those grazing pure grass. Also, when expressed as energy lost through eructation expressed as a percentage of GE intake, the cattle grazing the alfalfa-grass pasture lost less energy than the cows grazing pure grass (7.1% and 9.5% of GE, respectively).

Measuring Gas Emissions

Technologies to measure gas emissions from ruminants are necessary to the assessment of mitigation strategies. Technologies have been developed to measure daily CH_4 production, which include respiration chambers (**RC**), sulfur hexafluoride tracers (**SF_6**), and open-circuit gas quantification systems (**GQS**). Precise measurements of CH_4 emission can be obtained by the use of RC, but are impractical for grazing applications (Hammond et al., 2015). Estimations of eructed and expired CH_4 emissions can be obtained using SF6 technique, from grazing animals (Johnson et al., 1994). Short-term spot measurements from grazing animals can be obtained using

the GQS (Gunter and Beck, 2018). Each technology has its benefits and weaknesses (Jonker et al., 2016).

Respiration chambers are an enclosed system that measures the total gas exchange by the animal including CO_2 and CH_4 that is produced (Hammond et al., 2015), this includes the CH_4 produced in the hindgut (Munoz et al., 2012). Hindgut CH_4 production can account for 2 to 3% of the total CH_4 emitted (Munoz et al., 2012). Cattle are placed in an enclosed chamber and air is drawn through the chamber at set intervals and continuously monitored by using differential pressure within a venture flowmeter, while concentration change of the air coming in and leaving the system is measured (Brown et al., 1994). Outlet gasses from the chamber and background air is continuously sampled into a multiport gas switching unit and the air stream is directed to a continuous emission analyzer to determine CH_4 and CO_2 concentrations by infrared technology (Jonker et al., 2016). Respiration chambers allows for direct and accurate measurements of the total CO_2 and CH_4 (Hammond et al., 2015). However, cattle can stay in respiration chambers for 2 to 3 days of measurement at one time, and the animal is in an artificial setting, which impacts the animal's behavior and emissions (McGinn et al., 2006). This system cannot be used in grazing scenarios.

The sulfur hexafluoride (**SF_6**) tracer method uses a bolus filled with SF_6 (an inert tracer gas) that is placed into the rumen (Jonker et al., 2016). The release rate of SF_6 through a permeation tube is measured before the bolus that is filled with SF_6 is inserted into the rumen. A halter is placed on the animal's head that has been fitted with a capillary tube that is connected to a sampling canister. These canisters hang around the animal's nose and mouth, and when the animal eructates, the gases are captured into the PVC canisters. Methane and SF_6 concentrations are analyzed in a laboratory using gas chromatography (Hammond et al., 2015). Methane emission rate is calculated as follows: $QCH_4 = QSF_6 \times [CH_4]/[SF_6]$, where QCH_4 is the emission rate of CH_4 in liters per hour, QSF_6 is the known release rate of SF6 from the permeation tube in

the ruminal bolus, and [CH_4] and [SF_6] are the measured concentrations from the canister air (Johnson and Johnson, 1995). The SF_6 tracers can be used in a grazing environment (Johnson et al., 1994), however, the animals must be handled often, they must wear a halter with canisters, and a bolus will remain in their rumen. These factors could have a negative impact on the animal's grazing behavior. This method also does not measure CH_4 production from the hindgut (Gunter and Beck, 2018). Diurnal variation of emissions are also not measurable using the SF_6 system (Gunter and Beck, 2018).

Open Circuit Gas Quantification System

A relatively new system that takes spot measurements of CH_4 and CO_2 emissions from ruminants and uses those measurements to estimate daily CH_4 production (Dorich et al., 2015) is called the GreenFeed emission monitoring system (C-Lock Inc., Rapid City, South Dakota). The GQS accuracy of cattle's estimated CO_2 and CH_4 emission has been validated by recent research (Alemu et al., 2017; Dorich et al., 2015; Huhtanen et al., 2015b). The GreenFeed system is a head chamber with a feed hopper that baits cattle to visit throughout the day. The bait feeding events can be evenly spaced throughout the day to control for circadian variation in CO_2 and CH_4 emissions rates (Gunter and Bradford, 2015). When an animal approaches the GreenFeed system, the system reads the animal's radio frequency identification (RFID) tag. The GreenFeed system entices animals to visit by dropping bait in 6 to 8 allocations over a 3 to 8 min period. Animals are enticed to keep their head in the chamber for at least 3 minutes so that the system can capture emissions from multiple eructation events (Velazco et al., 2016). While the animal is consuming the bait, the GreenFeed system captures the animal's breath cloud. The breath cloud is then analyzed for CO_2, CH_4, and O_2 concentrations. The measurements are stored in the system and then uploaded hourly to a server and processed further. Using ideal gas laws and mass airflow estimates, algorithms are used to determine each animal's total daily emissions and O_2 consumption. Compared to other systems, a relatively large number of animals can use the

GreenFeed during a trial. In confinement, 20 to 25 animals can be sampled, and in a grazing environment, 15 to 20 animals can be sampled (Dorich et al., 2015; Hammond et al., 2015). However, training to the GreenFeed system can take 4 to 8 wk (Gunter and Beck, 2018). Some animals will never use the GreenFeed system, so a researcher will need to train 20% to 30% more animals at the beginning of the training period (Gunter and Beck, 2018).

The GQS has been used in research for several years and on different types of research. Méo-Filho et al. (2020) conducted a study using the GQS to determine CH_4 emissions of different lines of beef steers that were reared on pastures and finished in feedlots. The GQS has also been used to determine if enteric CH_4 was mitigated in beef feedlot cattle fed dietary nitrate (Velazco et al., 2013) as well as daily CH_4 emissions and emission intensity of grazing beef cattle that are genetically divergent for residual feed intake (Velazco et al., 2017). In addition to measuring emissions, the GQS can also be used to administer external markers to cattle (Beck et al., 2021). The researchers concluded that the external marker, titanium dioxide, when administered through the GQS feed hoppers is an acceptable method to measure fecal output.

