Does Supplemental Protein and Rumen-Protected Methionine Improve Performance and Digestibility During Late Gestation in Beef Cows?

by

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ABSTRACT

Does Supplemental Protein and Rumen-Protected Methionine Improve Performance and Digestibility During Late Gestation in Beef Cows?

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The objective of this study was to determine if supplemental protein and rumen-protected methionine (MET) improve cow performance and apparent total tract digestibility (TTD) during late gestation. Pregnant beef cows (n=99) and heifers (n=39) were randomly assigned to one of six diets formulated to meet 90, 100, or 110% of metabolizable protein (MP) requirements (NRC, 2016), with(without) 9 g/d of rumen-protected MET (Smartamine®M, Adisseo Inc.). Cows fed at 90% MP requirements lost body weight (BW), pregnancy corrected BW, and had reduced TTD (P<0.05). Supplemental MET did not affect cow performance; however, MET supplementation increased glucose and reduced EAA, BCAA, and ketogenic AA serum concentrations (P≤0.02). Calf birth weights were not impacted by dietary treatment. Thus, feeding cows below their MP requirements may limit late gestation performance and TTD. Supplemental MET may increase amino acid utilization but did not improve beef cow performance or digestibility parameters measured in late gestation.
DEDICATION

I would like to dedicate this to my mother, Susan Darrach. She instilled in me the importance of education and the power of learning. Although she didn’t make it to see me go to University, I have always felt her support.
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LIST OF ABBREVIATIONS

AA: Amino acid
ADF: Acid detergent fibre
ADG: Average daily gain
BW: Body weight
BCAA: Branch chain amino acid
BHBA: β-hydroxybutyrate
CP: Crude protein
DM: Dry matter
DMI: Dry matter intake
MCP: Microbial crude protein
MP: Metabolizable protein
uNDF: Indigestible neutral detergent fibre
NDF: Neutral detergent fibre
NEAA: Non-essential amino acids
NEFA: Non-esterified fatty acids
NEm: Net energy maintenance
PC-BW: Pregnancy corrected body weight
RDP: Rumen degradable protein
RUP: Rumen undegradable protein
TDN: Total digestible nutrients
TMR: Total mixed ration
3MH: 3- methyl histidine
1 Literature review

1.1 Introduction

Beef cattle production is a major component of the Canadian livestock production sector. Beef farms are the second most common farm type in Canada and as of July 2018, there was approximately 10.5 million beef cattle in Canada (Statistics Canada, 2018). Feed is the most significant contributor to the costs associated with beef production in Canada, accounting for ~50% of total costs of production which can increase in the winter (Manitoba Agriculture, 2017). Therefore, research to find more cost-effective ways to feed beef cattle while upholding cattle performance would greatly increase producer profits.

One particularly important period for beef cattle producer profits is during late gestation. In Canada, this period of the production cycle often occurs during the winter months, in which the producer is supplying feed to the animal. Late gestation is a crucial period for the cow to maintain body condition, body weight, while supporting rapid fetal development (Bauman and Currie, 1980). The nutrient requirements for cattle during this period are approximately 75% greater than a non-pregnant cow (Bauman and Currie, 1980). This increased demand is due to development of the fetal membranes, gravid uterus, mammary glands, and towards fetal growth with the fetus gaining approximately 60% of total birth weight during late gestation (Bauman and Currie, 1980). During this period, the dam needs to be able to repartition absorbed nutrients to the fetus without greatly compromising her own body reserves, through homeorhetic mechanisms. Homeorhesis is defined as the “orchestrated changes for the priorities of a
physiological state, i.e. coordination of metabolism in various tissues to support a physiological state” (Bauman and Currie, 1980). However, many of these homeorhetic mechanisms are not well characterized in beef cattle.

In North American beef production systems, cows are often fed low-quality forages during gestation with limited protein content. Traditionally, a protein supplement may also be provided and has been shown to improve cow performance during late gestation (Larson et al., 2014). Some producers may feed pregnant cattle above protein requirements in an attempt to improve dam health and calf growth. Recent studies have explored how the amount and quality of protein fed to a beef cattle dam can subsequently affect the progeny’s performance as well as the dam’s recovery time after pregnancy (Larson et al., 2014; Stalker et al., 2014).

Although protein supplementation has proven to benefit cow-calf performance, feeding high amounts of protein can have negative environmental impacts. Overfeeding protein increases the nitrogenous waste excreted via feces and urine (Burgos et al., 2010). Currently, there is pressure on the beef cattle industry to reduce environmental emissions, creating motivation for increased nitrogen utilization efficiency. Thus, an alternative for the over-supply of crude protein in the diet should be explored to reduce nitrogen pollution from the beef cattle industry while upholding dam and calf health and performance. One possible method to reduce nitrogen emissions is by improving the efficiency of protein utilization by more closely meeting amino acid (AA) requirements for cattle at their specific stage of production.
Although AA requirements for pregnant beef cows are not well known, the most likely limiting amino acid in forage-fed cattle is methionine (Waterman et al., 2012). However, there has been limited research exploring rumen-protected methionine’s effect on the dam and her progeny in beef cattle. This, review will address protein and rumen-protected methionine requirements and the influence they have on cow performance, circulating blood metabolites, protein metabolism, and total tract digestibility in gestating beef cattle.

1.2 Protein supplementation

1.2.1 Protein requirements for gestating beef cattle

Protein content in the diet is assessed in several ways such as: crude protein (CP), metabolizable protein (MP), rumen degradable protein (RDP), and rumen undegradable protein (RUP). Crude protein percentage is a common way to describe the nitrogen (N) content in the particular feedstuff. However, MP is considered to be the actual protein that is absorbed and used by the animal (NRC, 2016). This protein absorbed in the small intestine is primarily comprised of microbial crude protein (MCP) and RUP (NRC, 2016). Therefore, the model to calculate MP requirements is more complex than using dietary CP% as the standard for N requirements for cattle. Metabolizable protein requirements for gestation are calculated as follows:

*Equation one: the net protein retained by the conceptus (Ypn) can be calculated using the calf birth weight (CBW) and days pregnant (DP; NRC, 2016).*

\[
Ypn = CBW \times (0.001669 - 0.00000211xDP) \times e^{0.0278xDP - 0.0000176xDP^2} \times 6.25
\]
Equation two: metabolizable protein requirements for pregnancy (MPy) in g/d can be calculated using net energy acquired by the conceptus (Ypn), and the assumed fixed efficiency of 0.65 (NRC, 2016).

\[ MPy = \frac{Ypn}{0.65} \]

The MPy calculated in these equations can then be added to the cow’s maintenance requirements to acquire the cow’s total protein requirements during pregnancy estimated using expected calf birth weight and day of gestation. For calculating a heifer’s MP requirements, growth must also be taken into consideration as heifers are most often not at their mature weight during gestation (NRC, 2016).

1.2.2 Protein supplementation’s influence on cow performance

Since the dam metabolically prioritizes the fetal requirements over her own, meeting maintenance and pregnancy requirements is vital to limit severe body reserve mobilization (Bauman and Currie et al., 1980). During late gestation, the effect of nutrition on cow performance can be accessed by changes in body weight (BW), and body condition score (BCS) over gestation.

Previous research on protein supplementation during late gestation found that cows fed a protein supplement (containing 28% CP at 0.45 kg/d) to a ≤6.8%CP basal diet, had greater pre-calving and pre-breeding BW than cows fed the basal diet alone (Larson et al., 2014). This demonstrates that not only were there improvements in BW in cattle fed the supplement during gestation, but this effect is also carried over into the breeding season, supporting subsequent reproductive success. In another study, researchers fed a supplement containing a significantly higher level of protein
supplementation (42% CP at 0.45 kg/d) during gestation and found that the non-supplemented cows (≤8.7% CP) diet lost 29 kg BW during the pre-partum period while the supplemented cows maintained their body weight (Stalker et al., 2014). Feeding the protein supplement pre-partum also improved BCS at calving and subsequent rebreeding (Stalker et al., 2014). This study also found that birth weights were not affected by pre-partum protein supplementation; however, weaning weights were heavier in calves from supplemented dams (Stalker et al., 2014). These results also indicate that pre-partum supplementation may also have longer-term impacts on calf performance, which may support an economic advantage for producers. Body condition scores were higher for cows fed supplemental protein in the form of dried distillers grain-based cubes (24% CP) three times a week compared to cattle only grazing corn stalk residue during gestation (Warner et al., 2011). However, this study also found that calf birth weight, calf weaning weight, calving interval, percentage of cows cyclic before breeding, and final pregnancy rates did not differ between cows fed only cornstalks and those fed the supplement (Warner et al., 2011). This may indicate that although BCS increased throughout pregnancy, it was not enough to impact breeding success or calf performance. Conversely, when cows were either fed a diet meeting protein and energy requirements or a diet exceeding protein requirements (129% requirements), there were no differences in BCS, calf birth weight and post-calving BW as well as no differences in calving adjusted BW (Wilson et al., 2016). This demonstrates that once protein requirements are met, there may be no benefits from further protein supplementation.
Thus, although dam fertility or calf birth weight was not impacted by protein supplementation, most studies conclude that the dam’s BCS and BW can be increased by supplementing protein during late gestation. Although lower protein diets may increase nitrogen efficiency, research has suggested that it can negatively impact cow performance by reducing BW and BCS at calving and rebreeding (Larson et al., 2014; Stalker et al., 2014; Warner et al., 2011). However, in all these studies the addition of a protein supplement also increased the amount of new energy supplied to the cows. Therefore, it is difficult to determine if these effects were observed from N supplementation alone, or if the combination of N and energy contributed to the observed benefits of supplementing a low-quality forage diet in late gestation.

1.2.3 Protein supplementation’s influence on total tract digestibility

The digestive tract in a ruminant animal is much more complex than that of a monogastric animal. This is primarily due to the extensive fermentation that occurs in the forestomach where cattle rely on a symbiotic relationship with rumen microbes (Dehority, 2002). This mutually beneficial environment in the rumen allows cattle to utilize complex cell wall carbohydrates which can be summarized into three main types: neutral detergent fibre (NDF) that contains cellulose and hemicellulose, and lignin, and acid detergent fibre (ADF) composed of cellulose and lignin and lignin alone. While lignin is mainly resistant to digestion, ruminal digestion of cellulose and hemicellulose is dependent on many factors including animal species, supplements (grain or protein), forage processing, environmental factors and forage maturity (NRC, 2016). These cell wall carbohydrates (excluding lignin) can be utilized by the rumen microbes, in turn
increasing rumen MCP production. The rumen microbes do so by utilizing enzymes to produce microbial biomass and end products of fermentation (McAllister et al., 1994). Acetate, one end product of microbial fermentation, can contribute 50-60% of a cows’ energy requirements (Harfoot, 1978). Additionally, microbial crude protein can make up to ¾ of a cow’s total AA uptake through absorption in the small intestine (NRC, 2016). While most diets meet the RDP requirements to sustain a healthy rumen microbial population, low-quality forage diets fed to beef cows may not meet rumen microbial nitrogen and/or energy requirements (NRC, 2016; Fu et al., 2001). Therefore, research has explored the potential positive impact a protein supplement can have on cow total tract digestibility.

Protein supplementation is documented to crucially influence digestibility in cattle. Even when energy requirements are met or exceed, if dietary protein requirements are not met, DMI and digestibility will be reduced (DelCurto et al., 1990). Protein supplementation for cattle increases DM digestibility by enhancing N availability to rumen microbes, meeting microbial N requirements for growth and metabolism (May et al., 2014; Bohnert et al., 2002). For ruminant species, fibre digestion happens almost exclusively in the reticulorumen, whereas only about 10% of NDF is degraded in the hindgut (NRC, 2016). Ruminal digestion of fibre is mainly controlled by the microbial population as well as the chemical and physical characteristics of the fibre; however, protein supplementation can also influence fibre digestibility (NRC, 2016). Research clearly demonstrates that as protein supplementation increases, NDF digestibility of the diet will also increase (Hare et al., 2019; May et al., 2014; Bohnert et al., 2002).
Increasing the protein content in the diet of these cows is most likely supporting rumen microbial digestion of fibre. Since pregnant beef cattle in North America are typically fed low-quality forages, a protein supplement could be beneficial to enhance fibre digestibility and allow cattle to meet their nutrient requirements for growth, maintenance, and pregnancy. This is particularly important in late gestation beef cows as the growing calf competes with rumen capacity for space. This reduced rumen capacity, increasing the importance of the diets digestibility to optimize nutrient uptake (Stanley et al., 1993).

