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ENERGY AND AMINO ACID METABOLISM IN LACTATING JERSEY COWS CONSUMING FEED BYPRODUCTS

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Animal protein byproducts are high bypass proteins commonly used in the dairy industry. These bypass proteins can escape the rumen to supply additional amino acids needed to support milk and protein yield. Two of the more popular animal protein byproducts used in the dairy industry are blood meal and hydrolyzed feather meal.

In the first experiment, two flow meters were compared using headbox-style calorimeters. The objectives of the first study were to test mass flow meter (**MFM**) and volumetric flow meter (**VFM)** by measuring O_2 consumption and CO_2 production and to illustrate the effects of incomplete gas recovery on estimated energy partitioning. The gas recoveries were observed to be lower for the VFM than the MFM. The MFM resulted in higher performance than the VFM that was determined by the flow rate. Incomplete gas recovery can result in underestimates of heat production, thereby affecting estimates of whole-animal energy use. Our results indicate that MFM may be better suited for headbox-style indirect calorimetry to estimate heat production in lactating cows.

In the second experiment, 12 multiparous lactating Jersey cows were used to evaluate the effect of feeding hydrolyzed feather meal with or without blood with rumen protected lysine on milk protein and energy utilization. Treatments were composed of hydrolyzed feather meal

without blood and no rumen protected Lys (**RP-Lys**), hydrolyzed feather meal with blood and no RP-Lys, hydrolyzed feather meal without blood and RP-Lys, and hydrolyzed feather meal with blood and RP-Lys. Results suggest the hydrolyzed feather meal containing blood produces more milk and milk protein than hydrolyzed feather meal alone, which may be due to the increase supply of essential amino acids, observed by the blood plasma. Even though, total tract crude protein digestibility maybe lower for the hydrolyzed feather meal containing blood than hydrolyzed feather meal alone. Energy supply did not seem to be a factor in the increase production of milk and protein yield.

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INTRODUCTION

Over the last century the U.S dairy industry has advanced in all areas of production including technology, genetics, management, and nutrition. In the field of nutrition continued focus has been placed on identifying and understanding how alternative feedstuffs can be used for proper ration balancing to increase the health and profitability of dairy cattle. In 2007, the United States dairy population was 9.2 million cows, with a total milk production of 84.2 billion kg, compared to 25.6 million cows and a milk production of 53.0 billion kg in 1944 (Capper et al., 2009). Consequently, compared to 1944, the dairy industry today is producing 59% more milk with 64% fewer cows, while consuming 77% less feed and 65% less water (von Keyserlingk et al., 2013). Also, this is being achieved with considerably less land with a 10% reduction between 1945 and 2007 (von Keyserlingk et al., 2013). Due to reduction of land use, the dairy industry must investigate alternative feeds like animal protein byproducts from the rendering industry. Research suggests that the use of by-products, efficient energy partitioning, and efficient use of bypass protein and AA has increased animal health, milk production and milk components, and had a positive environmental impact (Vandehaar and St-Pierre, 2006, White and Hall, 2017).

Bypass protein is the fraction of protein contained in a feedstuff that escapes digestion in the rumen. These proteins reach the small intestine and are broken down into small peptides and AA. These may be absorbed by the small intestine to be utilized by the cow. Amino acids are commonly referred to as the "building blocks of life" because they are the foundational organic compounds for which protein structures are formed. Supplying AA's by feeding high-protein byproducts such as animal protein feeds are often economically advantageous. Animal protein byproducts such as blood meal (**BM**) and hydrolyzed feather meal (**HFM**) are high in rumen

undegradable protein (**RUP**), which contain a RUP content of 77% and 65%, respectively (NRC, 2001).

There are ten essential AA (**EAA**) that need to be provided to the dairy cow in a diet. These ten EAA are Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. Both methionine and lysine are considered to be frequent limiting AA for dairy cow (Schwab et al. 2005). If the supply of lysine becomes limiting, the synthesis of milk protein may be reduced (Vyas and Erdman, 2009). Reduction in Lys supply can reduce milk protein yield, which can cause a reduction in profitability to the producer because in many parts of the US revenue is based upon milk solids.

The U.S. poultry industry is the second largest broiler meat export in the world (USDA, 2018). In 2018, the U.S. poultry industry processed over 2.5 billion kg of chicken meat, which produced about 79 million kg of feathers (USDA, 2018). As this industry continues to grow the supply of HFM will also increase along with the supply of feathers. In comparison to BM, in addition to a lower digestibility, HFM is considered to have a poor AA profile in terms of Met and Lys, which may lead to a lower protein content of milk (Cunningham et al, 1994, Harris et al, 1992, Santos et al, 1998) when used as an AA supplement. Santos et al. (1998) reported that AA profile score in relationship to AA within milk protein was 13% for Lys, which is one of the poorest Lys sources on the market for bypass proteins. Blood meal has the highest Lys content to Lys content in milk protein (91%) of bypass protein, while fish meal (80%) is in close second (Santos et al, 1998). In order to increase the Lys profile of HFM, a portion of poultry blood may be included, and this addition has been investigated in beef cattle rations (Goedeken et al, 1990) but not dairy cattle.

Given the Lys content of blood, its addition to HFM should improve its nutrient value. Additionally, compared to other animal proteins HFM usually contains a greater concentration of fat. Surprisingly, only a few studies have sought to evaluate the inclusion of feather meal on overall protein and energy nutrient utilization in dairy cattle. The addition of blood to the HFM should increase the Lys supply of the cow and increase milk protein. Morris et al. (2020a) observed a decrease in milk protein yield using increasing inclusions of HFM containing no blood due to low EAA supply. Therefore, the objectives of this study were to evaluate nutrient composition and digestibility of HFM with blood when fed to lactating dairy cattle.

CHAPTER 1

LITERATURE REVIEW

Protein

Crude protein. Dietary protein within feedstuffs being fed to dairy cattle is generally referred to as CP, which is determined by the N content multiplied by a factor of 6.25. This factor is based on the structure of most proteins containing 16 % nitrogen (Mariotti et al., 2008). The estimated CP content includes both protein and non-protein nitrogen (**NPN**) and was one of the original 6 proximate nutrients established over 150 years ago for dairy cattle (NRC, 2001, Schwab and Broderick, 2017). In the past 60 years, protein nutrition research of dairy cattle has advance beyond the use of CP as a target nutrient with focus on meeting the ammonia and AA needs of ruminal fermentation for microbial protein synthesis and AA requirements of the cow (Patton et al. 2014; Schwab and Broderick, 2017). Although the use of CP remains, optimizing the efficiency of dietary CP to supply adequate AA balance can be beneficial to the environment by reducing total N excretion (Patton et al., 2014). Therefore, the main focus is to supply adequate amounts of rumen degradable protein (**RDP**) that will meet the N requirement of the ruminal microbes for maximal synthesis of microbial crude protein (**MCP**) and RUP that will help meet the metabolizable protein (**MP**) requirement of the cow for efficient AA balance (Figure 1, Patton et al. 2014; Schwab and Broderick, 2017). Metabolizable protein is defined as the true protein that is digested postruminally and the component of AA absorbed by the intestine (NRC, 2001).

RDP and RUP. Rumen degradable protein is the portion of dietary CP that is degraded by rumen microbes thereby providing a mixture of peptides, free AA, and ammonia and these

can be used for microbial growth and synthesis of MCP (Russell et al., 1992). Increasing microbial CP is a cost effective way of providing MP. Although being low in His and Met, microbial CP has an AA profile similar to milk protein (Patton et al. 2014). The two main sources of MCP, bacteria and protozoa, contribute approximately 66.5% and 11.0% of the total N, respectively (Clark et al., 1992; Orskov, 1992). The remaining MCP is from the plant cell wall NPN. For bacteria and protozoa to produce MCP, these microbes require an adequate rumen fiber mat for rumen function with ruminally fermentable carbohydrates and the level and degradability of RDP to provide them with AA's and ammonia (Patton et al., 2014). Low-quality forage may reduce MCP synthesis but may be improved by increasing RDP in the rumen. Köster et al. (1996) observed a linear increase in microbial and ammonia N to the duodenum when supplementing increasing amounts (0 to 720 g/d) of RDP with low quality tallgrass prairie forage in beef cows. In dairy cattle supplemented with increasing amounts of RDP using urea with corn silage based diets resulted in a quadratic effect on MCP flow and efficiency of MCP synthesis with a maximum response at dietary RDP concentrations of 10.8 and 10.0% of DM, respectively, compared to the control diet (9.2 % of diet DM, Boucher et al., 2007). According to Kalscheur et al. (2006) the increased MCP flow from increased RDP inclusions (6.8 to 11.0% of diet DM) increased milk, fat, and protein yield linearly by 6.3, 10.0, and 10.5%, respectively. However, not all RDP sources have the same concentration or degradability and can contribute differentially towards MCP synthesis. The most used sources of RDP in the US are soybean meal and canola meal with an RDP value of 65 and 64% of CP, respectively (NRC, 2001).

In 1966, it was thought that all AA's by ruminants could be provided by MCP; however, improvements in growth rates, milk production, and feed efficiency may be better supported by increasing AA supply (Schwab and Broderick, 2017). Annison, (1956) observed that proteins

like zein were slowly degraded by ruminal microbes, leading to the development of techniques to increase ruminal bypass protein (Schwab and Broderick, 2017). This ruminal bypass protein to the small intestine is known as RUP. Rumen undegradable protein sources are commonly found in byproducts. Typically, many byproducts that are fed to dairy cows are created from the removal of desired material (i.e. ethanol from corn or meat processing from livestock) that usually undergoes high temperatures to be used for other human resources. The exposure of feed to high temperatures usually increases the rumen bypass value of the feed protein (Schwab and Broderick, 2017). For example, DDGS is a by-product of the ethanol industry that has a RUP content of 51% of CP (NRC, 2001). Animal by-products such as hydrolyzed feather meal are high in RUP sources (65% of CP, NRC, 2001). Microbial N flow decreases when RUP is increased but this also increases non-ammonia non-microbial nitrogen (**NANMN**) flow to the duodenum due to decreased degradability in the rumen (Santos et al., 1998). Replacing soybean meal with high RUP sources has been shown to reduce MCP flow and doing so excessively may result in an inadequate supply of RDP (Santos et al., 1998). Cunningham et al. (1994) observed a linear increase in NANAM with increased inclusion of RUP using HFM plus BM (0, 33, 67, and 100% of CP) but a numerical decrease in microbial N, which can influence milk production and components. Wright et al. (1998) observed an increase in milk production and protein with feeding increased concentrations of RUP at different restrictive feed concentrations of the basal diet. It was suggested that this increased inclusion of RUP provided more AA's available to the small intestine and in turn, absorbed by the animal to increase milk production and protein.

Also, supplying high amounts of protein may elevate plasma urea nitrogen (**PUN**) and milk urea nitrogen (**MUN**), which could affect reproductive efficiencies of the cows (Canfield et al., 1990; Butler et al, 1996). Baker et al. (1995) fed four different diets to dairy cows: 1)

excessive RDP with deficient RUP 2) balanced RDP and RUP but not AA balanced 3) balanced RDP, RUP and AA and 4) excessive RDP, balanced RUP using the NRC and Cornell Net Carbohydrate and protein system. Results determined diet 4 had the highest PUN and MUN with 23.4 and 23.3 mg/dl, respectively. This is because the excess protein is being excreted as waste and requires energy to eliminate the excess nitrogen from the cow. The effects of feeding N in excess of the requirements on energy balance and heat production is 6.5 to 7.4 Mcals/kg of excess N (Tyrrell et al., 1970; Reed et al., 2017). Diet 3 produced the least amount of PUN (16.0 mg/dl) and MUN (15.1 mg/dl) while maintaining milk production and producing the highest amount of milk true protein (3.01 %). This research indicated that MUN concentrations are sensitive to changes in CP, RDP, and RUP, which can be elevated by ruminal microbes, ruminant tissues, or both and the true protein content of milk is influenced by supply of RUP and AA balance (Baker et al., 1995). In order to obtain high producing cows, it is recommended to achieve a CP concentration of 15 to 17%, RDP concentration around 9.0 to 10.0% of DM with an MUN in the 9 to 12 mg/dl range (Patton et al., 2014).

Metabolizable Protein. Microbial CP, RUP, and endogenous CP (**ECP**) contribute to the passage of MP to the small intestine (Patton et al., 2014). Metabolizable protein is defined as the true protein that is digested postruminally and the component AA absorbed by the intestine (NRC, 2001). The goal of ruminant protein nutrition is to provide optimum MP supply to the cow with a high nutritive value of essential AA's that contains the N available for improved milk yield and milk protein yield. Protein research has demonstrated that providing adequate MP increased, milk yield, and milk protein yield (Giallongo et al, 2016, Lee et al., 2012). The current NRC (2001) uses the MP system to determine protein requirements for maintenance and

production (growth, pregnancy, and lactation) of the cow. Metabolizable protein requirements of maintenance and lactation are calculated as:

$$MP = 4.1 \times BW^{0.50}(kg) + 0.3 \times BW^{0.60}(kg) + [(DMI\ (kg) \times 30) - 0.50\ ((bacterial\ MP/0.8) - bacteria\ MP)] + endogenous\ MP / 0.67 \quad (1)$$

$$MPLact = ((milk\ production\ (kg/d) \times (milk\ true\ protein\ /100)) / 0.67) \times 1000 \quad (2)$$

Where BW is body weight (kg) and DMI is dry mater intake (kg). According to the NRC (2001) it is assumed that the efficiency of converting MP to net protein is 67%, which is contributed by the amount of MP from MCP, RUP, and ECP. Microbial CP provided by bacteria and protozoa is assumed to contain 80% true protein and 20% NPN with a true protein digestibility of 80% and the conversion of MCP to MP is 64%. Rumen undegradable protein is assumed to be 100% true protein but the intestinal digestibility varies among feedstuffs, ranging from 50 to 100%. Therefore, the contribution of RUP to MP is variable and dependents on the feedstuff and formulation itself. Endogenous CP is assumed to contain 50% true protein with a true protein digestibility of 80% and the conversion of ECP to MP is assumed to be 40% (NRC, 2001). However, there is major controversy over assuming that the efficiency of MP to net protein is 67% for maintenance and lactation. For example, the NRC (2001) and CNCPS will often underestimate MP allowable milk at low MP supplies and overestimate at high MP supplies (Lapierre et al., 2007). Moraes et al., 2018 concluded that a nonlinear relationship between protein yield and MP supply, using the fixed efficiency of 67%, would likely overestimate MP requirements at low yields and underestimate requirements at high yields. Many studies have attempted to further study the assumption of consistent efficiency. Metcalf et al. (2008) suggested that the efficiency of utilizing supplied MP for milk true protein synthesis decreased from 77 to 50% when the MP supply varied from 25% below and 25% above the predicted MP

requirements. Daniel et al. (2016) observed a similar trend when MP above maintenance decreased from 82 to 58% and MP supplies increased from -400 g/d to +300 g/d in comparison to the MP supply needs of 67% efficiency. The differences in these efficiencies have sparked interest in removing the MP system and shifting to an individual AA system to predict protein requirements (Arriola Apelo et al., 2014, Lapierre et al., 2014). The problem is not all requirements have been determined for individual AA's and it is difficult to measure due to recycling of N from splanchnic and peripheral tissues. However, using an individual AA system could increase N efficiency (Haque et al., 2015).

Amino Acids. Ruminally synthesized MCP, RUP, and ECP begin to be digested in the abomasum where they are exposed to hydrochloric acid and pepsin. This breaks down the peptide bonds between AA's into polypeptides and some free AA's (Harmon, 1993). When digesta reaches the small intestine, it is buffered back to a neutral pH and pancreatic trypsin, chymotrypsin, and elastase begin the breakdown where carboxypeptidases A and B complete the digestion of polypeptides into AA's (Harmon, 1993; Patton et al., 2014). The main goal of protein digestion is for 1) production of absorbable free AA's, di- and tripeptides 2) resynthesize proteins and 3) immune processes, which are beneficial for dairy cattle heath, milk production, and milk protein. There are twenty primary AA's that are separated into two groups: essential or nonessential AA. Essential AA are AA that either cannot be synthesized by the animal or if they can (Arg and His), not at rates sufficient to meet requirements, which include Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. Leucine is the only AA that do not serve as a precursor for gluconeogenesis and cannot be converted to fatty acids or serve as immediate sources of metabolic energy when oxidized to carbon dioxide (NRC, 2001). Nonessential (**NEAA**) are those AA's readily synthesized from metabolites of intermediary metabolism and amino groups from

surplus AA, which include Ala, Asn, Asp, Gln, Glu, Gly, Pro, Ser, Tau, and Tyr. Nonessential AA requirements are met for growth and milk protein production before the requirements for the most limiting EAA, which has resulted in limited research of NEAA and focus on the first limiting EAA (NRC, 2001).

Lysine and Met have been considered the first limiting EAA in lactating dairy cattle (NRC, 2001). Schwab et al., 1992 determined Lys as one of the first limiting AA in a corn-based diet. Armentano et al., 1997 fed a total mixed ration containing high forage (alfalfa hay) with animal proteins that limited Met but contained adequate Lys. The increased inclusion of Met (5.25, 10.5, and 11.5 g/d RP-Met; Smartamine-M; Rhone-Poulenc Animal Nutrition, Commentry, France) resulted in an increase of milk protein yield; however, when 14.7 g/d of RP-Lys (Smartamine-ML; Rhone-Poulenc Animal Nutrition, Commentry, France) was introduced with 11.5 g/d RP-Met there was no effect on milk protein yield (Armentano et al., 1997). This lack of response may have been contributed by the adequate supply of Lys to the duodenum from the basal diet (Armentano et al., 1997). Polan et al., 1991 observed the opposite affect when RP-Lys inclusions were increase with RP-Met and corn gluten feed in addition to the basal diet, where Lys increased milk protein yield and Met had no effect. However, corn gluten feed is high in Met and low in Lys, and the additional Met could have been in excess (Polan et al., 1991). Overton et al (1998) observed the same effect when feeding RP-Met (Mepron M85; Degussa Corp., Allendale, NJ) with corn gluten feed. Since, the NRC (2001) many research studies have confirmed that Lys and Met may be limiting AA and have a positive effect on milk protein (Appuhamy et al., 2011; Lee et al., 2012b; Giallongo et al., 2016). Vyas and Erdman, 2009 suggested that supplying increasing amounts of Lys and Met may increase milk production and milk protein yield. However, in practice, Lys may not be as limiting (Paz et al., 2013). Lean

et al. (2017) published a meta-analysis to predict the effects of metabolizable AA on dairy cattle performance and determined that predicted metabolizable Lys (g/d) did not increase responses in production outcomes. However, this could be because mean metabolizable Lys supply (6.36% MP and 6.38% MP) was less than what was recommended by CNCPS models (6.68% MP). Predicted metabolizable Met was associated with milk yield and milk protein yield (Lean et al., 2017). However, there has been some speculation that His may also be a limiting EAA and could be more limiting than Lys (Lee et al., 2012a; Giallongo et al., 2016). Histidine is now available commercially as a rumen protected product.

Vanhatalo et al. (1999) infused His into the duodenum of the dairy cattle alone and in combination with Met and Lys and concluded that His was the first limiting amino acid in grass silage-based diets supplemented with cereal grains. Infusion of His alone increased milk yield and milk protein yield by 3.0 and 3.6%, respectively (Vanhatalo et al., 1999). Giallongo et al., 2017 compared His adequate diets to His inadequate diets determining that milk yield and milk protein yield increased in His adequate diets, which could be contributed to the increase in plasma His concentration. Histidine has been observed to increase DMI, which can increase milk production (Giallongo et al., 2016; Giallongo et al., 2017; Zang et al., 2019). Lee et al., 2012b observed no effect on milk yield and milk protein yield when comparing adequate MP to deficient MP with RP-Lys (Aminshure-L; Balchem Corportation, New Hampton, NY) and RP-Met (Mepron; Evonik Industries AG, Hanau, Germany), which could be due to the 42% decrease in plasma His concentration. The addition of His to a diet formulated to be deficient in MP, Lys, and Met increased milk yield (Lee et al., 2012a) and milk protein yield (Giallongo et al., 2016). The concentrations of plasma His and Lys also increased with the inclusion of His, which could have contributed to the increased milk yield (Lee et al., 2012a). Giallongo et al. (2016) observed

the same increase in plasma His and Lys concentration; but also, observed an increase in plasma Met concentration, contributing to the increase in milk protein yield. However, Morris et al. (2020a) observed an increase in plasma His, Lys, and Met concentration when feeding HFM with RP-His (Balchem Corp., New Hampton, NY) and RP-Lys (Ajipro; Ajinomoto Co., Inc., Tokyo, Japan) but no response in milk yield or milk protein yield. Lean et al. (2017) indicated that His is positively associated to milk production. The research provided indicates that inclusions of His and increasing amounts may increase milk yield or milk protein yield (Zang et al., 2019).

Milk protein. Dairy producers are paid on the production of milk solids as well as receive premiums on milk quality. Consequently, increasing milk production and components can potentially increase a dairy producer's profitability. Milk protein price is determined by the value of cheese and butter. Currently, the demand for cheese is increasing in the United States, causing an increase in the value of milk protein (USDA, 2020), which places increased emphasis on adequate protein nutrition.

Amino acid availability to the mammary gland is important in the production of milk protein synthesis; reduction in availability may in turn reduce the production of milk protein (Lapierre et al., 2012). The availability of EAA to the mammary gland is highly dependent on the uptake and catabolism of EAA by the splanchnic tissue (portal-drained viscera (**PDV**) and liver) and recycling of the peripheral tissues (Arriola Apelo et al., 2014, Figure 2). Hanigan et al., 2004 observed that the PDV clearance rate of EAA varied for each EAA, specifically Thr, Phe, Leu, and His were the highest and Lys and Arg were the lowest. Methionine and Val had an intermediate PDV clearance rate. This information can be used to determine the potential EAA that could be utilized for milk protein synthesis.

After deamination in the liver, direct sources of AA utilized for milk protein synthesis in the mammary gland epithelial cells come from free AA in the arterial blood supply (Patton et al., 2014). Amino Acids are used in transcription and translation to form milk protein (Arriola Apelo et al., 2014). DNA is formed into mRNA (transcription), which is regulated by lactogenic hormones prolactin and glucocorticoids (Doppler et al., 1989). Translation of milk protein (mRNA to protein) is regulated by the activity of initiation and elongation factors (Arriola Apelo et al., 2014). The mTORC1 complex regulates the rate of protein translation and cellular growth, which is made up of eIF4E-binding protein 1 (**4EBP1**), ribosomal protein S6 kinase 1 (**S6K1**), and eukaryotic elongation factor 2 (**eEF2**, Figure 3). Essential AA, especially Leu and Ile, activate the mTORC1 complex (Han et al., 2012), which can phosphorylate mTOR and 4EBP1 to increase casein synthesis rate, whereas, eEF2 phosphorylation can cause a decrease (Appuhamy et al., 2011).

However, the amount of EAA supplied to the mammary gland to activate the mTORC1 complex is highly dependent on the blood flow of the mammary gland. Essential AA, NEAA, and glucose have been observed to have an effect on mammary blood flow (Doepel and Lapierre 2010, Rulquin et al., 2004). Doepel and Lapierre (2010) observed a decrease in blood flow to the mammary by 10% when infusing EAA (359 g/d) and increase by 7% when infusing NEAA (356 g/d) in Holstein cows fed a 13.9% CP diet. Rulquin et al. (2004) observed an increase in mammary gland blood flow linearly with the provision of glucose in Holstein cows. It is interesting to think that glucose and NEAA would increase blood flow, whereas, EAA would reduce blood flow. However, mammary blood flow is negatively correlated to the ATP:ADP ratio within the cell, which is determined by blood nutrients (AA, acetate, glucose). Mammary gland blood flow is reduced when the ATP:ADP ratio increases (Cant and McBride, 1995,

Arriola Apelo et al., 2014). Inadequate dietary EAA would reduce the ATP:ADP ratio, causing an increase in mammary blood flow in order to supply EAA at low concentrations in the blood to support protein synthesis as a survive response. However, increasing blood flow can increase milk production (Hanigan et al., 2002).

Insulin has been shown to be a regulator of milk protein synthesis in lactating dairy cows (McGuire et al, 1995). Using the hyperinsulinemic-euglycemic clamp technique (1.0 μg·kg BW^{-1} ·h^{-1} of insulin infused) and infusing casein, branched-chain AA, and water, resulted in an increase of milk protein yield. The addition of the insulin clamp with water and insulin clamp with casein and branch-chain AA increased milk yield by 15 and 25%, respectively (Mackle et al., 2000). Winkelman and Overton (2013) observed an increase in milk protein yield with a subcutaneous injection of long-acting insulin by 5%. Supplying more insulin can potentially increase milk protein yield. In the mammary gland, insulin has been shown to phosphorylate insulin receptor substrate 1, Akt, and downstream protein (Arriola Apelo et al., 2014). The activation of Akt and AMP-activated protein kinase signals other kinases that interact with the mTORC1complex, while being activated by EAA (Arriola Apelo et al., 2014, Figure 3). Providing both energy and EAA is essential for the function of the mTORC1complex to promote milk protein synthesis.