In a study conducted by Jonker et al. (2016), enteric CH_4 and CO_2 emissions were measured using RC, SF_6, and GQS from beef heifers fed alfalfa silage at 3 feeding levels and 4 feeding frequencies. Their study concluded that in general, the 3 technologies provided means for CH_4 yields that were the same. Studies (Cole et al., 2020; Hammond et al., 2015), have reported that mean emissions of enteric CH_4 measured using the RC and GQS were not different. Studies (Arthur et al., 2017; Cole et al., 2020; Hammond et al., 2015; Jonker et al., 2016) have also shown that estimates of CH_4 and CO_2 emissions obtained from the GQS from beef cattle individually fed forage-based diets, are acceptable estimates when sufficient numbers of data points are obtained and sufficient numbers of animals are used. Studies (Boadi et al., 2002; McGinn et al., 2006; Pinares-Patino et al., 2008; Ulyatt et al., 1999) comparing RC to SF_6 found no difference between CH_4 emissions in beef cattle and sheep.

Summary of Literature

Global atmospheric concentrations of greenhouse gases are rising (IPCC, 2013). In 2015, the U.N. reached an agreement to combat climate change, called the Paris Agreement. The central aim of the agreement is to limit global warming to 2° C above pre-industrial levels. Ruminants contribute to the increasing GHGs while consuming fibrous feedstuffs that are unusable by humans and converting the fibrous feedstuffs into highly nutritional foods and consumable products. Globally, the demand for such products is increasing (Steinfeld et al., 2006). Therefore, mitigating emissions from ruminant animals is necessary to achieve the 2°C goal of the Paris Agreement.

There are several factors that impact the amount of enteric CH_4 produced from ruminants. One of the factors is forage quality. Forage quality encompasses the nutrients, energy, protein, digestibility, and fiber found in the forage, which impacts intake potential, digestibility, and partitioning of metabolized products (Buxton, 1996). The stage of maturity is a significant factor affecting forage quality. As the plant matures, lignin increases, causing the ADF value to increase, which decreases digestibility. Also, due to the increase in fiber, dry matter intake will decrease. The decrease in digestibility and decrease in intake causes acetate to increase, which causes CH_4 production to increase. Generally, as the plant ages, the nutrient levels begin to decline as well (Horrocks and Vallentine, 1999).

Enteric CH_4 is produced in the rumen during the microbial fermentation of feeds. This results in 2 to 12% of gross energy from the feed being lost. This lost energy could have been used by the animal for growth and production. Hence, reducing the environmental footprints of ruminants will not only help with reducing GHGs but will also reduce energy losses from the ruminants, thus potentially increasing the animal's performance, which in return has the potential to increase the producer's bottom line.

CHAPTER III

Effects of hay maturity on intake, digestion, metabolism, and enteric methane emissions by beef heifers

E. J. Friend[1,2], P.A. Beck[2], S.A. Gunter[1], and C.A. Moffet[1]

[1]Oklahoma and Central Plains Agricultural Research Center, United States Department of Agricultural, Agricultural Research Service, 2000 18th Street, Woodward, OK 73801

[2]Animal Science Department, Oklahoma State University, Stillwater, OK 74074

Abstract: The objective of this experiment was to investigate the effects of ad libitum feeding long-stemmed wheat hay cut at three different maturities on intake, digestion, metabolism, and enteric CH_4 emissions by beef heifers. Twelve heifers (initial BW = 296 ± 30 kg) were used in a 34-day feeding experiment fed over three periods. Heifers were randomly assigned to 1 of 3 treatments including an immature wheat (*Triticum aestivum* L.) hay harvested at the stem elongation stage [Zadok score (Tottman, 1987) = 3.1, 37% NDF], an intermediate maturity wheat hay harvested at the booting stage (Zadok score 3.9-4.1, 55% NDF), and a mature wheat hay harvested at the milk stage (Zadok = 7.9, 63% NDF) of maturity. Hay was offered at ad libitum along with a daily supplement of about 0.60 kg of pellets (12% CP) via an automated head-chamber system that measures CO_2 and CH_4 emissions, and O_2 consumption while the supplement was consumed. Data were analyzed as a completely random design and beginning BW as a covariate; means were separated using orthogonal contrasts. Dry matter intake (DMI) decreased linearly as the wheat matured ($P < 0.01$). Daily CH_4 emissions decreased as the hay matured ($P = 0.03$) but did not have an effect on CH_4 yield (expressed as g of CH_4/kg of DMI; P

= 0.13). However, CO_2 emissions and O_2 consumption decreased linearly ($P < 0.01$, $P < 0.01$) as the wheat matured. As the hay matured, heat production decreased linearly ($P < 0.01$). Hay maturity linearly decreased metabolizable energy intake and digestible energy intake ($P < 0.01$, $P < 0.01$). Digestibility decreased linearly ($P = 0.02$) with maturity, with digestibilities of 76.22%, 68.01%, and 58.57% for immature, intermediate, and mature hay, respectively. These results indicate that as forage matures it has a negative effect on DMI, CO_2 emissions, O_2 consumption, HP, digestible energy intake, and metabolizable energy intake, but did not have an effect on CH_4 yield.

Introduction

Globally, wheat (*Triticum aestivum* L.) is grown on more than 240 million hectares, which is larger than any other crop (Curtis, 2022). In places lacking rainfall and winter-spring temperatures up to 25°C, wheat is the main forage used as hay, silage or straw to provide feed to ruminants (Shaani et al., 2017). Up to 7 million head of stocker cattle graze wheat pasture during the winter in Oklahoma and the southern Great Plains each year (Horn, 2006). The practice of grazing wheat for pasture is also common in Argentina, Australia, Morocco, Pakistan, Syria, and Uruguay (Epplin et al., 2000). Wheat offers opportunities for harvesting excess biomass as preserved forages such as hay or silages.

As forages grow, the proportion of stems and cell wall constituents increase as the proportion of leaves decreases (Jung and Allen, 1995). Typically the leaves are of higher quality than the stems (Van Soest, 1987). As most plants mature, they produce more stems in relation to leaves. Stems are lower in digestibility than leaves, and digestibility declines more rapidly with increased plant maturity than that of leaves (Jung and Allen, 1995); thus fibrous cell wall constituents increase. Also, with increasing maturity, cell wall constituents become more lignified, which causes digestibility to decrease (Minson, 1990). When digestibility is decreased,

dry matter intake is reduced, due to the forages remaining in the rumen longer because of their slow rate of digestion (Jung and Allen, 1995). Increased fiber content is also related to an increase of the acetate: propionate ratio, which favors an increase in CH_4 production (McAllister et al., 1996).