1.2.4 Protein supplementation’s influence on circulating metabolites and markers of protein synthesis and degradation

Circulating metabolites can be an indicator of a cow’s physiological and metabolic states. Several circulating metabolites are particularly important indicators for an individual cow’s nutritional status including urea, glucose, insulin, NEFA (non-esterified fatty acids) and 3-methylhistidine. Protein supplementation in cattle can impact these circulating metabolites indicating the impact on underlying metabolism.

Circulating blood urea N concentrations can be one indicator of nitrogen status in the cow, as it is a measure of nitrogen recycling and ruminant species can transport nitrogen around the body in the form of urea that can re-enter the rumen (Reynolds and Kristensen, 2008). Rumen microbes can then use this form of non-protein nitrogen for MCP synthesis, which the animal can then use to meet AA requirements (NRC, 2016). One recent study found that oversupplying MP by approximately 140% of requirements to beef heifers during late gestation increased plasma urea-nitrogen concentrations (Hare et al., 2019). Another study supplemented primiparous beef cattle fed an annual rye hay with soybean meal during the final trimester and for 58 d following parturition
(Hess et al., 1998) and found that serum urea nitrogen concentrations tended to be elevated, and milk urea nitrogen concentrations with increased with protein supplementation post-partum (Hess et al., 1998). Similarly, plasma urea concentrations increased when gestating beef cattle were fed a soybean meal supplement vs. cows fed the basal diet containing 8.7% CP diet (Cappellozza et al., 2014). The researchers postulated that the elevated serum urea nitrogen was attributed to enhanced ruminal protein breakdown resulting in increased ammonia absorption by the rumen (Cappellozza et al., 2014). This allowed for increased urea formation in the liver, promoting urea transport into the blood. Therefore, these studies agree that protein supplementation can positively impact nitrogen status in cattle.

Additionally, serum and plasma NEFA concentrations are also an indicator of an animal’s physiological status. Blood NEFA concentrations are one of the most reliable indicators of lipolysis in cattle (González et al., 2011). High levels of circulating fatty acids are transported to the liver, and if the liver becomes overwhelmed by high NEFA from excessive lipolysis, fatty liver disease and ketosis can occur in severe cases (González et al., 2011). Past studies have documented an increase in blood NEFA concentrations in times of energy and/or protein restriction for cows (Amanlou et al., 2017; Wood et al., 2013).

Circulating concentrations of glucose and insulin can be utilized to assess a cow’s energy status. Insulin is essential for nutrient uptake and protein synthesis in animals (Brockman, 1978). In one study, gestating beef cattle grazing native dormant range containing ≤6% CP, were fed 1.82 kg/d of a protein supplement (38% CP) during
early gestation to assess insulin concentrations (McLean et al., 2018). Researchers found greater plasma insulin concentrations when cattle were fed the protein supplement compared to cows fed just dormant range (McLean et al., 2018). Mclean et al. (2018) postulated that this increased insulin concentration in supplemented cattle was in response to an increased in available nutrients to the animal. Another study evaluated pregnant beef heifers fed a diet primarily containing low-quality cool-season forages, and fed either no protein supplementation (CON), or supplemented with soybean meal (PROT) at 0.50% of shrunken BW (Cappellozza et al., 2014). Heifers fed PROT had higher concentrations of plasma glucose and insulin (Cappellozza et al., 2014). Thus, these studies suggest that blood glucose and insulin concentrations can be influenced by protein supplementation during late gestation, potentially increasing the animal’s nutrient uptake, rate of protein synthesis, and(or) repartitioning of nutrients.

In addition to markers of protein synthesis, markers of protein degradation can also be used to assess protein metabolism in cattle. The post-translationally modified amino acid 3- methylhistidine (3MH) can be utilized as a biomarker for skeletal protein degradation as approximately 90% of protein-bound 3MH is found in skeletal muscle in actin and myosin (McKeran et al., 1979; Haverberg et al., 1975). After 3MH is released from bound actin and myosin, it can be found in the blood and is eventually excreted in the urine (McKeran et al., 1979). In dairy cattle serum, 3MH concentrations spike during the start of lactation, most likely due to skeletal protein breakdown to meet protein demands for milk production (Phillips et al., 2003; Blum et al., 1985). However, there is
a lack of research exploring 3MH responses in beef cattle blood or urine when fed a protein-restricted diet.

1.3 Methionine supplementation

1.3.1 Methionine supplementation’s influence on cow performance

Amino acids are the building blocks of protein that are responsible for supporting many biological processes in growth and metabolism. If one or more essential amino acid is limiting in the diet, important biological pathways can be limited, and animal performance may be impacted. For ruminant species, there are 10 essential amino acids: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, arginine, leucine, and lysine (Nichols et al., 2017). Methionine comprises 6.4 grams per 100g of empty body bovine protein, making it the 6th most prevalent amino acid (NRC, 2016). Methionine is a sulfur-containing amino acid that plays a vital role in many biological processes including: protein synthesis, antioxidation, and DNA methylation (Martínez et al., 2017; Cauvoto and Fenech, 2012). In particular, methionine is a significant methyl donor in the ruminant animal as it acts as a promoter for phospholipid synthesis and a precursor for apolipoproteins, required for the secretion of VLDL from the liver (Grummer, 1993). Without the secretion of fat from the liver, ruminant animals can develop fatty liver, in severe cases leading to ketosis (Grummer, 1993). Additionally, methionine also plays a vital role in hundreds of one-carbon methylation reactions via the synthesis of glutathione and taurine, which are important sulfur-containing antioxidants (Schwab and Broderick, 2017). Therefore, methionine not only
plays an important role in protein synthesis but also fat mobilization and disease prevention.

Although it is a very important AA for many biological processes, methionine is the likely first limiting amino acid in forage-fed cattle as well as for the rumen microbes (Waterman et al., 2012; Salter et al., 1979). When forages are fed to cattle, they rely on MCP to meet AA requirements, MCP only contains a small amount of methionine reducing the cow’s ability to meet methionine requirements (Liker et al., 2005; Clark et al., 1992). In order to determine the first limiting AA in the ruminant diet, Klemesrud et al., (2000) increased methionine content in the diet and using a non-linear analysis to find the breaking point where ADG had no further changes with an increasing methionine supplementation.

Since methionine is such a vital, yet limiting amino acid, a methionine supplement may be beneficial for adding to ruminant diets. However, ruminal microbes can degrade methionine. To avoid this, methionine can be rumen-protected via encapsulation or the addition of an isopropyl ester (Baghbanzadeh-Nobari et al., 2016). This allows the cow to uptake methionine directly from the small intestine instead of being lost to protein degradation in the rumen.

Although protein requirements have been extensively researched in cattle, AA requirements for pregnant beef cows are not fully understood. Feeding low levels of RUP (found in forage-fed cattle) can reduce the passage of methionine into the small intestine. This is primarily due to the fact that MCP produced in the rumen contains only small amounts of methionine, reducing the cow’s ability to meet methionine
requirements (Liker et al., 2005; Clark et al., 1992). Methionine supplementation has been studied in dairy cattle (Alharthi et al., 2018; Batistel et al., 2017; Vyas and Erdman, 2009; Leonardi et al., 2003; Armentano et al., 1997) and growing beef cattle (Archibeque et al., 2002; Klemesrud et al., 2000); however, only limited work on rumen-protected methionine supplementation has been explored in gestating beef cows.

Methionine supplementation in steers has been used to explore its effects on nitrogen retention and ADG (average daily gain). In ruminant species, nitrogen can be recycled within the body and utilized by rumen microbes for the synthesis of microbial crude protein (NRC, 2016). In a symbiotic relationship, the animal can then use this microbial protein to meet their own protein requirements. When D-L methionine was supplemented to steers infused with isotopic urea, steers tended to have decreased daily urinary nitrogen levels in comparison to controls, and tended to have increased daily nitrogen retention, and a greater percentage of N retained from total nitrogen digested in the steers (Archibeque et al., 2002). Thus, this study demonstrated that methionine was effective at increasing nitrogen retention. Additionally, researchers have reported quadratic relationships between increased methionine infusion levels in the abomasum and nitrogen retention in Holstein bull calves and 4-month old Charolais x Dorset male sheep (Li and Zhao, 2011; Schwab et al., 1982). These studies suggest that methionine plays an intrinsic role in nitrogen efficiency in ruminant species; thus, certain forms of dietary nitrogen may have reduced utilization for protein synthesis if methionine requirements are not being met.
The impact of methionine on ADG in growing cattle has also been studied. When a rumen-protected methionine supplement was fed with meat and bone meal (MBM) as a dietary protein source, increased ADG was found vs. cattle fed just MBM or urea protein supplementation (Klemesrud et al., 2000). This suggests that MBM may be limiting in digestible methionine and does not provide adequate levels of methionine to cattle (Klemesrud et al., 2000). After methionine supplementation exceeded 2.9 g/d, there was no further improvement in ADG providing evidence that the growth requirements for methionine were met at this amount (Klemesrud et al., 2000). Thus, this suggests that supplementing methionine may only be beneficial to increasing ADG in cattle when the diet is limiting in methionine.

Although methionine supplementation has been explored in steers, limited research has been completed on methionine’s effect on cow performance during late gestation, and in the few published studies findings are often contradicting. Several studies completed on dairy cattle found milk protein percentage increased when a rumen-protected methionine was supplemented (Vyas and Erdman, 2009; Leonardi et al., 2003; Armentano et al., 1997). These studies suggest that a methionine supplement improves protein repartitioning to the milk in dairy cows. Conversely, Clements et al. (2017) fed mature beef cows a methionine hydroxyl analog for 23 ± 7 days and found cow BW and BCS did not differ at 27 ± 7 days post-partum. There were no differences in BW or BCS at weaning nor any differences in milk production (Clements et al., 2017). Thus, there is still conflicting results and a lack of research on the impact of
supplemental methionine on cow performance, specifically regarding changes in BW and BCS over gestation, and milk synthesis.

There is limited research on how maternal methionine supplementation impacts calf performance and this research is almost exclusively found in dairy cow literature. Two studies fed dairy cow dams a rumen-protected methionine supplement during late gestation and found that progeny had increased birth weights compared to calves from unsupplemented dams (Alharthi et al., 2018; Batistel et al., 2017). Batistel et al. (2017) also found that the rumen-protected methionine supplement increased glucose and AA transporters in placental tissues, which they postulated was one of the reasons for the increase in calf birth weights with methionine supplementation (Batistel et al., 2017). A study conducted on cows supplemented with a methionine hydroxyl analog at 23 ± 7 d prior to calving, and 73 ± 7 d postpartum found no differences in calf birth weights or weaning weights compared to calves from unsupplemented dams (Clements et al., 2017). Another study in beef cattle found that when late gestation heifers were fed a rumen-protected supplement during a period of weight loss, it did not influence calf birth weights compared to feeding the control diet (Waterman et al., 2012). Thus, there are conflicting results on whether or not calf birth weight is influenced by late gestation methionine supplementation. However, the amount and type of methionine (rumen-protected vs non-protected methionine) is not consistent between trials. Thus, further research is required to understand at what dose and type of methionine supplement increases calf birth weight consistently.
1.3.2 Methionine supplementation’s influence on circulating metabolites

To further investigate methionine’s influence on cow performance, circulating blood metabolites such as glucose, urea and NEFA concentrations have been evaluated. Methionine supplementation has previously been found to influence glucose production. In ewes and beef cattle supplemented with DL-methionine, serum glucose levels were greater in comparison to unsupplemented ewes and cattle (Baghbanzadeh-Nobari et al., 2016; Liker et al., 2005). Alternatively, when dairy cattle were fed a rumen-protected methionine supplement or a 2-hydroxy-4-(methylthio)-butanoic acid (HMB), there was no influence on serum and plasma glucose concentrations compared to unsupplemented cattle (Osorio et al., 2014, Wang et al., 2010). In one study, over-wintering beef cows were fed 15 g of DL-rumen protected methionine daily for the last 102 days of gestation. Glucose concentrations were lower for cows fed methionine 34 and 68 days before parturition (Liker et al., 2005). Methionine supplementation also tended to decrease plasma urea (mmol/L) concentrations at the end of the trial (68 days prior to parturition; Liker et al., 2005). Thus, methionine’s influence on glucose production requires further investigation to fully understand the mechanism behind the contradicting results. Similar to protein supplementation, as methionine supplementation (HMB) decreased serum NEFA concentrations increased in lactating dairy cows, indicating that methionine supplementation could reduce lipolysis or improve energy status (Wang et al., 2010). Thus, blood metabolite concentrations can be influenced by methionine supplementation, although further investigation is required as there are still conflicting results in the literature on effects of methionine supplementation in
1.4 Conclusion

This review suggests that feeding supplemental protein during late gestation may improve cow performance, circulating blood metabolites, and total tract digestibility. Beef cow performance can be positively affected by late gestation protein supplementation; however, there is little evidence to suggest that calf birth weights are influenced by moderate protein supplementation during late gestation. Additionally, researchers have found that protein supplementation can impact serum urea, glucose, insulin and NEFA concentrations and increase digestibility. Methionine supplementation in ruminants can increase nitrogen efficiency and ADG, and may increase calf birth weight. There is minimal research on the response of gestating beef cattle to methionine supplementation and the literature often reports contradictory results. There is also a lack of research which integrates protein content and rumen-protected methionine supplementation in which energy supplied is also held constant in the diet on the performance, circulating blood metabolites and total tract digestibility of beef cows and heifers in late gestation.