Animal Protein Byproducts

Rendering Industry and Processing. In the United States, approximately 100 million hogs, 35 million cattle, and 8 billion chickens are produced and slaughtered annually (Meeker and Hamilton, 2009). However, 49%, 44%, and 37% of the live weight of cattle, pigs, and broilers, respectively, is not consumable by humans, contributing to nearly 25.5 billion kg of raw material which is then diverted to the rendering process (Meeker and Hamilton, 2009). This

material includes the raw material from hides, skins, hair, feathers, hooves, horns, feet, heads, bones, toe nails, blood, organs, glands, intestines, muscle and fat tissues, shells, and whole carcasses that can be used to produce animal by-product (Meeker and Hamilton, 2009). The rendering industry contributes to the sustainability of animal food production because these animal tissues are used for productive purposed and not placed in a landfill (Meeker and Hamilton, 2009). The rendering industry use the raw material primarily to produce meat and bone meal, meat meal, poultry meal, HFM, BM, fish meal, and animal fats that are used in dairy cow diets. In 2009, the European Union placed a ban on the use of animal by-product because it was valued as a potential risk to the public and animal health from increased crises related to foot-and-mouth disease, bovine spongiform encephalopathy (**BSE**), and the occurrence of dioxins within the feedstuff (European Commission, 2009). However, animal byproducts are regulated by the United States Food and Drug Administration, where they prohibited certain ruminant proteins from being used in ruminant diets to prevent the spread of BSE (Meeker and Hamilton, 2009). This means that bovine blood meal cannot be fed to dairy cows; but porcine blood meal can be fed to dairy cows. The rendering industry provides quality proteins and fats that have become an important aspect to society and without this industry, the accumulation of unprocessed animal byproducts would impede the meat industries and pose a serious potential hazard to animal and human health (Meeker and Hamilton, 2009).

The rendering process takes place where raw material (non-consumable food to human) is physically and chemically transformed by various equipment and processes (Meeker and Hamilton, 2009). Typically, all rendering processes involve the application of heat, extraction of moisture, and the separation of fat. However, the process can change with the raw material. The raw material is ground to a consistent particle size and is cooked with steam in a continuous-flow

or batch system at 115 to 145 °C for 40 to 90 minutes, depending on the type of material (Meeker and Hamilton, 2009). Temperature and length of time are critical during the cooking process, which determines the quality of the product. Also, the raw material composition can affect the animal by-product (Blasi et al., 1991). During the cooking process, the melted fat is separated from the protein and bone solids, while a large portion of the moisture is removed and microbes are deactivated (Meeker and Hamilton, 2009). Fat is separated from the cooking material by using a screw press with a closed vessel. After cooking and fat separation, the remaining material is known as “cracklings” or “crax”, which contains protein, minerals, and some residual fat that is further processed and ground to make other animal byproducts (Meeker and Hamilton, 2009).

Blood meal. Blood meal is a common feed ingredient used in dairy nutrition that is high in by-pass protein. Blood meal is an animal byproduct from the rendering industry, which is heated at high temperatures to remove the water (Meeker and Hamilton, 2009). Blood meal contains 96% of CP with 71% as RUP and is viewed as a high valued product because of its high Lys (8.98% of CP) and His (6.36% of CP) concentration (NRC, 2001). However, BM has a low concentration of Met (1.17% of CP, NRC, 2001). Blood meal is a very expensive protein source when calculated as a percent of CP; however, when the price is calculated per kg of RUP, high yielding dairy cows can be more economical (Pires et al., 1996). This is because of the RUP Lys and His that becomes available to the small intestine of the cow. Santos et al. 1998 reported percentage of Lys and His within different feed protein compared to the percentage within milk protein in a 12-year literature review, where BM had a percentage for Lys (91%) and His (100%, indicating BM could promote milk protein synthesis.

Blood meal has been extensively researched in dairy cows. Santos et al. (1998) review did 8 comparisons from 5 trials on BM or BM and DDGS replaced with soybean meal in corn silage or alfalfa silage diets, which showed that there was no effect on DMI, milk yield, or milk protein yield. However, there are concerns with palatability in high BM diets. Pires et al. (1996) reported a 9.8% decrease in DMI when feeding BM at 2.7% of the diet, compared to the control diet (soybean meal), which had a negative numerical effect on milk yield (soybean meal). Supplying high quantities of BM could result in a reduction of DMI, causing a reduction in milk yield.

Blood meal is a rumen by-pass protein compared to soybean meal (NRC, 2001). This allows higher amounts of N from the BM to potentially reach the small intestine that can be farther digested and absorbed by the cow. Waltz et al. (1987) observed a 33% decrease in bacterial N and 6.0% increase in dietary N flow to the duodenum when replacing soybean meal with BM in duodenum cannulated Holsteins. This indicated that BM was more resistant to ruminal degradation than soybean meal. However, not all sources of BM are the similar. Paz et al. (2014) used the mobile bag technique to determine intestinal digestibility of common RUP sources, including 3 sources of BM where one was spray dried and the others were ring dried, which were observed to be very different. The RUP content of the 3 BM's were 14.7, 70.2, and 59.3% of CP, indicating that the drying methods used had an effect on RUP content of the BM. Residue from BM 1 and BM 3 were limited and intestinal digestibility was not obtained but BM2 had a RUP digestibility of 87.9% (Paz et al., 2014). The CP digestibility and AA compositions of BM can be affected by the drying method (Meeker and Hamilton, 2009). Spray dry methods use an anti-coagulation liquid that sprays in a warm chamber on the blood and transfers it into powder, whereas, ring drying uses steam to coagulate the blood and the coagulum is centrifuged

and dried with hot gas in a ring drier (Jaydip Mulik, 2014). Bureau et al. (1999) observed that the CP digestibility of spray dried BM was significantly higher than the ring dried BM. As a dairy nutritionist, it is critical to understand how the BM is manufactured to insure proper AA nutrition to the dairy cow.

Hydrolyzed Feather Meal. Hydrolyzed feather meal is another animal byproduct from the rendering industry that is higher in RUP commonly used in dairy diets. Hydrolyzed feather meal contains 92% CP, which is mostly RUP, 65% (NRC, 2001). However, HFM is a poor source of Met, Lys, and His (NRC, 2001). Santos et al. 1998 reported percentage of Met, Lys and His within different feed protein compared to the percentage within milk protein in a 12-year literature review, where HFM had the lowest percentage for Met (23%), Lys (13%), and His (11%), indicating HFM could reduce milk protein. Harris et al. (1992) observed a decrease in milk protein as the inclusion of HFM increased (0, 3, and 6% dietary DM) in 14% and 18% CP diets. Morris et al. (2020b) observed a similar decrease in milk protein yield with increasing inclusions of HFM at 0, 6.7, and 10% dietary DM. However, milk yield was observed to increase for the 0 to 6.7% HFM but decreased with 10% HFM. The decreased milk protein indicates that the AA profile of HFM lacks the EAA to promote milk protein synthesis. Research has used HFM to induce His deficient diets, which result in decreased DMI, milk yield, and milk protein (Stahel et al., 2014).

The hydrolysis process is the most critical step when producing HFM in the rendering industry (Meeker and Hamilton, 2009). Hydrolysis time and temperature have been shown to have an impact on the quality (degradation and digestibility) of HFM (Blasi et al., 1991; Meeker and Hamilton, 2009). Feather meal is hydrolyzed using high temperature and pressure to disrupt the keratin bonds within raw feathers to improve digestibility (Douglas Anderson, 2006).

Feathers and hair are the only process that requires pressurization (Douglas Anderson, 2006). However, pressurization has a negative effect on the availability of AA's, which can cause a reduction in milk protein of dairy cows (Meeker and Hamilton, 2009).

Because of the poor AA profile of HFM, the rendering industry also offers an HFM that contains blood that has been used in dairy diets. As we known, blood is high in Lys and His (NRC, 2001). Hydrolyzed FM with blood is 85% CP with RUP of 70% and Lys and His concentration of 2.90% and 1.33% of CP compared to HFM alone, which is 2.57% and 1.15% of CP (NRC, 2001). However, HFM with blood has not been extensively researched in dairy cows but has been researched in beef cattle. Goedeken et al. (1990) determined that adding blood to HFM may improve the quantity and quality of the ruminal escape protein in beef cattle. However, Grant and Haddad (1998) increased milk and protein yield by 10% and 12% respectively for 17.6% CP diet with BM and HFM, but both decreased by 10% and 17% for diets containing 19.6% CP. The increase in milk and protein yield with HFM and BM in 17.6% CP diets could have been due to the 13% increase in protein efficiency, whereas the 19.6% observed no effect on protein efficiency (Grant and Haddad, 1998). This indicates that adding blood to hydrolyzed feather instead of BM to hydrolyzed feathers could influence milk protein synthesis. However, similar issues occur when feeding either HFM alone and HFM containing blood because different rendering plants use different methods that can affect the chemical composition and AA profile of the feedstuff (Cotanch et al., 2020).

Energy and Nitrogen Utilization

Energy Balance. Energy is a limiting nutrient in dairy cow production systems (Coppock 1985; Brun- Lafleur et al., 2010; Weiss, 2019), and a cow may derive energy both from that which is consumed and that which is mobilized from body stores. Energy utilization can be

evaluated by calculating energy balance. Gross energy intake (**GEI**), is the amount of energy that an animal consumes and is calculated by multiplying the gross energy of feed ingredients by feed intake of the animal, which is determined by the combustion of feed in a bomb calorimeter (Eq. 3). This energy consumed by the animal is the potential energy available to maintain body functions, growth, and milk production. In order to determine digestible energy (**DE**), fecal energy is removed from GEI (Eq. 4). Metabolizable energy (**ME**) is calculated by the removal of both urinary and gaseous energy from DE (Eq. 5). Net energy of lactation (**NEL**) is calculated by the removal of heat increment from ME (Eq. 6). The NEL is the energy required for maintenance, lactation, gestation, and growth. According to Weiss (2007), the NE system is considered the most thorough method for differentiating feeds when formulating rations for dairy cows.

$$\text{GEI (Mcal/d)} = \text{intake of feed} \times \text{GE of feed} \quad [3]$$

$$\text{DE (Mcal/d)} = \text{GEI} - \text{fecal energy} \quad [4]$$

$$\text{ME (Mcal/d)} = \text{DE} - \text{urinary energy} - \text{gaseous energy} \quad [5]$$

$$\text{NEL (Mcal/d)} = \text{ME} - \text{heat increment} \quad [6]$$

Bomb calorimeters are used to determine the energy of feed ingredients, feces, and urine. This is calculated by the increase in water temperature inside the bomb and multiplied by the heat capacity of the water to estimate the amount of heat produced (Figure 4, Blaxter, 1989).

Energy Losses. Calculation of energy balance uses the laws of thermodynamics (Brody, 1945). The first Law of thermodynamics states that energy cannot be created nor destroyed, meaning that the energy is conserved. Thus, the energy input of an animal must equal the energy output plus or minus any change in body energy (Weiss, 2007). When a cow consumes energy, a portion of that energy is lost through the feces, urine, gas, milk, and heat (Blaxter, 1989). The

second law of thermodynamics states that the entropy of the universe is always increasing, meaning that the transformation of energy is 100% efficient and inefficiencies are lost as heat (Weiss, 2007). Small amounts of energy occur at the body surface; however, it only accounts for <0.5% of the energy consumed and is typically ignored (Weiss, 2007). However, heat energy is one of the largest contributors to energy lost (Coppock, 1985). For example, a 600 kg cow producing 40 kg of 4% milk produces about 25.5% lactation energy, 31.1% heat energy, 2.8% urinary energy, 5.3% gaseous energy, and 35.3% fecal energy.

Heat energy, also known as heat increment (**H_iE**), is the increase in heat production associated with an increase in consumption of food (Moe, 1981). Heat increment contributes to one-third of the loss of ME (VandeHaar, 1998) and is the increase in heat production between a fasted animal and a fed animal in a thermoneutral environment that includes heat from digestion and absorption, product formation, fermentation, waste formation and excretion (Morris et al., 2020c). Heat increment is difficult to determine accurately because it cannot be measured directly. Heat increment is the difference between the maintenance and heat production of an animal and the heat production that is not attributed to net energy of maintenance (**NEm**). In other words, H_iE is described as the difference in heat production when dietary nutrients are metabolized compared to the heat production of nutrients metabolized by body stores (Weiss, 2007). Energetic efficiency, heat production, and H_iE are affected by production (Belyea and Adams, 1990), forage inclusion (Reynolds et al., 1991), and N intake in excess of requirements (Tyrrell et al., 1970, Reed et al., 2017). Dairy nutritionist and researchers want to decrease heat increment to partition more energy toward NEL, which could be used to increase milk production. This can be achieved by feeding adequate amounts of fat, concentrate, and protein (Coppock, 1985; Holter et al., 1970; Omphaluis et al., 2019a).

Effect of Protein on Energy Utilization. Protein and energy metabolism are inter-related. Providing high energy or protein increases milk and protein yield but has no energy and protein interaction (Omphaluis et al., 2019a). However, an increased milk protein yield was observed when both energy and MP increased from deficient to excess by 0.18 kg/d (Brun-Lafleur et al. 2010). This may have been due to using later lactation dairy cows rather than the early lactation dairy cows used by Omphaluis et al (2019a). Energy is required for the efficiency of converting AA into milk protein, and protein is needed by cows to efficiently convert GE into NEL. Protein influences energy utilization in three different ways: 1) dietary protein is associated with increased fiber digestibility, 2) increased dietary protein increases urinary energy loss, 3) increasing dietary protein increases heat increment.

Increasing the digestibility of feedstuffs within an animal often increases the concentration of DE. Supplying adequate RDP is important for ruminal NDF digestibility (Lee et al., 2012c). Rumen degradable protein degrades in the rumen and supplies N to the rumen microbes to promote growth. Increasing the growth of the fiber digesting microbes increases NDF digestibility. Lee et al. (2011) fed diets to dairy cows 200g/d deficient in RDP and observed a 20% reduction in total-tract apparent NDF digestibility compared to a diet that contained 27 g more RDP than required. Broderick et al. (2008) increased CP content of diets from 14.8 to 18.6% by increasing RDP from 10.0 to 12.3% and observed an increase of 7 % in NDF digestibility.

The conversion of DE to ME accounts for the loss of gas (methane) and urinary energy (Figure 5). Dietary protein is highly correlated to urinary energy loss, which affects the efficiency of converting DE to ME (Weiss, 2019). In other words, feeding excess protein increases the urinary energy loss. Excessive amounts of N may be toxic to the animal and is thus

excreted as urea in the urine. When excess N is not utilized by the animal it enters the urea cycle from transdeamination of Glu and transamination of Asp to form urea and this process requires energy. On average, one gram of urinary N is associated with approximately 14.3 kcals of energy. Feeding excess dietary CP will increase the urinary energy loss of the animal. Theoretically, two cows at a similar intake (22.7 kg/d) fed either a 15% or 17% CP diet, the CP intake of the 17% diet would increase by 0.45 kg compared to the 15% CP diet, which equals 73g of N. If milk protein yield remains constant, the 17% CP diet would excrete 51 g more of N in urine, which is a 0.73 Mcal increase in urinary energy (Table 1). Increasing CP intake increases urinary urea excretion (Spek et al., 2013) and feeding protein in excess of requirements has been associated with increased HP (Reed et al., 2017) and decreased energy balance (Tyrrell et al., 1970, Reed et al., 2017).

The conversion of NE from ME accounts for heat increment. Increased metabolic heat decreases the efficiency of converting ME to NEL because heat increment increases with changes in protein turnover. Unfortunately, increasing dietary CP also increases heat increment, reducing the efficiency of ME conversion to NEL (Reed et al., 2017). Energy required for the removal of N as urea produces a measure of heat. By using the above example, the 51g of urinary N excreted would equal a total of 0.8 Mcal/d of heat production (Table 5). If this cow produced 34.0 kg/d of milk and consumed DMI of 22.7 kg of feed each day it would produce on average 22 Mcal/d of heat. Additionally, the energetic efficiency of protein deposition is lower than fat deposition, and this is believed to be mostly due to the energy cost of protein turnover (Moe et al, 1981).

Effect of Feeding Energy and Protein on Nitrogen Efficiency. Dietary protein is the main factor determining milk N efficiency and N losses in dairy cows (Huhtanen and Histov,

2009) Lactating dairy cows have a low dietary N efficiency (milk N/ N intake) of about 40% that proposes a challenge to increase without a detrimental effect on milk protein yield. However, research has determined factors that can be manipulated to improve N efficiency by balancing diets for AA that may increase MP efficiency (Haque et al., 2015). Lee et al. (2012b) compared a diet formulated with adequate MP supply and inadequate MP supply but containing RP-Met and RP-Let. In this study cows consuming a diet deficient in MP containing RP-Lys and MP deficient diets with RP-Lys and RP-Met increased in N efficiency by 7.3 and 11.3%, respectively, compared to the adequate MP diet. It was suggested that this occurred because the efficiency of dietary AA utilization for milk protein synthesis decreases with the increasing AA supply due to increased oxidation rates in the splanchnic tissues and liver (Arriola Apelo et al., 2014).

Energy and protein supply may influence N efficiency. Increasing the NEL supply may increase MP efficiency (Hanigan et al., 1998) and increases in dietary CP may decrease N efficiency (Ruis et al., 2010a). Ruis et al. (2010a) evaluated the relationship between diets high in protein (6.6 vs 4.6 % RUP of DM; RDP constant at 10.1% of DM) and energy (1.54 vs 1.44 of NEL Mcal/kg) on whole animal N efficiency and determined high energy diets had a higher N efficiency than high protein diets (Table 2). This resulted in a 21.9 and 15.5% increase in milk and protein yield. Metabolizable energy intake often has a positive effect on the amount of milk N as expressed in proportion (%) to feces and urine N (Kebreab et al., 2010). Ruis et al., (2010a) suggest this is because the postaborptive N efficiency in lactating dairy cows has high variation, independently affecting N and energy supply. Also, that feeding less RUP than NRC (2001) recommendations in combination with high dietary energy concentration may be useful to increase the capture of N in milk protein (Ruis et al., 2010a). Omphaluis et al. (2019a) observed

an increase in N efficiency by 13% in high energy diets (32.5 vs 25.0 Mcal/d of NEL) and 24% decrease in high protein diets (2,254 vs 1.266 g/d of MP) and milk and protein yield increased for either diet by 8.7 and 12.3%, respectively. At the mammary gland, feeding a high energy diet increased mammary gland uptake of total-AA N because of the increase in mammary plasma flow, whereas the atrial-venous differences of the total-AA N and EAA N decreased (Omphaluis et al., 2019a). For the high protein diets, total-AA N uptake in the mammary gland increased with increased mammary gland atrial-venous concentration of total-AA N, which was driven by EAA and no changes in mammary plasma flow. Omphaluis et al. (2019b) observed similar results for N efficiency, milk and protein yield with starch and casein infuses into the abomasum of lactating dairy cows. However, not only did the mammary gland increase blood flow but the portal drained viscera and splanchnic tissues increased in high energy diets, which means supply of more AA's for protein synthesis. This could mean an increase in protein turnover. Infusion of AA's in Holstein steers resulted in an 8.0% increase in whole-body protein turnover compared to water infusion (Wessels et al., 1997). Protein synthesis increased by 9.9% and protein degradation was enhanced by 6.4%, meaning an increase in protein accretion occurred. Ruis et al. (2010b) observed an interesting result in milk protein when infusing casein and starch into the abomasum of dairy cattle. The infusion of starch increased milk protein yield and not casein because of mammary plasma flow; but also, observed an increased in phosphorylation of ribosomal protein S6 and endothelial nitric oxide synthase, which promote milk protein synthesis (Ruis et al. 2010b). There was an interaction between starch and casein infusion with increased milk protein yield; however, this was due to the phosphorylation of mTOR, which also promotes milk protein synthesis. Overall, assessing energy and protein supply will help with N efficiency.

University of New Hampshire energy studies. The world of energy research has had a plethora of animal nutritionist that have dedicated their lives to determine the energy requirements of animals and how energy is utilized within the animal. The names that come to mind when conducting energy metabolism research break throughs include Henry Armsby (Penn State University), Max Kleiber (University of California-Davis), Samuel Brody (University of Missouri), Kenneth Blaxter (Cambridge University), Paul Moe (US Department of Agriculture), and Henry Tyrrell (US Department of Agriculture). One site of energy research which is less frequently mentioned is the Ritzman Laboratory at the University of New Hampshire (Durham, NH). Ernest Ritzman, Francis Benedict, Nicholas Colovos, and James Holter have conducted successful and impactful energy research that have benefited the energy metabolism research community (Table 3).

Ernest Ritzman and Francis Benedict developed the Ritzman Laboratory at the University of New Hampshire. The focus of their research was to determine basal metabolism and fasting heat production in multiple species and to determine the effects of fasting heat production on behavior and environmental changes. In order to determine heat production of their animals they used indirect calorimetry respiration chambers. Benedict and Lee (1936) conducted an experiment on the basal heat production of mice ranging from 8 to 59 g. In this study the investigators determined that the basal heat production per unit of surface area of the fat mice was double that of the dwarf mice, indicating that heat production is affected by size of the animal. Ritzman extended this knowledge by testing the dogma at the time which stated that fasting heat production of mammals is proportional to heat loss by surface radiation, and that species with more hair covering or insulation would have a lower basal metabolism (Ritzman and Benedict, 1938). However, Ritzman argued that this was not true, because they observed that

the basal metabolism of sheep with high amounts of cover, had a higher basal metabolism than goats with lower cover of hair (32 cal/kg/d vs 21 cal/kg/d, respectively). In dairy cows, Ritzman and Benedict were interested in the fasting heat production at different environmental temperatures, physical behavior, and with feeding different feedstuffs. These studies were conducted using indirect calorimetry respiration chambers. These data concluded that basal metabolism varies by feedstuff, environment, behavior, and even varies within species.

Nicholas Colovos conducted studies to determine the nutrient value of different feedstuffs (Table 3). This research was based on similar methodology as that conducted at the dairy metabolism facility at the University of Nebraska-Lincoln today. Foth et al. (2015) conducted an energy balance study to determine the energy content of reduced-fat dried distillers grains with solubles with lactating Jersey cows using headbox-style calorimeters at the University of Nebraska-Lincoln. Also, determining whole-body energy balancing of feedstuffs like corn oil and calcium sulfate with the same headboxes (Judy et al., 2019). Although Colovos conducted most of his energy metabolism studied with dairy heifer of different breeds. In the studies of Colovos, feed, orts, feces, and urine were collected to determine the GE using a bomb calorimetry. An indirect calorimetry respiration chamber was used to collect gases to determine estimated heat production that was created by Benedict (Colovos et al., 1949). However, a Haldane apparatus using Carpenter modification was used to analysis the respiration chamber gas concentration of O_2, CO_2 and CH_4 that was also used by Benedict (Figure 5). The Carpenter modification improves the removal of O_2 from the apparatus by using potassium pyrogallate. This apparatus is used to measure the concentration of oxygen, carbon dioxide, and methane. Colovos conducted many nutritive value energy metabolism studies and these studies included wood molasses, cane molasse, urea and timothy grass hay.

James Holter conducted most of his energy metabolism work on fasting heat production and heat increment. In 1970, Holter determined the heat increment of acetate, propionate, and butyrate (40, 18, and 18 kcal/100 kcal metabolizable energy, respectively) by infusing 32 kcal/kg of body wt. $^{0.75}$ daily in the rumen of fistulated rumen cannulated dairy cows (Holter et al., 1970). He also determined that the fasting heat production was dependent upon milk production (Holter et al., 1976). In these studies, dairy cows were fasted for 4 days and heat production measurements were made 12 h after fasting using open-circuit indirect calorimetry respiration chambers. Cows were fed at maintenance before the fasting period. Holter also conducted research in predicting methane production in dairy cows (Table 3; Holter and Young, 1992).

Calorimetry

Calorimetry is the measurement of heat, but physiologically it is defined as the art of measuring the transfer of heat between an animal and the surrounding environment (Nienaber et al., 2009). Lavoisier was the first to use calorimetry to define "oxygen" using the combustion process. Johnson et al. (2003) noted three broad objectives in the study of nutritional energetics are to 1) establish relationships between gas exchange and heat production 2) to devise basis for evaluation of feed or foods that could be related to energy requirements and energy expenditure and 3) to establish causes of variation within energy expenditure. There are two general methods in determining heat production: direct and indirect calorimetry. Both methods have been accepted and are accurate in determining heat production.

Direct calorimetry. Direct calorimetry measures the sensible and evaporative heat loss of the animal (Nienaber et al., 2009). Lavoisier and Laplace were the first to use direct calorimetry by confining a guinea pig in a chamber which contained ice. While doing so they estimated the heat production by measuring the amount of ice which melted (Brody, 1945). They

observed that the melting of the ice was correlated to the exhalation of the amount of carbon dioxide (Brody, 1945). Typically, direct calorimetry with dairy cattle has not often been used because of the high cost of respiratory chambers. In 1902, the first experiment with an animal was conducted in the Armsby Respiratory Calorimeter at Penn State University but was retired in 1960 because it was very complex and labor intensive to operate and new technologies were developed (Reynolds 2000; Nienaber et al., 2009). A majority of the direct calorimetry work has been done in poultry or small ruminants.

Indirect calorimetry. Indirect calorimetry measures the heat production of the animal (Nienaber et al., 2009). Indirect calorimetry estimates heat production by measuring oxygen consumption, carbon dioxide, methane, and urea production (Foth et al, 2015). In the 1940's and 1950's coefficients were derived from the complete oxidation of carbohydrate, protein, and fat using data from the 19th and 20th century (Gerrits and Labussière, 2015). Estimation of heat production is justified by the Law of Hess, which states that the total enthalpy change during a chemical reaction is the same regardless of the number of steps. This implies that it does not make a difference whether the substrate is completely oxidized, or whether intermediate products are produced from being transformed or oxidized at a later stage (Gerrits and Labussière, 2015).