Ruminants produce enteric methane during the process of microbial digestion of feed. Methane produced from cattle constitutes 2-12% of gross energy loss (Johnson and Johnson, 1995). The composition of the diet and the amount of feed the animal consumes are the main factors affecting the amount of CH_4 emitted (Johnson and Johnson, 1995). Methane is a potent greenhouse gas that has 28 times the global warming potential of CO_2 (IPCC, 2013). Ruminants are significant sources of enteric CH_4 (Gill et al., 2009) and contribute around 11.6% of all the anthropomorphic greenhouse gas emissions (FAOSTAT, 2022), and twenty-five percent of the U.S. methane emissions (E.P.A., 2022). Ruminants are one of the few sources of CH_4 production we can manipulate (Johnson and Johnson, 1995) and are also a primary source of food security (Herrero et al., 2015). The combined economic benefits to the livestock industry and environmental benefits to society by decreasing ruminant CH_4 emissions makes research on mitigating enteric CH_4 important (Johnson and Johnson, 1995). With growing concerns of global climate change and the vast amount of wheat that is grown globally and used as hay or pastures, it is important to research methods to lessen the enteric emissions by ruminants consuming wheat forage.

The purpose of our study was to investigate the effects that feeding heifers long-stemmed wheat hay cut at three different maturities have on forage intake, forage digestibility, energy metabolism and enteric methane emissions when offered on an ad libitum basis.

Materials and Methods

Animals used in this experiment were under the care standards describes in the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2021). These standards were reviewed and approved by the Southern Plains Range Research Station Animal Care and Use Committee (Protocol number, AUP-028).

Experiment Date and Location

The experiment was conducted from July 19, 2021, to August 21, 2021, at the Southern Plains Experimental Range, Ft. Supply, OK (36° 37' N, 99° 35' W). Heifers were housed in a pen equipped with individual feed bunks that were equipped with Calan gates (American Calan Inc., Northword, NH), 101.2 m^2 of dirt surface/heifer, and 3.25 m^2 of shade/heifer.

Training and Acclimation Period

Twenty-five Red Angus heifers were trained to an automated head chamber system (AHCS; GreenFeed; C-Lock, Inc.) used for measuring gas exchange of O_2, CO_2, and CH_4. The training period started 15 wk before the start of the trial and proceeded as follows. The AHCS requires panels to be attached to both sides of the front of the unit to prevent air contamination from other animals. The AHCS also requires a narrow alleyway to prevent two animals from using the AHCS at one time. During the first part of the trial period, the panels and alleyway were removed to encourage use of the AHCS. The side panels and alleyway were replaced after 2 wk when the majority of the heifers started visiting the AHCS regularly. After a 7 wk training period, 14 heifers that most consistently used the AHCS were selected.

The 14 heifers were then trained to feed bunks using 12 Calan gates. During week one, latches on the Calan gates were held open to assist heifers with learning how to open the gates. At wk 2, the 12 heifers that were using the Calan gates with ease were randomly assigned a gate key, which was placed around their neck, and latches on the gates were released. After 2.5 wk, heifers

were sorted and penned in groups of four to assist the heifers with finding their gate. After 3 wk, heifers were then randomly assigned to 1 of 3 treatments.

During the 14-d adaptation period, heifers were fed their randomly assigned treatments of different maturities of long-stemmed wheat hay (*Triticum aestivum* L.). They were fed individually on an *ad libitum* basis using Calen gates. During this 2 wk period, heifers had access to the AHCS.

Dietary Treatments, Animals, and Feeding

The 12 heifers (initial BW = 296 ± 30 kg) were randomly assigned to one of three treatment diets and remained on the same treatment throughout the study; 1) immature maturity hay cut at the stem elongation stage (Zadok score=3.1); 2) intermediate maturity hay cut at the stem elongation stage/booting stage (Zadok score=3.9-4.1); and 3) mature hay cut at the milk development stage (Zadok score=7.9) (Table 3.1 and Figure 3.1). Further, heifers were supplemented daily with 0.60 kg of 12% calf creeper pellets (Table 3.2) (Stillwater Milling Company, Stillwater, OK) delivered via the AHCS.

The wheat (*Triticum aestivum* L.) was obtained from the USDA-ARS Southern Plains Experimental Range near Ft. Supply, Ok. The wheat was grown on a Lesho clay loam and Lincoln clay loam soil with a slope of 0 to 1%. In March, liquid urea ammonium nitrate (UAN) was applied. The wheat was cut on three different dates and baled into small square bales. The first cutting was an immature hay cut at the stem elongation stage. This wheat had a Zadok score (Tottman, 1987) of 3.1 and NDF of 37%. The second cutting was intermediate maturity cut at the stem elongation /booting stage, which had a Zadok score of 3.9-4.1 and NDF of 55%. The third cutting was mature stage cut at the milk development stage, which had a Zadok score of 7.9 and NDF of 63%. The bales of hay were placed on pallets on a concrete floor in an enclosed metal building.

Body weights were collected on day 0, 15, 22, 29, and 35. Body weights were recorded before the morning feeding to minimize variation associated with gastrointestinal fill. After body weights were collected, fecal samples were taken, via rectal grab, approximately every 3 hours for the first 24 hours (0, 3, 6, 9, 12, 15, 18, 21, and 24). After the initial 24 hours, fecal samples were taken at approximately 30, 36, 42, 48, 60, 72, 84, 96, and 108 hr.

Heifers were fed on an ad libitum basis and orts, if present, were collected at 0800 daily before feeding and weighed on a platform scale (0.05 lb. readability; Ohaus SD 35, Ohaus, Parsippany, NJ).

Carbon Emissions Sampling and Analyses

Emissions of CH_4 and CO_2 were measured using the AHCS. The principles of CH_4 and CO_2 measurements by the AHCS used in this experiment are described in Hristov et al. (2015). In brief, the heifers can move freely in and out of the AHCS and emissions are measured when the heifer's head is in the proper position in the chamber with its muzzle in front of the manifold as signaled by an infrared nose position sensor (Gunter et al., 2017). At each visit, a radio frequency identification tag (RFID) is read by the unit, and if a meal is allowed, bait is dropped. The AHCS bait used for this experiment was 12% creeper pellets that were dropped at 24-s intervals up to 8 times/feeding event (25.65 g/drop) with a maximum of 4 feeding events/d. Once a feeding event occurs, the AHCS was programmed to not allow a feeding event for 4.5 h to evenly space events throughout the day to control for circadian variation in CH_4 and CO_2 emission rates (Gunter and Bradford, 2015). The AHCS was also programed for a 24-s interval between drops of bait to keep the heifer's head in the unit for an adequate amount of time (approximately 3 min) to capture 3 or more eructations and to achieve a representative measurement of emissions (Huhtanen et al., 2015a). To ensure near complete tidal breath sampling, the air to be drawn in to the manifold was at a minimum rate of 26 L/s (Gunter et al., 2017).