1.5 Hypothesis

Supplementing a low metabolizable protein TMR with an additional protein source will increase diet digestibility and cow and heifer performance during late gestation.
Additionally, rumen-protected methionine supplementation will benefit late gestation beef cattle by promoting protein synthesis and gluconeogenesis, reducing the need for the cow to compromise her own body reserves to support pregnancy. Late gestation methionine supplementation may ensure essential AA requirements are met without feeding unnecessary amount of dietary protein.

1.6 Objectives

This study investigates the effects of methionine supplementation for late gestation beef cows, fed below, at, or above MP requirements (NRC, 2016). This study will evaluate the impact that rumen-protected methionine supplementation and MP intakes have on beef cattle during late gestation (8-weeks prior to calving) through the evaluation of:

1) Cow performance: focusing on changes in BW, BCS, ultrasound fat thickness and REA and calf birth weight

2) Blood metabolites and amino acid concentrations before calving

3) Apparent total tract digestibility to assess DM, CP, NDF, ADF and NEm digestibility
2 Materials and Methods

2.1 Introduction

Late gestation is one of the most crucial periods for beef cow-calf producers as fetal nutrient requirements are greatest during late gestation and may impact maternal energy and protein stores (Bauman and Currie, 1980). Therefore, proper nutrition during this period is vital for profitable cow-calf operations. Since feed is the most significant contributor to the cost associated with raising beef cattle, increasing feed digestibility may also improve feed efficiency for producers (Manitoba Agriculture, 2017). Although protein supplementation is documented to increase digestibility and cow performance, there is limited research on the effect of rumen-protected methionine on apparent total tract digestibility and cow-calf performance in beef cattle (Larson et al., 2014; Stalker et al., 2014; May et al., 2014; Warner et al., 2011; Bohnert et al., 2002). Therefore, the objectives of this study were to assess how level of MP intake and rumen-protected methionine supplementation influence beef cow performance, blood metabolites and amino acid concentrations, and total tract digestibility during late gestation.

2.2 Animals and experimental design

This experiment followed the recommendations of the Canadian Council on Animal Care (1993) and met the approval of the University of Guelph Animal Care Committee. One-hundred-thirty-eight multiparous (n=99) and primiparous (n=39) cattle, primarily of Simmental x Angus breeding were bred to bulls of similar genetic merit and housed at the Elora Beef Research Center (EBRC). Cattle were artificially inseminated to commercially available bulls and expected calving date were determined through 3
veterinary pregnancy checks throughout gestation. The cattle were separated into 3 blocks based on expected calving date. Within each block, cattle were separated by parity (heifer or cow) and then randomly placed into pens of 6 cows. Pens were equipped with Calan gates (American Calan, Inc., USA) allowing for each cow to be individually fed. This study used a 3 x 2 factorial arrangement of dietary treatments (3 levels of MP, with or without rumen-protected MET supplementation) with each pen containing one cow per dietary treatment.

After weaning, during early to mid-gestation and prior to the start of the trial, cattle were fed a common diet consisting of straw and alfalfa haylage ad libitum. Approximately 8-weeks before their expected calving date, the cows were introduced to one of 6 dietary treatments (Table 1). Treatments were designed to be isocaloric. In order to achieve this all treatments were few a common base ration composed of alfalfa haylage and millet straw. For cows, the ration consisted of 60% haylage and 40% millet straw (DM basis); for heifers, the ration was 70% haylage and 30% millet straw, with a greater % of haylage used for heifers in order to meet greater nutrient requirements vs. multiparous cows. This base TMR was limit-fed at a rate of 1.15% and 1.21% of BW (DM basis) for cows and heifers, respectively. This was done for each individual animal and adjusted to changes in BW assessed biweekly. Each cow was then provided a top-dressed supplement package based on their assigned dietary treatment. Diets were formulated to meet 90, 100, or 110% of MP requirements (NRC, 2016) for late gestation beef cows based on a DM basis (Table 1). Diet differences in metabolizable protein (MP) content were achieved by varying the amounts of soybean meal (SBM) in the diet.
To ensure all diets were isocaloric, palm fat was added at 5, 4 and 3% on a DM basis for 90, 100 and 110% MP diets respectfully to balance the additional dietary energy that soybean meal added. In addition, half of the cows on each MP treatment were supplemented with 9 g/d of rumen-protected methionine (MET+; Smartamine®M, Adisseo Inc.) while remaining cows on each MP treatment were not supplemented with rumen-protected methionine (MET-), therefore 6 dietary treatments were: 1) 90% MP (MET-), 2) 90% MP (MET+), 3) 100% MP (MET-), 4) 100% MP (MET+), 5) 110% MP (MET-), 6) 110% MP (MET+). For cows, the 110% MP diet consisted of 9% soybean meal added on top of the base TMR; to meet 100% MP requirements, 4.5% soybean meal was added to the base TMR; for the 90% MP protein diet, no SBM was added to the base TMR. For heifers, the 110% protein diet consisted of 9.5% soybean meal added to the base TMR for heifers; for 100% MP diet, 5% soybean meal was added to the base TMR for heifers; for the 90% MP diet, no SBM was added to the base TMR for heifers. Soybean meal, palm fat and methionine were top-dressed onto the base TMR ration of each animal’s bunk according to dietary treatment, then mixed manually into the TMR. In addition, a commercially available beef cow vitamin and mineral premix (Floradale Feed Mill Limited, Floradale, ON; see appendix 2) was added to the TMR to meet or exceed all NRC (2016) mineral requirements.

2.2.1 Feed and feed refusal analysis

A TMR sample was taken weekly for all cow and heifer rations and SBM and palm fat samples were taken monthly throughout the trial period. Approximately 200
grams of feed samples were collected on-farm and then frozen at -20°C for later chemical analysis.

Prior to the nutrient analysis of feed samples, %DM was determined. Frozen feed samples were separated into two 100 gram samples ±10 g which were then dried at 65°C for 48 hours weighed. The samples were then dried for another 24 hours, and weighed again to ensure they were fully dried (no further weight change). After obtaining the %DM, feed samples were sent to A&L Canada Laboratories Inc. (London, ON) for analysis. Nitrogen content of diets and feedstuffs were determined through combustion with a LECO FP628 nitrogen analyzer (AOAC 990.3). Crude protein content was then calculated by multiplying the nitrogen content by 6.25. ADF content was measured with an Ankom 200 using Ankom Method 5; NDF content was determined via an Ankom 200 using Ankom Method 6 (AOAC 973.18, AOAC 2002.04). Ash content was measured via Blue M Electric programmable muffle furnace in which samples were ashed at 550°C for 3-hours. Minerals were analyzed via aquaregia digestion, inductively coupled plasma and atomic emission spectroscopy. Net energies were calculated using equations from Nutrient Requirements of Dairy Cattle 7th Edition (NRC, 2001).

If orts were present, they were weighed for each individual cow and heifer from their Calan gate bunk every 2 weeks, and a 500g sample was taken from each bunk and then frozen at -20°C. The samples were split in half and then dried for four days at 65°C; samples were then re-weighed to determine the %DM. Daily feed intake was determined by totaling the feed delivered to each individual cow on a DMB over 2-
weeks and then subtracting the amount of orts recorded on a DMB over approximately a 2-week period to get the total intake on a DM basis. This was then divided by the appropriate number of days in the feeding period to determine the average daily DMI. Metabolizable protein intake (g/d) was calculated for each 2-week period on the trial diet in cattle and heifers (NRC, 2016).

2.3 BW, BCS, ultrasound and calf birth weight

Body weights were taken bi-weekly until calving on the same weigh scale throughout the trial period. Body weight was taken on 2 consecutive days at the start of the trial, and last body weight was taken within 5 ± 3.62 days of parturition. The BW change was also analyzed via subtracting the final BW from the initial average BW.

Body weights without the weight of the fetus, gravid uterus, fetal membranes, and fluids were calculated using Silvey and Haydock's (1978) calculation and are summarized in equations 3-5. This calculation takes into consideration calf birth weight and day of gestation to calculate fetus, gravid uterus, fetal membranes and fluids. The sum of which is then subtracted from actual BW cow on the given day of gestation. To calculate pregnancy corrected BW change, pregnancy corrected BW was subtracted from the final pregnancy corrected weight.

Equation 3: Regression to calculate the fresh weight of the foetus (F). Fresh foetal weight in kg can be defined as the proportion of the calf birth weight (Fp) at the time in gestation (t).

\[ F = 0.00000181F_p e^{(0.115t-0.000368t^2+0.000000448t^3)} \]
Equation 4: Regression to calculate the gravid uterus, foetal membranes and fluids (U).

\[ U = 0.00473F_{p} e^{(0.0349t-0.0000610t^2)} \]

Equation 5: Calculation for pregnancy corrected weight (PCW) by taking into consideration gravid uterus, foetal membranes and fluids (U), fresh foetal weight (F) and cow body weight (BW).

\[ PCW = BW - (U + F) \]

Using a PCW equation allows researchers to calculate the changes in the dam’s body weight without the additional variation of the calf, associated tissues and fluid.

The potential energy stores for each pregnant cow/heifer were assessed via BCS scoring and ultrasound measures and the change in both. Body condition score was measured by the same trained individual, blind to treatments. The BCS was assessed on a 5-point scale, 1= emaciated to 5= extremely fat (BCRC, 2019). Ultrasound measures of subcutaneous fat cover (cm) over the rump and ribs as well as longissimus muscle area (LMA; cm\(^2\)), were collected by a trained ultrasound technician using an EXAGO ultrasound machine (Echo Control Medical, Angouleme, France) with an 18cm transducer. Cow and heifers’ hair was clipped down to the hide from the spine downward and between the 12\(^{th}\) and 13\(^{th}\) ribs to measure rib fat depth over the Longissimus dorsi muscle and LMA (Wood et al., 2013). Hair was also clipped between the hook and pin bones, and fat depth over the gluteus medius muscle was measured. Canola oil was used between the cow’s hide and the transducer to facilitate the capture of a clear image. The rump and rib fat depths (cm), as well as LMA (cm\(^2\)), were measured using ImageJ software (Maryland, USA; version 1.51, 2015).
Within 24-hours of birth, calf BW was measured. These measurements will allow assessment for potential effects of the fetal programming aspect of this study to be evaluated for progeny of the dams that were fed one of the 6 diets.