In 1958, Brouwer developed a formula that is widely used to calculate heat production by livestock and integrates measurements of indirect calorimetry (Brouwer, 1965):

$$HP, kcal/d = 3.866 \times O_2\,(L/d) + 1.200 \times CO_2\,(L/d) - 0.518 \times CH_4\,(L/d) - 1.431 \times N\,(g/d) \quad [7]$$

This formula was based on measurements of oxygen consumption (L), carbon dioxide production (L), and heat produced by the combustion of 1 g of fat, carbohydrates, and protein.

The ratio of carbon dioxide produced to oxygen consumed is commonly known a respiratory quotient (RQ) and can be used as a gross predictor of the body substance being oxidized (Nienaber et al., 2009). In general, the oxidation of lipids, protein, and carbohydrates results in an RQ of 0.71, 0.81, and 1.00, respectively, whereas an RQ greater than 1 is associated with lipid synthesis (Blaxter, 1989). Morris et al. (2020c) fed high starch (30.8 % of DM and 1.9% fatty acids) and high fat diets (16.8% starch of DM and 4.1% fatty acids) and observed an increase in RQ when feeding high starch diets, suggesting that lipid synthesis was greater in high starch diets. Nicholas et al. (2019) abdominally infused glucose and isoenergetic palm olein (primarily palmitic, oleic, and linoleic acid) that increased RQ and decreased RQ, respectively. Measuring RQ allows researchers to have an idea of what metabolic substrate is being utilized by the animal for energy and energy utilization can be predicted more accurately when using indirect calorimetry.

Previous research has shown that indirect calorimetry is comparable to direct calorimetry. In 1967, research at the Rowett Research Institute employed both methods with sheep and the estimated of heat production was ± 5% of each another (Gerrits and Labussière, 2015). Indirect calorimetry has the advantage of being more versatile allowing environment modification, easily investigative changes, and could be less costly. (Nienaber et al., 2009).

There are two types of indirect calorimetry systems namely closed or open circuit. In a closed-circuit respiration chamber, the system is airtight, which is maintained with circulated air through scrubbers for removing carbon dioxide and water while oxygen is being introduced into the system. Oxygen use is based on the required input, while carbon dioxide production is obtained from the change in weight (Reynolds 2000). This system is not recommended for the use of large animals and for accurate methane production (Reynolds 2000). Typically, an open-

circuit system is used for large animals like dairy cows. In an open-circuit system, respiratory exchange is based on the concentration of gases entering and leaving the system, which is then multiplied by the flow rate through the system after correcting for temperature, humidity and pressure (Reynolds 2000). An example of an open-circuit system are headbox-style indirect calorimeters.

Headbox-style indirect calorimeters have been used to estimate heat production by collecting a volume of oxygen consumed, carbon dioxide and methane produced while the animal is in the headbox. Urine is also collected from the animal to determine nitrogen content to calculate heat production using the Brouwer (1965) equation. The headbox is equipped with feed and water and is large enough that the animal can move freely. This technique can be less expensive to construct than a whole-animal chamber given that the headbox is only surrounding the head versus the whole body (Johnson and Johnson, 1995). Headbox-style indirect calorimeters are an advantage when doing energy utilization research on lactating dairy cows because the cows can be milk while in the headbox. A disadvantage is that they do not account for the hindgut fermentation losses of gas, which is approximately less than 2%. However, 89% of the hindgut methane is absorbed in the blood and expired through the lungs, which is collected (Boadi and Wittenberg, 2002).

SUMMARY OF LITERATURE REVIEW

Protein is an important nutrient for lactating dairy cows. Dietary CP is divided into RDP and RUP, where RDP is used to support rumen microbial growth to maximize synthesis of MCP. In contrast, RUP directly supplies protein with additional AA's for the animal. Microbial CP, RUP, and ECP contribute to the passage of MP to provide EAA's (especially common limiting AA of Lys, Met, and His) available for digestion and absorption in the small intestine of the cow. These EAA are important in supporting milk and protein yield. Currently, the MP efficiency of converting MP to net protein is 67% (NRC, 2001). However, it has been suggested that this MP efficiency can easily overestimate or underestimate the requirement for MP when based on production and stage of lactation. The differences in these efficiencies has sparked interest in removing the MP system (Arriola Apelo et al., 2014) and shifting to an individual AA system to predicted protein requirements, this path however has challenges and limitations. One challenge is that not all requirements have been determined for individual AA's and total supply is difficult to measure due to recycling of N from splanchnic and peripheral tissues. However, using individual AA balancing may increase the N efficiency with increase milk protein yield (Haque et al., 2015).

Animal protein byproducts are byproducts created by the rendering industry such as BM and HFM, these are easily available high RUP sources and commonly used in the dairy industry. Blood meal is blood removed from animal carcasses that has been heated at high temperatures to remove the water. Blood meal contains high concentrations of Lys and His that can be utilized by the animal for milk protein synthesis, and these are two of the three most commonly limiting AA. However, HFM has a poor AA composition because of the hydrolyzing process to increase digestibility. Hydrolyzed feather meal is low in Lys and His, which have been shown to reduce

milk protein yield (Harris et al., 1992). The rendering industry does produce HFM that contains some blood, improving the AA composition by increasing Lys and His concentration. This additional Lys and His may potentially improve milk protein yield. However, because of differences in rendering processing methods at rendering plants the chemical and AA composition of these animal protein byproducts may be associated with high variation (Cotanch et al., 2020).

Energy is also an important nutrient for lactating dairy cows. Researchers have partitioned energy into GE, DE, ME, and NE. However, energy is lost through urine, gas, feces, milk and heat. Protein influences energy utilization in three different ways: 1) dietary RDP is needed to support fiber digestibility, 2) increased dietary protein increase urinary energy loss, 3) increasing dietary protein increases heat increment (Weiss, 2019). Rumen degradable protein increases microbial growth in the rumen, which increases fiber digestion. Excess protein utilized by the animal is excreted as urea, which is an energy cost. Energy required for the removal of N as urea produces a measure of heat, which increases the heat increment of the animal. Also, feeding energy and protein influences N efficiency (Ruis et al. 2010). This can be achieved by supplying adequate AA or supplying higher energy than protein. Both situations have been shown to result in an increase in milk and protein yield due to increased N efficiencies.

Calorimetry is a method used to measure heat and there are two methods used to measure heat production in cattle namely, direct and indirect calorimetry. Direct calorimetry measures the sensible and evaporative heat loss of the animal, whereas indirect calorimetry measures the heat production of the animal by measuring O_2 consumption, CO_2, CH_4, and urea production. Open circuit indirect calorimetry is the most common method to measure heat production in lactating dairy cattle. Headbox-style indirect calorimeters are an advantage when conducting energy

utilization research on lactating dairy cows because the cows can eat and be milked while in the headbox. A disadvantage is that they do not account for the hindgut fermentation losses of gas. However, 89% of the hindgut methane is absorbed in the blood and expired through the lungs, which is collected. This results in an accurate measure for heat production (Boadi and Wittenberg, 2002).

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TABLES AND FIGURES

Table 1.1. Example of how an increase in CP concentration could affect urinary N values when dry matter intake was 22.4 kg/d with no difference diets[1,2]

	15% CP	17% CP
CP intake, kg/d	3.40	3.86
CP-GE intake, Mcal/d	19.3	21.8
CP-DE intake, Mcal/d	12.5	14.2
Change in urinary N, g/day	0	51
Change in urinary energy N, Mcal/d	0	0.73
Change in urinary energy N lost as heat energy, Mcal/d	0	0.8

[1] Assumed apparent digestibility of CP, starch, and NDF as 65, 92, and 48% and no negative or positive associative effects were applied. The energy content of CP was assumed to be 1.67 Mcal/kg and 0.87 Mcal/kg for starch and NDF, respectively.

[2] Table was adapted from Weiss, 2019.

Table 1.2. Nitrogen utilization in cows fed varying amounts of energy and protein[1]

	Experimental diets[2]					*P* value[3]		
Items	HE/HP	HE/LP	LE/HP	LE/LP	SEM	E	P	E × P
N supplied in MP, g/d	470	372	468	364	14	0.71	< 0.01	0.84
Milk protein N, g/d	176	160	143	136	8	< 0.01	0.19	0.63
Predicted urinary N, g/d[4]	292	216	338	202	11	0.20	< 0.01	0.01
N efficiency, %[5]	37.1	43.0	31.0	38.5	1.4	< 0.01	< 0.01	0.53

[1] Adapted from Ruis et al., 2010a.

[2] HE and LE = high energy and low energy, respectively; HP and LP = high protein and low protein, respectively.

[3] E = energy; P = protein, and E ×P = energy and protein interaction.

[4] Estimated urine N output= 0.026 × MUN (mg/dL) × BW (kg); Kauffman and St-Pierre (2001).

[5] N efficiency % = 100 × milk N (g/d) / N supplied in MP (g/d).

Table 1.3. Energy metabolism research from the University of New Hampshire-Durham.

Name	References
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J.B. Holter	J.B Holter. 1976. Fasting heat production in “lactating” versus dry dairy cows. J. Dairy Sci. 59:755-759.
J. B. Holter	Holter, J. B., C.W. Heald, N.F. Colovos. 1970. Heat increments of steam-volatile fatty acids infused separately and in a mixture into fasting cows. J. Dairy Sci. 53:1241-1247.
J. B. Holter	Holter, J. B. and A. J. Young. 1992. Methane prediction in dry and lactating Holstein cows. J. Dairy Sci. 75:2165-2175.

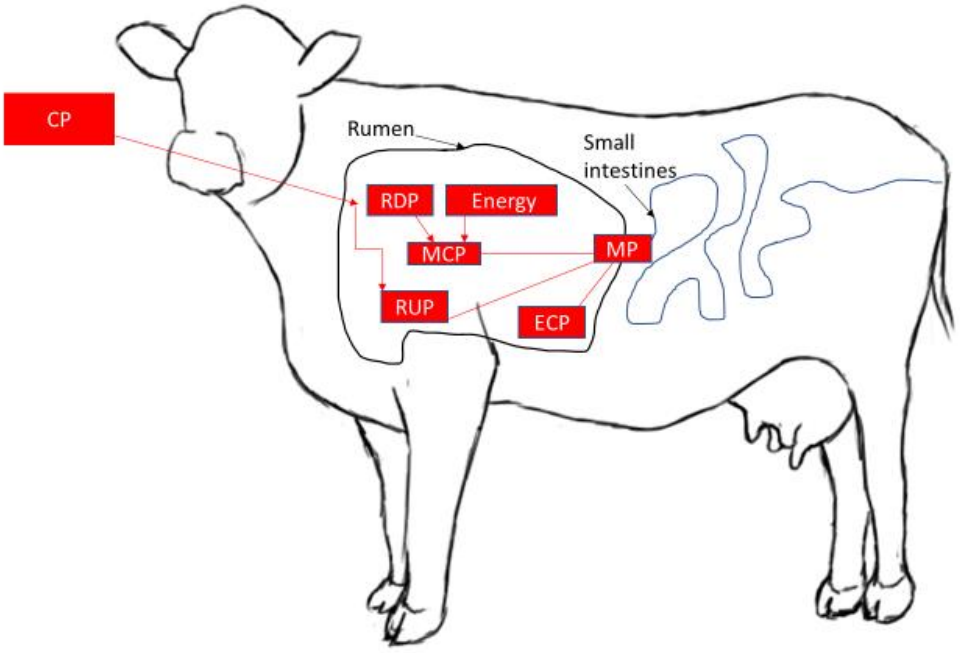

Figure 1.1. Diagram of the metabolizable protein system. Crude protein (CP) divide in two fractions in the rumen: rumen degradable protein (RDP) and rumen undegradable protein (RUP). RDP and energy increase growth of microbe, creating microbe CP (MCP). Endogenous CP (ECP) is protein available from sloughed rumen cells. MCP, RUP, and ECP are available as metabolizable protein (MP).

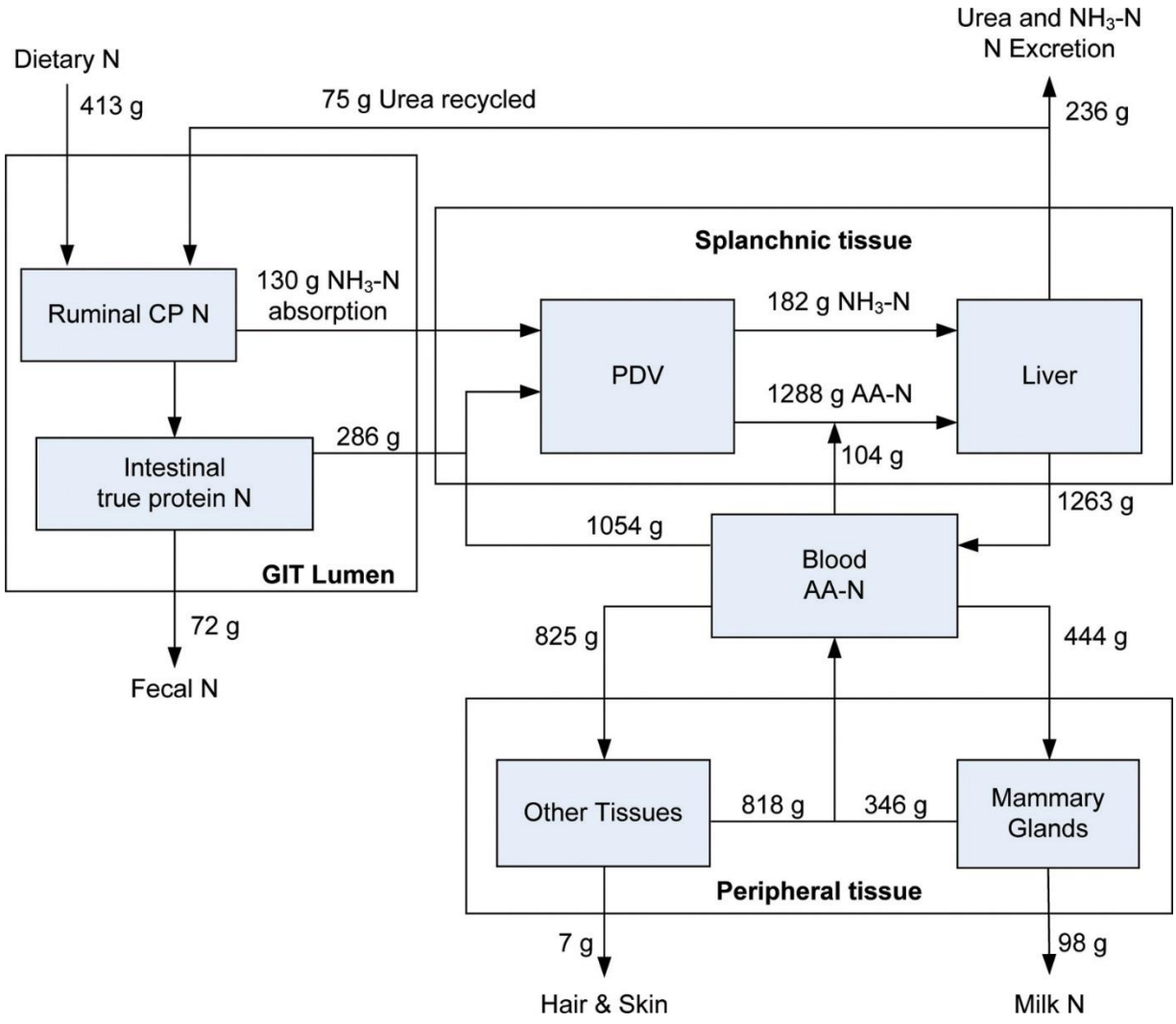

Figure 1.2. Nitrogen flux diagram acquired from Arriola Apelo et al. (2014) feeding 413 g of dietary N. Solid boxes represent pools, open boxes represent compartments, and numbers indicate fluxes (g of N/d). GIT=gastrointestinal tract; PDV=portal-drained viscera. Intestinal AA flux was obtained from NRC (2001). Urine and fecal N excretion, and other tissue N losses were calculated by difference.

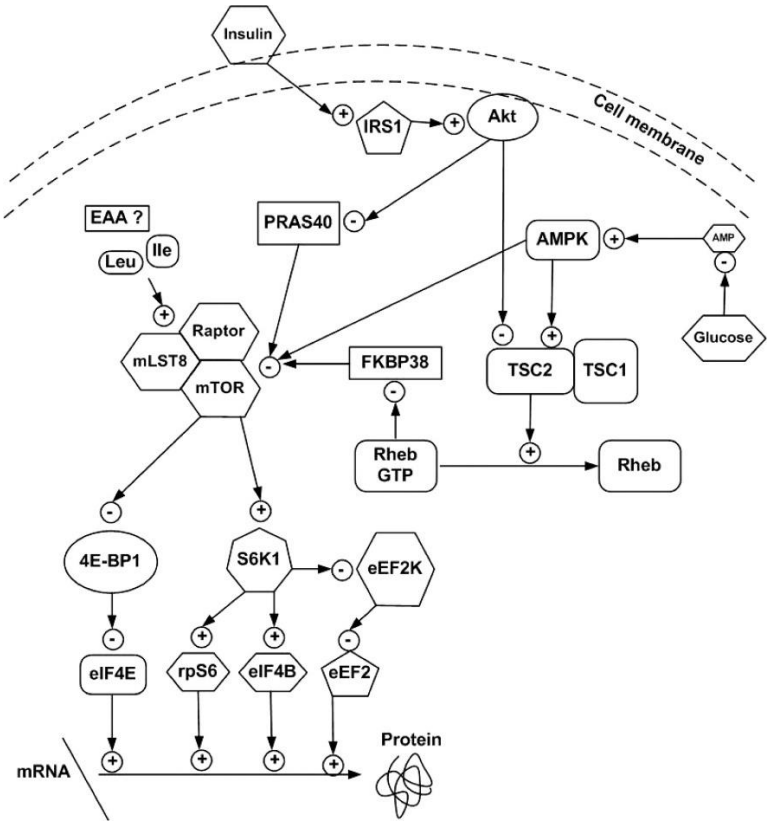

Figure 1.3. Acquired by Arriola Apelo et al., 2014. Mammalian target of rapamycin complex 1 (mTORC1) translation regulation pathway. Depicted are the effects of insulin, AMP, and identified AA, as well as other potential essential AA, on mTORC1 pathway. Several effects of the mTORC1 downstream protein on translation regulation are also represented (Bellacosa et al., 1998; Hardie, 2004; Mahoney et al., 2009). 4EBP1=eukaryotic initiation factor 4 E binding protein 1; AMPK=AMP kinase; Akt=protein kinase B; eEF2=eukaryotic elongation factor 2; eEF2K=eukaryotic elongation factor 2 kinase; eIF4E=eukaryotic initiation factor 4 E; FKBP38=FK506 binding protein 38; IRS1=insulin receptor substrate 1; mLST8=mammalian lethal with SEC3 protein 8; PRAS40=proline-rich Akt substrate 40 kDa; Rheb=Ras homolog enriched in brain; rpS6=ribosomal protein S6; TSC=tuberous sclerosis complex; S6K1=ribosomal protein S6 kinase 1. Stimulatory effects are designated by + and inhibitory effects by −.

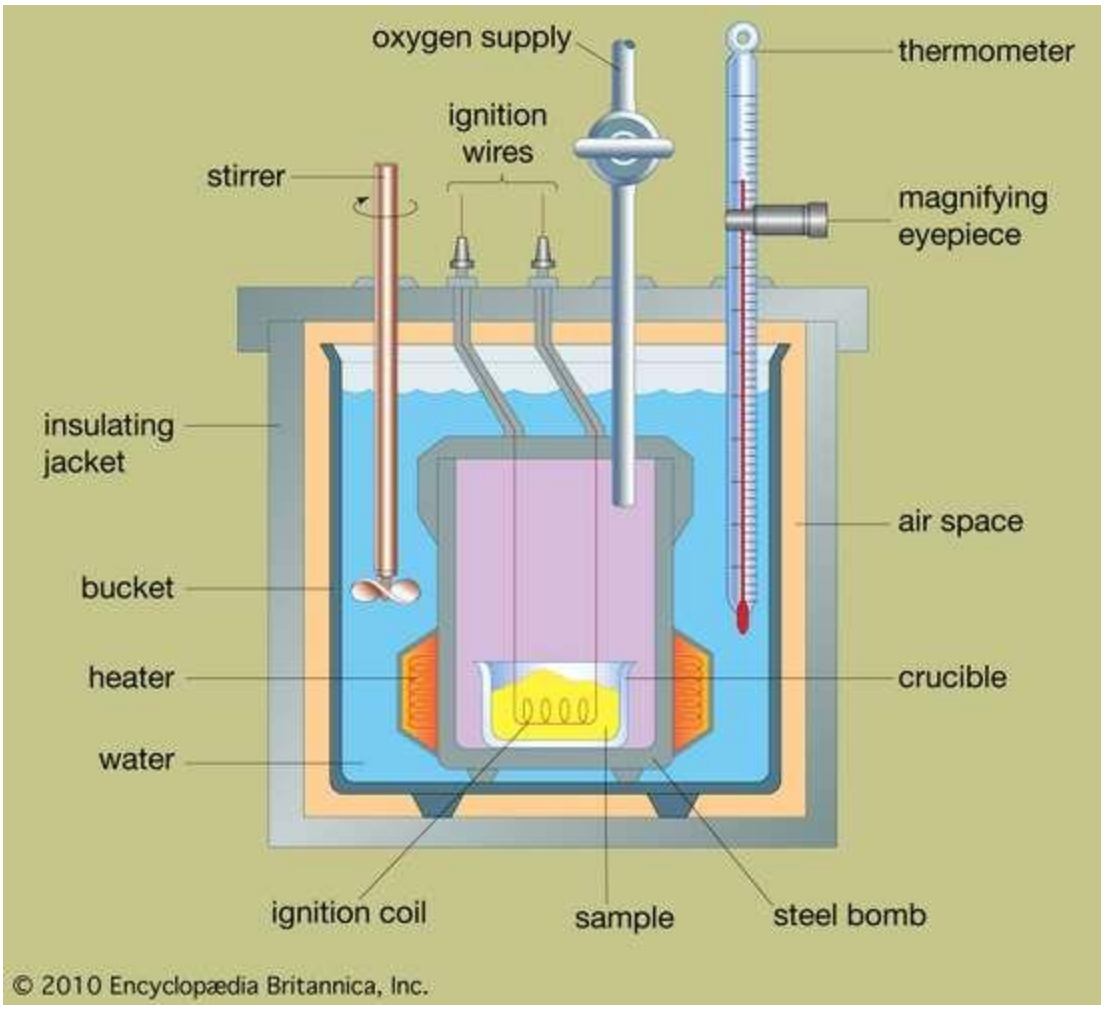

Figure 1.4. Diagram of a bomb calorimeter acquired by Encyclopaedia Britannica (2010).

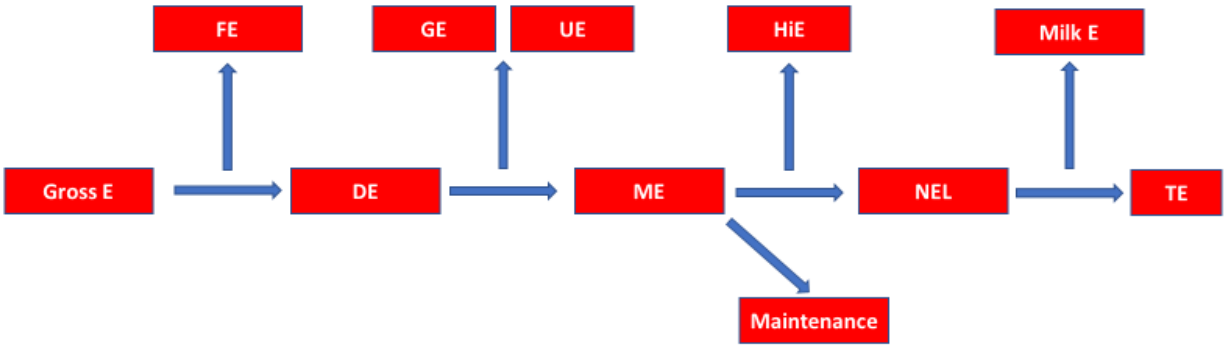

Figure 1.5. Energy balance diagram. Gross E = gross energy, FE = fecal energy, DE = digestible energy, GE = gaseous energy, UE = urinary energy, ME = metabolizable energy, HiE = heat increment, Maintenance = maintenance energy, NEL = net energy of lactation, Milk E = milk energy, and TE = tissue energy.

CHAPTER 2

Use of a mass flow meter in headbox-style indirect calorimetry, and the effects of gas recovery on estimated energy partition in lactating dairy cows

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ABSTRACT

Headbox-style indirect calorimeters can robustly estimate heat production (**HP**) of cattle. Volumetric flow meters (**VFM**), originally designed to measure gas flow in residential homes, have proven to be a cost effective method to measure airflow. Effective operation of these meters requires frequent maintenance and recalibration. Additionally, the rate of airflow of a lactating dairy cow is outside of the meters designed range. The objectives of this study were to test mass flow meters (**MFM**) and VFM by measuring O_2 consumption and CO_2 production and to quantify the effects of incomplete gas recovery on estimated energy partitioning. Two headboxes were initially equipped with VFM and later replaced with MFM. To determine the effects of type of airflow meter on O_2 consumption and CO_2 production, ethanol (100%) was burned for 2 h. Efficiency was calculated as the proportion of O_2 and CO_2 recovered from the amount of alcohol burned. As airflow was estimated by both methods, a subsample of gas was collected into a bag. Air in each bag was analyzed using gas chromatography. Data were analyzed using a paired t-test. Recovery of O_2 was greater for MFM than VFM (100.0 ± 1.44% vs. 86.7 ± 1.44%, P = 0.01). Recovery of CO_2 was greater ($P < 0.01$) when using the MFM than VFM (98.1 ± 2.58 % vs. 85.7 ± 2.58 %). These results suggest that MFM may yield more precise measures needed for indirect calorimetry. Incomplete gas recovery can result in underestimates of HP, thereby affecting estimates of whole-animal energy use. For example, for a typical Jersey cow (assuming body weight = 450 kg, dry matter intake = 18.5 kg/d, milk yield = 25.0 kg/d, urinary N excretion = 225 g/d and tissue energy = 0.00 Mcal/d) a 5.0 % decrease in gas recovery results in a reduction in HP by 1.30 Mcal/d and increase in tissue energy by 1.45 Mcal/d. This tissue energy translates into an increased net energy of 1.46 Mcal/d. Our results indicate that in striving

for estimates of gas recovery of 95.0 ± 5.00 %, MFM may be better suited for headbox-style indirect calorimetry to estimate HP in lactating cows.