Methane, CO_2 and O_2 concentrations were continuously measured in the outgoing air by a non-dispersive infrared analyzer (CH_4 and CO_2) and a paramagnet analyzer (O_2) and simultaneous measurements were made of airflow, temperature, and relative humidity. All data were uploaded hourly to the server at C-Lock, Inc. Algorithms were used to calculate CH_4, CO_2 and the consumption of O_2 for each visit were calculated as described in Gunter and Bradford (2017). The CO_2, CH_4, and O_2 sensors were calibrated at the beginning of each of the 3 periods and at the end of period 3. After a clean air filter (K & N Engineering; Riverside, CA) was installed, the sensors were flushed with a zero gas (0.001 ppm of CO_2, 0.001 PPM NO_x, 0.001 PPM sulfur dioxide, 20.80% O_2) for approximately 2 min followed by a span gas (0.4998% CO_2, 20% O_2, and 500.1 PPM CH_4). At the beginning of each of the 3 periods and at the end of period 3, the air flux sensor was calibrated gravimetrically 3 times by releasing CO_2 into the unit using a 90-g prefilled CO_2 cylinder (SigSauer CO_2 Cylinder, SigSauer, Newington, NH) for 3 min, then compared the gravimeter release to calculated capture. (102.45 ± 0.30% CO_2 recovery, SD = 1.05, n=12). The CO_2:CH_4 ratio was calculated by dividing moles of CO_2 emitted by moles of CH_4 emitted (Madsen et al., 2010).

The GLIMMIX procedure of SAS (SAS 9.4; SAS Inst., Inc., Cary, NC.) was used to identify outliers by a model using treatment, airflow rate, and hour of the day designed to calculate a studentized residual for each observation. Any residual gas flux with a calculated studentized residual of greater than 3.0 or less than -3.0 were removed. Further, only individual gas measurements from heifers lasting 3-min or longer were used so three eructations per spot-measure were present (Caetano et al., 2018; Huhtanen et al., 2015a) and necessary to minimize standard errors (Gunter and Bradford, 2017).

Feed Sampling and Analysis

Fecal samples were dried in a 60° C drying oven and ground to pass through a 2-mm screen (Thomas A. Wiley Laboratory Mill, model 4). Fecal samples were composited, by weight (5 g), for each heifer and period.

Samples of the hays were collected by coring every bale and drying in a forced-air oven at 60° C for 48 h to adjust dietary ingredients to a DM basis. Samples of the hays were sent to a commercial lab (Dairy One Inc., Ithaca, NY) for analysis of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), starch and minerals (Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and Mo). A subsample was retained for analysis at the USDA-ARS Southern Plains Range Research Station in Woodward, OK. The retained samples and pellets collected from the AHCS were ground to pass through a 2-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) in preparation for analysis.

Hay and fecal samples were analyzed for absolute dry matter (AOAC International, 2000), ash (AOAC International, 2000), and total N using a combustion method (Vario Max CN; Elementar Americas, Mount Laural, NJ, USA). Minerals (P, Ca, K, S, Co, Cu, Fe, Mn, Se, and Zn) in pellets were analyzed using a Delta Premium portable x-ray florescent spectrometer (Olympus America, Inc.; Newton, MA). The hay, pellets, and fecal samples were analyzed for gross energy (GE) using a combustion calorimeter (AC600, LECO; St. Joseph, MI). The hay, pellets, and composited fecal samples were additionally analyzed for acid insoluble ash (AIA) (Liu, 2022). Digestibility was then calculated as follows (Merchen, 1988):

$$Fecal\ DM\ Output = \frac{DMI\ x\ Diet\ AIA}{Fecal\ AIA} \quad [1]$$

$$DM\ Digestibility\ (\%) = \frac{DMI - Fecal\ DM\ Output}{DMI} * 100 \quad [2]$$

Energy Calculations

Urinary N excretion was predicted for each heifer and period using the following equation developed by (Dong et al., 2014) :

$$N_{urine}\left(\frac{g}{d}\right) = 2.39 + 0.55\ X\ N_{intake}\left(\frac{g}{d}\right) - 3.36\ X\ DMI\left(\frac{kg}{d}\right) \quad [3]$$

where N_{urine} is the urine N excretion rate, N_{intake} is the daily N intake, and DMI is the daily dry matter intake. A regression analysis for Equation 3 by Waldrip et al. (2013) showed close agreement between predicted N excretions and actual values, and in testing showed a 49 to 81% agreement with validation data sets.

Heat production (HP) was calculated using the equation of Brouwer (1965) as recommended by (NRC, 1981):

$$\text{HP}\left(\frac{Mcal}{d}\right) = \frac{3.8660\ x\ O_2\left(\frac{L}{d}\right) + 1.200\ \text{x}\ CO_2\left(\frac{L}{d}\right) - 1.44\ \text{x}\ N_{urine}\left(\frac{L}{d}\right) - 0.518\ \text{x}\ CH_4\left(\frac{L}{d}\right)}{1{,}000} \quad [4]$$