### 2.4 Blood sampling and analysis

Blood plasma and serum samples were obtained pre-calving prior to feeding at approximately 800 hours via jugular vein puncture. The last blood sample was taken within 7 ±4.67 days of calving. Plasma samples were collected into lithium heparinized tubes (BD Vacutainer®, Franklin Lakes, NJ) and immediately stored on ice prior to centrifugation. Serum samples were collected into serum separator tubes (BD Vacutainer®, Franklin Lakes, NJ) and kept at room temperature for 30 minutes to allow for clot formation and then placed on ice prior to centrifugation. Blood serum and plasma samples were centrifuged at 3,000 x g for 25 minutes; serum and plasma were then removed and pipetted into 5 ml cryotubes and frozen at -20 °C for further analysis. The serum samples were then analyzed for a bovine serum profile to assess total protein, albumin, globulin, urea, NEFA, BHBA, glucose, haptoglobin, cholesterol, calcium, phosphorous, magnesium (Mg), sodium (Na), potassium (K) and chlorine (Cl) levels at the Animal Health Laboratory, University of Guelph. The analysis was completed using a Cobas 6000 c501 biochemistry analyzer (Roche Diagnosis, Laval, QC). The procedures used to determine the metabolites were: TP2: ACN678 for total protein, ALB2: ACN413 for albumin, UREAL: ACN418 for urea, A RX monza analyzer (Randox Laboratories-US, Ltd, Kearneysville, West Virginia) was used for the determination of BHBA (using Ranbut) and NEFA, GLUC3: ACN717 for glucose
concentration, haptoglobin determination was based on methods from Makimura and Suzuki (1982), CHO2I: ACN798 for cholesterol, CA2: ACN698 for calcium, PHOS2: ACN714 for phosphorus, MG-2: ACN701 for magnesium, NA-K-Cl Gen.2 for sodium, potassium, and chloride concentrations.

The plasma samples were then analyzed for insulin levels using a commercially available Bovine Insulin ELISA kit and protocol (Mercodia Bovine Insulin ELISA, Mercodia, Uppsala, Sweden). The plates had two replicates per sample and was read on a Biotek spectrophotometer (BioTek Instruments, Inc., VT, USA).

Serum AA concentrations were analyzed using ultra-performance liquid chromatography (UPLC Waters Corporation, Milford, MA, USA). Each serum sample (100 μL) was deproteinized via adding 100 μL of 10% sulfosalicylic acid (Sigma-Aldrich operation, St Louis, MO) and then subsequently centrifuged at 12,000 rpm for 5 minutes (Mansilla et al., 2018). Next, 10 μL of the sample supernatant were derivatized by an ACCQTag Ultra derivatization kit (Waters Corporation, Milford, MA, USA; Mansilla et al., 2018). A UPLC with a UV detection of 260 nm was then used to separate the remaining derivatized AA in a column at 55 °C (Mansilla et al., 2018). Amino acid concentrations were compared to standards, then analyzed using Waters Empower 2 Software (Waters Corporation, Milford, MA, USA; Mansilla et al., 2018). In addition to analyzing individual AA, the AA were also assessed in groups of gluconeogenic, ketogenic, BCAA, EAA and NEAA (Waterman et al., 2012).
2.5 Apparent total-tract collection and analysis

Apparent total tract digestibility of the diets was assessed to determine the amount nutritional components of the diet absorbed by the animal. This was measured for all cows (n=35) and heifers (n=25) in the second block. During the fourth-week on the trial diet, feces collection occurred every nine hours over a three-day period to allow for a sample every 4 hr in a 24-hr cycle in accordance to methods for collection by Ferraretto et al. (2015). Fecal grab samples were taken via a plastic grab bag inserted into the rectum. Bags were then labeled and frozen at -20 °C for later analysis. Feed samples were taken daily during the apparent total tract collection period and analyzed according to methods described in section 2.2.1.

Fecal samples for each animal were dried in a forced-air oven at 55 °C for 4 days then weighed. The samples were then placed back into the dryer for another 24 hours and then weighed again to ensure complete drying of the sample. The %DM was then determined, and fecal samples were composited for each animal, ground in a coffee grinder and mixed. Similar to feed samples, the dried and ground fecal samples were analyzed at A&L Canada Laboratories Inc. (London, ON; section 2.2.1). Undigested neutral detergent fibre (uNDF) was used as a digestibility marker using the feed and fecal samples taken during the three-day sampling period. The amount of uNDF is unaffected by digestion and feed uNDF concentrations should be the same as in feces. Therefore, can be used to identify the % digestibility of the other dietary components. The equations used for %DM digestibility and digestibility % of nutrients (N) are shown below.
Equation 6: Calculations for determining %DM digestibility and % nutrient (N) digestibility using the uNDF marker and nutrient concentrations found in feed and fecal.

\[
DM\% = 100 - \left(\frac{uNDF_{feed}}{uNDF_{ecal}} \times 100\right)
\]

\[
N\% = 100 - \left(\frac{uNDF_{feed} \times N_{ecal}}{uNDF_{ecal} \times N_{feed}} \times 100\right)
\]
2.6 Statistics

The data was analyzed as a randomized complete block design using the PROC GLIMMIX in SAS version 9.4 (SAS University Edition SAS Institute Inc., Cary, NC, USA), with the fixed effects, metabolizable protein level, methionine, and their interaction. The Kenward-Rogers correction was added after the model statement to adjust the statistic and the denominator degrees of freedom. Parity (heifer or cow) was used as a covariate in the model. For this experiment, cows and heifers are the experimental units, block (3 blocks based on similar calving date) was used as a random variable in this study. The means were separated for protein, methionine and their interaction using the pdiff option. If no interaction was present, main effects (protein or methionine) results were pooled. Results for all analyses are considered as a tendency between $P<0.10$ and $P>0.05$ and significant if $P<0.05$. 
3 Results

This study began with 151 late gestating beef cattle (40 heifers and 111 cows). Three cows were found to be not pregnant after starting treatment and one cow aborted her calf before it reached full term; therefore, these cattle were removed from the trial. Out of the remaining 147 cattle, 9 gave birth to twins; creating a 6% herd twinning rate. The cattle that had twins were removed from the data set to reduce added variation the additional fetus would introduce; therefore, the final numbers were 99 cows and 39 heifers (N=138).

3.1 Effects of protein and methionine supplementation on cow performance

Initial weight, and BCS were not ($P \geq 0.07$) different between main effects of protein and methionine treatments (Table 2). However, there was a metabolizable protein level by methionine interaction on initial body weight, as the 110% MP (MET+) cattle had heavier ($P < 0.02$) initial body weights and pregnancy corrected weights than all other treatment groups (see appendix 1 for all interactions). Additionally, there was a metabolizable protein requirements by methionine interaction for initial BCS, where 110% MP (MET+) cattle had higher ($P = 0.02$) BCS than 110% MP (MET-) and 100% MP (MET+) cattle.

While there were no effects of metabolizable protein level on body weight changes in weeks 2, 4, and 8, body weight change was different ($P \leq 0.03$) between metabolizable protein levels during week 6 (Table 2). During week 6, cows fed at 90% MP requirements lost weight ($P = 0.02$), while cattle fed at 100% or 110% MP
requirements gained or maintained weight. Body weight change was not affected \((P >0.2)\) by providing supplemental methionine during the trial. Body weight and pregnancy corrected BW changes over the trial period were more negative \((P<0.01)\) in cattle fed at 90% MP requirements than cattle fed at 100 or 100% MP requirements. Final BW and pregnancy corrected BW changes in cattle were not affected \((P>0.2)\) by providing supplemental methionine in the diets. There were no interactions between metabolizable protein level and methionine for any of the body weight change measurements \((P>0.05)\).

Final BW and pregnancy corrected BW were higher \((P\leq0.04)\) for cattle fed 110% MP requirements than cattle fed at 100% or 90% MP requirements (Table 2). Final BW and pregnancy corrected BW were not different \((P>0.2)\) for cattle fed MET+ than those that were fed MET-. There was an interaction between methionine and protein on final BW and pregnancy corrected BW; 110% MP (MET+) cattle had heavier \((P\leq0.01)\) final BW, and pregnancy corrected BW than cattle from all other treatment combinations. There were no differences in final BCS or BCS changes from the trial initiation between the main effects or their interactions \((P\geq0.06)\).

For ultrasound results, there were no differences \((P>0.06)\) between any main effects or their interaction on REA/BW or changes in rib fat thickness, rump fat thickness and REA.

The cows and heifers were formulated to be fed (kg/d) at 1.15% and 1.21% BW TMR respectively. The actual amount of TMR delivered to cows was $1.15 \pm 0.05\%$BW.
and 1.26±0.08% BW for heifers. When actual DMI was calculated including TMR, and supplements, the cattle on average were fed at 1.26%±0.07 for cows and 1.34%±0.07 for heifers on a % of final BW basis. As designed by the trial, MP intake (g/d) and MP intake as a %BW was different (P<0.001) between protein treatments, where cattle fed 110%MP requirements had the highest MP intake and MP intake as a %BW, followed by cattle fed at 100%MP requirements and lowest for cattle fed 90%MP requirements (Appendix 3 and 4; Table 2). Dry matter intake (kg/d) was different (P<0.001) between protein treatment groups, where cows fed at 110% requirements had highest DMI, followed by cattle fed 100%, and cattle fed 90% of MP (Table 2). There was no effect (P>0.4) of supplementing methionine on DMI during the trial. There was a metabolizable protein by methionine treatment interaction on DMI where cattle fed 110% MP (MET+) had higher (P<0.01) DMI than any other treatment group and cattle fed 110% MP (MET-) had higher (P<0.05) DMI than 100% (MET-), 90% MP (MET+) and 90% (MET-) fed cattle.

There were no main treatment effects or interaction between treatment on calf birth weights (P>0.3; Table 2).

3.2 Effects of protein and methionine supplementation on blood metabolites and amino acid concentrations

Urea levels were different (P<0.001) between cows fed at varying MP levels with urea highest for the cows fed at 110% MP and lowest for cows fed at 90% MP requirements (Table 4). NEFA concentrations were lower (P=0.03) for cows fed at 110% MP requirements when compared to cattle fed at 90% MP requirements. Serum
cholesterol levels were different \((P<0.001)\) between the protein treatment groups, with the highest values for cattle fed at 90% MP requirements followed by lower values for cattle fed at 100% MP requirements with the lowest cholesterol concentrations found for cattle fed at 110% MP requirements. Total serum protein concentration tended to be higher \((P=0.09)\) for cattle fed at 110% MP requirements compared to cattle fed at 100% MP requirements. Methionine supplementation tended to increase \((P=0.06)\) total serum protein concentration compared to MET- cattle.

Methionine supplementation increased \((P\leq0.04)\) serum calcium, sodium, and glucose concentrations compared to MET- cattle. Albumin serum concentrations also tended to be greater for MET+ fed cows \((P=0.08)\). There were no interactions between methionine and protein supplementation for any serum metabolites \((P\geq0.10)\).

For circulating amino acids, alanine (Ala) concentrations in cattle fed at 110% MP requirements tended to be lower \((P=0.08)\) than cattle fed at 100% MP requirements (Table 5). Serum serine concentrations for cattle fed at 90% MP requirements tended to be greater \((P=0.06)\) than serine concentrations for cattle fed at 100% or 110% MP requirements. Glycine (Gly) serum concentrations were impacted by protein treatment; cattle fed at 90% MP requirements had the highest levels followed by cattle fed at 100% and 110% MP requirements \((P<0.001)\). Cattle fed above 110% MP requirements had reduced \((P=0.01)\) total NEAA and glucogenic AA compared to cattle fed at 90% MP requirements.
Serine (Ser; \( P=0.01 \)), Ile (isoleucine; \( P=0.02 \)), Leu (leucine; \( P=<0.001 \)), Lys (lysine; \( P=0.03 \)), Thr (threonine; \( P=0.02 \)) and Val (valine; \( P=0.04 \)) serum concentrations were lower for MET+ supplemented cattle than MET-. Serum concentrations for Ser, Ile, Leu, Lys, Thr, and Val, were lower (\( P \leq 0.04 \)) in cattle supplemented with MET+ than concentrations for cattle fed MET-. Serum concentrations for Asp (aspartate), His (histidine), Phe (phenylalanine), Tyr (tyrosine) tended to be lower (\( P \leq 0.09 \)) for MET+ fed cattle. Methionine supplementation also reduced (\( P \leq 0.02 \)) total serum EAA, BCAA, and ketogenic AA concentrations. There were no interactions (\( P>0.15 \)) between metabolizable protein level and methionine impacting serum AA concentrations.