Keywords: energy partition, gas recovery, indirect calorimetry

INTRODUCTION

Indirect calorimetry is a tool to estimate heat production and today's use of this tool is usually based on the assumptions of the Brouwer (1965) equation. In indirect calorimetry oxygen consumption, carbon dioxide, methane, and urea production are used to indirectly estimate body heat production. Although there is an array of devices used to make these physiological measures, headbox-style indirect calorimeters are common practice because they are relatively simple to use and operate (Foth et al., 2015). According to the Brouwer equation once the physiological measure is taken, heat production can be estimated with the following equation: $3.866 \times O_2$ consumption + $1.2 \times CO_2$ production -$0.518 \times CH_4$ production -$1.431 \times$ urinary N excretion.

In previous research, headbox-style indirect calorimeters equipped with volumetric flow meters (**VFM**) were used to determine flow rate and estimated gas recovery (Foth et al., 2015; Morris et al, 2020). This method has proven to be a cost effective method to measure airflow. The VFM used in these studies were designed to measure an operational flow rate of approximately 200 L/m; however, for dairy cattle the flow rate usually ranges between 850 and 1200 L/m. While these VFM can been operated adequately for a short periods of time opportunities to improve analytical estimates should be sought. Additionally, new methods may be advantageous if they require less maintenance and recalibration. Mass flow meters (**MFM**) are a style of gas meters that calculate the mass rather than volume of airflow and have been previously used in measuring gas production of group-house chickens (Xin and Harmon, 1996). Mass flow meters require fewer operational measures because they automatically adjust measures by accounting for changes in barometric pressure and temperature. The objectives of this study were to firstly, evaluate new MFM and compare them to the VFM by conducting a

series of procedures in which the recovery of gasses are estimated in a controlled environment and secondly to quantify and discuss the effects of incomplete gas recovery on estimated energy partitioning. We hypothesize that gas recovery will improve with the use of the MFM resulting in less error in estimated energy partitioning in lactating dairy cows.

MATERIALS AND METHODS

Headbox-style indirect calorimeters (n=3) were equipped with VFM (Model AL425, American Meter, Horsham, PA, Figure 1) and later replaced with an MFM (MCW Whisper, Alicat Scientific, Tucson, AZ). The effects of the type of airflow meter on oxygen consumption and carbon dioxide was determined by burning 100% ethyl alcohol for two hours, which we refer to as a "lamp run." During a Lamp Run (2 per headbox), we place three lamps (Eisco TM Alcohol Lamp, Eisco, Ambala, India) containing about 50 ± 10g of 100% ethyl alcohol and placed in an 10" × 18" tray on top a half full, 19 L bucket containing ice, inside the headbox. Headboxes were operated for two h while the lamps burned. Temperature and dew point inside the headbox were measured every minute during the two h collection interval using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) and recorded using a data logger (Model XR440, Pace Scientific Inc.). Line pressure was measured using a u-tube manometer (Item # 1221–8, Park Supply of America, Inc., Minneapolis, MN) and barometric pressure of the room was measured using a barometer (Chaney Instruments Co., Lake Geneva, WI). When using the VFM the total volume of gas flow through the headbox was measured and corrected to standard temperature and pressure (0 ºC, 760 mmHg) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). No correction was needed when using MFM and data logger recorded the flow rate of gas. Continuous samples of incoming and outgoing air from the headbox were collected into separate bags (44 L, LAM-JAPCON-NSE; Pollution Measurement

Corp., Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate "50," Brooks Instruments, Hatfield, PA). Gas bags were analyzed for O_2, CO_2 and CH_4 using an Emerson X-stream 3-channel analyzer (Solon, OH) according to the method of Nienaber and Maddy (1985).

After two h, the flame was extinguished and the headbox continued to run for 10 min so that the airflow meter would recover any remaining O_2 and CO_2. Then, lamps were removed and weighed, and final dial reading on VFM was recorded. Flow rate of the VFM was determined by the difference in final and initial dial readings. The difference in final and initial lamp weight was equivalent to the amount of ethyl alcohol burned. The efficiency of each headbox to measure gas flow was calculated as the proportion of oxygen and carbon dioxide recovered. The respiratory quotient (**RQ**) was calculated using the ratio of CO_2 produced to O_2 consumed. This number is used because the conversion of CO_2 produced to O_2 consumed from the combustion of ethanol is 0.66 (Gerrits and Labussière, 2015) thus a successful Lamp run was considered with a RQ 0.66 ± 0.01.

In order to determine the differences in measured flow rate between VFM and MFM flow rate was determined simultaneously at 5 different flow rates. Flow rates were adjusted by increasing voltages from 20, 30, 40, 50 and 60 volts on each headbox ($n = 2$). At each flow rate, initial and final dial readings for the VFM were recorded and flow rate was recorded on the data logger for the MFM with a duration of 10 minutes ($n = 2$). Barometric pressure, dew point and temperature were used the same as gas recovery measures for the corresponding airflow meter.

Data was analyzed using a paired t-test of SAS and comparisons were made between the gas recoveries of the VFM and MFM. Additionally, the data were analyzed using a paired t-test of SAS to determine the difference from 100% gas recovery from each meter and its corresponding percent gas recovery. Regression of MFM and VFM flow rate in liters per minute

versus a slope of one and an intercept of zero were analyzed using proc reg of SAS. Significance was declared at $P \leq 0.05$ and trends were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Gas recovery is presented in Table 1. After each lamp run was completed, we observed that the MFM had a higher O_2 recovery than the VFM (100.0 vs 86.7 ± 1.44 %, $P = 0.01$) and a higher CO_2 recovery (98.1 vs 85.7 ± 2.58 %, $P < 0.01$). This was a 13.3% and 12.4% unit difference in O_2 and CO_2 recovery, O_2 and CO_2 recovery for the VFM was significantly different from 100% gas recovery ($P < 0.05$). Mass flow meters may yield more precise measures of estimated heat production with increase of gas recovery. Previous research at the University of Nebraska-Lincoln Dairy Research Center using headbox-style indirect calorimeters with VFM have observed O_2 and CO_2 recoveries of 101.0 % and 100.8%, respectively (Morris et al., 2020a) and 93.7% and 91.4 %, respectively (Morris et al., 2020b). Overtime, it is possible that gas recoveries are reduced (Figure 3).

The differences in the measured flow rates between the VFM and MFM flow rate were determined simultaneously at five different flow rates and evaluated with a linear regression (Figure 3). The slope versus 1 and the intercept versus 0 of the linear regression models indicates that there were differences in flow rates between the VFM and MFM ($P = 0.01$ and $P = 0.02$, respectively). At an operational flow (600 L/min for lactating Jerseys), the VFM was 98.7% the flow rate of the MFM (Figure 3). Typically, when an energy utilization study is conducted with headbox-style indirect calorimeters the cow is inside the headbox for 23 h. A 1.3% reduction in flow rate of the VFM, leads to a lower computed heat production (Table 2). This reduction in flow rate of the VFM may be due to a wearing of the functional parts and perhaps because it is at conditions beyond capabilities.

Because we observed the VFM to have a reduced flow rate and gas recoveries, we further computed how this could affect estimated energy partitioning on an average lactating Jersey cow. The following assumptions were made for this animal; BW = 450 kg, DMI = 18.5 kg/d, milk yield = 25 kg/d, milk fat = 5.70%, milk protein = 3.60%, milk lactose = 4.85%. We suggest that incomplete gas recovery can result in a troublesome underestimate of heat production (Table 2) as speculated by Gerrits et al. (2018). This affects important estimates of whole–animal energy use as a 5.0% decrease in gas recovery results in a reduction in heat production by 1.30 Mcal/d and increases tissue energy by 1.45 Mcal/d, resulting in an increased net energy intake of 1.46 Mcal/d (Table 2). Metabolizable energy intake slightly increased due to the reduction in methane energy of 0.19 Mcal/d. Gardiner et al. (2015) determined that there were three potential sources of error in collection gases to estimate heat production: 1) analyzer error, 2) ducting efficiency from the chambers to analyzer including measurements of airflow, and 3) chamber mixing. Ducting includes the measurement of airflow, which was the largest source of error of gas recoveries. The respiration chambers used by Gardiner et al. (2015) varied between 59 and 115%, affecting energy partitioning. Nineteen publications in Journal of Dairy Science from volumes 99 and 100 failed to report gas recoveries (Gerrits et al., 2018). Hammond et al. (2016) stated that regardless of the method used, gas recovery tests are required for method development and should be reported with routine operation. Our data illustrates the importance of precise and accurate gas recovery to estimate heat production as an underestimate of heat production could lead to the erroneous conclusion that this energy is accounted for in tissue energy. Overall, either flow meter type can be used in energy utilization studies and although VFM's can be used with confidence (Morris et al. 2020a) we suggest that the MFM is more precise and superior. Gas flow is measured in the VFM by mechanical movement from air

flowing through the meter which in turn, rotates a crankshaft. We believe that overtime this crankshaft weakens resulting in an imprecise measure of airflow. In comparison, the MFM employs an electronic sensor to indicate airflow. This allows for more accurate readings of airflow and may improve gas recovery. Maintenance (recalibration) is still required for the MFM but less frequent (annually) than VFM. Calibration is recommended for long term energy utilization studies. Also, the MFM offers an advantage of having easy to record voltage output, a built in temperature, and pressure compensation, which makes it simpler and easier to use than the VFM.

CONCLUSION

In general, we strive for gas recoveries of 95-105% and lamp runs are conducted prior to using the headboxes experimentally. Results of this study suggest that the MFM are superior for use in headbox-style calorimetry to estimate heat production of lactating dairy cows. Additionally, the MFM offers the advantage of having an easy to record voltage output, a built in temperature, and pressure compensation.

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TABLES AND FIGURES

Table 2.1 Effect of oxygen and carbon dioxide recovery (%) using volumetric and mass flow meters when burning ethanol for 2 hours in indirect calorimetry headboxes

	Flow meter[1]		SEM	*P*-value
Items	VFM	MFM		
O_2 recovery, %	86.7*	100.0	1.44	0.01
CO_2 recovery, %	85.7*	98.1	2.58	<0.01

* Difference from 100 % gas recovery ($P < 0.05$).

[1] VFM = Volumetric flow meter (Model AL425, American Meter, Horsham, PA), MFM = Mass flow meter (MCW Whisper, Alicat Scientific, Tucson, AZ).

Table 2.2. The effects of gas recovery on estimated energy partition in lactating Jersey cows[1]

	Gas recovery, %						
Items[2]	100	95	90	85	80	75	70
Gases							
O_2 consumption, L/d	5000	4750	4500	4250	4000	3750	3500
CO_2 production, L/d	5250	4988	4725	4463	4200	3938	3675
CH_4 production, L/d	400	380	360	340	320	300	280
Energy[3]							
ME intake, Mcal/d	48.1	48.3	48.5	48.6	48.8	49.0	49.2
NE_l intake, Mcal/d	32.7	34.2	35.7	37.1	38.6	40.0	41.5
NE_l /ME	0.68	0.71	0.74	0.76	0.79	0.82	0.84
Energy fractions							
Methane, Mcal/d[4]	3.78	3.59	3.40	3.21	3.02	2.84	2.65
Heat production, Mcal/d[5]	25.1	23.8	22.6	21.3	20.0	18.7	17.5
Recovered energy, Mcal/d[6]	23.0	24.4	25.9	27.4	28.8	30.3	31.7
Tissue energy, Mcal/d[7]	0.00	1.45	2.91	4.37	5.83	7.29	8.75
Tissue/GE	0.00	1.79	3.58	5.37	7.17	8.96	10.8
Tissue/ NE_l	0.00	4.44	8.90	13.4	17.8	22.3	26.7

[1] Jersey cow, Production - BW = 450 kg, DMI = 18.5 kg, milk yield = 25 kg/d, milk fat % = 5.70, milk protein % = 3.60, milk lactose % = 4.85, urinary nitrogen = 225 g/d, Energy - gross energy intake = 81.4 Mcal/d, digestible energy intake = 55.4 Mcal/d, Energy fractions – fecal energy = 26.0 Mcal/d, urine energy = 3.5 Mcal/d, milk energy = 23.0 Mcal/d, maintenance energy = 9.77 Mcal/d.

[2] ME = metabolizable energy, NE_l = net energy of lactation, GE = gross energy.

[3] NE_l intake = milk energy + tissue energy + maintenance energy.

[4] Methane = CH_4 X 0.00945.

[5] Brower equation. Heat production = 3.866 × O_2 consumption + 1.2 ×CO_2 production - 0.518 ×CH_4 production - 1.431 × urinary N excretion of urea.

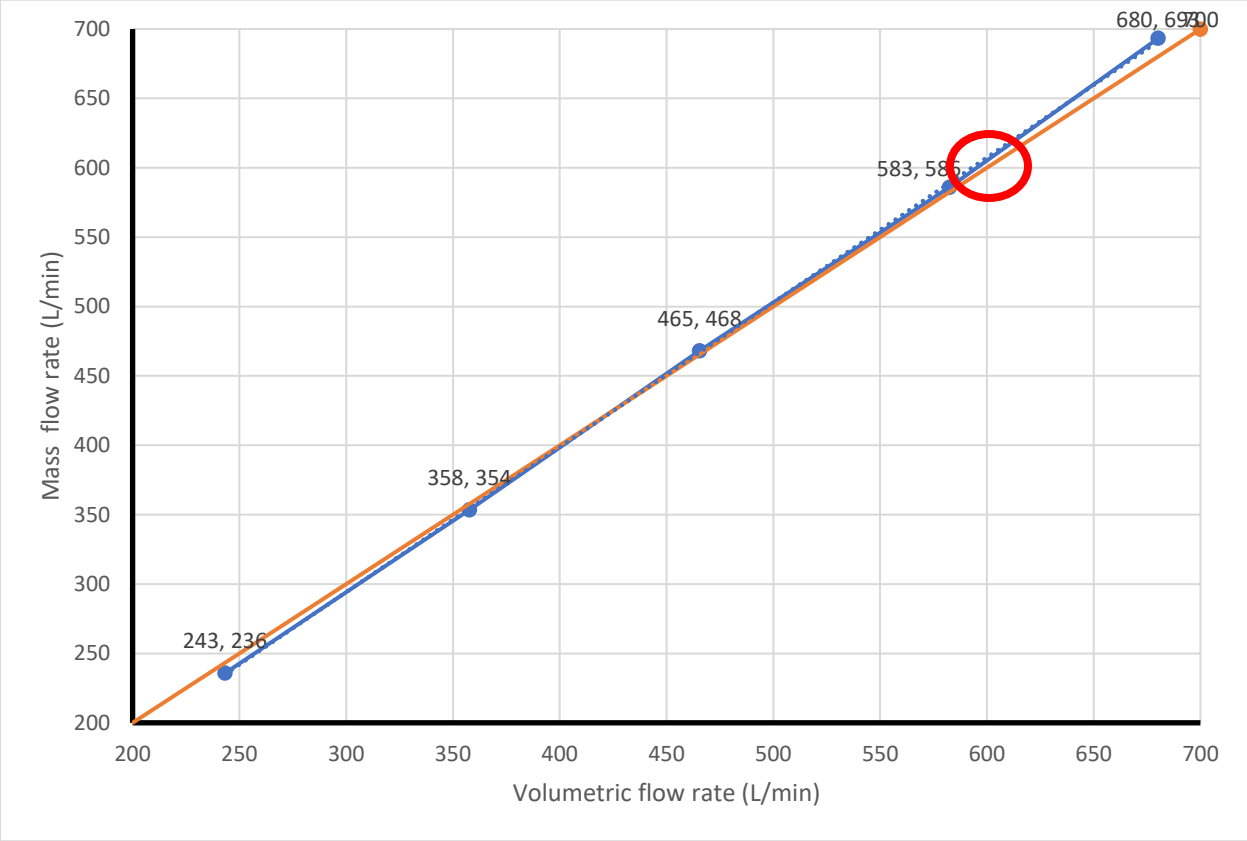
[6] Recovered energy = metabolizable energy – heat production.

[7] Tissue energy = recovered energy – milk energy.

Figure 2.1. Picture of the volumetric flow meter.

Figure 2.2 Picture of the mass flow meter.

Figure 2.3 Regression of MFM flow rate on the VFM flow rate in liters per minute. y = 1.05x ± 7.53 – 22.4 ± 0.02, Slope vs. 1: *P* = 0.01, Intercept. vs. 0: *P* = 0.02.



CHAPTER 3

Effect of feeding hydrolyzed feather meal with or without blood with rumen protected lysine on milk protein and energy utilization in late lactation dairy cows

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ABSTRACT

Hydrolyzed feather meal (**HFM**) is a feed by-product that is high in bypass protein; however, in some studies when HFM is fed to lactating dairy cows a negative effect on milk protein is observed. It is possible that this response is due to a shortage of Lys. The objective of this study was to determine the effects of feeding HFM while adjusting the concentration of Lys. In this study, 12 multiparous Jersey cows were enrolled in a triplicated 4 x 4 Latin square with 4 periods for 28 d. Cows were fed two TMRs that differed by source of HFM. The HFM was included at 4.5% of the diet DM, and one source contained blood while the other did not. Cows were randomly assigned to one of 4 treatments: HFM without blood and no rumen protected Lys (**RP-Lys**), HFM with blood and no RP-Lys, HFM without blood and RP-Lys (22 g of digestible Lys), and HFM with blood and RP-Lys. The source of HFM containing blood tended to increase ($P = 0.06$) DMI (18.2 and 17.7 ± 0.72 kg/d for HFM with and without blood respectively), and this effect was accompanied with an increase ($P < 0.01$) in the concentration of both Lys and His in plasma (83.9 and 70.5 ± 4.06; 30.3 and 16.1 ± 3.15 μM for HFM with and without blood respectively). Milk yield and protein yield also increased ($P < 0.01$) when cows consumed HFM containing blood (20.6 and 18.8 ± 1.31 kg/d; 0.79 and 0.70 ± 0.040 kg/d for HFM with and without blood respectively), but the inclusion of RP-Lys did not affect milk and protein yield ($P \geq 0.55$). The addition of RP-Lys had no effect on the concentration of either plasma Lys or His ($P \geq 0.63$). The concentration of GE tended to increase ($P = 0.07$) for HFM containing blood (81.8 and 79.1 ± 3.29 Mcal/d); however, no difference was observed in the concentration of DE (52.7 ± 2.20 Mcal/d), ME (46.4 ± 2.02 Mcal/d), and NEL (31.2 ± 1.66 Mcal/d, $P \leq 0.39$). Similar to DMI, N intake increased ($P = 0.03$) with the inclusion of HFM containing blood, but CP digestibility decreased by 6.4% ($P < 0.01$). In conclusion, based on a positive effect of feed

intake, feeding sources of HFM containing blood may improve milk protein yield due to the increased supply of EAA and His could be more limiting than Lys.

Keywords: hydrolyzed feather meal, lysine, milk protein

INTRODUCTION

Hydrolyzed feather meal (**HFM**) is a byproduct of the poultry processing industry and is a widely available source of feed protein for dairy cows. The U.S is the second largest poultry producer in the world, processing over 2.5 billion chickens, in 2018 (USDA, 2018). Assuming that an average chicken average weighs 3.63 kg, 7% of which is feathers this industry contributes to over 79 million kg of feathers being produced each year (USDA, 2018). Hydrolyzed feather meal contains 92% CP, a high proportion of which is RUP, 65% (NRC, 2001). Feather meal is hydrolyzed using high temperature and pressure to disrupt the keratin bonds and this improves digestibility (Douglas Anderson, 2006). Unfortunately, it is believed that the process of hydrolysis may have a negative effect on the availability of AA's, when used as a dairy feed which may cause a reduction in milk protein (Douglas Anderson, 2006; Harris et al., 1992). In addition to the reduction in the availability of AA, this decrease in milk protein could also be due to the low concentration of some AA in HFM (Stahel et al., 2014).

The reduced supply of lysine may limit the synthesis of milk protein (Vyas and Erdman, 2009). Compared to blood meal, HFM is lower in Lys (2.57% vs. 8.98 of CP, NRC, 2001). Santos et al. (1998) reported a percentage of Lys within different feed protein compared to the percentage of Lys within milk protein in a 12-year literature review, where HFM had the lowest percentage for Lys (13%). The supply of Lys in dairy diets may also be increased by supplementing with rumen protected Lys (**RP-Lys**) sources (Giallongo et al., 2016). Because routine analysis of AA is costly, nutritionists often rely upon table values of feed composition, but it is known than some feeds may vary in AA content. For example, HFM can be produced by containing varying amounts of blood and this in turn, may affect the AA concentration of the feedstuff, most notably Lys (NRC, 2001). When blood was added to HFM and fed to beef cattle

both the quantity and quality of the ruminal escape protein was increased (Goedeken et al., 1990).To date the effect of source of HFM has not been investigated in dairy cows.

The objective of this experiment is to determine the effects of feeding HFM with or without blood with an RP-Lys on milk production, milk protein, and energy and N utilization. We hypothesized that feeding a source of HFM containing blood with RP-Lys to late lactation dairy cows would result in an increased and additive effect on milk protein.

MATERIALS AND METHODS

Animals and Treatments

Animal care and experimental procedures were approved by the University of Nebraska–Lincoln Animal Care and Use Committee. Twelve multiparous cows averaging 226 ± 22 DIM at the beginning of the experiment were used. Cows were housed in individual tie-stalls equipped with rubber mats in a temperature-controlled (20°C) barn at the Dairy Metabolism Facility in the Animal Science Complex at the University of Nebraska–Lincoln (Lincoln, NE) and milked at 0700 and 1800 h. These same cows were also used in a previous study observing the effect of rumen protected Lys and His on milk production, energy, and N utilization in diets containing HFM alone (Morris et al, 2020a). All cows were less than 70 ± 8 d at the start and 161 ± 8 d pregnant at the end of the last experimental period. Thus, fetal energy was assumed to be zero (NRC, 2001).

The experimental design was three times replicated 4 × 4 Latin square with 4 periods of 28 d. Cows were fed one of two TMRs that included HFM originating for one of two sources. HFM was included in the diet at 4.5% of diet DM. These HFM differed by source of origin and by the amount of blood included in the feed. Cows were grouped by milk yield and DMI and

were assigned randomly to three groups and one of 4 treatments: HFM without blood and no supplemental Lys (**FM LYS0**; American Proteins Inc., Cumming, GA), HFM with blood and no supplemental Lys (**FMB LYS0**; Mountaire Farms of Delaware, Inc, Millsboro, DE), HFM without blood and supplemental Lys (**FM LYS+**; 65 g/d of Ajipro; Ajinomoto Co., Inc., Tokyo, Japan), HFM with blood and supplemented Lys (**FMB LYS+)**. Rumen-protected Lys was top-dressed after feeding. Dietary ingredients for the TMR diets (Table 1), were added to a Calan Data Ranger (American Calan, Inc., Northwood, NH), mixed, and fed as a TMR once daily at 0930 h with a target refusal rate of 5%. Each period included 24 d of ab libitum diet adaptation, followed by 4 d of collection at which time diets were feed at 100% of the prior week's intake to limit refusal.

The supply of digestible Lys from RP-Lys product (Ajipro; Ajinomoto Co., Inc., Tokyo, Japan) was estimated using the mobile bag technique (Paz et al., 2014). Briefly, each product was placed in the rumen (16 h; the remaining residue was assumed to be RUP), a pepsin HCl bath (3 h), and then inserted into the duodenum and allowed to pass with digesta. The entire procedure was replicated in 2 lactating Jersey cows. Rumen and duodenal incubated samples were analyzed for N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI) and the disappearance was assumed to be RDP and digestible RUP, respectively. The AA content of RP Lys was determined as the N content of the product divided by the N content of Lys (19.16%).

Sample Collection and Analysis

Individual feed ingredients were sampled daily during collection periods and frozen at −20°C. All feeds were dried at 60ºC for 48 h, and then were ground to pass a 1-mm screen (Wiley Mill; Arthur A. Thomas Co., Philadelphia, PA). A subsample of ground feed was sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis and ADF (method

973.18; AOAC International, 2000), acid detergent lignin (Goering and Van Soest, 1970), sugar (Dubois et al., 1956), starch (Hall, 2009), crude fat (2003.05; AOAC International, 2000), minerals (985.01; AOAC International, 2000). Additionally, feed ingredients were analyzed for DM (935.29; AOAC International, 2000), ash (942.05; AOAC International, 2000), N (990.03; AOAC International, 2000; FlashSmart N/Protein Analyzer CE Elantech, Inc. Lakewood, NJ), and gross energy (**GE**) content (Parr 6400 Calorimeter, Moline, IL) in the nutrition laboratory of the University of Nebraska-Lincoln and for complete AA profile by the Missouri–Columbia Agricultural Experiment Station Chemical Laboratory for complete AA profile (method 982.30; AOAC International, 2000). At University of Nebraska-Lincoln, NDF analysis was done using the procedure described by Van Soest et al. (1991). Additionally, 2 doses (0.5 mL/dose) of alpha amylase (Catalog # FAA, Ankom Technologies, Macedon, NY) were added during the hour boil in NDF solution. NDF was corrected for ash contamination (**NDF_{OM}**). Total mixed rations were sampled on d 1 of each collection period and used to determine particle size using the Penn State particle separator (Heinrichs and Kononoff, 2002) on an as-is and DM basis (60ºC for 48 h). During each d of the collection period, refusals were sampled and composited on a weight basis. Refusals were analyzed for N, NDF, NDFom, starch, ash, and GE via the same methods as feeds.