Intake of ME was estimated according to the following equation:

$$ME\ intake\left(\frac{Mcal}{d}\right) = HP\left(\frac{Mcal}{d}\right) + Tissue\ Energy\ Balance\ \left(\frac{Mcal}{d}\right) \quad [5]$$

where tissue energy balance was calculated according to the need to gain 1 kg of shrunk body weight (BW). Because the actual body composition and the mature BW at 25% body fat are unknown, BW was adjusted to a BW at which they are equivalent in body composition to heifers in the (Garrett, 1980) database as described by (Tylutki et al., 1994):

$$EQSBW = SBW\ x\ \left(\frac{SRW}{FSBW}\right) \quad [6]$$

where EQSBW is the BW equivalent used in the medium-framed steer equation (NRC, 1984), SBW is the shrunk BW (94.6% of full BW) being evaluated, SRW is the standard reference BW for the expected final body fat (478 kg and 27.8%, respectively) (NASEM, 2016), and FSBW is

the final shrunk BW at the expected final body fat ((600 kg; (NASEM, 2016)). To calculate the empty BW (EBW) of the heifers, the EQSBW was substituted into Equation 7 from SBW (kg):

$$EBW = 0.891\ X\ SBW \quad [7]$$

Also, to calculate empty BW gain (EBG) of the heifers, shrunk average daily gain (ADG) over each 7-d period was used as an estimate of SWG (kg):

$$EBG = 0.956\ X\ SWG \quad [8]$$

To compute the retained energy (RE), the energy content of BW gain for standard medium-framed steer equation (NRC, 1984) (Equation 7) using the EBW and EBG is from Equations 7 and 8, respectively:

$$RE\left(\frac{Mcal}{d}\right) = 0.0635\ X\ EBW^{0.75}\ X\ EBG^{1.097} \quad [9]$$

Digestible energy (DE) intake was estimated as the sum of metabolizable energy (ME) intake and energy emitted as CH_4 and excreted as urine:

$$DE\ intake\ \left(\frac{Mcal}{d}\right) = ME\ intake\left(\frac{Mcal}{d}\right) + CH_4 - energy\ output\left(\frac{Mcal}{d}\right)$$

$$+urinary - energy\ output\ \left(\frac{Mcal}{d}\right). \quad [10]$$

Total digestible nutrients was estimated by using the equation of Weiss (1993):

$$TDN = 0.98\ x\ (100 - NDF_N - CP - ash - FA - 1) + 0.93\ x\ CP + 2.25\ x\ FA +$$

$$0.75\ x\ (NDF_N - lignin)\ x\ \left[1 - \left(\frac{lignin}{NDF_N}\right)^{0.667}\right] - 7 \quad [11]$$

Where FA is fatty acid and NDF_N is N-free NDF, and all values are expressed as a percentage of DM.

Statistical Analysis

All response variables were analyzed using the ANOVA procedure of SAS (SAS 9.4; SAS Inst., Inc., Cary, NC) for a completely random design in a repeated measure. Hay maturity was the main plot. The following model was used for the dependent variables:

$Y_{ijk} = trt + Time + trt*Time + \varepsilon_{ijk}$

Where Y_{ijk} is the dependent variable, trt is the forage quality, Time is the treatment effect of sequence, trt*Time is their interaction, and ε_{ijk} is the residual overall error. The independent variable, NDF of the 3 forages, was separated using contrast statements for unequally spaced treatments (Robson, 1959). If a significant F-test was detected ($P < 0.05$), least square means for treatments were analyzed for linear and quadratic effects.

Results and Discussions

One heifer was removed during the last period of the study due to a shortage of hay. This heifer had been offered the immature hay. Also, the last period lasted 6 days, instead of 7 due to hay shortage.

Diet Nutrient Composition

As the wheat matured, CP, organic matter and total nitrogen of the hays decreased (Table 3.1). The immature hay consisted of 25.6% CP; the intermediate hay contained 19.0% CP while the mature hay consisted of 11.7% CP. The NDF and ADF concentrations increased as the maturity of the wheat hay increased. The immature hay had the highest value of TDN, and the mature hay had the lowest value. A one-unit increase in NDF was associated with a 0.31 decrease in TDN ($P = 0.04$; $R^{2 =}$ 0.996). Starch was less than 1% in the immature and intermediate wheat hays and was 3.3% in the mature wheat hay.

A major factor that affects forage quality is maturity of the plant. As the plants mature, the proportion of stems and cell wall constituents increase as the proportion of leaves decreases (Jung and Allen, 1995). Leaves are of higher quality than the stem (Van Soest, 1987). Stems are lower in digestibility than leaves, and digestibility declines more rapidly with increased plant maturity (Jung and Allen, 1995); thus fibrous cell wall constituents increase. Also, fibrous cell wall constituents become more lignified. There is a strong correlation between the decline of forage quality and the proportion of lignified structural tissue (Van Soest, 1987). As grasses mature, NDF increases while other nutrient levels decline (Horrocks and Vallentine, 1999). In our study, ADF, NDF increased, and CP decreased as maturity increased which corresponds with the finding of Lang et al. (2022), who reported that NDF and ADF of wheat in the dough, late milk, and flowering stage ranged between 32.95-51.09% and 17.17-28.79%, respectively, and increased with increasing maturity.

An analysis of the total starch of each of the hays was performed to determine if the amount of starch was of significant amounts, due to the bacteria that ferments the starch producing a higher proportion of propionic acid than the bacteria that ferments cellulose or hemicellulose (Orskov, 1986). Propionic acid serves as a hydrogen sink, and therefore hydrogen is captured in a metabolizable form rather than lost as methane (Orskov, 1986). Furthermore, rapidly-fermenting diets also reduce methane production by increasing the PR by reducing the pH of the rumen, which affects the growth of methanogens and protozoa (Gómez et al., 2016).

Intake, Digestibility, and Performance

Dry matter intake of hays fed at ad libitum declined linearly ($P < 0.01$; Table 3.3) as the maturity of forage offered increased. Heifers that were fed the immature hay consumed 3.40 kg more than the heifers fed the mature hay. A one-unit increase in NDF was associated with a 0.128 kg decrease in DMI ($P = 0.04$; $R^2 = 0.980$). Since DMI and CP values declined with increasing

maturity, CP intake decreased linearly ($P < 0.01$). Heifers consuming the immature hay consumed fewer creeper pellets and therefore visited the AHCS less often, and heifers consuming the intermediate hay consumed the most creeper pellets ($P < 0.01$). Total DMI decreased linearly as the wheat hay matured ($P < 0.01$). Heifers consuming the immature hay consuming 3.17 kg more than the heifers consuming the mature hay and 1.77 kg more than the heifers consuming the intermediate hay.

There was a correlation between the NDF values and the DMI that caused DMI to decrease as the grass matured. Neutral detergent fiber values are related to digestibility, feed intake, and rate of digestion (Mertens, 1997). The NDF component is digestible in the rumen but is more resistant to microbial degradation than soluble nutrients. This leads to a slower digestion rate, slower passage rate (PR), increase in ruminal residence time, and thus lowers intake (Van Soest, 1987). Forage digestibility decreases as ADF increases (Horrocks and Vallentine, 1999). Thus, ADF is used to predict the digestibility and therefore the energy content of feeds.