### 3.3 Apparent total tract digestibility

Diet DM digestibility was greatest (\( P<0.001 \)) for cattle fed at 110% MP requirements, followed by cattle fed at 100% and 90% MP requirements (Table 6). Crude protein % digestibility was different (\( P<0.001 \)) between metabolizable protein levels. Cattle fed at 110% MP requirements had the highest CP digestibility value, followed by cattle fed at 100% and 90% MP requirements. Calculated Net energy maintenance (NEm; Mcal/kg) digestibility was greater (\( P<0.01 \)) in cows fed at 110% MP requirements in comparison to those fed at 100% or 90% MP requirements. Cattle fed at 110% MP requirements had increased NDF digestibility (\( P<0.05 \)) compared to cattle fed at 100% MP requirements. Cattle fed at 110% MP requirements also had increased (\( P<0.03 \)) ADF% and DM% digestibility compared to cattle fed at 100% or 90% MP protein requirements. Methionine supplementation reduced DM (\( P=0.04 \)) and NEm (mcal/kg; \( P=0.02 \)) digestibility values, but did not impact CP, ADF and NDF digestibility.
values. There were no interactions between protein and methionine for any digestibility measurement ($P \geq 0.5$).
4 Discussion

There is currently a lack of research completed which integrates dietary MP content and rumen-protected methionine supplementation in the diets of gestating beef cows and heifers to assess supplemental methionine’s potential to replace excess dietary protein. Therefore, the objective of this study was to assess the impacts of dietary MP levels and supplemental methionine on late gestation beef cows.

4.1 Cow performance

Prior to the initiation of the study, cattle were fed an ad libitum haylage-based diet, which greatly exceeded energy requirements early-to-mid gestation. As a result, initial BCS average was 3.8 and rump fat and rib fat thickness were 11 and 9 mm respectively. Therefore, these cattle started the trial in an over-conditioned state, since the ideal BCS for beef cows on a 5-point scale is approximately 3. Initial body weight weights were not different between cattle placed in the methionine or control group. Although cattle were randomly allocated to dietary treatment groups, the initial BW tended to be heavier in cattle fed at 110% MP requirements than cattle fed at 90% or 100% MP requirements. There was also an interaction between % MP requirements and methionine on initial BW where cattle placed on the treatment 110% MP (MET+) had higher initial body weights than all other treatment groups. Additionally, there were interactions between treatments on initial BCS. Because initial BW and BCS had an interaction and were not initially block based on similar BW and BCS, change in BW and BCS and ultrasound are the main focus of cow performance in this experiment.
Since the trial diets were formulated to meet energy requirements and were limit fed in order to meet these requirements, some cattle lost weight over the trial. Cattle on the diet formulated to 90% MP requirements lost more absolute BW over the period prior to calving. The reduction in DMI is to be expected as the study was designed to restrict feed cattle to their energy requirements as calculated based on their bi-weekly measured BW. The cows and heifers were formulated to be fed (kg/d) at 1.15% and 1.21% BW respectively. Although the diet met energy requirements, the cattle fed 90% MP requirements lost more BW due to dietary MP restriction. Alternatively, cattle fed to 110% MP requirements gained weight and had higher DMI prior to calving. This supports findings from previous researchers that found protein supplementation improved BW gain in beef cattle during late pregnancy (López-Valiente et al., 2018; Larson et al., 2014; Stalker et al., 2014). Therefore, previous research supports the present findings that BW gain is reduced in cattle fed below protein requirements compared to meeting, or exceeding requirements. Although there were differences in BW gain, there were no differences between BCS, back fat and rump fat thickness or REA changes in the present study. This suggests that this BW change may be associated with changes in tissues such as fetal associated membranes and tissues, or visceral fat. However, when adjusting for pregnancy corrected body weight (PCBW) change, all dams lost weight over the trial period. Cattle fed at 90% MP requirements lost more PCBW (-30 kg), over the trial period than cattle fed at 100% (-22 kg) or 110% MP requirements (-23 kg). This demonstrates that although cattle fed at 100% or 110% MP requirements gained absolute BW over the trial period, they still may be
compromising their own body reserves to support fetal growth during late pregnancy. During late gestation when fetal growth is exponentially increased, partitioning of nutrient is of high priority by the homeorhetic controls (Bauman and Currie et al., 1980); this may explain the loss in PCBW, but maintaining or gaining BW in cattle fed at 100 or 110% MP requirements in the present experiment.

Although dam BW changes were observed due to treatment, calf birth weights were not impacted by main treatment effects or their interactions. This result is supported by multiple studies that found that protein supplementation during pregnancy did not influence calf birth weights (Wilson et al., 2016; Stalker et al., 2014; Warner et al., 2011; Wood et al., 2010). Studies that found dam nutrition influenced calf birth weights primarily involved severe energy restriction during gestation (Taylor et al., 2016 and Gao et al., 2012). Since the diets for this study were isocaloric and formulated to meet dam energy requirements, and protein restriction/oversupply was small, calf birth weight differences were not expected.

The current experiment also found no differences in calf birth weight when cattle were fed the methionine supplement. Alternatively, a study conducted on dairy cows demonstrated that late gestation methionine supplementation can increase calf birth weight (Batistel et al., 2017). However, Batistel et al. (2017) did not restrict dairy cows to energy requirements and found that methionine also increased DMI. Unlike several studies in dairy cattle which reported increase in ad libitum feed intake (Batistel et al., 2017; Fisher et al., 1972), the cattle in the current trial were restricted fed to meet energy requirements, thus we were unable to assess methionine’s full impact on ad
libitum DMI. Although increased DMI with methionine supplementation can partially explain the increase in calf birth weight found in previous research, scientists also found that methionine supplementation increased glucose and AA transporters in placental tissues, which may also contribute to increased calf birth weight (Batistel et al., 2017). This may allow for more nutrients to reach the fetus during high demands for growth.

Additionally, final weight gain was not different for cattle fed the methionine supplement. This is consistent with previous research that found no effects on final weight gain when steers and heifers were fed a supplemental methionine with soybean meal as a protein source (Tripp et al., 1998). Alternatively, when cattle were supplemented with meat and bone meal as a protein source, the cattle fed supplemental methionine had greater ADG compared to the control (Klemesrud et al., 2000). This suggests that in the present study, and the diet in Tripp et al. (1998), may not be as limiting in methionine in comparison to the cattle in the Klemesrud et al. (2000) study that were fed meat and bone meal as a protein source. Another potential reason for the lack of performance changes for methionine supplemented cattle in the present experiment is the dose of rumen protected methionine implemented (9g/d), as diets may still have been limiting in methionine. Although this dose is similar to dairy studies based on level of intake (Phillips et al., 2003; Armentano et al., 1997), this dose may not be equitable for beef cattle. However, previous research that found changes in cow performance feeding a methionine supplement have also found increases in voluntary DMI, which is also most likely driving these performance changes (Batistel et
al., 2017; Fisher et al., 1972). This is supported by the present study where energy intake was held constant, and no cow performance changes occurred.

However, there was an interaction between metabolizable protein level and methionine on final BW. Cows fed 110% MP (MET+) had higher final body weights than any other treatment combination. Since the initial BW had the same interaction, these results are not significant indicators that the treatment interaction was impactful on BW. Although initial and final BCS and BW were significant for a metabolizable protein level and methionine interaction, there were no interactions between metabolizable protein level and methionine for any BW or BCS changes during the experiment.

Therefore, feeding cattle a moderately MP restricted diet while meeting energy requirements during late gestation promotes increased BW losses; however, this approach may not have been severe enough to impact fat thickness assessed via ultrasound or BCS. Additionally, supplemental MET+ had little impact on cow performance changes when cattle had restricted DMI.

4.2 Effects of protein and methionine supplementation on plasma and serum parameters

Serum and plasma blood metabolites were utilized in this experiment to give insight on how dietary MP and methionine inclusion impacts: mineral, energy, insulin, glucose, and protein status within the animal. During pregnancy, the dam may utilize her own body reserves to provide AA and glucose for fetal development (Freetly et al., 2000). Increased concentrations of blood NEFA can be indicative of lipolysis of adipose tissues. In the current study, NEFA concentrations were lower for cows fed at 110% MP
requirements when compared to cattle fed at 90% MP requirements. This is supported by various studies that found increases in NEFA concentrations when cattle are fed below their energy or protein requirements (Amanlou et al., 2017; Wood et al., 2013). As cattle also lost the least amount of pregnancy corrected BW when fed at 110% MP requirements, this suggests that these cattle were metabolizing less of their body reserves to meet nutritional demands for late gestation. Previous research has shown that when a partially rumen-degradable methionine analog source (2-hydroxy-4-(methylthio)-butanoic acid: HMB) was supplemented to lactating dairy cattle, NEFA concentrations were reduced (Wang et al., 2010). This may indicate that HMB could reduce lipolysis; however, the supplemented cattle still had increased milk fat production (Wang et al., 2010). Conversely, MET+ supplementation did not impact serum NEFA concentrations in the present study demonstrating that MET+ may not significantly impact lipolysis of adipose tissues, which is further supported by the lack of changes found in ultrasound and BCS between MET + and MET - cattle.

Similar to NEFA, an increase in serum urea concentrations can be indicative of cattle mobilizing body reserves (in the form of muscle tissues), or alternatively, the breakdown of dietary protein (Dimiski, 1994). In the present study, serum urea concentrations were different between MP treatment groups. With urea concentrations greatest for the cows fed at 110% MP requirements and lowest for cows fed at 90% MP requirements. Since cattle that had the highest serum urea concentrations were fed above protein requirements, the increase in serum urea nitrogen is most likely due to increased dietary protein catabolism, rather than muscle tissue catabolism (Dimiski,
1994). This increase in serum urea is consistent with previous studies that found when protein supplementation increased, so did serum urea concentrations (Tahuk et al., 2018; Cappellozza et al., 2014).

Conversely, serum cholesterol concentrations decreased with the increase in MP provided. Similar results were found in pigs fed below protein requirements (5% protein) who developed hypoproteinemia and had elevated serum cholesterol concentrations compared to pigs fed a high protein diet (Gupta et al., 1974). This increased cholesterol concentration may be a source of carbon for hepatic metabolism allowing for the pregnant dam to provide nutrients to the fetus. Interestingly, maternal protein restriction in rats has also resulted in elevated cholesterol levels in adult offspring due to permanent suppression of Cholesterol 7α-Hydroxylase Promoter (Cyp7a1) leading to decreased ability to catabolize cholesterol into bile acids (Sohi et al., 2011). This suggests that not only dam cholesterol levels can be affected by protein restriction but also her offspring. However, in the present study this result is likely a result of dietary palm fat added inversely to protein supplementation to create the isocaloric diets. Fat supplementation in ruminants is well documented to increase serum cholesterol concentrations (Nestel et al., 1978; Bitman et al., 1973; Bohman et al., 1962) and may also cause the decrease in serum cholesterol levels noted with increasing %MP inclusion.

In addition, some serum minerals concentrations were impacted by methionine supplementation, including calcium (Ca) and sodium (Na). However, since the difference between Ca concentrations in response to MET+ (2.33) and MET- (2.39) is
small, it is unlikely that it has any biological significance. Similarly, serum Na concentrations were higher in MET+ (141.64) in comparison to MET- (140.95) fed cattle, also with unlikely any biological significance.

Methionine supplementation also tended to increase total serum protein concentrations. Previous research on piglets found that with a methionine supplement, piglets had increased serum total protein concentrations which the researchers postulated was due to superior utilization of nitrogen in methionine supplemented piglets (Tian et al., 2016). Although there is limited research on methionine’s impact on total serum protein in beef cows, methionine may be allowing for an increase in overall protein synthesis (Cavuoto and Fenech, 2012). However, further research investigating N balance would need to be completed to confirm these results.