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive d. To do so, a 137- × 76-cm rubber mat was placed behind the cow to aid in fecal collect. Feces were manually collected by personnel during defecation or were picked up from the rubber mat and deposited into a trash can (Rubbermaid, Wooster, OH) with a trash bag covering the top to minimize N losses prior to subsampling. Daily feces were subsampled (~500g as-is), composited on a weight basis and frozen between collection events. After collections, feces were dried at 60°C for 48 h and ground to pass through a 1-mm screen

(Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces samples were analyzed as described for refusals. Total urine was collected by inserting a 30 French Foley catheter into each cow's bladder with a stylus. The balloon was inflated to 55 mL with physiological saline. The catheter was drained into a 55-L plastic container via Tygon tubing (Saint Gobain, La Defense, Courbevoie, France). Acid (50% HCl) was added to the urine collection container at the beginning of the collection d to maintain a pH < 5. Urine was subsampled daily and composited on a wet weight basis. Urine samples were frozen (−20°C) until analysis for GE as described previously. Urine subsamples were sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis of N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI).

Milk production was measured daily, and milk samples were collected during both the morning and evening milking of the collection periods. Milk from individual milkings were preserved with 2-bromo-2-nitropropane-1, 3 diol and sent to Heart of America DHIA (Kansas City, MO). Milk samples were analyzed for fat, protein, lactose, SNF, MUN, and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). Additionally, milk from each milking event was composited on a weight basis. Composited milks samples were analyzed for GE and N as described previously for urine.

Blood samples were collected into evacuated K_2EDTA tubes from the tail vein approximately 3 h after feeding for 2 d during collection week on days when cows were not in a headbox. Plasma was immediately separated by centrifugation at 1,500 × g at 4°C for 15 min and frozen at –20°C until later analysis. An aliquot of 3 mL of plasma was deproteinized with 15% sulfosalicylic acid (4 parts plasma to 1 part 15% sulfosalicylic acid). Samples were then placed in an ice bath for 10 min before centrifuging at 1,500 × g at 4°C for 15 min. The supernatant was

collected, and 0.75-mL aliquots were placed into Nunc CryoTube vials (Nalge Nunc International, Roskilde, Denmark) and stored at −80°C. Plasma samples collected on the second day were processed the same way and added to CryoTubes. Plasma samples were submitted to the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratory for free AA analysis (Deyl et al., 1986, Fekkes, 1996). Plasma AA concentrations (μgM) were adjusted for the use of 15% sulfosalicylic acid.

Heat production was determined through the headbox-type indirect calorimeters as described previously (Freetly et al., 2006; Foth et al., 2015). For each cow, a collection period of 23-h was used to measure O_2 consumption and CO_2 and CH_4 production. Feed was placed in the bottom of the headbox and cows were allowed ad libitum access to water from a water bowl placed inside the headbox. Free water intake was measured using a water meter (Model DLJSJ75, Daniel L. Jerman Co., Hackensack, NJ) while each cow was inside the headbox. Within the headbox, temperature and dew point were measured every minute during the 23-h collection interval using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) and recorded using a data logger (Model XR440, Pace Scientific Inc.). Line pressure was measured using a u-tube manometer (Item # 1221–8, Park Supply of America, Inc., Minneapolis, MN) and barometric pressure of the room was measured using a barometer (Chaney Instruments Co., Lake Geneva, WI). Total volume of gas flow through the headbox was measured using a volumetric gas meter (Model AL425, American Meter, Horsham, PA) and corrected to standard temperature and pressure (0 ºC, 760 mmHg) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). In addition to volumetric flow meters, mass flow meters were also used (MCW Whisper, Alicat Scientific, Tucson, AZ). No correction was needed when using mass flow meters. From the headbox, continuous samples of incoming and outgoing air were collected

into separate bags (44 L, LAM-JAPCON-NSE; Pollution Measurement Corp., Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate "50," Brooks Instruments, Hatfield, PA). Gas bags were analyzed for O_2, CO_2 and CH_4 using an Emerson X-stream 3-channel analyzer (Solon, OH) according to the method of Nienaber and Maddy (1985). Heat production was estimated as follows (Brouwer, 1965):

Heat production (HP, kcal/d) = 3.866 × O_2 (L/d) + 1.200 × CO_2 (L/d) – 0.518 × CH_4 (L/d) – 1.431 × Urinary N excretion (g/d) (1)

The respiratory quotient (**RQ**) was calculated using the ratio of CO_2 produced to O_2 consumed. Methane energy was estimated by multiplying CH_4 production by its enthalpy (9.45 kcal/L). Tissue energy was calculated and partitioned into body protein and fat deposit as follows (Freetly et al., 2006; van Knegsel et al., 2007):

Tissue energy (**TE**; Mcal/d) = ME (Mcal/d) – HP (Mcal/d) – Milk energy (Mcal/d) (2)

Tissue energy as body protein (**TE_P**; Mcal/d) = retained N (g/d) × 5.88 (g of protein/g of N) × 0.0057 (Mcal/g of protein) (3)

Tissue energy as body fat (**TE_F**; Mcal/d) = TE (Mcal/d) – TE_P (Mcal/d) (4)

Prior to the start of the experiment and between collection periods, system efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O_2 and CO_2 were (average ± SD) 87.3 ± 2.93 and 86.6 ± 2.54%, respectively, for the volumetric flow meters and 100.0 ± 1.00 and 98.1 ± 1.99% for the mass flow meters. Because the gas recoveries were much lower for the volumetric flow meters than the mass flow meters, each headbox equipped with volumetric flow meters were corrected using

an average of oxygen and carbon dioxide recovered from burning 100% ethyl alcohol (n=3), then averaging oxygen and carbon dioxide recovery together. Each gas in volume was divided by this correction factor to represent the gas recovery of the mass flow meter. The mass flow meter was not corrected as it had close to 100% gas recovery.

Statistical Analysis

Data were analyzed in SAS (9.4). The model included the fixed effects of blood and Lys level and interaction of blood and Lys and the random effect of period, square, and cow nested in square. A type III analysis of variance with Kenward-Rodger's denominator degrees of freedom was completed using the PROC GLIMMIX function of SAS. All data are presented as least-squares means ± largest standard error. Significance was declared with a P-value ≤ 0.05.

RESULTS

Fourty-five out of the 48 planned observations were obtained for gas related energy calculations and 46 out of 48 were used for remaining variables. During the second period, one cow became ill (pneumonia, on treatment FMBLYS+) and the data were removed prior to statistical analysis. During the third period, one cow (on treatment FMBLYS+) exhibited a dramatic reduction in dry matter intake (17.2 vs 7.5 kg/d) whilst in the headbox. This resulted in low respiratory quotient (0.95 < 1.00). However, when removed from the headbox normal DMI was observed thus only gas production data were removed. Another cow (on treatment FMBLYS0) during the fourth period DMI was dramtaically reduced when placed inside the headbox and this intake never recovered when removed and all data were removed from the final dataset.

Diet Composition

Diet composition of the two basal diets are listed in Table 1. Crude protein for the diets with HFM containing blood were slightly higher than HFM alone (17.5 ± 0.59% and 17.1 ± 0.62%, respectively). The ADF in the HFM alone diets were slightly lower than HFM with blood (21.5 ± 1.05% and 22.0 ± 0.94%, respectively). Dry matter, NDF, sugar, starch, crude fat, and ash were similar among diets. Particle size of the diets (as fed basis) was similar among treatments. The top screen (>19.0 mm) contained 4.70 ± 2.98% and 3.30 ± 0.81% of DM for HFM alone and HFM containing blood, respectively. The second screen (8.0–19.0 mm) contained 33.6 ± 3.20% and 34.0 ± 3.18% of DM for HFM alone and HFM containing blood, respectively. The third screen (1.18–8.0 mm) contained 44.3 ± 4.40% and 44.3 ± 5.91% of DM for HFM alone and HFM containing blood, respectively. The final pan (<1.18 mm) contained 17.4 ± 3.41% and 17.1 ± 3.47% of DM for HFM alone and HFM containing blood, respectively.

Chemical composition of corn silage, alfalfa hay, concentrate mixes, and HFM's are listed in Table 2. The orgins of feather meal used in this study were different and it should be noted that although they differ by the concentration of blood, they may also differ by production process. In the current study HFM containing blood was higher in CP (90.7 vs 87.2% of DM), Lys (3.12 vs 2.08% of DM), and His (1.53 vs 0.72% of DM) and lower in crude fat (6.46 vs 10.9% of DM), and ash (1.84 vs. 3.13% of DM). Additionally, based on the moblie bag assay TTDCP was higher in the HFM not containing blood (81.9 ± 1.91% vs 69.2 ± 7.95%). The RUP content and RUP digestiblilty were higher in the HFM not containing blood (66.5 ± 1.27% vs 64.6 ± 6.51% and 72.7 ± 3.40% vs 52.8 ± 7.54%, respectively).

Dietary Energy, Metabolizable Protein, and Lysine Balance

The estimated dietary energy, metabolizable protein (**MP**), and Lys balance are listed in Table 3. Based on simulations using the NRC (2001), all diets were formulated to be isoenergetic (average NEL of 1.58 Mcal/kg) when using the estimated treatment means for DMI, milk yield and composition, and dietary nutrient composition. Using the same estimated treatment means for each treatment, simulations suggested that all treatments contained adequate amounts of MP averaging 1821 g/d. Diets that were not supplemented with RP-Lys were 3 and 8 g/d below the requirements for digestible Lys. Diets supplemented with RP-Lys were 20 and 16 g/d above the requirements for digestible Lys, based on requirements recommended by Schwab et al. (2005).

Feed Intake, Milk Yield and Composition

Milk yield and components, DMI, BW, and BCS are listed in Table 4. Dry matter intake tended to be higher with the inclusion of HFM containing blood (18.2 vs 17.7 ± 0.72 kg/d, P = 0.06), and the addition of supplemental Lys had no effect on DMI (P = 0.53). Milk yield was higher with inclusions of blood in HFM (20.6 kg/d) compared to HFM alone (18.8 ± 1.31 kg/d, P = 0.01). The inclusion of RP-Lys had no effect on milk yield (P = 0.55). Milk fat percent was lower for HFM with blood compared to the inclusion of HFM alone (5.89 vs 6.32 ± 0.33%, P = 0.02). However, fat yield was not different ($P \geq 0.27$) averaging 1.16 ± 0.06 kg/d. Milk protein and lactose percent were not affected ($P \geq 0.12$) by treatments averaging 3.88 ± 0.12% and 4.72 ± 0.06% across treatments, respectively. Protein yield was significantly higher with HFM containing blood compared to than HFM alone, both without RP-Lys (0.79 vs 0.70 ± 0.04 kg/d, $P < 0.01$). The increase in milk and protein yield resulted in an increase of ECM when cows were fed HFM containing blood (28.0 vs 26.6 ± 1.42 kg/d, P = 0.02). The inclusion of RP-Lys increased MUN (17.0 vs 16.2 ± 1.03 mg/dL, P = 0.04), whereas HFM containing blood

decreased MUN compared to HFM alone (15.0 vs 16.2 mg/dL, $P = 0.01$). Free water intake was not affected ($P \geq 0.19$) for any treatments averaging 73.2 ± 5.45 L/d.

Plasma AA Concentration

The plasma AA concentration are listed in Table 5. Source of HFM had no effect on the EAA except for Arg ($P = 0.04$), His ($P = <0.01$), Lys ($P = <0.01$), and Thr ($P = 0.02$). The inclusion of RP-Lys had no effect on EAA except for Thr ($P = 0.02$) and Trp ($P = 0.09$). Supplementation of RP-Lys did not affect the plasma concentration of Lys (70.8 ± 4.06 μM, $P = 0.63$); however, the concentration of plasma Lys was higher for HFM containing blood compared to HFM alone (83.9 vs 70.5 μM, $P = <0.01$). The plasma concentration of His has been observed to be higher with inclusion of HFM containing blood compared to HFM alone (30.3 vs 16.1 ± 3.15 μM, $P = <0.01$) and RP-Lys had no effect on His ($P = 0.69$). Additionally, HFM with blood and RP-Lys decreased plasma concentration of His compared to HFM with blood (25.3 vs 30.3 ± 3.15 μM, $P = 0.03$). Plasma concentration of Arg increased with HFM with blood compared to HFM alone (83.0 vs 74.8 ± 4.30 μM, $P = 0.04$) and plasma concentration of Thr was higher in HFM with blood (97.2 ± 4.67 μM vs 88.2 ± 4.67 μM, $P = 0.02$). Plasma concentration of Thr and Trp decreased and tended to decreased, respectively, with RP-Lys in comparison to blood (81.0 vs 88.2 ± 4.67 μM and 40.6 vs 43.5 ± 3.07 μM, respectively).The non-EAA were not affected by source of HFM except for Ser, which was lower for HFM with blood (105 vs 125 ± 9.01 μM, $P = 0.01$). The inclusion of RP-Lys had no effect on non-EAA except for Gly ($P = 0.02$), Ser ($P = 0.09$), Tau ($P = 0.07$), and Tyr ($P = 0.06$). Plasma concentration of Gly was lower for supplemental RP-Lys compared to no supplementation of RP-Lys (337 vs 382 ± 25.6 μM). Plasma concentration of Ser, Tau, and Tyr tended to be lower for supplemental RP-Lys compared to no supplementation of RP-Lys (113 vs 125 ± 9.0 μM, 34.2

vs 37.1 ± 2.65 μM, and 44.3 vs 46.9 ± 2.89 μM, respectively). Plasma concentration of carnosine was higher with HFM with blood compared to HFM alone (10.8 vs 8.55 ± 0.84 μM, P <0.01).

O_2 consumption, CO_2 and CH_4 production, and Respiratory Quotient

Results of oxygen consumption, carbon dioxide and methane production, and respiratory quotient (**RQ**) are listed in Table 6. Oxygen consumption, carbon dioxide and methane production were not affected ($P \geq 0.75$) by treatment averaging 4660 ± 174, 4988 ± 188, and 426 ± 22.2 L/d respectively. Across treatments, there were no effects on methane per unit of DMI ($P \geq 0.49$) averaging 24.0 ± 0.93 L/kg and methane per ECM ($P \geq 0.35$) averaging 16.0 ± 0.83 L/kg. Methane per unit OM digested was higher when fed HFM containing blood in comparison to HFM alone (6.39 vs 6.17 ± 0.322, $P = 0.02$). Additionally, methane per unit of NDF_{OM} digested increased with HFM containing blood in comparison to HFM alone (8.67 vs 8.05 ± 0.458, $P < 0.01$). RP-Lys had no effect on methane per OM digested and methane per NDF_{OM} digested ($P = 0.94$ and $P = 0.64$, respectively). The RQ was not observed to be different across treatments ($P \geq 0.33$) averaging 1.07 ± 0.00.

Energy Utilization

Energy partitioning is listed in Table 7. Fecal energy was higher with HFM containing blood compared to HFM alone (28.2 ± 1.74 vs 26.6 ± 1.74 Mcal/d, $P < 0.01$) but RP-Lys did not affect fecal energy ($P = 0.99$). Gaseous energy was not affected by treatment ($P \geq 0.28$) averaging 4.00 ± 0.213 Mcal/d. Urinary energy tended to be lower when feeding HFM containing blood compared to HFM alone (2.29 and 2.45 ± 0.094 Mcal/d, $P = 0.08$). Heat production was unaffected by either source of HFM ($P = 0.15$) or inclusion of RP-Lys ($P = 0.16$). However, heat production as a proportion of MBW had a tendency to be higher when

cows consumed HFM containing blood (229 vs 221 ± 8.34 Mcal/d, $P = 0.09$). Milk energy was higher when cows consumed HFM containing blood compared to HFM alone (19.1 vs 18.2 ± 0.98 Mcal/d, $P = 0.02$) but the inclusion of RP-Lys had no effect ($P = 0.57$). Supplemental RP-Lys had no effect ($P = 0.12$) on tissue energy and HFM containing blood had no effect ($P = 0.59$) on tissue energy. However, it seemed to increase protein TE ($P = 0.09$). Energy fractions (digestible (**DE**) and metabolizable energy (**ME**)) were not different across treatments ($P \geq 0.39$). Gross energy (**GE**) was different when feeding HFM containing blood ($P = 0.07$). Gross energy tended to be higher when cows consumed HFM containing blood compared to HFM alone (81.1 vs 79.1 ± 3.29 Mcal/d). As a proportion of kg of dry matter, GE and DE tended to decrease with HFM containing blood compared to HFM alone (4.46 vs 4.48 ± 0.04%, $P = 0.08$ and 2.91 vs 2.97 ± 0.05%, $P = 0.02$, respectively). All measures of energy utilization efficiencies were unaffected by treatment ($P \geq 0.13$).

Fecal and Urinary output, Nitrogen Excretion, Secretion, and Partitioning

Fecal and urinary outputs are listed in Table 8. Fecal output was higher for cows consuming HFM containing blood compared to than HFM alone (6.20 vs 5.84 ± 0.31 kg/d DM, $P < 0.01$). RP-Lys did not affect fecal output ($P = 1.00$). Urinary output was not affected by treatment averaging 22.6 ± 1.34 kg/d as is ($P \geq 0.11$).

Measures of nitrogen excretion and utilization are listed in Table 8. Nitrogen intake increased from 474 to 491 ± 22.8 g/d with HFM containing blood in comparison to HFM alone ($P = 0.03$). Fecal N was higher ($P < 0.01$) and urinary N lower ($P = 0.01$) when cows consumed HFM containing blood compared to HFM alone (187 vs 160 ± 22.8 g/d and 146 vs 164 ± 6.70 g/d, respectively). Milk N increased when cows consumed HFM containing blood compared to HFM alone (136 vs 124 ± 7.10 g/d, $P < 0.01$). Nitrogen balance was unaffected ($P \geq 0.43$) by

treatment averaging 21.1 ± 1.35 g/d. RP-Lys did not affect N intake, fecal N, urinary N, milk N, and N balance ($P \geq 0.31$). When expressed as proportion of N intake, fecal N increased from 34.0 to 38.2 ± 1.35% when feeding HFM containing blood compared to HFM alone ($P < 0.01$). Urinary N expressed as proportion of N intake was lower when cows consumed HFM containing blood compared to HFM alone (30.0 vs 35.0 ± 1.78%, $P < 0.01$). When expressed as a proportion of N intake milk N was higher when cows consumed HFM containing blood compared to HFM alone (27.5 vs 26.1 ± 0.76 %, $P = 0.03$). N balance as a proportion of N intake was unaffected ($P \geq 0.56$) across treatments averaging 4.00 ± 2.851%. Supplemental RP-Lys had no effect on fecal N, urinary N, milk N, and N balance as a proportion of N intake ($P \geq 0.41$).

Nutrient Digestibility

Nutrient digestibility results are listed in Table 9. Compared to HFM alone, the addition of HFM containing blood was lower or tended to be lower for dry matter, organic matter, NDF, NDF_{om}, CP, and energy digestibility ($P \leq 0.07$). One exception of this trend was starch digestibility which was unaffected by treatment ($P \geq 0.25$). Supplemental Lys had no effect on most measures of nutrient digestibility except for NDF digestibility ($P = 0.05$) which decreased from 46.1 vs 44.3 ± 1.91%.

DISCUSSION

Lysine supply can limit the synthesis of milk protein (Vyas and Erdman, 2009). In the current study, late lactation Jersey cows were used, and it should be noted that deficiencies in both energy and protein often result in more dramatic effects in early lactation. Nonetheless, deficiencies can still be observed in late lactation and in the current study the supply of Lys was varied by feeding different sources of HFM (one containing blood and another without) and by manipulating the inclusion of RP-Lys. Compared to feathers alone, blood is higher in Lys (NRC,

2001); therefore, we expected that FMB would contain more Lys (Table 2). Previous research has demonstrated that milk protein may decrease as the source of HFM containing no blood is increased (Harris Jr et al., 1992; Morris et al, 2020a). It has been suggested that this response is due to the low concentration and bioavailability of Lys in HFM (NRC, 2001). Consequently, we hypothesized that dairy cattle consuming HFM containing blood and RP-Lys would result in cows consuming more Lys and that these two factors would result in an additive effect on milk protein yield. Surprisingly, this was not observed by consumed RP-Lys and a positive effect on milk protein was only observed when cows consumed HFM containing blood.

Dietary Energy, Metabolizable Protein, and Lysine Balance

In a previous study where the effects of RP-Lys were tested, diets were formulated to be deficient in MP and with a low supply of Lys (Giallongo et al., 2016). In the current study, diets were formulated (NRC 2001) to be adequate, but not in gross oversupply of MP (+53 g/d FM and +67g/d FMB) (assuming: 18.3 kg/d DMI, 22.2 kg/d milk yield, 5.70% milk fat, and 3.39% milk true protein). Unfortately due to lower than expected milk production the balance of MP for HFM alone averaged 94.5 g/d, whereas HFM with blood averaged 52 g/d. Assuming the requirement for Lys is 6.6 % of MP (Schwab et al., 2005), the non-RPLys supplemented treatments remained below the recommendations. According to Schwab et al. (2005), diets fed either HFM without RP-Lys were 3 to 7% deficient in digestible Lys. The RP-Lys used in this study contained 44.4% Lys, 80.3 ± 5.31% RUP with a RUP digestibility of 95.8 ± 1.23%. These estimates were determined using the mobile bag method (Paz et al., 2014). Therefore, 65 g of RP-Lys was estimated to supply 22.0 g of digestible Lys, which exceeded the recommendations when supplementaled with RPLys.

Feed Intake, Milk Yield and Compostition, and Plasma AA Concentration

Practically it is generally believed HFM is an ingredient of low palatability and may negatively affect DMI however experiemntal observations have been variable. Harris Jr. et al. (1992) did not observe an effect of HFM on DMI with increasing inclusions of HFM (0, 3, 6% of dietary DM), whereas (Morris et al. 2020b) observed an increase in DMI when HFM was included to 6.7% of dietary DM but then this decreased when it was inculded at 10% dietary DM. In the current study, DMI tended to increase by 2.7% with the addition of blood in HFM (Table 4). In the current study, the concentration of both plasma Lys and His concentration increased by 16% and 47% respectively when consuming HFM containing blood. It is possible the the increase supply of the AA could have stimulated the increase DMI. This response may have been a result of the increase of His which was higher in HFM containing blood compared to HFM alone (Table 2). Stahel et al. (2014) fed HFM alone to induce a defienciey of His , which tended to decrease in DMI. Another study supplied adaquate Lys and His to lactating dairy cows with HFM in the diet and resulted in an increase of DMI (Giallongo et al., 2017). Deficiencies in His can have a negative impact on DMI. However, differenecs in DMI from the two sources of HFM could have been a result of differences in palatability. Morris et al. (2020a) observed a quadratic increase in milk yield when increasing the proportion of HFM 0 to 6.7% FM, but milk protein yield decreased. Grant and Haddad (1998) supplemented diets containing 17.6% and 19.6% CP with a mixture of HFM and bloodmeal. Milk and protein yield increased by 10% and 12% respectively for the 17.6% CP diet, but decreased in cows consuming diets containing 19.6% CP. The authors suggested that the increase in milk and protein yield with HFM and bloodmeal in diets containing lower protein diets may have been due to an increase in protein efficiency. It is unclear why milk and protein yield decreased with supplemental HFM and BM in 19.6% CP diets; however, it may have been due to a reduction in palatability as DMI

was reduced by 13% (Grant and Haddad, 1998). In the current study, feeding HFM with blood increased milk yield by 8.7% and milk protein yield by 11.4 % (Table 4). These observations were supported by an increase in dry matter intake. However, we also observed a 6.4% reduction in CP digestibility (Table 9) with an increase in plasma concentration of Lys and His in cattle fed HFM with blood (Table 5). This increase in Lys and His could have in part supported the increase in milk and protein yield. The sources of the HFM alone and HFM containing blood used for this study contained a plasma Lys concentration of 2.08 and 3.12% DM, respectively. Also, the sources of the HFM alone and HFM containing blood contained a plasma His concentration of 0.72 and 1.53% of DM, respectively. The HFM containing blood had a 33% and 53% increase in Lys and His concentration compared to HFM alone and this may have been beneficial for milk and protein yield. However, processing methods among rendering plants producing HFM are variable and could affect the Lys and His content (Cotanch et al. 2020).

Previous research has demonstrated that deficiencies in Lys and His may be overcome by supplementing the diet with rumen protected AA when feeding HFM (Stahel et al., 2014 and Giallongo et al., 2017). However, we did not observe an increase in either milk yield or milk protein with supplemental RP-Lys and HFM containing blood. Interestingly, when HFM with blood was fed we observed an increase in plasma His but not Lys concentration (Table 5). It is possible that in the current study all cows were consuming adequate amounts of Lys, thus the additional Lys was not needed. This is supported by an increase in MUN with the addition of RP-Lys which may indicate that these animals excreted additional Lys through the milk (Table 4). Even though, research has indicated that increasing Lys intake increases milk protein yield (Vyas and Erdman, 2009) this effect is not always observed (Morris et al, 2020b). Plasma AA concentration may be a gross indicator of physiological status of AA’s in the lactating dairy cow;

however, plasma AA may not always be reflective of dietary supply of AA as plasma concentrations can be affected by metabolism of AA in the gut, liver, peripheral tissue, and mammary gland (Arriola Apelo et al., 2014; Morris et al., 2020b).