Digestibilities of hay decreased as the plants matured ($P = 0.02$). The digestibility of the immature hay was 76.22%, intermediate hay was 68.01%, and the mature hay's digestibility was 58.57%. These values are closely related to the ADF values of the wheat hays.

There was a tendency for the average daily gain to be linear ($P = 0.06$). Heifers consuming the immature hay gained 0.54 kg more than the heifers consuming the mature hay and 0.01 kg more than the heifers consuming the intermediate hay.

Digestibility refers to the fraction of a diet which disappears during passage through the gut. This implies that the assessment of nutritive value also involves the absorption process (Merchen, 1988). Since total fecal collection would be difficult to obtain, an internal marker was used to calculate digestibility. Acid-insoluble ash is naturally present in all feedstuffs (Liu, 2022).

Acid-insoluble ash is the ash that is insoluble in a dilute HCL solution (Liu, 2022). Feces contain considerable quantities of material of non-dietary origin, therefore coefficients of digestibility determined by calculation of the difference in amount of a given nutrient consumed and amount excreted in feces is considered to be apparent digestibility (Merchen, 1988). Therefore, the digestibility of the forages used in this experiment is an apparent digestibility and not true digestibility.

Energy Metabolism

As hay maturity increased, heat production decreased linearly ($P < 0.01$; Table 3.3). Energy retention of the mature hay was 0.8221 Mcal/day, while the energy retention values of the immature and intermediate hays were 2.3190 Mcal/day and 2.4615 Mcal/day, respectively. Predicted metabolizable energy intake decreased linearly as wheat matured ($P < 0.01$). Predicted digestible energy intake also decreased linearly as the wheat matured ($P < 0.01$).

The magnitude of HP is determined by many variables, which includes the quality and quantity of feed (MacRae and Lobley, 1982). Diets high in fiber are linked to higher HP than a high-concentrate diet. (Van Soest, 1987). Heifers consuming the immature wheat hay consumed 3.40 kg more than the heifers that consumed the mature hay, which could have caused the HP to decrease linearly as the hay maturity increased. Retained energy is the net energy for gain. It is the fraction of dietary energy that is deposited in body protein, body fat, conceptus, milk, hair, etc. (Ferrell and Oltjen, 2008). The body weight gains are used in the calculation of RE. In this study the ADG of the heifers consuming the immature and intermediate hay were 0.73 kg and heifers consuming the mature hay was 0.19 kg. Therefore, in our study, the RE was greatest for the immature and intermediate hay. Cole et al. (2020) had similar results and stated the differences in energy retention are based on differences in ME intake. This is due to the fact that

relationships between RE, proportion of fat in gain, and the proportion of protein in gain are influenced by dietary ME concentration (NASEM, 2016).

Metabolizable energy describes the amount of accessible energy a feed provides to the animal. Metabolizable energy is the digestible energy minus the energy lost in the urine and gases produced, which is mostly CH_4 (NASEM, 2016). Metabolizable energy intake was predicted by adding heat production to the retained energy values. Heat production was calculated using the measured O_2 consumption, CO_2 and CH_4 emissions and using 1.066674 as the factor for urinary energy loss of 6.674% (Shreck et al., 2017). Metabolizable energy intake decreased as the forage matured due to the increase in O_2 consumption and CO_2 production as well as the decline in intake.

Digestible energy is the gross energy of the food minus the energy lost in the feces (NASEM, 2016), which most of the energy lost from feed is via the feces. Digestible energy reflects diet digestibility (NASEM, 2016) and ADF is used to predict the digestibility of feeds. Therefore, as ADF increased with increasing maturity, DE intake decreased.

Methane Emissions

Daily CH_4 production was affected by hay maturity ($P = 0.03$; Table 3.4). Daily CH_4 was greatest for heifers consuming the immature hay and lowest for the heifers that consumed the mature hay. The average CH_4 emissions for mature hay was 160.26 g CH_4/day, intermediate hay was 169.62 g CH_4/day and immature hay resulted in 198.40 g CH_4/day being produced. Methane yield as expressed as g of CH_4/kg of DMI was not affected by maturity of hay ($P = 0.13$). Methane expressed as g of CH_4/kg of DMI ranged from 22.55 to 29.75. Cole et al. (2020) reported similar values of CH_4/kg of DMI 26.1-32.2g. As the wheat matured, methane reported as CH_4/unit of TDN was not linearly or quadratically effected by hay maturity ($P = 0.26$). Heifers consuming the mature hay emitted 2.84 g CH_4 /TDN intake, heifers consuming the intermediate

maturity hay emitted 2.96 g CH_4/TDN intake, while the heifers consuming the immature hay emitted 3.11 g CH_4 /TDN intake. Moss et al. (2000), stated that when energy intake is increased via increased forage quality there is an increase in digestion, which results in greater energy loss as CH_4. When expressed as CH_4/unit of CP and, we saw a linear ($P < 0.01$) increase in CH_4 production as the wheat hay matured. When CH_4 was expressed as g of CH_4/NDF intake and CH_4/ADF intake, we saw a linearly decrease as the hay matured ($P < 0.01$, $P < 0.01$). We did not see an effect of quality on CH_4 production when expressed as g of CH_4/ADG ($P = 0.61$).

Our results of the daily CH_4 production agrees with the results of Ominski et al. (2006) whose results showed that CH_4 production increased as forage quality decreased. Unlike in our study, however, Cole et al. (2020) found that CH_4 production per kg of digestible organic matter and NDF decreased as the forage quality increased. Boadi and Wittenberg (2002) also found that as forage quality declined, CH_4 production per kg of digestible organic matter intake (DOMI) increased as the quality of forages decreased.

The NDF values for Cole et al. (2020) study ranged from 65.7 – 73.6% and the low and medium quality hays consisted of Old World bluestem and the high quality hay consisted of alfalfa. They were fed at 90% ad libitum. The NDF values for Ominski et al. (2006) study ranged from 46.4 to 60.8%. Steers were fed alfalfa-grass silage diets at ad libitum. In Boadi and Wittenberg (2002) study, the NDF values ranged from 38.5 – 61.5%. Heifers were fed ad libitum. The low and medium quality hays consisted of chopped grass hay and the high-quality hay consisted of a legume/grass chopped mixed hay. In our study the NDF values ranged from 37.1 – 62.7%. In all the studies mentioned, a legume was used in the diets. Legumes have a higher digestible organic matter content and a faster PR which might shift fermentation against ruminal methanogenesis (Boadi and Wittenberg, 2002). It has been shown that alfalfa reduces enteric emission by 10% compared to non-legume forages (McCaughey et al., 1999).