Serum glucose concentrations were higher for cows fed methionine compared to the control. Similar results have previously found an increase in serum glucose concentrations in pregnant beef cattle fed DL-methionine in comparison to the control (Liker et al., 2005). Since cattle can synthesize glucose in the liver from VFA, and glucogenic AA, methionine may have increased hepatic glucose synthesis (Lager and Jordan, 2012). Since methionine is very sensitive to most forms of reactive oxygen species, it is a precursor to products with beneficial anti-oxidative capabilities (Bin et al., 2017). One way this can impact serum glucose concentrations is by reducing potential oxidative stress and inflammation that could limit hepatic gluconeogenesis (Osorio et al., 2014). However, studies in cattle fed a methionine supplement do not limit DMI and have found increases voluntary DMI (Batistel et al., 2017; Fisher et al., 1972). The
current study suggests the increase in circulating glucose concentrations in methionine supplemented cattle may result from MET supplementation directly, and not only explained by an elevated DMI response. This suggests that methionine supplementation may favour repartitioning of energy towards glucose production when energy intake is held constant in late gestating beef cows.

Therefore, this study suggests that protein restriction increases serum markers related to body mobilization to meet pregnancy requirements (NEFA), and reduced dietary protein catabolism (total protein and urea). Additionally, MET+ supplementation increased glucose concentrations independently of DMI changes.

4.3 Serum amino acid concentrations

Amino acid concentrations in serum and plasma can give indications of an animals’ physiological protein status. An increase in NEAA (non-essential amino acids) is indicative of an animal in a state of protein deficiency leading to muscle catabolism (Fujita et al., 1981). In the current study when cattle were fed 90% MP requirements, they had increased NEAA concentrations of Gly and tended to have higher Ser concentrations compared to cattle fed 100% or 110% their MP requirements. Cattle fed 90% MP requirements had increased total NEAA and gluconeogenic AA concentrations compared to cattle fed 110% MP requirements. These results support the notion that animals fed an MP deficient diet may be experiencing protein deficiencies, that could lead to increased muscle catabolism, and potential fat mobilization. This is also supported by changes in NEFA serum concentrations found in the cattle fed below % MP requirements, which is also suggestive of an increase in fat mobilization. However,
the MP restriction in the current study was likely not extreme enough to see changes in body fat thickness measured in ultrasound or BCS.

In beef cattle, there are 10 EAA (essential amino acids: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, arginine, leucine and lysine). In the current experiment, methionine supplementation decreased the concentrations of 5 EAA including: Ile, Leu, Lys, Thr and Val. Similarly, a study on growing beef steers found that methionine supplementation decreased serum Val concentrations; however, unlike the present study, there was no impact of methionine supplementation on His, Cys, Asp, Gln and Tyr (Waggoner et al., 2009). An additional study that fed cattle a protein supplement low in methionine (meat and bone meal) with increasing methionine concentrations (0, 0.45, 0.9, 1.35, 3 and 6 g/d) found that as methionine supplementation increased, plasma concentrations of Ile and Val linearly decreased (Klemesrud et al., 2000). Similar to individual EAA, the total EAA concentrations were lower for MET+ than MET- fed cattle in the present study. Several studies in dairy cattle when fed a rumen-protected methionine or a pelleted methionine hydroxy analog found no differences in EAA totals compared to unsupplemented cows (Giallongo et al., 2016; Wang et al., 2010). The current study also found that MET+ reduced ketogenic AA totals. However, a previous study completed on gestating beef cows fed a post ruminal DL-methionine supplement increasing in dose (from 0, 5, 10 to 15 g/d) found no linear, quadratic or cubic influence on ketogenic AA, EAA or BCAA (Waterman et al., 2007). In addition to EAA, methionine supplementation reduced one NEAA, Ser. Serine along with Ala and Gly are important glucogenic AA that comprise the majority of hepatic AA
catabolized for glucogenesis during the periparturient period (Larsen and Kristensen, 2013). Therefore, this may have contributed the increased serum glucose concentrations found in MET+ fed cattle; however, future research would need to be explored on the possible connection.

Methionine supplementation also collectively decreased BCAA in the present study. Branch chain amino acids (Leu, Ile and Val) are particularly important for muscle protein synthesis (Monirujjaman and Ferdouse, 2014). Since the present study found methionine supplementation decreased serum BCAA, it may indicate there could be more BCAA being utilized for muscle protein synthesis as a result of methionine supplementation (Kimball and Jefferson, 2000). The BCAA, leucine, in particular, can increase protein synthesis via the upregulation of mRNA translation (Kimball and Jefferson, 2000). The current study found that methionine supplementation decreased not only serum BCAA totals, but also Leu concentrations. Therefore, this may indicate that MET+ fed cattle had an up-regulation of mRNA translation due to the utilization of Leu; however, we were unable to measure this in the current study. Additionally, there is limited research in cattle fed MET+ finding changes in serum EAA, BCAA, and ketogenic AA.

Although the AA concentrations in MCP remain fairly consistent, the methionine composition in MCP varies (Atasoglu and Wallace, 2003). Therefore, in the current experiment, the de novo synthesis of sulphur containing AA from microbes is unknown and could have influenced some of the observed results. Although, the rumen-protected methionine supplemented was likely by-passing the rumen and absorbed in the small
intestine, it is not well known how dietary MP influences microbial AA production in beef cows. Therefore, further research will have to be completed to confirm these novel observations found in the current study.

As previously mentioned, recent research has shown that methionine supplementation can increase glucose and AA transporters in the placenta (Batistel et al., 2017). The decline in EAA, BCAA, and ketogenic AA observed with methionine supplementation in maternal serum in the current study may be a result of repartitioning maternal AA towards the growing fetus. However, in the present study, this did not result in birth weight differences, and feeding to 90% MP requirements may not have been a severe enough protein restriction to examine the potential for a positive fetal programming influence with methionine supplementation.

Blood concentrations of the amino acid 3- methyl histidine (3MH) can be utilized as a marker for skeletal protein catabolism (McKeran et al., 1979). Concentrations of serum 3MH have been documented to spike during the start of lactation in dairy cattle, demonstrating a need for skeletal protein breakdown to meet the increased protein demands during the early stages of lactation (Phillips et al., 2003; Blum et al., 1985). Although MP deficient cattle in the current study lost BW, there were no changes in 3MH serum concentrations. This may be because our protein restricted diet (90% MP) was not severely limiting enough to provoke significant changes in muscle catabolism, and as a result not change 3MH serum concentrations. There were however numerical decreases with increased %MP supplementation.
Additionally, MET+ had no influence on 3MH concentrations, which was expected given the lack of differences in animal performance. However, future research should be completed to estimate if 3MH concentrations are affected with a more severe MP restriction in combination with MET+. This would be particularly important to research as the present experiment suggests that methionine may improve AA utilization which could reduce the need for skeletal muscle mobilization in a state of protein malnutrition.

4.4 Total tract digestibility

Protein supplementation is inherently important when cattle are fed low-quality forages, as increasing energy in the diet with inadequate dietary protein levels has been found to reduce DMI and digestibility (DelCurto et al., 1990). Increasing protein supplementation has been well documented to increase N availability to rumen microbes allowing for an increase in DM digestibility (May et al., 2014; Bohnert et al., 2002). This is in agreement with the present experiment that found that DM digestibility was higher in cows fed 110% MP requirements compared to feeding at 100% or 90% MP requirements. This increase in DM digestibility allows producers to maximize the nutrient value from the feed as low-quality (low CP and high NDF) diets are often used for gestating beef cattle. Feeding above MP requirements may have also promoted the increase in BW gain compared to MP restricted cattle that had reduced DM digestibility and BW gain in the present experiment.

Crude protein and nitrogen digestibility values have been documented in previous research to increase as a result of feeding a protein supplement in ruminants (Hare et
al., 2019; Amanlou et al., 2017; Knowlton et al., 2010). Similarly, the present study found an increase in CP digestibility when feeding 100% or 110% MP requirements. This increase in CP digestibility may also support the increased serum urea concentrations found in the cattle fed 100% or 110% MP requirements discussed above. Although we did not measure total N balance or N excretions, previous studies have suggested that when protein is in excess in the diet (in this experiment 110% MP), there is elevated nitrogenous waste excreted in the feces and urine (Hare et al., 2019; Burgos et al., 2010). Since there is currently increased pressure on the beef cattle industry to reduce their environmental impact, the mild benefit in cow performance noted in this study with increasing N supplementation may not outweigh the negative environmental impact and waste of usable dietary protein.

Metabolizable protein content in the diet impacted fibre digestion in the present study in the forms of ADF and NDF. Acid detergent fibre takes into consideration two cell wall components: cellulose and lignin. In the present study, cattle fed 110% MP requirements had increased ADF digestibility compared to cattle fed 100 or 90% MP requirements. Alternatively, previous research in ruminants found no differences in ADF digestibility when cattle were fed 133% MP requirements compared to those meeting MP requirements (Hare et al., 2019). Although Hare et al. (2019) did not find any changes in ADF digestibility, the researchers fed their cattle a greater amount of feed (over 1 kg more head/d) than the present study with lower levels of ADF and NDF which may change passage rate and subsequent digestibility. Therefore, further research is needed to understand the results found in the current study with regards to ADF
digestibility. Although the present study yielded only differences in NDF digestibility between cattle fed at 100% or 110% MP requirements, there is most likely little biological significance (less than 1.5% difference). However, previous research on ruminants found that when a protein supplement is added to the diet there is, or tends to be an increase in NDF digestibility (Hare et al., 2019; May et al., 2014; Bohnert et al., 2002). Fibre digestibility is particularly important for pregnant beef cattle in North America as their diet contains a significant quantity of low-quality forages. For example, in the current study, the diets contained between 47-53% NDF and maximizing fibre digestibility is vital to allow the cattle to meet their nutritional requirements for pregnancy when fed a traditional beef cow diet.

Since the pregnant beef cows diet contains such a high quantity of forage it also contributes to a significant amount of energy content in the diet. Net energy maintenance (Mcal/kg) digestibility is vital for cow performance as this component of the feed is what allows the cow to meet her maintenance requirements to limit bodily reserve losses during pregnancy. Net energy maintenance (Mcal/kg) digestibility was elevated in cow’s fed at 110% MP requirements in comparison to those fed at 100% or 90% MP requirements. This is similar to a digestibility trial conducted on steers fed a DIP restricted diet, or urea/DIP supplementation which saw a decrease in dietary digestible energy (DE) with decreased urea supplementation (May et al., 2014). An increase in protein available for rumen microbes can enhance MCP production (NRC, 2016). When microbes escape the rumen, they can be utilized by the cow to meet protein and energy requirements (NRC, 2016). Therefore, dietary protein
supplementation may also promote the ability of gestating cows to meet their energy requirements during late gestation.

Overall, increasing MP content in the diet is most likely supporting improved ruminal digestibility. The decrease in digestibility of nutrient components such as CP, ADF and NEm in cattle fed at 90% MP requirements likely prevented the cattle from meeting their overall nutrient requirements. This could also explain why cattle fed below MP requirements lost more weight over the trial period compared to cattle fed at 100% or 110% MP requirements.