We did observe a 36% increase in plasma concentration of His when feeding HFM containing blood with RP-Lys. A similar interaction was observed when RP-Lys and RP-His were supplemented in HFM diets (Morris et al., 2020b). Unfortunately, the reason for this observation is not clear. However, in the current study we observed a numerical increase in carnosine when feeding HFM with blood and RP-Lys (Table 5). Carnosine (β-alanyl-His) is an endogenous source of His, which acts as an intracellular buffer in skeletal muscle at low concentrations and is found in plasma (Lapierre et al., 2011). Ten m*M* of muscle carnosine in a dairy cow could contribute approximately 420 g of His supply that could be used for milk protein synthesis (Lapierre et al., 2011). The slight numerical increase in carnosine could have contributed to the increase of plasma His concentration when feeding HFM with blood and RP-Lys; but there was no increase in milk protein yield.

Energy Utilization

Energy supply to the mammary gland has shown to be beneficial in improving milk protein synthesis by increasing the uptake of AA's by the mammary gland (Raggio et al., 2006; Ruis et al., 2010). In the current study, we observed an increase in milk energy by 4.7% when feeding HFM containing blood than HFM alone, which was caused by increased protein and lactose content within the milk (Table 4). We observed a decrease in GE and DE concentration (Mcal/kg of DM) when feeding HFM with blood compared to HFM alone. Gross energy intake had a 2.5% increase when cows were fed HFM containing blood; however, it did not translate to a difference in DE, ME, and NEL. This implies that the supply of energy was not responsible for

the increase in milk protein and the supply of EAA's from the HFM with blood influenced the milk protein response. The increase of GE intake was supported by the increase in DMI. The inclusion of Lys had no effect on energy utilization (Table 7). These observations are similar to Morris et al. (2020a), who observed a similar energy utilization results when supplementing Lys with different blends of HFM with no blood.

Nitrogen Utilization and Nutrient Digestibility

In the current study differences were observed in N utilization. Nitrogen intake was increased with HFM with blood, this in turn increased total fecal and milk N (Table 8). This could be supported by the increase in DMI, resulting in an increase in milk N and milk protein yield. Crude protein of the HFM containing blood was slightly higher than the HFM alone, and this may have resulted in the observed increase in N intake. When feeding HFM containing blood we observed an increase in N intake, but the proportion of N digested by the cow was reduced by 6.4 % (Table 9). This in turn resulted in an increase fecal N (as proportion of N intake) by 11% while urinary N decreased by 14 %, and milk N increased by 5.0%. Both sources of feather meal were evaluated using the mobile bag method to determine total tract CP digestibility and RUP digestibility (Paz et al., 2014). Feeding HFM containing blood resulted in a 16% decline in total tract CP digestibility compared to HFM alone using the mobile bag method, and this decrease in total tract CP digestibility was also observed in vivo (Table 9). Buse et al (2019) assessed different sources of HFM and tested whether the addition of blood affected the digestibility of protein but did not observed any differences in total tract CP digestibility. Using an in-situ bag method, Waltz et al. (1989) did not observe an effect on total tract N digestibility as a percent of N intake when feeding HFM in addition to BM. We suggest that

differences in CP digestibility were not a result in the addition of blood per se but may have been due to source and production method of HFM.

CONCLUSIONS

Hydrolyzed FM is a cost effective source of bypass protein to be used in dairy cattle rations; however the perception is that the use of this feed must be limited because of the low concentration of Lys and His. Feeding dairy cows HFM with blood may increase milk and milk protein yield because of higher concentration of EAA. Results of this study suggest that His may also play an important role in increasing milk and milk protein yield. Future research should seek to evaluate key differences in the rendering process that may affect digestibility of HFM.

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TABLES

Table 3.1. Ingredients, chemical composition and particle size distribution of each experimental diet used to feed experimental cows (% of DM)

Items	FM[1]	FMB[1]
Ingredients		
Corn silage	39.9	39.9
Alfalfa hay	18.6	18.6
HFM[2]	4.51	0.00
HFM with blood	0.00	4.51
Corn grain, ground	16.6	16.6
Soybean meal	1.11	1.11
Soybean hulls	9.53	9.53
Molasses, sugarcane	2.23	2.23
Whey	2.72	2.72
Rumen-protected Met[3]	0.09	0.09
Fat[4]	1.24	1.24
Urea	0.52	0.52
Mineral-vitamin mix[5]	3.05	3.05
Chemical composition		
DM	68.7 (0.01)	68.7 (0.01)
CP	17.1 (0.62)	17.5 (0.59)
ADF	21.5 (1.05)	22.0 (0.94)
NDF	28.9 (0.01)	28.8 (0.01)
NDFom	27.6 (0.01)	27.3 (0.01)
Starch	27.0 (1.40)	26.9 (0.98)
Sugar	6.16 (0.569)	6.15 (0.598)
Crude fat	3.89 (0.280)	3.76 (0.267)
Ash	8.57 (0.247)	8.57 (0.634)
Ca	1.09 (0.091)	1.15 (0.289)
P	0.38 (0.040)	0.37 (0.025)
Mg	0.38 (0.028)	0.39 (0.027)
K	1.50 (0.042)	1.48 (0.013)
S	0.28 (0.010)	0.27 (0.008)
Na	0.46 (0.046)	0.49 (0.083)
Cl	0.54 (0.023)	0.57 (0.064)
Particle Size		
>19.0 mm, % as-is	4.70 (2.984)	3.30 (0.809)
8.0–19.0 mm, % as-is	33.6 (3.20)	34.0 (3.18)
1.18–8.0 mm, % as-is	44.3 (4.40)	44.3 (5.91)
<1.18 mm, % as-is	17.4 (3.41)	17.1 (3.47)
>19.0 mm, % of DM	4.30 (3.047)	3.30 (0.928)
8.0–19.0 mm, % of DM	28.2 (2.33)	28.6 (2.38)
1.18–8.0 mm, % of DM	46.9 (3.85)	48.2 (5.20)
<1.18 mm, % of DM	20.5 (3.93)	19.9 (3.83)

[1] FM = Hydrolyzed feather meal diet, FMB = Hydrolyzed feather meal with blood diet.

[2] HFM = Hydrolyzed feather meal.

[3] Smartamine M (Adisseo, Alpharetta, GA).

[4] Energy Booster 100 (Milk Specialties, Eden Prairie, MN).

[5] Contained per kg: 311 g of $CaCO_3$. 269 g of $NaHCO_3$, 177 g of Ca_2PO_4, 111 g of MgO, 102 g of salt, 16 g of vitamin premix (14,850 IU/g Vitamin A, 3,850 IU/g Vitamin D, and 90 IU/g of Vitamin E), and 13 g of trace mineral premix (180,000 mg/kg Zn, 150,000 mg/kg Mn, 25,000 mg/kg Cu, 2,600 mg/kg I, 2,300 mg/kg Co, 1,000 mg/kg Fe, and 820 mg/kg Se).

Table 3.2. Chemical composition of corn silage, alfalfa hay, concentrates mixes, and hydrolyzed feather meal fed to late lactating Jersey cows in a TMR (% of DM)[1]

	Corn silage		Alfalfa hay		CONM1[2]		CONM2[2]		FM[2,6]	FMB[2,6]
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Mean
DM, % as is	36.8	0.02	88.3	0.00	90.4	0.00	90.4	0.00	91.7	90.0
CP	9.20	0.458	20.2	1.51	23.2	1.26	24.2	1.71	87.2	90.7
ADF	20.9	2.57	37.7	2.99	14.9	1.62	16.1	0.36	[3]-	-
NDF	32.7	0.04	41.2	0.05	19.8	0.03	19.7	0.02	-	-
NDFom	31.0	0.04	40.1	0.05	18.8	0.03	18.1	0.02	-	-
ADICP[4]	0.70	0.213	1.29	0.103	2.26	0.169	2.25	0.193	-	-
NDICP[5]	0.83	0.284	1.55	0.056	3.37	0.228	3.46	0.271	-	-
Lignin	2.96	0.428	9.21	0.777	3.35	0.980	3.41	0.634	-	-
Sugar	0.77	0.709	6.60	0.200	11.3	1.04	11.3	1.12	-	-
Starch	38.9	1.92	0.98	0.320	27.2	2.59	27.0	0.99	-	-
Crude fat	3.04	0.073	1.48	0.136	5.79	0.600	5.49	0.564	10.9	6.46
Ash	5.20	0.845	10.4	0.55	11.0	0.32	11.0	1.71	3.13	1.84
Ca	0.21	0.024	1.01	0.140	1.97	0.173	2.12	0.677	0.76	0.24
P	0.22	0.017	0.29	0.018	0.57	0.082	0.54	0.049	0.43	0.24
Mg	0.17	0.017	0.21	0.013	0.67	0.056	0.68	0.067	-	-
K	1.02	0.047	3.39	0.050	1.11	0.067	1.08	0.022	-	-
S	0.15	0.006	0.25	0.025	0.43	0.024	0.41	0.017	0.14	0.13
AA										
% Lys	-	-	-	-	-	-	-	-	2.08	3.12
% Met	-	-	-	-	-	-	-	-	0.65	0.71
% His	-	-	-	-	-	-	-	-	0.72	1.53

[1] n=4 corn silage, alfalfa hay, CONM1, and CONM2, n=2 Hydrolyzed feather meal.

[2] CONM1 = Concentrate mix 1, CONM2 = Concentrate mix 2, FM= Hydrolyzed feather meal without blood (American Proteins Inc., Cumming, GA; Pilgrim's), FMB= Hydrolyzed feather meal with blood (Mountaire Farms of Delaware, Inc, Millsboro, DE).

[3] Not recorded.

[4] Acid detergent insoluble CP.

[5] Neutral detergent insoluble CP.

[6] Mobile bag data for FM and FMB: RDP = 33.5 and 35.4% of CP, respectively; RUP = 66.5 and 64.6 % of CP, respectively; RUP digestibility = 72.7 and 52.8%, respectively; Total Tract CP digestibility; 81.9 and 69.2, respectively.

Table 3.3. Estimated dietary energy, MP, and Lys balance in late lactating Jersey cows when fed hydrolyzed feather meal with or without blood with rumen protected (RP) Lys[1]

	Treatments[2]			
	FM		FMB	
Items	LYS0	LYS+	LYS0	LYS+
NEL, Mcal/kg[3]	1.59	1.60	1.56	1.57
MP, g/d				
Requirements	1709	1638	1849	1794
Supply	1809	1727	1897	1849
Balance	100	89	48	56
dLys, g/d				
Requirements[4]	113	108	122	118
Supply from the diet	110	106	114	112
Supply from the RP Lys	0	22	0	22
Balance	-3	20	-8	16

[1] All values estimated with NRC, 2001 using mean production and measured feed composition values to determine MP and dLys.

[2] FM = hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 = 0 g/d of RP Lys, LYS+ = 65 g/d RP Lys.

[3] NEL = $0.080 \times BW^{0.75}$ + milk energy + tissue energy (NRC, 2001).

[4] Requirements of digestibility Lys (dLys) were calculated as 6.6% (Schwab et al., 2005) of MP requirements.

Table 3.4. Effects of feeding hydrolyzed feather meal with or without blood and rumen-protected Lys on intake, milk production and components, free water intake, BW, and BCS of late lactation Jersey cows

	Treatment[1,2]				SEM	*P*-value[3]		
	FM		FMB					
Item	LYS0	LYS+	LYS0	LYS+		B	L	B x L
DMI, kg/d	17.7	16.9	18.2	18.3	0.72	0.06	0.53	0.34
Milk yield, kg/d	18.8	18.0	20.6	20.4	1.31	0.01	0.55	0.61
ECM, kg/d[4]	26.6	25.6	28.0	28.1	1.42	0.02	0.56	0.48
ECM/ DMI	1.50	1.51	1.53	1.53	0.043	0.36	0.91	0.70
Fat, %	6.32	6.33	5.89	5.95	0.341	0.02	0.86	0.88
Fat, kg/d	1.16	1.12	1.18	1.19	0.065	0.27	0.64	0.43
Protein, %	3.82	3.82	3.89	3.89	0.119	0.12	0.97	0.98
Protein, kg/d	0.70	0.68	0.79	0.79	0.040	<0.01	0.66	0.68
Lactose, %	4.71	4.72	4.73	4.72	0.058	0.76	0.83	0.71
Lactose, kg/d	0.89	0.85	0.97	0.97	0.067	0.01	0.56	0.66
MUN, mg/dL	16.2	17.0	15.0	16.0	1.03	0.01	0.04	0.83
Free water intake, L/d	69.2	73.1	74.7	75.7	5.45	0.19	0.43	0.63
BW, kg	484	479	482	481	16.9	0.95	0.41	0.56
BCS[5]	3.44	3.27	3.36	3.27	0.157	0.42	0.01	0.40

[1] FM = hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 = 0 g/d of RP Lys, LYS+ = 65 g/d; n = 46 for all variables.

[2] Least squares means; largest standard error of treatment mean is shown.

[3] B =Blood, L = Lys, and B x L = Blood x Lys interaction.

[4] ECM = 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × true protein (kg) (Tyrrell and Reid, 1965).

[5] Scored 1-5 by 2 independent observations.

Table 3.5. Effects of feeding hydrolyzed feather meal with or without blood and rumen-protected Lys on plasma AA of late lactation Jersey cows (μM)

	Treatments[1,2]							
	FM		FMB		SEM	*P*-value[3]		
Item	LYS0	LYS+	LYS0	LYS+		B	L	B x L
EAA[4]								
Arg	74.8	74.3	83.0	79.5	4.30	0.04	0.53	0.64
His	16.1	19.6	30.3	25.3	3.15	<0.01	0.69	0.03
Ile	117	114	129	119	7.4	0.15	0.26	0.49
Leu	139	140	153	138	8.8	0.32	0.25	0.19
Lys	70.5	71.1	83.9	80.3	4.06	<0.01	0.63	0.51
Met	25.8	23.7	26.6	25.7	1.18	0.17	0.16	0.57
Phe	50.6	53.3	52.6	48.0	2.41	0.30	0.56	0.03
Thr	88.2	81.0	97.1	87.9	4.67	0.02	0.02	0.74
Trp	43.5	40.6	45.0	43.0	3.07	0.17	0.09	0.76
Val	277	272	292	269	17.9	0.60	0.23	0.44
Non-EAA								
Ala	253	237	259	255	11.2	0.09	0.18	0.41
Asn	39.0	44.3	41.5	41.1	5.13	0.97	0.60	0.55
Asp	1.12	1.35	1.44	1.31	0.152	0.34	0.73	0.23
Gln	226	204	216	214	12.1	1.00	0.17	0.24
Glu	46.2	47.3	47.1	47.9	2.85	0.67	0.60	0.95
Gly	382	337	357	335	25.6	0.33	0.02	0.41
Pro	101	93.3	95.2	91.6	4.69	0.34	0.15	0.63
Ser	125	113	105	100	9.0	0.01	0.09	0.47
Tau	37.1	34.2	40.0	35.3	2.65	0.32	0.07	0.66
Tyr	46.9	44.3	45.8	42.2	2.89	0.32	0.06	0.78
Carnosine	8.55	8.81	10.9	9.85	0.854	<0.01	0.35	0.12
3-Methylhistidine	3.32	3.60	3.54	3.59	0.354	0.52	0.29	0.44

[1] FM= hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 =0 g/d of RP Lys, LYS+ = 65 g/d; n = 46 for all variables.

[2] Least squares means; largest standard error of treatment mean is shown.

[3] B =Blood, L = Lys, and B x L = Blood x Lys interaction.

[4] BCAA = branched-chain AA (Ile, Leu, Val).

Table 3.6. Effects of feeding hydrolyzed feather meal with or without blood and rumen protected Lys on O_2 consumption, CO_2 and CH_4 production, and respiratory quotient of late lactation Jersey cows

	Treatment[1,2]				SEM	*P*-value[3]		
	FM		FMB					
Items	LYS0	LYS+	LYS0	LYS+		B	L	B x L
O_2 consumption, L/d	4504	4637	4637	4863	174	0.16	0.15	0.71
CO_2 production, L/d	4830	4943	4964	5215	188	0.15	0.19	0.61
CH_4 production, L/d	425	406	431	442	22.2	0.14	0.75	0.29
CH_4/DMI, L/kg	24.1	24.3	23.7	23.7	0.93	0.49	0.94	0.91
CH_4/ECM, L/kg	16.1	16.2	15.6	15.8	0.83	0.35	0.74	0.91
CH_4/OM digested, L/kg	6.17	5.91	6.39	6.63	0.322	0.02	0.94	0.20
CH_4/NDF_{OM} digested, L/kg	8.05	7.85	8.67	9.16	0.458	<0.01	0.64	0.27
RQ[4]	1.07	1.07	1.07	1.07	0.012	1.00	0.76	0.33

[1] FM= hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 = 0 g/d of RP Lys, LYS+ = 65 g/d RP Lys; n= 45 for all variables.

[2] Least squares means; largest standard error of treatment mean is shown.

[3] B =Blood, L = Lys, and B x L = Blood x Lys interaction.

[4] RQ = respiratory quotient, CO_2 production/O_2 consumption.

Table 3.7. Effects of feeding hydrolyzed feather meal with or without blood and rumen protected Lys to late lactation Jersey cows on energy partitioning

	Treatments[1,2]				SEM	*P*-value[3]		
	FM		FMB					
Item[4]	LYS0	LYS+	LYS0	LYS+		B	L	B x L
Components, Mcal/d								
Feces	26.6	25.5	28.2	29.3	1.74	<0.01	0.99	0.22
Methane	4.01	3.83	4.06	4.08	0.213	0.28	0.56	0.44
Urine	2.45	2.34	2.29	2.28	0.094	0.08	0.33	0.43
HP[5]	22.8	23.4	23.4	24.6	0.88	0.15	0.16	0.70
kcal/MBW	221	229	230	240	8.3	0.09	0.12	0.88
Milk	18.2	17.5	19.1	19.2	0.98	0.02	0.57	0.49
TE	5.16	2.79	3.97	2.77	1.283	0.59	0.12	0.61
TE_P	0.31	0.31	0.31	0.31	0.001	0.09	0.69	0.69
TE_F	4.86	2.48	3.66	2.46	1.284	0.59	0.12	0.61
Fractions, Mcal/d								
GE	79.1	75.4	81.1	81.8	3.29	0.07	0.50	0.32
DE	52.6	49.9	52.7	52.5	2.20	0.41	0.39	0.46
ME	46.1	43.7	46.4	46.2	2.02	0.39	0.41	0.48
NE_L[6]	31.6	28.5	31.2	30.2	1.66	0.64	0.16	0.44
Fractions, Mcal/kg of DM								
GE	4.48	4.47	4.46	4.46	0.038	0.08	0.94	0.69
DE	2.97	2.95	2.91	2.87	0.050	0.02	0.44	0.72
ME	2.60	2.58	2.56	2.53	0.050	0.13	0.49	0.79
NE_L	1.77	1.67	1.73	1.65	0.062	0.48	0.07	0.88
Efficiencies								
ME/DE	0.88	0.87	0.88	0.88	0.004	0.31	0.95	0.91
Milk/ME	0.40	0.40	0.41	0.41	0.016	0.13	0.77	0.80
HP/ME	0.50	0.54	0.51	0.53	0.020	0.71	0.10	0.55
TE/ME	0.10	0.05	0.08	0.06	0.027	0.76	0.18	0.63
TE_P/ME	0.01	0.01	0.01	0.01	0.000	0.30	0.56	0.49
TE_F/ME	0.09	0.05	0.07	0.05	0.028	0.77	0.18	0.63

[1] FM= hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 = 0 g/d of RP Lys, LYS+ = 65 g/d RP Lys, n = 46 for feces, urine, milk, GE, and DE, TE_P, n = 46 for all other variables.

[2] Least squares means; largest standard error of treatment mean is shown.

[3] B =Blood, L = Lys, and B x L = Blood x Lys interaction.

[4] HP = heat production, MBW = metabolic body weight, GE = gross energy, DE = digestible energy, TE = tissue energy, TE_P = tissue energy as body protein, TE_f = tissue energy as body fat.

[5] $HP = 3.866 \times O2 + 1.200 \times CO2 – 0.518 \times CH4 – 1.431 \times N$ (Brouwer, 1965).

[6] $NE_L = 0.080 \text{ x } BW^{0.75}$ + milk energy + tissue energy (NRC, 2001).

Table 3.8. Effects of feeding hydrolyzed feather meal with or without blood and rumen protected Lys to late lactation Jersey cows on fecal and urinary output, N excretion, secretion, and partitioning

	Treatmeants[1,2]				SEM	*P*-value[3]		
	FM		FMB					
Item	LYS0	LYS+	LYS0	LYS+		B	L	B x L
Output, kg/d (DM)								
Feces	5.84	5.61	6.20	6.43	0.307	<0.01	1.00	0.23
Urine	22.4	22.9	21.5	23.4	1.34	0.78	0.11	0.37
Mass, g/d								
N intake	474	456	491	501	22.5	0.03	0.78	0.32
Fecal N	160	154	187	193	22.8	<0.01	1.00	0.34
Urinary N	164	162	146	156	6.70	0.01	0.31	0.15
Milk N	124	119	136	138	7.10	<0.01	0.72	0.48
N balance	26.4	21.1	22.7	14.0	13.3	0.54	0.43	0.85
As proportion of N intake, %								
Fecal N	34.0	34.0	38.2	38.5	1.35	<0.01	0.83	0.87
Urinary N	35.0	35.8	30.0	31.4	1.78	<0.01	0.41	0.85
Milk N	26.1	26.0	27.5	27.5	0.76	0.03	0.93	0.85
N balance	4.91	4.19	4.27	2.64	2.851	0.58	0.56	0.82

[1] FM= hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 = 0 g/d of RP Lys, LYS+ = 65 g/d RP Lys, n = 46 for all variables.

[2] Least squares means; largest standard error of treatment mean is shown.

[3] B =Blood, L = Lys, and B x L = Blood x Lys interaction.

Table 3.9. Effects of feeding hydrolyzed feather meal with or without blood and rumen protected Lys to late lactation Jersey cows on total-tract digestibility (%)

	Treatment[1,2]				SEM	*P*-value[3]		
	FM		FMB					
Item	LYS0	LYS+	LYS0	LYS+		B	L	B x L
DM	66.8	66.6	65.9	65.1	0.87	0.07	0.47	0.66
OM	69.1	69.0	68.0	67.3	0.82	0.03	0.53	0.65
NDF	46.1	44.3	44.8	41.8	1.91	0.11	0.05	0.61
NDFom	49.2	48.3	46.8	44.9	1.76	0.01	0.21	0.66
CP	66.0	66.0	61.8	61.4	1.35	<0.01	0.83	0.87
Starch	96.9	97.2	96.9	96.7	0.58	0.38	0.91	0.25
Energy	66.3	66.1	65.2	64.5	1.27	0.05	0.46	0.72

[1] FM= hydrolyzed feather meal, FMB = hydrolyzed feather meal with blood, LYS0 = 0 g/d of RP Lys, LYS+ = 65 g/d RP Lys, n = 46 for all variables.

[2] Least squares means; largest standard error of treatment mean is shown.

[3] B =Blood, L = Lys, and B x L = Blood x Lys interaction.

GENERAL SUMMARY AND CONCULSION

Headbox-style calorimeters fitted with volumetric flow meter (**VFM**) used at the dairy metabolism facility at University of Nebraska-Lincoln are routinely evaluated for gas recoveries by burning ethyl alcohol. Internally some on the precision of gas measures existed. Additional VFM are designed with many moving parts and concerns over wear of these parts and effects on our measures of heat production also existed. The objectives of the first study were to test mass flow meter (**MFM**) and VFM by measuring O_2 consumption and CO_2 production and to illustrate the effects of incomplete gas recovery on estimated energy partitioning. As hypothesized, gas recoveries were observed to be lower for the VFM than the MFM by about 13%. The MFM resulted in higher performance than the VFM that was determined by the flow rate. Incomplete gas recovery can result in underestimates of heat production (**HP**), thereby affecting estimates of whole-animal energy use. Our results indicate that in striving for estimates of gas recovery of 95.0 ± 5.00 %, MFM may be better suited for headbox-style indirect calorimetry to estimate HP in lactating cows. The implications for this study emphasize the importance of precision in gas recovery has on energy utilization studies using headbox-style indirect calorimeters. Future research should conduct routine lamps runs to ensure precision gas recoveries are maintained and to determine if maintenance is in need of the meters or the headbox.

Hydrolyzed feather meal (**HFM**) is a widely available source of feed protein for dairy cows that is a high bypass protein. Unfortunately, the HFM has poor AA profile that is low in Lys, where low supply of Lys may limit the synthesis of milk protein. In this study, the supply of Lys was varied by feeding two sources of HFM (one containing blood and another without) and diets also varied by the inclusion of rumen-protected Lys (**RP-Lys**). In this study HFM containing blood resulted in a positive effect on feed intake, this feeding sources of HFM

containing blood may improve milk production and milk protein yield due to the increased supply of essential AA. The concentration of Lys and His plasma of cows consuming HFM containing blood were higher than HFM alone, while a reduction in CP digestibility was also observed. Energy utilization was not observed to be affected as was milk and protein yield. The addition of RP-Lys has no effect on milk production, milk protein, and energy and N utilization.