In the current study, our range of NDF values in the forage was greater than the values utilized in Cole et al. (2020) and Ominski et al. (2006). Boadi and Wittenberg (2002) NDF values had a range of 23% and the current study had a range of 25.6%. The values of enteric CH4 emissions in our study are lower than that of Ominski et al. (2006) and Boadi and Wittenberg (2002).

Johnson and Johnson (1995) stated that cell wall constituents yielded greater CH4 production than cell solubles. They contributed this to the greater acetate: propionate ratios in the rumen produced during fermentation.

Carbon Dioxide Emissions and Oxygen Consumption

Carbon dioxide emissions had a linear decrease in response to hay maturity ($P < 0.01$; Table 3.5). When expressed as CO_2 per kg of DMI, hay maturity did not have an effect on CO_2 emissions ($P = 0.17$). Oxygen consumption also had a linear decrease in response to hay maturity ($P \leq 0.01$). The respiratory quotient (RQ) was not impacted by hay maturity ($P = 0.35$).

Although CO_2 is not as potent as CH_4, it is still a concern from an environmental standpoint regarding livestock production. Microbes in the rumen ferment and digest plant cell wall components and break them down into sugars and carbohydrates. The fermenting of sugars produces volatile fatty acids, which include acetate, propionate, and butyrate. Carbon dioxide, hydrogen, CH_4, and ammonia are also byproducts. The byproducts are either absorbed through the rumen wall, flow out into the lower tract, or is removed by eructation (Neel et al., 2019). In addition to CO_2 being produced during enteric fermentation, the largest part is produced during normal intermediary metabolism in the animals (Madsen et al., 2010).The RQ is a dimensionless number and used as an indicator of basal metabolic rate. It is defined as the volume of CO_2 released over the volume of O_2 absorbed during respiration (Patel et al., 2022). The RQ provides

information as to if carbohydrates or fats are being utilized (Van Soest, 1987). In this study, the RQ was similar among all treatments and above 1.0, indicating high rates of lipid synthesis for all heifers.

Fecal Analysis

Total fecal nitrogen decreased quadratically as the hay the heifers consumed matured ($P < 0.01$). Fecal crude protein was calculated from the nitrogen content of the sample, therefore fecal crude protein followed the same patterns ($P < 0.01$). Fecal NDF quadratically increased as the wheat hay consumed increased in maturity ($P < 0.01$). Fecal ADF also quadratically increased as the wheat hay consumed increased in maturity ($P < 0.01$). Fecal GE was not affected by quality ($P = 0.93$). Ash quadratically decreased with the increasing maturity of the wheat hay ($P < 0.03$).

Conclusions

Reducing the amount of CH_4 produced from beef cattle during the microbial digestion of feed not only reduces the carbon footprint of the cattle industry but also reduces the amount of energy lost that could have been used more effectively by the animal. This experiment looked at feeding beef heifers three different maturities of wheat hay. As the wheat hay matured the quality of the hay decreased. This study showed that as wheat hay matures, there is a negative effect on DMI, CO_2 emissions, O_2 consumption, gain of body weight, predicted ME intake, predicted DE intake, and heat production. Heifers consuming the mature hay emitted less daily CH_4, but maturity did not have an effect on CH_4 yield. However, other studies do not agree with the findings (Boadi and Wittenberg, 2002; Cole et al., 2020; Ominski et al., 2006). McAllister et al. (1996) stated that as forage digestibility decreases, forage intake decreases and the acetate: propionate ratio increases, which favors increased CH_4 production per unit of forage consumed.

Table 3.1 Nutrient composition of three different maturities of wheat hays offered to growing beef heifers

Item	Immature hay	Intermediate hay	Mature hay
DM, % of fresh matter	90.7	91.2	93.5
Zadok's Maturity Score	3.1[a]	3.9-4.1[b]	7.9[c]
		DM basis	
CP	25.6	19	11.7
ADF	25.9	32.7	40.6
NDF	37.1	55.5	62.7
Starch, %	0.8	0.8	3.3
TDN, %	64	58	56
NEm, Mcal/kg	0.63	0.53	0.49
NEg, Mcal/kg	0.37	0.28	0.24
Ash	19.66	14.38	11.48
Gross energy, cal/g	4,087.77	4,206.87	4,226.61
Total, N%	3.91	3.06	2.03
Ca, %	0.87	0.56	0.31
P, %	0.29	0.21	0.2
Mg, %	0.27	0.2	0.15
K, %	3.76	2.48	1.59
Na, %	0.02	0.01	0.01
Fe, %	0.17	0.16	0.07
Zn, ppm	28	28	21
Cu, ppm	10	12	8
Mn, ppm	85	86	65
Mo, ppm	1.18	0.98	1.7

[a] 3.1 maturity score corresponds with the stem elongation stage of development
[b] 3.9-4.1 maturity score corresponds with the stem elongation/booting stage of development
[c] 7.9 maturity score corresponds with the milk development stage of development

DM = Dry matter
CP = Crude protein
ADF = Acid detergent fiber
NDF = Neutral detergent fiber
TDN = Total digestible nutrient
NEm = Net energy for maintenance
NEg = Net energy for gain
Mcal = Megacalories
Kg = Kilograms

Table 3.2 Nutrient composition of creeper pellets offered to growing beef heifers

Item	Creeper pellets
DM, % of fresh matter	92.41
	DM basis
Ash	13.51
Gross energy, cal/g	4,087.77
CP, %	16.69
NDF	35.28
ADF	12.98
Ca, %	0.45
P, %	0.44
K, %	1.55
S, %	0.28
Fe, %	0.22
Zn, ppm	88.48
Cu, ppm	7.2
Mn, ppm	214.29

DM = Dry matter
CP = Crude protein
NDF = Neutral detergent fiber
ADF = Acid detergent fiber

Table 3.3 Intake, digestibility, performance and energy metabolism of beef heifers fed three different maturities of wheat hay