Unlike protein supplementation, methionine supplementation decreased DM and NEm (Kg/d) digestibility values in the present study. However, since the differences between MET+ and MET- means for these digestibility’s are less than 1%, there is likely no biological significance to this result. Since cattle are foregut fermenters, most feedstuff digestibility occurs in the rumen, since the MET+ supplement fed in this experiment is rumen-protected, very little of this methionine supplement would have been degraded by the rumen microbes. Thus, it is unlikely that the MET+ would greatly influence rumen digestibility. However, future research should be conducted to confirm MET+ influence on digestibility in the hind gut.
5 Overall conclusion

Overall, this study has demonstrated that feeding beef cattle during late gestation below their MP requirements negatively impacts cow performance in the form of increased maternal weight loss. However, MP restriction did not affect body fat thickness changes or calf birth weight. Additionally, feeding below MP requirements reduced total tract digestibility, and may limit the amount of absorbed nutrients available to help support the growth of the fetus during pregnancy which agrees with the experimental hypothesis. However, opposite to our hypothesis, rumen-protected methionine may not to be able to replace additional dietary protein during late gestation, as it does not improve cow performance or digestibility. However, methionine does impact glucose and AA blood concentrations, suggesting repartitioning of nutrients, although no performance changes were noted. During pregnancy, nutrient partitioning favours the conceptus at the expense of the dam (Wallace, 2000). Therefore, methionine supplementation may increase AA and glucose partitioning to the fetus via placental transfer. However, the exact mechanism for the changes in serum AA concentrations when pregnant beef cattle are fed rumen-protected methionine will require further research.
5.1 Implications

Feeding a restricted MP diet was detrimental to cow-performance and digestibility. The weight loss due to MP restriction may negatively influence future fertility costing producers, especially if cattle go into lactation under-weight, and under condition, challenging the dam’s ability to lactate and rebreed successfully. Additionally, the reduced digestibility in MP restricted cattle would cause producer losses from wasted feedstuffs due to limited absorption of nutrients. Therefore, the conclusions of this experiment support the notion that feeding pregnant beef cattle below their MP requirement would not be beneficial for producers via decreased feed digestibility and body weight losses.

Although methionine supplementation did not affect cow performance or digestibility parameters, serum markers indicate that methionine is causing a change in AA metabolism and possibly nutrient repartitioning in late gestation. However, we found no evidence that methionine could replace additional protein in the diet in order to improve cow performance or apparent total tract digestibility during late gestation.

5.2 Future research

Continuous pressure on the beef cattle industry to reduce their environmental footprint requires further research to investigate alternatives to overfeeding protein to cattle. To do so, this trial should be replicated with the exception of controlling DMI to energy requirements (as completed in dairy research) and the addition of a nitrogen
balance experiment. This would allow for further evidence if methionine could influence DMI in beef cows and how that could impact performance and nitrogenous waste.

Additionally, since beef producers rely on an annual healthy calf crop, a high percentage of live and healthy calves at weaning is vital for the industry. Following calves from methionine and(or) protein supplemented dams could investigate if the influence of these supplements during late gestation have carry-over effects into weaning. Previous research has suggested that protein supplementation during late gestation can produce calves with a higher weaning weight (Stalker et al., 2014) and less calves needed to be treated for bovine respiratory disease in the feedlot (Larson et al., 2014). However, there is a gap of knowledge on how methionine and protein supplementation may interact to potentially influence calf performance and health.

Furthermore, to continue to test methionine’s impact on AA metabolism, future research should test the effect methionine supplementation has on muscle protein turnover and fetal AA flow. This may help explain AA repartitioning found in the current study. To do so, regulatory protein expression related to muscle protein catabolism could be assessed in dam skeletal muscle tissues, such as calpain and ubiquitin as they can be indicators of protein status (Smith and Dodd, 2007; Lecker et al 2006). Other indicators associated with muscle protein metabolism or reduction in catabolism, such as mTOR and associated proteins could be utilized to further investigate the serum AA disappearance in MET+ fed cattle (Saxton and Sabatini, 2017). Therefore, although this experiment did find some novel impacts of dietary methionine supplementation on serum metabolites and AA concentrations, further research is required to fully
understand the mechanisms behind the results found. Additionally, it would be beneficial to understand how this mechanism could be utilized to improve beef cow performance and profitability.
### TABLE 1: BASE TMR CHEMICAL COMPOSITION OF COW AND HEIFER RATIONS, FED AT 90%, 100% OR 110% METABOLIZABLE PROTEIN (MP) REQUIREMENTS ONCE A DAY AT 0800 H

<table>
<thead>
<tr>
<th>Metabolizable Protein Level&lt;sup&gt;z&lt;/sup&gt;</th>
<th>90%</th>
<th>100% MP</th>
<th>110% MP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cows&lt;sup&gt;x&lt;/sup&gt; (n=99)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>48.8</td>
<td>50.1</td>
<td>53.3</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>11.5</td>
<td>13.3</td>
<td>15.0</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>39.9</td>
<td>38.8</td>
<td>37.9</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>53.2</td>
<td>51.9</td>
<td>50.7</td>
</tr>
<tr>
<td>Crude Fat, %DM</td>
<td>7.8</td>
<td>6.7</td>
<td>5.8</td>
</tr>
<tr>
<td>NEm, Mcal/kg</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

| **Heifers<sup>x</sup> (n=39)**         |      |         |         |
| DM, %                                  | 45.8 | 47.3    | 48.5    |
| CP, % DM                               | 12.4 | 14.3    | 16.0    |
| ADF, % DM                              | 39.0 | 37.9    | 36.5    |
| NDF, % DM                              | 50.6 | 49.2    | 48.1    |
| Crude Fat, %DM                         | 7.9  | 6.9     | 5.9     |
| NEm, Mcal/kg                           | 1.4  | 1.4     | 1.5     |

<sup>z</sup>Protein treatments: Cattle fed at 110% MP, 100% or 90% metabolizable protein (MP) requirements

<sup>x</sup>60% grass haylage, 40% millet straw for cows

<sup>x</sup>70% grass haylage, 30% millet straw for heifers
<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Metabolizable Protein content</th>
<th>Methionine</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90% MP</td>
<td>100% MP</td>
<td>110% MP</td>
<td>SE</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>698</td>
<td>690</td>
<td>726</td>
<td>12.1</td>
</tr>
<tr>
<td>Weight change at 2 weeks, kg</td>
<td>-8.9</td>
<td>-9.0</td>
<td>-9.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Weight change at 4 weeks, kg</td>
<td>-9.02</td>
<td>-5.42</td>
<td>-5.94</td>
<td>2.0</td>
</tr>
<tr>
<td>Weight change at 6 weeks, kg</td>
<td>-6.79a</td>
<td>0.62b</td>
<td>0.31b</td>
<td>2.5</td>
</tr>
<tr>
<td>Weight change 8 weeks, kg</td>
<td>-3.37</td>
<td>5.92</td>
<td>6.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>692b</td>
<td>693b</td>
<td>731a</td>
<td>12.0</td>
</tr>
<tr>
<td>Final change, kg</td>
<td>-5.7a</td>
<td>2.8b</td>
<td>4.5b</td>
<td>3.2</td>
</tr>
<tr>
<td>Pregnancy corrected weight, kg</td>
<td>630b</td>
<td>631b</td>
<td>666a</td>
<td>11.8</td>
</tr>
<tr>
<td>Pregnancy corrected weight change, kg</td>
<td>-30.8a</td>
<td>-21.9b</td>
<td>-22.9b</td>
<td>3.1</td>
</tr>
<tr>
<td>Calf birth weight, kg</td>
<td>37.6</td>
<td>37.7</td>
<td>39.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>3.7</td>
<td>3.6</td>
<td>3.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Final BCS</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Final BCS change</td>
<td>0.19</td>
<td>0.35</td>
<td>0.23</td>
<td>0.08</td>
</tr>
<tr>
<td>Rump fat thickness change, mm</td>
<td>-0.27</td>
<td>-1.05</td>
<td>-0.48</td>
<td>0.37</td>
</tr>
<tr>
<td>Rib fat thickness change, mm</td>
<td>-0.84</td>
<td>-0.70</td>
<td>-0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>REA change, cm²</td>
<td>-3.1</td>
<td>-3.4</td>
<td>-0.15</td>
<td>1.9</td>
</tr>
<tr>
<td>REA*BW, cm²/BW</td>
<td>0.13</td>
<td>0.13</td>
<td>0.12</td>
<td>0.002</td>
</tr>
<tr>
<td>DMI kg/d</td>
<td>8.6c</td>
<td>9.0b</td>
<td>9.8a</td>
<td>0.14</td>
</tr>
<tr>
<td>TMR% BW, TMR (DM basis), Kg/ BW, Kg</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.01</td>
</tr>
<tr>
<td>MP% BW, MP (DM basis), kg/ BW, Kg</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

a,b,c Means within a dependent variable that have uncommon letters differ (P<0.05)

Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable protein (MP) requirements with(without) 9 g/d supplemental methionine (Smartamine®M, Adisseo Inc.) MET+ (MET supplemented) or MET- no supplemental MET

yBCS= Body condition score

xREA= Rib eye area
*TMR=Total mixed ration containing haylage and straw (kg/d)
*MP= Metabolizable protein
### TABLE 4. PRE-PARTUM SERUM AND PLASMA METABOLITE CONCENTRATIONS IN CATTLE FED AT 90, 100 AND 110% METABOLIZABLE PROTEIN (MP) REQUIREMENTS WITH(WITHOUT) SUPPLEMENTAL RUMEN PROTECTED METHIONINE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Metabolizable protein content</th>
<th>Methionine</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90% MP</td>
<td>100% MP</td>
<td>110% MP</td>
</tr>
<tr>
<td>Total Protein g L⁻¹</td>
<td>69.2</td>
<td>68.7</td>
<td>70.5</td>
</tr>
<tr>
<td>Albumin g L⁻¹</td>
<td>37.6</td>
<td>37.5</td>
<td>37.6</td>
</tr>
<tr>
<td>Globulin g L⁻¹</td>
<td>31.6</td>
<td>31.2</td>
<td>32.8</td>
</tr>
<tr>
<td>Urea mmol L⁻¹</td>
<td>3.42ᵃ</td>
<td>3.96ᵇ</td>
<td>4.56ᶜ</td>
</tr>
<tr>
<td>BHBA μmol L⁻¹</td>
<td>312</td>
<td>302</td>
<td>280</td>
</tr>
<tr>
<td>NEFA mmol L⁻¹</td>
<td>0.68ᵃ</td>
<td>0.65ᵇ</td>
<td>0.55ᵇ</td>
</tr>
<tr>
<td>Haptoglobin g L⁻¹</td>
<td>0.14</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Cholesterol mmol L⁻¹</td>
<td>5.05ᵃ</td>
<td>4.44ᵇ</td>
<td>3.79ᶜ</td>
</tr>
<tr>
<td>Insulin μg L⁻¹</td>
<td>0.20</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Glucose mmol L⁻¹</td>
<td>3.66</td>
<td>3.61</td>
<td>3.70</td>
</tr>
<tr>
<td>Insulin Glucose ratio</td>
<td>5.76</td>
<td>5.70</td>
<td>5.38</td>
</tr>
<tr>
<td>Ca mmol L⁻¹</td>
<td>2.31</td>
<td>2.30</td>
<td>2.30</td>
</tr>
<tr>
<td>P mmol L⁻¹</td>
<td>2.17</td>
<td>2.18</td>
<td>2.13</td>
</tr>
<tr>
<td>Mg mmol L⁻¹</td>
<td>0.98</td>
<td>0.97</td>
<td>0.96</td>
</tr>
<tr>
<td>Na mmol L⁻¹</td>
<td>141</td>
<td>141</td>
<td>142</td>
</tr>
<tr>
<td>K mmol L⁻¹</td>
<td>4.64</td>
<td>4.69</td>
<td>4.68</td>
</tr>
<tr>
<td>Cl mmol L⁻¹</td>
<td>100</td>
<td>100</td>
<td>101</td>
</tr>
</tbody>
</table>

ᵃᵇᶜ Means within a dependent variable that have uncommon letters differ (P<0.05)

²Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable protein (MP) requirements with(without) 9 g/d supplemental methionine (Smartamine®M, Adisseo Inc.) MET+ (methionine supplemented) or MET- no supplemental methionine
TABLE 5. PRE-PARTUM SERUM AMINO ACID METABOLITE CONCENTRATIONS IN COWS AND HEIFERS FED AT 90%, 100% OR 110% METABOLIZABLE PROTEIN (MP) REQUIREMENTS WITH(WITHOUT) RUMEN-PROTECTED METHIONINE