It should be noted that, not all sources of HFM containing blood possess the same chemical and AA composition as difference in processing methods exist. In this study, only single sources of HFM were used. Future research should explore the variation in chemical composition and digestibility of HFM that exist within the US feed markets. This is because hydrolysis time, pressure, and cooking method have been shown to have effect of chemical composition of animal byproducts.

APPENDIX A: FM AND FMB DIETS ACCORDING TO THE NRC DAIRY MODEL (2001)

HYDROLZED FEATHER MEAL (FM) DIET:

Summary Report

Animal Inputs

Animal Type : Lactating Cow
Age : 54 months
Body Weight : 460 kg
Milk Fat : 6.32%
Days In Milk : 200

Milk Production : 18.8 (kg/day)
Days Pregnant : 0
Breed : Jersey
Milk True Protein : 3.82%

Diet Nutrient Balances

Requirements	NE1 (Mcal/day)	MP (g/day)	Ca (g/day)	P (g/day)	K (g/day)
Maintenance	7.9	637	15	19	125
Pregnancy	0.0	0	0	0	0
Lactation	18.9	1072	27	17	28
Growth	0.0	0	0	0	0
Total Required	26.8	1709	42	36	154
Total Supplied	28.1	1809	102*	47*	234*
Balance	1.3	100	60	11	81

* Note that these mineral supplied values are total *absorbable* supplied.

Animal Performance

DMI - Actual : 17.7 (kg/day)
DMI - Predicted : 18.7 (kg/day)

NE1 Allowable Milk : 20.1 (kg/day)
MP Allowable Milk : 20.6 (kg/day)

Milk Production : 18.8 (kg/day)

Days to gain one condition score : 281

Daily Weight Change due to Reserves : 0.2 (kg/day)

Protein Values

RDP Required : 1746 (g/d)
RDP Supplied : 1808 (g/d)
RDP Balance : 62 (g/d)

RUP Required : 943 (g/d)
RUP Supplied : 1083 (g/d)
RUP Balance : 140 (g/d)

MP - Bacterial : 950 (g/d)
MP - RUP : 775 (g/d)
MP - Endogenous : 84 (g/d)

CP - Diet : 16.3 (%DM)
CP - RDP : 10.2 (%DM)
CP - RUP : 6.1 (%DM)

Diet Concentrations

NDF : 28.2 (%DM)
Forage NDF : 20.7 (%DM)
ADF : 21.8 (%DM)
NFC : 44.5 (%DM)
Undiscounted TDN : 70 (%DM)
ME : 2.50 (Mcal/kg DM)
NE1 : 1.59 (Mcal/kg DM)
NEg : 1.08 (Mcal/kg DM)
Ca : 0.9 (%DM)
P : 0.4 (%DM)
Ether-Extract : 4.2 (%DM)
DCAD : 307 (mEQ/kg)

Target Diet Concentrations

NE1 : 1.43 (Mcal/kg)
MP : 91 (g/kg)

Ca : 2 (g/kg)
P : 2 (g/kg)

Diet Summary

Feed Name	kg/day (Dry Matter)	kg/day (As-Fed)	% (Dry Matter)
2018-19 UNL Corn Silage	7.05	19.17	39.85
2018-19 UNL Alfalfa Hay	3.29	3.72	18.56
Corn Grain, ground, dry	2.94	3.33	16.58
HFM American	0.80	0.87	4.51
Soybean, Meal, solv. 48% CP	0.20	0.22	1.11
Soybean, Hulls	1.69	1.86	9.53
Molasses, Sugarcane	0.40	0.53	2.23
WheyDepro	0.48	0.51	2.72
Smartamine CNCPS 6.5	0.02	0.02	0.09
Energy Booster 100	0.22	0.22	1.24
Urea	0.09	0.09	0.52
Agipro	0.00	0.00	0.00
Calcium Carbonate	0.17	0.17	0.95
Sodium Bicarbonate	0.15	0.15	0.82
Salt	0.06	0.06	0.31
Magnesium Oxide	0.06	0.06	0.34
Calcium Phosphate (Di-)	0.10	0.10	0.54
UNL Trace Mineral Premix	0.01	0.01	0.04
UNL Vitamin premix	0.01	0.01	0.05

HYDROLYZED FEATHER MEAL WITH BLOOD (FMB) DIET:

Summary Report

Animal Inputs

Animal Type : Lactating Cow
Age : 54 months
Body Weight : 460 kg
Milk Fat : 5.89%
Days In Milk : 200

Milk Production : 20.6 (kg/day)
Days Pregnant : 0
Breed : Jersey
Milk True Protein : 3.89%

Diet Nutrient Balances

Requirements	NE1 (Mcal/day)	MP (g/day)	Ca (g/day)	P (g/day)	K (g/day)
Maintenance	7.9	652	15	19	128
Pregnancy	0.0	0	0	0	0
Lactation	19.8	1196	30	19	31
Growth	0.0	0	0	0	0
Total Required	27.8	1849	44	38	159
Total Supplied	28.4	1897	104*	46*	240*
Balance	0.6	48	60	9	81

* Note that these mineral supplied values are total *absorbable* supplied.

Animal Performance

DMI - Actual : 18.2 (kg/day)
DMI - Predicted : 19.1 (kg/day)

NE1 Allowable Milk : 21.3 (kg/day)
MP Allowable Milk : 21.4 (kg/day)

Milk Production : 20.6 (kg/day)

Days to gain one condition score : > 305

Daily Weight Change due to Reserves : 0.1 (kg/day)

Protein Values

RDP Required : 1786 (g/d)
RDP Supplied : 1868 (g/d)
RDP Balance : 82 (g/d)

RUP Required : 1075 (g/d)
RUP Supplied : 1141 (g/d)
RUP Balance : 66 (g/d)

MP - Bacterial : 971 (g/d)
MP - RUP : 840 (g/d)
MP - Endogenous : 86 (g/d)

CP - Diet : 16.5 (%DM)
CP - RDP : 10.3 (%DM)
CP - RUP : 6.3 (%DM)

Diet Concentrations

NDF : 28.2 (%DM)
Forage NDF : 20.7 (%DM)
ADF : 21.8 (%DM)
NFC : 44.0 (%DM)
Undiscounted TDN : 70 (%DM)
ME : 2.46 (Mcal/kg DM)
NE1 : 1.56 (Mcal/kg DM)
NEg : 1.07 (Mcal/kg DM)
Ca : 0.9 (%DM)
P : 0.4 (%DM)
Ether-Extract : 4.0 (%DM)
DCAD : 337 (mEQ/kg)

Target Diet Concentrations

NE1 : 1.45 (Mcal/kg)
MP : 97 (g/kg)

Ca : 2 (g/kg)
P : 2 (g/kg)

Diet Summary

Feed Name	kg/day (Dry Matter)	kg/day (As-Fed)	% (Dry Matter)
2018-19 UNL Corn Silage	7.25	19.70	39.84
2018-19 UNL Alfalfa Hay	3.38	3.82	18.55
Corn Grain, ground, dry	3.02	3.42	16.57
HFM American	0.00	0.00	0.00
Soybean, Meal, solv. 48% CP	0.20	0.23	1.11
Soybean, Hulls	1.73	1.91	9.53
Molasses, Sugarcane	0.41	0.55	2.23
WheyDepro	0.50	0.52	2.72
Smartamine CNCPS 6.5	0.02	0.02	0.09
Energy Booster 100	0.23	0.23	1.24
Urea	0.10	0.10	0.52
Agipro	0.00	0.00	0.00
Calcium Carbonate	0.17	0.17	0.95
Sodium Bicarbonate	0.15	0.15	0.82
Salt	0.06	0.06	0.31
Magnesium Oxide	0.06	0.06	0.34
Calcium Phosphate (Di-)	0.10	0.10	0.54
UNL Trace Mineral Premix	0.01	0.01	0.04
UNL Vitamin premix	0.01	0.01	0.05
HFMBlood	0.83	0.92	4.55

APPENDIX B: 2019 ADSA ANNUAL MEETING POSTER:

Use of mass flow meter in headbox-style indirect calorimetry, and the effects of gas recovery on estimated energy partitioning in lactating dairy cows

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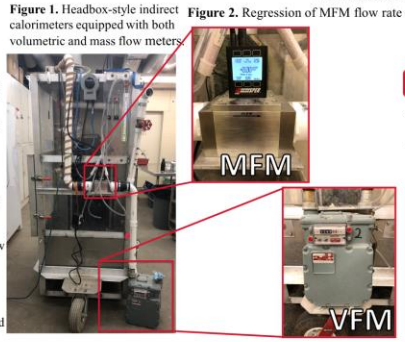
INTRODUCTION

- Headbox-style indirect calorimeters can estimate heat production (**HP**) of cattle.
- Indirect calorimetry calculates HP through O_2 consumption, CO_2 and CH_4 production, and urea production.
- The volume of gases collected are used in the Brouwer equation to estimate HP.
- Brouwer equation = 3.866 * O_2 consumption + 1.2 * CO_2 production - 0.518 * CH_4 production - 1.431 * urinary N excretion (E. Brouwer, 1965)
- Volumetric flow meters (**VFM**) have proven to be a cost effective method to measure airflow.
- Operational flow rate for VFM is 200 L/min.
- When using VFM for indirect calorimetry of dairy cattle, operational flow rate is typically operated between 600 and 1200 L/min.
- Proper operation of VFM's and accurate HP data require frequent maintenance and recalibration.
- Mass flow meters (**MFM**) are new meters that calculate the mass of airflow, instead of volume like the VFM's.
- MFM's are less labor intensive and account for barometric pressure and temperature.
- Objectives of this study were to test MFM and VFM by measuring O_2 consumption and CO_2 production and to quantify the effects of incomplete gas recovery on estimated energy partitioning.

MATERIALS and METHODS

- Headboxes were equipped with VFM and later replaced with MFM (n=3).
- Effects of the type of airflow meter on O_2 consumption and CO_2 production was determined by burning ethanol (100%) for 2 hours.
- Efficiency was calculated as the proportion of O_2 and CO_2 recovered from the amount of alcohol burned.
- A subsample of gas was collected in a bag and each were analyzed using gas chromatography .
- The differences in measured flow rate between VFM and VFM flow rate was determined simultaneously at 5 different flow rates (Figure 1) .
- Data was analyzed using a paired t-test and proc reg of SAS.

Figure 1. Headbox-style indirect calorimeters equipped with both volumetric and mass flow meters.



RESULTS

Table 1. Effect of O_2 and CO_2 recovery (%) using volumetric and mass flow meters while burning ethanol for two hours in indirect calorimetry headboxes

	Flow meter[1]		SEM	*P*-value
Items	VFM	MFM		
O_2 recovery, %	86.7*	100.0	1.44	0.01
CO_2 recovery, %	85.7*	98.1	2.58	<0.01

*Difference from 100 % gas recovery ($P < 0.05$), [1]VFM = Volumetric flow meter (Model AL425, American Meter, Horsham, PA), MFM = Mass flow meter (MCW Whisper, Alicat Scientific, Tucson, AZ).

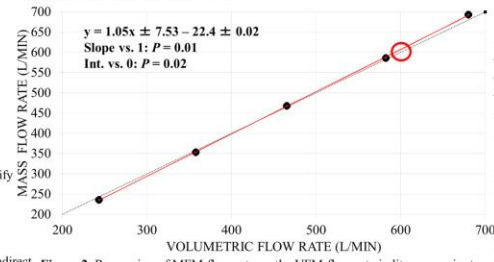


Figure 2. Regression of MFM flow rate on the VFM flow rate in liters per minute.

Figure 3. Headbox-style indirect calorimeter using VFM with a lactating Jersey cow while collecting gas (O_2, CO_2, CH_4) in bags for 23 hours using VFM.

Table 2. The effects of gas recovery on estimated energy partition in lactating Jersey cows[1]

	Gas recovery, %					
Items[2]	100	95	90	85	80	75
Gases						
O_2 consumption, L/d	5000	4750	4500	4250	4000	3750
CO_2 production, L/d	5250	4988	4725	4463	4200	3938
CH_4 production, L/d	400	380	360	340	320	300
Energy[3]						
ME intake, Mcal/d	48.1	48.3	48.5	48.6	48.8	49.0
NE_l intake, Mcal/d	32.7	34.2	35.7	37.1	38.6	40.0
NE_l /ME	0.68	0.71	0.74	0.76	0.79	0.82
Energy fractions						
Methane, Mcal/d[4]	3.78	3.59	3.40	3.21	3.02	2.84
Heat production, Mcal/d[5]	25.1	23.8	22.6	21.3	20.0	18.7
Recovered energy, Mcal/d[6]	23.0	24.4	25.9	27.4	28.8	30.3
Tissue energy, Mcal/d[7]	0.00	1.45	2.91	4.37	5.83	7.29
Tissue/GE	0.00	1.79	3.58	5.37	7.17	8.96
Tissue/NE_l	0.00	4.44	8.90	13.4	17.8	22.3

[1] Jersey cow, Production - BW = 450 kg, DMI = 18.5 kg, milk yield = 25 kg/d, fat % = 5.70, protein % = 3.60, lactose % = 4.85, urinary nitrogen = 225 g/d, Energy - gross energy intake = 81.4 Mcal/d, digestible energy intake = 55.4 Mcal/d, Energy fractions – fecal energy = 26.0 Mcal/d, urine energy = 3.5 Mcal/d, milk energy = 23.0 Mcal/d, maintenance energy = 9.77 Mcal/d, [2] ME = metabolizable energy, NE_l = net energy of lactation, GE = gross energy, [3] NE_l intake = milk energy + tissue energy + maintenance energy, [4] Methane = CH_4 * 0.00945, [5] Brouwer equation = 3.866 * O_2 consumption + 1.2 * CO_2 production - 0.518 * CH_4 production - 1.431 * urinary N excretion,[6] Recovered energy = metabolizable energy – heat production, [7] Tissue energy = recovered energy – milk energy.

DISCUSSION

- MFM may yield more precise measures HP (Table 1).
- At operational flow (600 L/min for lactating Jerseys), VFM is 98.7% the flow rate of MFM (Figure 2).
- Incomplete gas recovery can result in underestimates of HP. This affects estimates of whole-animal energy use, a 5.0% decrease in gas recovery results in a reduction in HP by 1.30 Mcal/d and increases tissue energy by 1.45 Mcal/d, resulting in an increased net energy of 1.46 Mcal/d (Table 2).

CONCLUSIONS

- Strive for estimates of gas recovery of 95-105%.
- MFM may be better suited for headbox-style indirect calorimetry to estimate HP in lactating cows.
- MFM offer the advantage of having an easy to record voltage output, a built in temperature, and pressure compensation.

REFERENCES

- Brouwer, E. 1965. Report of sub-committee on constants and factors. Pages 441-443 in Proc. Energy Metabolism. Publication no. 11. European Association for Animal Production, Ayr, Scotland.

Abstract # W159

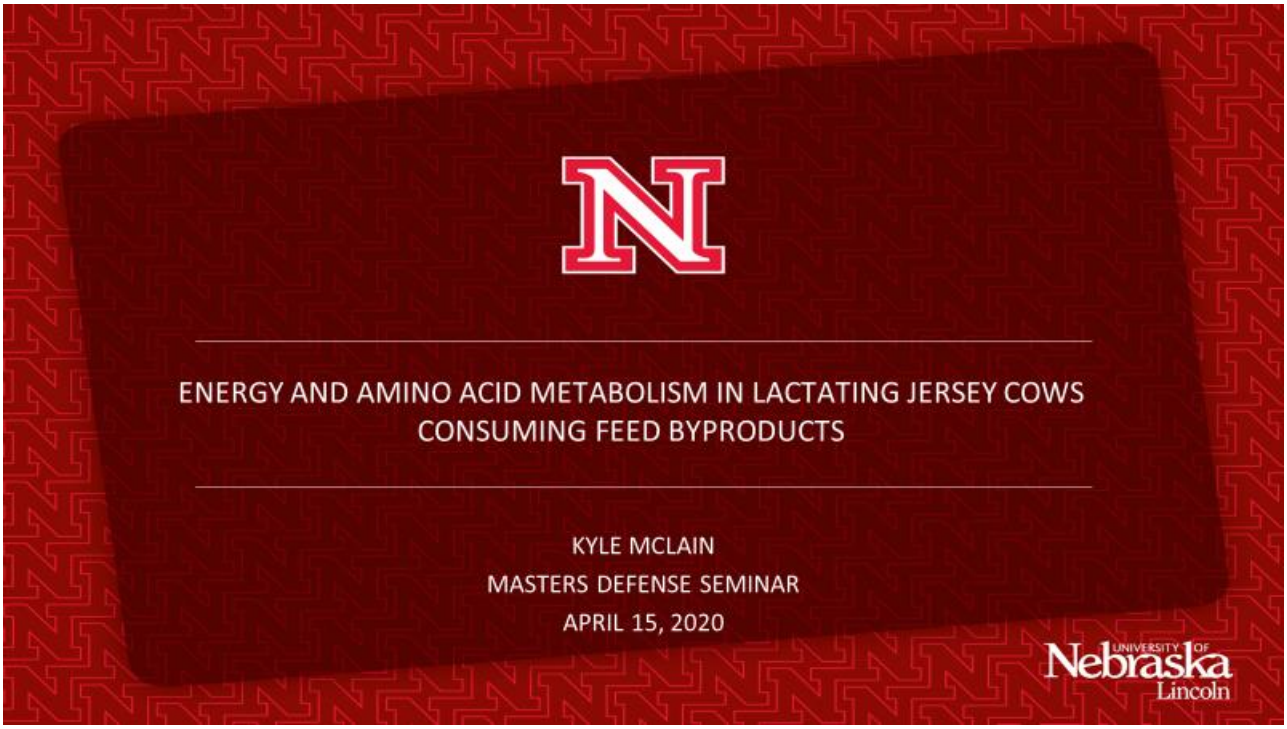
APPENDIX C: HEADBOX AND METER PARTS LIST:

ITEM	SUPPLIER	ADDRESS	MODEL No.	PHONE	PRICE	DETAILS
Fecal Bags/ Hoods	Hastings Canvas	230 Eastside Blvd. Hastings, NE 68901		402-462-6615		
Welding work	Wahoo Metal Products	130 W. 4th St. Wahoo, NE 68066		402-443-3448	$3200	
Gas meters	Central States Group/ Mueller Sales	520 50th Ave Dr. SW Cedar Rapids, IA 52404	American Meter, Horsham, PA AL425-TC 10#	800-332-0159	$479.80	Top connection size- 1 ¼ Index- odometer Drive output- 1 ft
Vacuum motor	Central Vacuum Factory	P.O. Box 9062 Baskersfield, CA 93389	Ametek Lamb Electric, Kent, OH 115923 2-stage 5.7" vacuum motor 120 volt	877-822-7868	$93.99	Max Air Watts: 447 Max Air Flow: 122 CFM Motor Speed: 23,700 RPM
U-tube manometer	Park Supply of America	2727 E. 26th St. Minneapolis, MN 55406	United Instruments, Westbury, NY Item #: 1221-8 Order #: 02000193	800-877-9449 ext. 228	$39.43	Range (in): 0-8
Variable Transformer	A-I Consolidated Inc.	4970 N. Manufacturing Way Ste 2 Coeur D Alene, ID 83815-6028	Staco Energy Products Co., Dayton, OH 3PN1010B	800-635-1545	$314.07	Input 120 V 50-60 Hz Output 0-140 V 10 Amp 1.4 KVA
GMI Caps	Central States Group/ Mueller Sales	520 50th Ave Dr. SW Cedar Rapids, IA 52404	21737P082	800-332-0159	$5.95 (x2)	

GMI Straight 30LT Swivel	Central States Group/ Mueller Sales	520 50th Ave Dr. SW Cedar Rapids, IA 52404	2897P084	800-332-0159	$10.50 (x2)	
Washer 30LT	Central States Group/ Mueller Sales	520 50th Ave Dr. SW Cedar Rapids, IA 52404	59061P005	800-332-0159	$3.85 (x2)	
Glass Tube Rotameter	The Meter & Valve Company	1195 S. Pierce St. Lakewood CO 80232	Brooks Instruments, Hatfield, PA 1350E Sho-Rate "50" Serial #: 01B20410079 Model #: 1350EJA6AEB1A	800-876-2826	$315 (x2)	Low-flow glass tube variable area meter Air Capacity: m3n/hr: 0.002-3.7 scfm: 0.001-2.33
Drierite Drying Tube	WA Hammond Drierite Co. Ltd.	PO Box 460 Xenia, OH 45385-0460	26930 30 g Drierite Max Flow Rate: 300 cm^3/min	937-376-2927	$6.30 (x2)	¾" o.d. x 8" length hose barbs for ¼" to 3/8" i.d. flexible tubing Water capacity:3 g.
Gas Sample Bags	PMC	1013 S. Lyman Ave. Oak Park, IL 60304		708-383-7794	$21.02	24" x 24" LAM-JAPCON-NSE 44L
Pocket Logger	Pace Scientific Inc.	PO Box 4418 Moorseville, NC 28117	XR440	704-799-0688	$399	Stores up to 32,256 readings Temp: -40 to 60°C/140F
Temperature/ Relative Humidity Probe	Pace Scientific Inc.	PO Box 4418 Moorseville, NC 28117	TRH-100	704-799-0688	$205	Accuracy ± 3% RH from 0-95% RH
Let-Up Udder	eNasco		C17683N		$68.25	

Support with Neck Strap						
Air/vacuum pump	Bianaca Products Inc.	41636 Enterprise Circle N., Unit A, Temecula, CA 92590	BP 202-1 115 VAC	951-296-3397	$137.71	Variable speed, with mount
Water bowls	Nebraska Dairy System	Norfolk, NE	S22	402-371-7293	$225	
Stopcocks	Optics Planet		Code: NL-LB-6460-0004 MPN: 6460-0004		$45 (x2)	Nalge Nunc Stopcocks, polypropylene with Teflon resin TFE plug
Water Meters	DLJ Watermeters		GLJGHT Garden Hose Water Meter		$79.95	
Alicat Flow Meter	Alicat Scientific	Alicat Scientific Inc. 7641 N Business Park Dr. Tucson, AZ	Whisper Series Mass Flowmeter MW-1000SLPM-D	520-290-6060	$4764.25	
Alicat Power Adapter	ALicat Scientific		PVPS24U Power Adapter	520-290-6060	$52.25	
Alicat Cable	Alicat Scientific	Alicat Scientific Inc. 7641 N Business Park Dr. Tucson, AZ	DC-61 6’ Single Ended mini-din cable 8 conductor	520-290-6060	$28.50	

APPENDIX D: THESIS PRESENTATION:



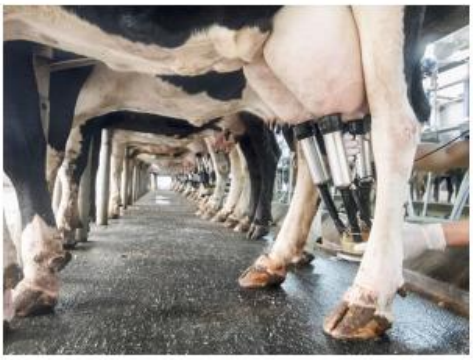
Background

- Grow up on a small beef cattle operation
  - But surrounded by dairy's
- The family owned and operated the local butcher shop
- Undergraduate degree from the University of New Hampshire-Durham

DAIRY RESEARCH

Introduction

- Dairy production systems have become more efficient in producing milk over the last century
- In 1944
  - Cow population = 25.6 million
  - Milk production = 53.0 billion kg
- In 2007
  - Cow population = 9.2 million
  - Milk production = 84.2 billion kg
- 59% more milk with 64% less cows



Capper et al. 2009

DAIRY RESEARCH

The Dairy Industry

- Modern dairy practices require less resources for milk production
  - Cows consume 77% less feed and 65% less water
  - 10% reduction in land
- Problem
  - Land is continuing to decrease
  - Need of alternatives like animal protein byproducts

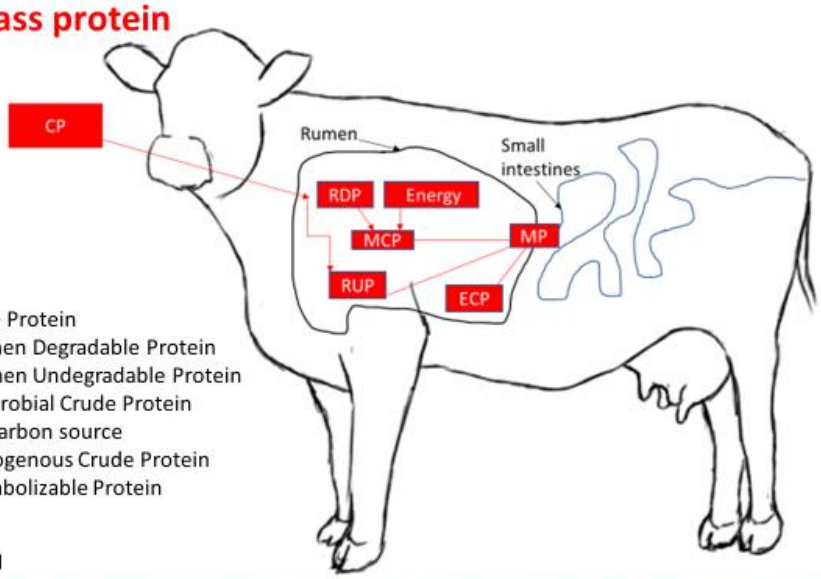
von Keyserlingk et al., 2013

The Dairy Industry

- Why an increase in milk production?
  - Byproducts
  - Efficient use of bypass protein and AA
  - Efficient use of energy partitioning