	Diet				Contrast	
Item	Immature	Intermediate	Mature	SEM	Linear	Quadratic
DMI, Kg	8.45	6.44	5.05	0.51	0.005	0.563
Pellet Intake, Kg	0.44	0.68	0.66	0.06	0.005	0.184
Total DMI, Kg	8.89	7.12	5.72	0.51	0.006	0.503
CPI, g	2199.72	1228.93	726.47	103.67	< 0.001	0.566
Digestibility, %	76.22	68.01	58.57	0.04	0.023	0.387
pME intake, Mcal	18.88	15.94	13.50	0.66	0.002	0.361
pDE intake, Mcal	21.50	18.18	15.61	0.76	0.003	0.427
RE, Mcal	2.32	2.46	0.82	0.47	0.094	0.128
HP, Mcal	16.59	13.45	12.68	0.39	0.001	0.541
ADG	0.73	0.73	0.19	0.15	0.060	0.132

DMI = Dry matter intake
CPI = Crude protein intake
pME = Predicted metabolizable energy
pDE = Predicted digestible energy
RE = Retained energy
HP = Heat production
ADG = Average daily gain
Mcal = Megacalories
Kg = Kilograms
G = Grams

Figure 3.1 Chart showing average daily gain of heifers consuming 3 different maturities of wheat hay

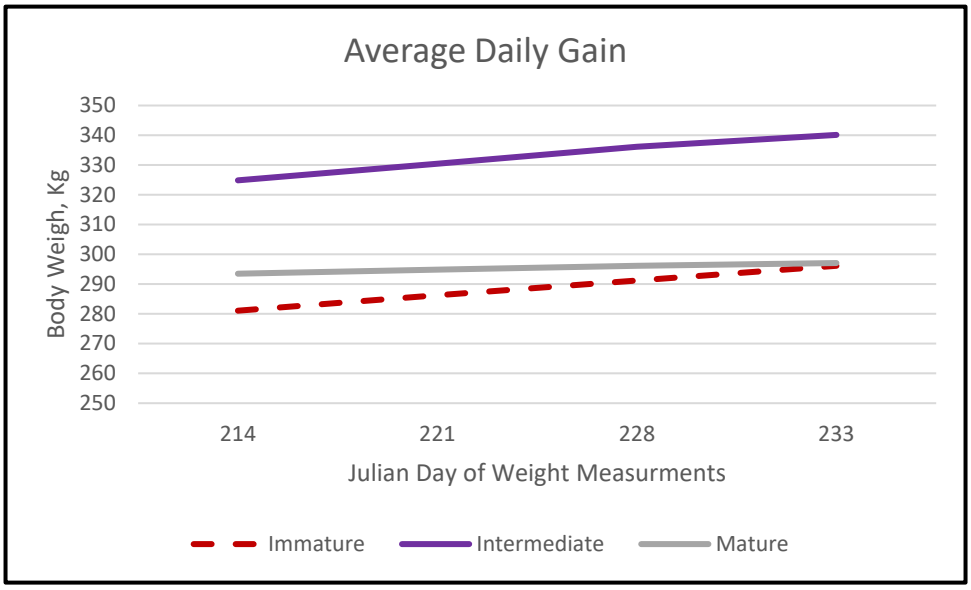


Table 3.4 Methane emissions of beef heifers fed three different maturities of wheat

	Diet				Contrast	
Item	Immature	Intermediate	Mature	SEM	Linear	Quadratic
CH_4 (g/day)	198.40	169.62	160.26	10.22	0.029	0.926
CH_4 (g/kg of DMI)	22.55	24.27	29.75	2.89	0.129	0.429
CH_4 (g/kg ADG)	370.60	197.01	330.15	129.17	0.613	0.457
CH_4 (g/TDN)	3.12	2.96	2.84	0.17	0.258	0.854
CH_4 (g/ADF intake)	0.78	0.53	0.44	0.04	0.001	0.880
CH_4 (g/NDF intake)	0.53	0.29	0.28	0.02	0.001	0.153
CH_4 (g/CP intake)	0.81	0.96	1.32	0.07	0.004	0.071

DMI = Dry matter intake
ADG = Average daily gain
TDN = Total digestible nutrients
ADF = Acid detergent fiber
NDF = Neutral detergent fiber
CP = Crude protein
Kg = Kilograms
G = grams

Table 3.5 Carbon dioxide emissions, oxygen consumption, and the respiratory quotient from beef heifers fed three different maturities of wheat hay

Item	Immature	Intermediate	Mature	SEM	Linear	Quadratic
O_2 (g/day)	4352.98	3532.66	3347.12	102.83	0.001	0.491
CO_2 (g/day)	6405.41	5198.71	4824.45	160.23	0.001	0.754
CO_2 (g/kg of DMI)	736.69	746.84	889.23	67.28	0.172	0.334
RQ	1.08	1.07	1.05	0.01	0.353	0.332

RQ = Respiratory Quotient
DMI = Dry matter intake
Kg = kilograms
G = Grams

Figure 3.2 Wheat cut at three different maturity levels

Zadok score=3.1[a]	Zadok score=3.9-4.1[b]	Zadok score=7.9[c]
Immature	Intermediate	Mature

[a] 3.1 maturity score corresponds with the stem elongation stage of development
[b] 3.9-4.1 maturity score corresponds with the stem elongation/booting stage of development
[c] 7.9 maturity score corresponds with the milk development stage of development

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VITA

Emalee Jean Friend

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF HAY MATURITY ON THE ENTERIC METHANE EMISSIONS, FORAGE INTAKE, ENERGY METABOLISM AND DIGESTION BY BEEF HEIFERS

Major Field: Animal Science

Biographical:

Education:

Completed the requirements for the Master of Science in Animal Science at Oklahoma State University, Stillwater, Oklahoma in May, 2023.

Completed the requirements for the Bachelor of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in December, 2002.

Experience: Worked at: United States Department of Agriculture, Oklahoma and Central Plains Agricultural Research Center in the Livestock, Forage, and Pasture Management Research Unit (December 2002-current); Oklahoma State University in the Small Grains Weed Science Department (August 2000-December 2002); Oklahoma State University in the Wheat Breeding and Genetics Department (May 1998-August 2000). Raised on a family farm near Canton, Oklahoma where we raised wheat, stocker calves, and had a cow/calf operation.

Professional Memberships: American Society of Animal Science