<table>
<thead>
<tr>
<th>Variable (μmol L⁻¹)</th>
<th>Treatment</th>
<th>Metabolizable protein content</th>
<th>MET</th>
<th>Protein</th>
<th>MET</th>
<th>Protein*MET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90% MP</td>
<td>100% MP</td>
<td>110% MP</td>
<td>SE</td>
<td>MET+</td>
<td>MET-</td>
</tr>
<tr>
<td>Ala</td>
<td>219</td>
<td>225</td>
<td>210</td>
<td>5.56</td>
<td>217</td>
<td>218</td>
</tr>
<tr>
<td>Arg</td>
<td>193</td>
<td>197</td>
<td>202</td>
<td>7.18</td>
<td>198</td>
<td>197</td>
</tr>
<tr>
<td>Asn</td>
<td>37</td>
<td>38</td>
<td>36</td>
<td>1.19</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Asp</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0.50</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cys</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0.39</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gln</td>
<td>282</td>
<td>296</td>
<td>296</td>
<td>8.54</td>
<td>296</td>
<td>287</td>
</tr>
<tr>
<td>Glu</td>
<td>184</td>
<td>182</td>
<td>183</td>
<td>4.52</td>
<td>183</td>
<td>183</td>
</tr>
<tr>
<td>Gly</td>
<td>572a</td>
<td>523b</td>
<td>476c</td>
<td>22.06</td>
<td>518</td>
<td>529</td>
</tr>
<tr>
<td>His</td>
<td>71</td>
<td>71</td>
<td>67</td>
<td>1.96</td>
<td>68</td>
<td>71</td>
</tr>
<tr>
<td>Ile</td>
<td>111</td>
<td>112</td>
<td>108</td>
<td>2.31</td>
<td>107</td>
<td>113</td>
</tr>
<tr>
<td>Leu</td>
<td>135</td>
<td>134</td>
<td>131</td>
<td>2.75</td>
<td>128</td>
<td>138</td>
</tr>
<tr>
<td>Lys</td>
<td>95</td>
<td>93</td>
<td>92</td>
<td>2.91</td>
<td>91</td>
<td>96</td>
</tr>
<tr>
<td>Met</td>
<td>24</td>
<td>25</td>
<td>24</td>
<td>0.63</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Phe</td>
<td>64</td>
<td>62</td>
<td>61</td>
<td>1.40</td>
<td>61</td>
<td>64</td>
</tr>
<tr>
<td>Pro</td>
<td>87</td>
<td>86</td>
<td>83</td>
<td>2.12</td>
<td>84</td>
<td>87</td>
</tr>
<tr>
<td>Ser</td>
<td>127</td>
<td>119</td>
<td>117</td>
<td>3.87</td>
<td>116</td>
<td>126</td>
</tr>
<tr>
<td>Thr</td>
<td>60</td>
<td>64</td>
<td>60</td>
<td>2.06</td>
<td>59</td>
<td>64</td>
</tr>
<tr>
<td>Trp</td>
<td>47</td>
<td>46</td>
<td>44</td>
<td>1.66</td>
<td>44</td>
<td>47</td>
</tr>
<tr>
<td>Tyr</td>
<td>56</td>
<td>54</td>
<td>54</td>
<td>1.47</td>
<td>53</td>
<td>56</td>
</tr>
<tr>
<td>Val</td>
<td>202</td>
<td>202</td>
<td>202</td>
<td>4.04</td>
<td>197</td>
<td>207</td>
</tr>
<tr>
<td>EAA totalsv</td>
<td>1000</td>
<td>1006</td>
<td>992</td>
<td>19.55</td>
<td>977</td>
<td>1022</td>
</tr>
<tr>
<td>NEAA totalsx</td>
<td>1575a</td>
<td>1531a</td>
<td>1466b</td>
<td>33.77</td>
<td>1514</td>
<td>1534</td>
</tr>
<tr>
<td>Glucogenicw</td>
<td>1816a</td>
<td>1775a</td>
<td>1705b</td>
<td>37.02</td>
<td>1750</td>
<td>1780</td>
</tr>
<tr>
<td>BCAAy</td>
<td>447</td>
<td>448</td>
<td>441</td>
<td>8.71</td>
<td>432</td>
<td>458</td>
</tr>
<tr>
<td>Ketogenicu</td>
<td>230</td>
<td>227</td>
<td>223</td>
<td>5.13</td>
<td>218</td>
<td>235</td>
</tr>
</tbody>
</table>

EAA = Essential Amino Acids; NEAA = Non-Essential Amino Acids; BCAA = Branched-Chain Amino Acids; Ketogenic = Ketogenic Amino Acids.
<table>
<thead>
<tr>
<th>3MH&lt;sup&gt;1&lt;/sup&gt;</th>
<th>25</th>
<th>24</th>
<th>22</th>
<th>1.68</th>
<th>23</th>
<th>24</th>
<th>1.46</th>
<th>0.30</th>
<th>0.58</th>
<th>0.90</th>
</tr>
</thead>
</table>

<sup>a,b,c</sup> Means within a dependent variable that have uncommon letters differ (P<0.05)

<sup>2</sup>Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable (MP) requirements with (without) 9 g/d methionine (Smartamine®M, Adisseo Inc.) MET+ (methionine supplemented) no supplemental methionine (MET-)

<sup>y</sup>Essential AA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val

<sup>x</sup>Nonessential AA = Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr

<sup>w</sup>Glucogenic AA = Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser, and Val

<sup>v</sup>Branch chain AA = Ile, Leu and Val

<sup>u</sup>Ketogentic AA = Leu and Lys

<sup>1</sup>3MH = 3- methyl histidine
<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Metabolizable protein content</th>
<th>Methionine</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tract digestibility (%)</td>
<td>90% MP (n=18)⁵</td>
<td>100% MP (n=18)⁵</td>
<td>110% MP (n=20)⁵</td>
</tr>
<tr>
<td>DM</td>
<td>66.8b</td>
<td>67.2b</td>
<td>68.7a</td>
</tr>
<tr>
<td>CP</td>
<td>64.1a</td>
<td>67.8b</td>
<td>72.0c</td>
</tr>
<tr>
<td>ADF</td>
<td>58.4b</td>
<td>58.5b</td>
<td>59.9a</td>
</tr>
<tr>
<td>NDF</td>
<td>60.1ab</td>
<td>59.6b</td>
<td>61.0a</td>
</tr>
<tr>
<td>Energy (NEm Mcal/kg)</td>
<td>72.1b</td>
<td>72.3b</td>
<td>73.6a</td>
</tr>
</tbody>
</table>

⁵Means within a dependent variable that have uncommon letters differ (P<0.05)
²Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable protein (MP) requirements with(without) 9 g/d supplemental methionine (Smartamine®M, Adisseo Inc.) MET+ (methionine supplemented) or MET- no supplemental methionine
⁶Obtained from block 2 cow and heifers
APPENDIX TABLE 1. PERFORMANCE INTERACTIONS IN COWS AND HEIFERS FED AT 90, 100 AND 110% METABOLIZABLE PROTEIN (MP) REQUIREMENTS WITH(WITHOUT) SUPPLEMENTAL RUMEN PROTECTED METHIONINE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>90% MP</th>
<th>100% MP</th>
<th>110% MP</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, kg</td>
<td>MET+</td>
<td>696&lt;sup&gt;b&lt;/sup&gt;</td>
<td>677&lt;sup&gt;b&lt;/sup&gt;</td>
<td>704&lt;sup&gt;b&lt;/sup&gt;</td>
<td>762&lt;sup&gt;a&lt;/sup&gt;</td>
<td>690&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MET-</td>
<td>699&lt;sup&gt;b&lt;/sup&gt;</td>
<td>676&lt;sup&gt;b&lt;/sup&gt;</td>
<td>703&lt;sup&gt;b&lt;/sup&gt;</td>
<td>760&lt;sup&gt;a&lt;/sup&gt;</td>
<td>690&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>MET+</td>
<td>689&lt;sup&gt;b&lt;/sup&gt;</td>
<td>684&lt;sup&gt;b&lt;/sup&gt;</td>
<td>703&lt;sup&gt;b&lt;/sup&gt;</td>
<td>768&lt;sup&gt;a&lt;/sup&gt;</td>
<td>694&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MET-</td>
<td>695&lt;sup&gt;b&lt;/sup&gt;</td>
<td>684&lt;sup&gt;b&lt;/sup&gt;</td>
<td>703&lt;sup&gt;b&lt;/sup&gt;</td>
<td>768&lt;sup&gt;a&lt;/sup&gt;</td>
<td>694&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnancy corrected BW, kg</td>
<td>MET+</td>
<td>626&lt;sup&gt;b&lt;/sup&gt;</td>
<td>620&lt;sup&gt;b&lt;/sup&gt;</td>
<td>642&lt;sup&gt;b&lt;/sup&gt;</td>
<td>702&lt;sup&gt;a&lt;/sup&gt;</td>
<td>629&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MET-</td>
<td>634&lt;sup&gt;b&lt;/sup&gt;</td>
<td>620&lt;sup&gt;b&lt;/sup&gt;</td>
<td>642&lt;sup&gt;b&lt;/sup&gt;</td>
<td>702&lt;sup&gt;a&lt;/sup&gt;</td>
<td>629&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Initial BCS&lt;sup&gt;y&lt;/sup&gt;</td>
<td>MET+</td>
<td>3.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MET-</td>
<td>3.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>MET+</td>
<td>8.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MET-</td>
<td>8.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means within a dependent variable that have uncommon letters differ (P<0.05)

<sup>z</sup>Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable protein (MP) requirements with(without) 9 g/d supplemental methionine (Smartamine®M, Adisseo Inc.) MET+ (methionine supplemented) or MET- no supplemental methionine

<sup>y</sup>BCS= Body condition score
## APPENDIX TABLE 2. CHEMICAL COMPOSITION OF MINERAL AND VITAMIN PREMIX

<table>
<thead>
<tr>
<th>Content</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (%)</td>
<td>14.13</td>
</tr>
<tr>
<td>Phosphorous (%)</td>
<td>10.01</td>
</tr>
<tr>
<td>Salt (%)</td>
<td>15.00</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>5.97</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Chloride (%)</td>
<td>9.15</td>
</tr>
<tr>
<td>Sulphur (%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>5.96</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>5,840</td>
</tr>
<tr>
<td>Fluorine (mg/kg)</td>
<td>373</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>2,994</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>619</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>2,028</td>
</tr>
<tr>
<td>Iodine (mg/kg)</td>
<td>82.08</td>
</tr>
<tr>
<td>Cobalt (mg/kg)</td>
<td>29.70</td>
</tr>
<tr>
<td>Vitamin D (IU/kg)</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin E (IU/g)</td>
<td>3,005</td>
</tr>
</tbody>
</table>
APPENDIX TABLE 3. METABOLIZABLE PROTEIN CONTENT IN COWS FED AT 90, 100 AND 110% METABOLIZABLE PROTEIN (MP) REQUIREMENTS

<table>
<thead>
<tr>
<th>Weeks on Trial Diet</th>
<th>90%MP Cows</th>
<th>100%MP Cows</th>
<th>110%MP Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>4</td>
<td>b</td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>6</td>
<td>c</td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>8</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Protein: P<0.001
MET: P>0.22
Protein*MET: P≥0.02

Means within a dependent variable that have uncommon letters differ (P<0.05)
Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable (MP) requirements
APPENDIX TABLE 4. METABOLIZABLE PROTEIN CONTENT IN HEIFERS FED AT 90, 100 AND 110% METABOLIZABLE PROTEIN (MP) REQUIREMENTS

Means within a dependent variable that have uncommon letters differ (P<0.05)

Treatments: Cattle fed at 110% MP, 100% or 90% metabolizable (MP) requirements

Protein: P<0.001
MET: P≥0.02
Protein*MET: P≥0.09

Means within a dependent variable that have uncommon letters differ (P<0.05)
REFERENCES


Oversupplying metabolizable protein in late gestation for beef cattle: effects on
prepartum BW, ruminal fermentation, nitrogen balance, and skeletal muscle catabolism.

Haverberg, L.N., Omstedt, P.T., Munro, H.N., and Young, V.R. 1975. Nτ-Methylhistidine

Supplemental protein plus ruminally protected methionine and lysine for primiparous

2000. Feeding stimulates protein synthesis in muscle and liver of neonatal pigs through


Knowlton, K. F., McGilliard, M. L., Zhao, Z., Hall, K. G., Mims, W., and Hanigan, M. D.
2010. Effective nitrogen preservation during urine collection from Holstein heifers fed