Vandehaar and St-Pierre, 2006

Bypass protein



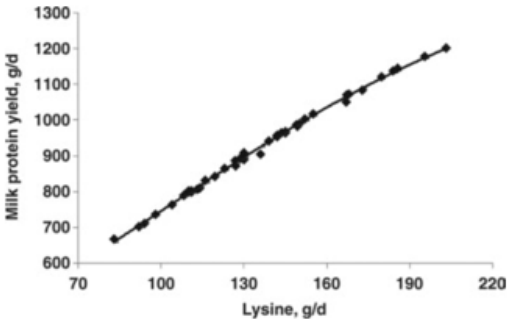
CP = Crude Protein
RDP = Rumen Degradable Protein
RUP = Rumen Undegradable Protein
MCP = Microbial Crude Protein
Energy = Carbon source
ECP = Endogenous Crude Protein
MP = Metabolizable Protein

DAIRY RESEARCH

Essential Amino Acids (EAA)

- Ten EAA
  - Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val
- Limiting AA Met and Lys (Schwab et al. 2005)
  - If Lys is limiting, milk protein synthesis may be reduced (Vyas and Erdman, 2009)
- Problem

↓ milk protein = ↓ profitability



Vyas and Erdman, 2009

DAIRY RESEARCH

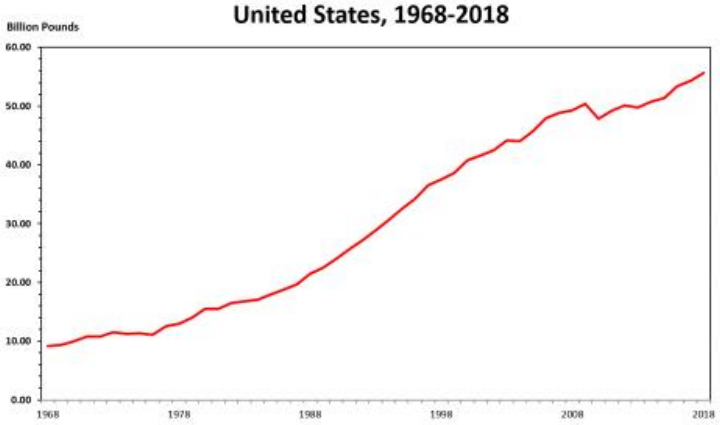
Animal Protein Byproducts

- Blood meal (**BM**)
  - CP = 96%
  - RUP = 77%
  - Lys = 8.98% of CP
- Hydrolyzed feather meal (**HFM**)
  - CP= 92%
  - RUP = 65%
  - Lys = 2.57% of CP

NRC, 2001

U. S. A. Poultry Industry

Broiler Pounds Produced
United States, 1968-2018



USDA-NASS
06/25/2019

Problems with HFM

- Poor AA profile in terms of Met and Lys
  - Lys = 2.57% of CP
  - Lead to lower milk protein content (Harris et al., 1992)
- Increase Lys profile with the addition of poultry blood
  - Has been investigate in beef cattle but not dairy cattle (Goedeken et al, 1990)

HFM Containing Blood

- Improve nutrient value of HFM
  - Blood = high Lys
  - Should increase Lys supply of the cow
  - Should increase milk protein
- ↑ inclusions of HFM with no blood, ↓ milk protein yield due to low supply of EAA (Morris et al., 2020)

Objectives

- The objectives of this study were to evaluate nutrient composition and digestibility of HFM with blood fed to lactating dairy cows

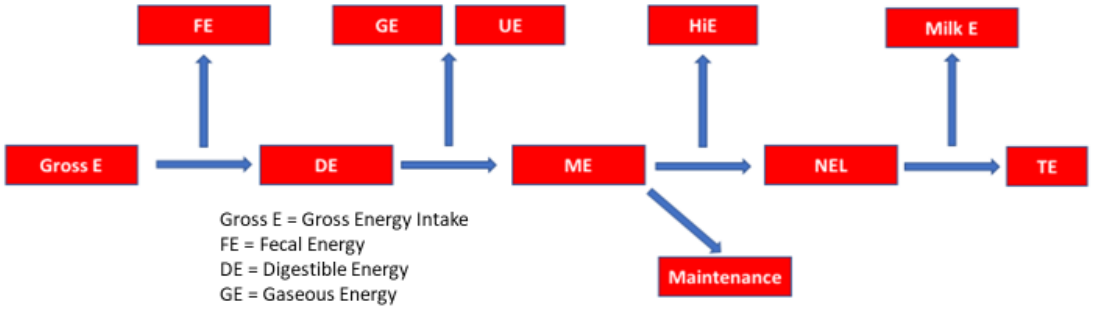
DAIRY RESEARCH

Use of a mass flow meter in headbox-style indirect calorimetry, and the effects of gas recovery on estimated energy partitioning in lactating dairy cows

K. A. McLain, K. K. Buse, T. M. Brown-Brandl, D.L. Morris, P. J. Kononoff

University of Nebraska Lincoln

Energy Balance



Gross E = Gross Energy Intake
FE = Fecal Energy
DE = Digestible Energy
GE = Gaseous Energy
UE = Urinary Energy
ME = Metabolizable Energy
HiE = Heat Increment
Maintenance = Maintenance Energy
NEL = Net Energy of Lactation
Milk E= Milk Energy
TE = Tissue Energy

DAIRY RESEARCH

Headbox-Style Indirect Calorimetry

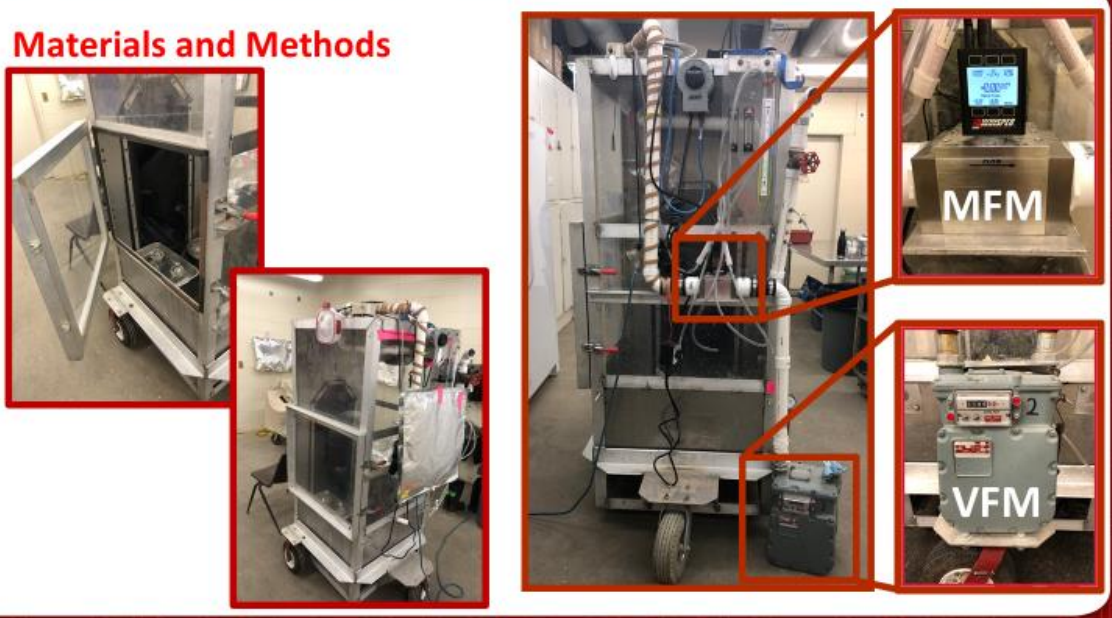
Introduction

- Volumetric flow meters (**VFM**)
  - Reduced gas recovery
  - Operational flow rate = 200 L/min
- Dairy cattle flow rate range between 850 and 1200 L/min
- Mass flow meter (**MFM**) may be an advantage
  - Less maintenance and recalibration
  - Account for changes in barometric pressure and temperature
- Cost
  - VFM = $500
  - MFM = $4800

Objectives

1. Evaluate new MFM and compare them to the VFM by conducting a series of procedures in which the recovery of gasses are estimated in a controlled environment
2. Quantify and discuss the effects of incomplete gas recovery on estimated energy partitioning.

Materials and Methods



Results

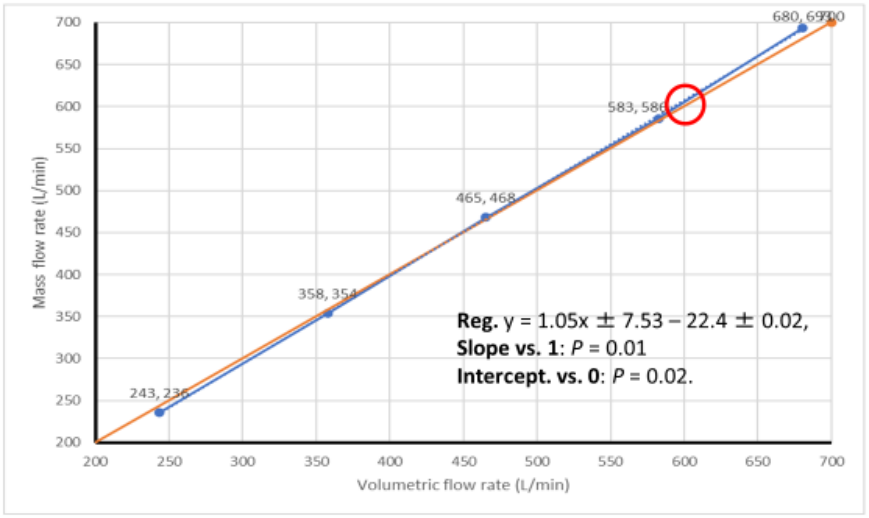
Table 1. Effect of oxygen and carbon dioxide recovery (%) using volumetric and mass flow meters when burning ethanol for 2 hours in indirect calorimetry headboxes

	Flow meter[1]		SEM	*P*-value
Items	VFM	MFM		
O_2 recovery, %	86.7*	100.0	1.44	0.01
CO_2 recovery, %	85.7*	98.1	2.58	< 0.01

* Difference from 100 % gas recovery ($P < 0.05$).
[1] VFM = Volumetric flow meter (Model AL425, American Meter, Horsham, PA), MFM = Mass flow meter (MCW Whisper, Alicat Scientific, Tucson, AZ).

DAIRY RESEARCH

Figure 1. Regression of MFM flow rate on the VFM flow rate in liters per minute.



At operational flow (600 L/min for lactating Jerseys), VFM is 98.7% the flow rate of MFM.

Table 2. The effects of gas recovery on estimated energy partition in lactating Jersey cows[1]

	Gas recovery, %			
Items	100	95	90	85
Gases				
O_2 consumption, L/d	5000	4750	4500	4250
CO_2 production, L/d	5250	4988	4725	4463
CH_4 production, L/d	400	380	360	340
Energy				
ME intake, Mcal/d	48.1	48.3	48.5	48.6
NE_l intake, Mcal/d	32.7	34.2	35.7	37.1
Energy fractions				
Heat production, Mcal/d	25.1	23.8	22.6	21.3
Tissue energy, Mcal/d	0.00	1.45	2.91	4.37

[1] Jersey cow, Production - BW = 450 kg, DMI = 18.5 kg, milk yield = 25 kg/d, fat % = 5.70, protein % = 3.60, lactose % = 4.85, urinary nitrogen = 225 g/d, Energy - gross energy intake = 81.4 Mcal/d, digestible energy intake = 55.4 Mcal/d, Energy fractions – fecal energy = 26.0 Mcal/d, urine energy = 3.5 Mcal/d, milk energy = 23.0 Mcal/d, maintenance energy = 9.77 Mcal/d.

Conclusions

- Strive for gas recoveries of 95 – 105%
- Conduct lamp runs before using headboxes
- MFM may be superior for using headbox style indirect calorimetry to estimate heat production
  - Advantages
    - Easy to record voltage output
    - Built in temperature and pressure compensation

DAIRY RESEARCH

Effect of feeding hydrolyzed feather meal with or without blood with rumen protected lysine on milk protein and energy utilization in late lactation dairy cows

K. A. McLain, D. L. Morris and P. J. Kononoff

University of Nebraska Lincoln

Introduction

- Hydrolyzed feather meal
  - Byproduct of the poultry industry
  - 92% CP and 65% RUP
  - Hydrolysis process requires high temperature and pressure to disrupt the keratin bonds to improve digestibility
- Issues
  - May cause a reduction in milk protein (Harris et al., 1992)
    - Hydrolysis process is believed to have negative effect on AA availability
    - Low concentration of some AA (Stahel et al., 2014)

DAIRY RESEARCH

Introduction

- Lysine supply may limit milk protein synthesis (Vyas and Erdman, 2009)
- HFM is lower in Lys compared to blood meal
  - 2.57% vs. 8.98% of CP (NRC, 2001)
- Rumen-protected Lys (RP-Lys) source can increase Lys supply (Giallongo et al., 2016)

DAIRY RESEARCH

Introduction

- AA analysis is costly
- Nutritionist will relay on book values
  - May vary in AA content
- HFM
  - Contain blood or no blood
    - affects AA concentration (especially Lys)
- Addition of blood to HFM improved both quantity and quality of the ruminal escape protein in beef cattle (Goedeken et al., 1990)

Objectives

- To determine the effects of feeding HFM with or without blood with an RP-Lys on milk production, milk protein, and energy and N utilization
- We hypothesized that feeding a source of HFM containing blood with RP-Lys to late lactation dairy cows would result in an increased and additive effect on milk protein

Materials and Methods- Experimental Design

- 3 × replicated 4 × 4 Latin square
- Each cow was randomly assigned to 1 of 4 treatments
- 28 day periods
  - 23 days of adaption
  - 5 days of collection
- Cows were grouped by
  - Milk yield
  - DMI

Materials and Methods-Animals

- 12 multiparous Jersey cows
  - 226 ± 22 DIM
  - 70 ± 8 d at the start and 161 ± 8 d pregnant at the end
    - Fetal energy assumed to be zero (NRC,2001)
- Milked twice daily
  - 0700 and 1800 h
- Housed in UNL Dairy Metabolism Facility
  - Environmentally control temperature

Materials and Methods – Treatments

	HFM without blood and no RP-Lys	HFM with blood and no RP-Lys	HFM without blood and RP-Lys	HFM with blood and RP-Lys
Blood or No Blood	No blood	Blood	No Blood	Blood
Lys or No Lys	No Lys	No Lys	Lys	Lys
Lys Amount (g/d)	0	0	65	65
Available Digestible Lys (g/d)	0	0	22	22

DAIRY RESEARCH

Materials and Methods- TMRs

Items	Hydrolyzed Feather Meal without Blood	Hydrolyzed Feather Meal with Blood
Ingredients		
Corn silage	39.9	39.9
Alfalfa hay	18.6	18.6
HFM	4.51	0.00
HFM with blood	0.00	4.51
Corn grain, ground	16.6	16.6
Soybean meal	1.11	1.11
Soybean hulls	9.53	9.53
Molasses, sugarcane	2.23	2.23
Whey	2.72	2.72
Rumen-protected Met	0.09	0.09
Fat	1.24	1.24
Urea	0.52	0.52
Mineral-vitamins mix	3.05	3.05

Materials and Methods- Collections

5 d collections at 95% ad libitum

- TMR
- Feed Ingredients
- Refusals
- Fecal and urine collections
- Milk samples (AM and PM)

23 d ADAPTATION

PERIOD START — PERIOD ENDS

28 d PERIOD

1 d collection

- Heat production (23h/ cow)

2 d collection

- Blood samples

DAIRY RESEARCH

Materials and Methods- Collections

DAIRY RESEARCH

Materials and Methods- Statistical Analysis

- PROC GLIMMIX function of SAS
- Fixed effects
  - Blood level
  - Lys level
  - Blood and Lys interaction
- Random effects
  - Period, square, and cows nested in square
- Significance declared with a *P*-value ≤ 0.05

Results and Discussion- Diet and HFMs Chemical Composition

	FM, TMR		FMB, TMR		FM, Ingredient	FMB, Ingredient
Item	Mean	SD	Mean	SD	Mean	Mean
DM, % as is	68.7	0.01	68.7	0.01	91.7	90.0
CP	17.1	0.62	17.5	0.59	87.2	90.7
ADF	21.5	1.05	22.0	0.94	-	-
Crude fat	3.89	0.280	3.76	0.267	10.9	6.46
Ash	8.57	0.247	8.57	0.634	3.13	1.84
AA						
% Lys	-	-	-	-	2.08	3.12
% Met	-	-	-	-	0.65	0.71
% His	-	-	-	-	0.72	1.53

Feed intake, Milk Production, and Milk Components

	Treatments				SEM			
	HFM without Blood		HFM with Blood			*P*-value		
Items	LYS0	LYS+	LYS0	LYS+		Blood	Lys	Blood x Lys
DMI, kg/d	17.7	16.9	18.2	18.3	0.72	0.06	0.53	0.34
Milk yield, kg/d	18.8	18.0	20.6	20.4	1.31	0.01	0.55	0.61
ECM, kg/d	26.6	25.6	28.0	28.1	1.42	0.02	0.56	0.48
Fat, %	6.32	6.33	5.89	5.95	0.341	0.02	0.86	0.88
Fat, kg/d	1.16	1.12	1.18	1.19	0.065	0.27	0.64	0.43
Protein, %	3.82	3.82	3.89	3.89	0.119	0.12	0.97	0.98
Protein, kg/d	0.70	0.68	0.79	0.79	0.040	<0.01	0.66	0.68
Lactose, %	4.71	4.72	4.73	4.72	0.058	0.76	0.83	0.71
Lactose, kg/d	0.89	0.85	0.97	0.97	0.067	0.01	0.56	0.66

Plasma EAA and Carnosine Concentration (µM)

	Treatments				SEM			
	HFM without Blood		HFM with Blood			*P*-value		
Items	LYS0	LYS+	LYS0	LYS+		Blood	Lys	Blood x Lys
His	16.1	19.6	30.3	25.3	3.15	<0.01	0.69	0.03
Lys	70.5	71.1	83.9	80.3	4.06	<0.01	0.63	0.51
Met	25.8	23.7	26.6	25.7	1.18	0.17	0.16	0.57
Carnosine	8.55	8.81	10.9	9.85	0.854	<0.01	0.35	0.12

DAIRY RESEARCH

Energy Utilization

	Treatments				SEM			
	HFM without Blood		HFM with Blood			*P*-value		
Items	LYS0	LYS+	LYS0	LYS+		Blood	Lys	Blood x Lys
Components Mcal/d								
Feces	26.6	25.5	28.2	29.3	1.74	<0.01	0.99	0.22
Methane	4.01	3.83	4.06	4.08	0.213	0.28	0.56	0.44
Urine	2.45	2.34	2.29	2.28	0.094	0.08	0.33	0.43
HP[5]	22.8	23.4	23.4	24.6	0.88	0.15	0.16	0.70
kcal/MBW	221	229	230	240	8.3	0.09	0.12	0.88
Milk	18.2	17.5	19.1	19.2	0.98	0.02	0.57	0.49
TE	5.16	2.79	3.97	2.77	1.283	0.59	0.12	0.61

DAIRY RESEARCH

Energy Utilization

	Treatments				SEM			
	HFM without Blood		HFM with Blood			*P*-value		
Items	LYS0	LYS+	LYS0	LYS+		Blood	Lys	Blood x Lys
Fractions, Mcal/kg of DM								
GE	4.48	4.47	4.46	4.46	0.038	0.08	0.94	0.69
DE	2.97	2.95	2.91	2.87	0.050	0.02	0.44	0.72
ME	2.60	2.58	2.56	2.53	0.050	0.13	0.49	0.79
NE_L	1.77	1.67	1.73	1.65	0.062	0.48	0.07	0.88

DAIRY RESEARCH

Nitrogen Utilization

	Treatments				SEM			
	HFM without Blood		HFM with Blood			*P*-value		
Items	LYS0	LYS+	LYS0	LYS+		Blood	Lys	Blood x Lys
N intake, g/d	474	456	491	501	22.5	0.03	0.78	0.32
Fecal N, % of N intake	34.0	34.0	38.2	38.5	1.35	<0.01	0.83	0.87
Urinary N, % of N intake	35.0	35.8	30.0	31.4	1.78	<0.01	0.41	0.85
Milk N, % of N intake	26.1	26.0	27.5	27.5	0.76	0.03	0.93	0.85

DAIRY RESEARCH

Total-Tract Digestibility

	Treatments				SEM			
	HFM without Blood		HFM with Blood			*P*-value		
Items	LYS0	LYS+	LYS0	LYS+		Blood	Lys	Blood x Lys
DM	66.8	66.6	65.9	65.1	0.87	0.07	0.47	0.66
OM	69.1	69.0	68.0	67.3	0.82	0.03	0.53	0.65
NDF	46.1	44.3	44.8	41.8	1.91	0.11	0.05	0.61
NDFom	49.2	48.3	46.8	44.9	1.76	0.01	0.21	0.66
CP	66.0	66.0	61.8	61.4	1.35	<0.01	0.83	0.87
Starch	96.9	97.2	96.9	96.7	0.58	0.38	0.91	0.25
Energy	66.3	66.1	65.2	64.5	1.27	0.05	0.46	0.72

Mobile bag data for FM and FMB (total tract CP digestibility); 81.9 and 69.2, respectively.

DAIRY RESEARCH

Summary

- Milk yield and milk protein yield increase with HFM with blood
  - ↑ DMI and N intake
  - ↑ Plasma concentration of Lys and His
  - Energy not a factor in ↑ milk protein
  - ↓ total-tract CP digestibility
- Issues
  - Rendering processing methods (Cotanch et al., 2020)

Conclusions

- Feeding dairy cows HFM with blood may increase milk and milk protein yield
  - Higher EAA concentration
- His may play an important role in increasing milk and milk protein yield

Future Research

- High variation in chemical and AA composition among HFM with or without blood
  - Differences in rendering processing methods
- Should seek to evaluate key differences in rendering process that may affect digestibility in HFM

Thank you!

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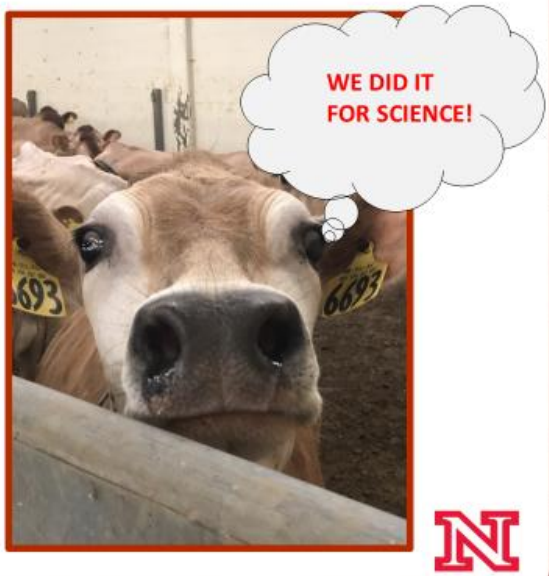

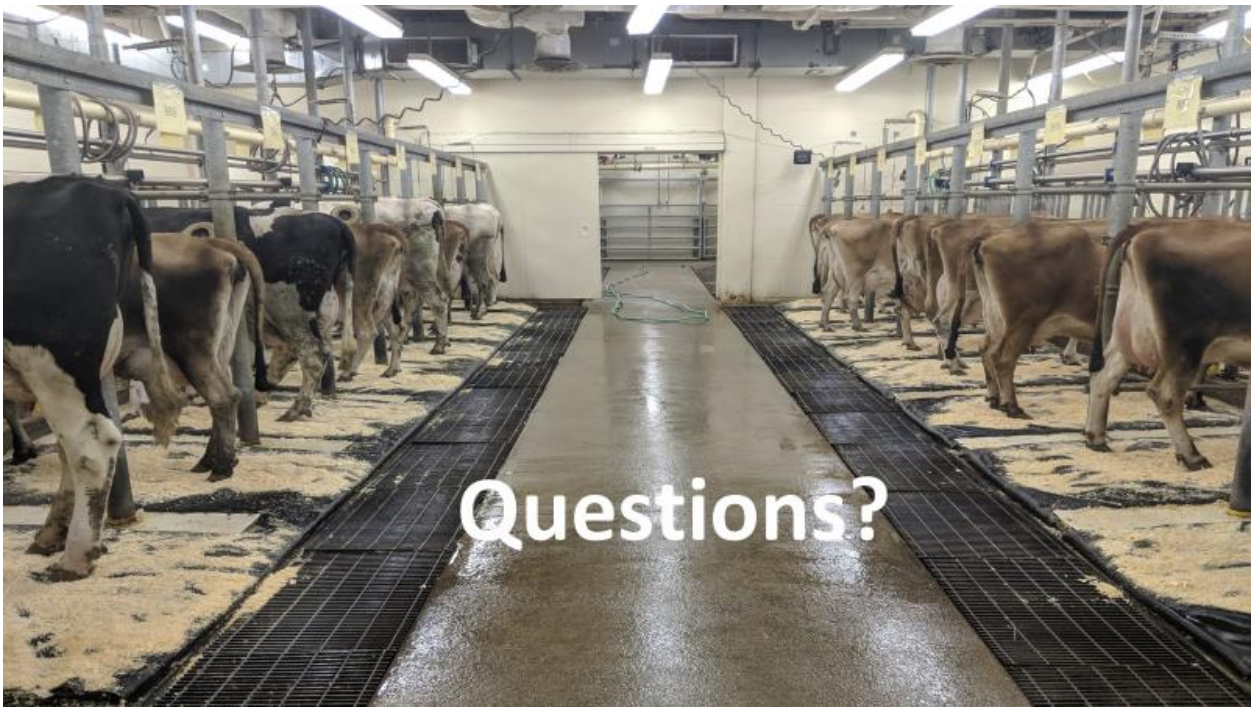

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