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ENERGY METABOLISM IN JERSEY COWS: IMPROVING OUR
UNDERSTANDING OF ENERGY REQUIREMENTS AND UTILIZATION

by

Dennis Logan Morris

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Animal Science

(Ruminant Nutrition)

Under the Supervision of Professor Paul J. Kononoff

Lincoln, Nebraska

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ENERGY METABOLISM IN JERSEY COWS: IMPROVING OUR
UNDERSTANDING OF ENERGY REQUIREMENTS AND UTILIZATION

Dennis L. Morris, PhD

University of Nebraska, 2020

Advisor: Paul J. Kononoff

Energy is the most limiting nutrient for high producing lactating dairy cows and improving our understand of factors affecting energy supply should improve our ability to estimate net energy of lactation (**NE_L**), milk production, and tissue accretion. To achieve this object, firstly, three experiments were completed to determine 1) maintenance energy requirements; 2) factors affecting heat production; and 3) the relationship between urinary N and energy excretion. Three additional experiments using lactating Jersey cows were completed to evaluate energy utilization of diets varying in 1) supply of Lys and His; 2) fatty acids (**FA**) and starch; and 3) FA, starch, and Lys. Maintenance energy requirement was 0.102 ± 0.0071 Mcal/kg of metabolic body weight (**MBW**). Heat production was most effectively explained by MBW and dry matter intake (**DMI**), and heat production associated with milk protein was two-fold that associated with milk fat. Urinary energy excretion was linearly associated with urinary N. Milk protein yield increased when rumen-protected His was added to a diet containing hydrolyzed feather meal. When adding rumen-protected Lys, milk protein yield was unaffected, but N balance increased. Feeding a high-starch compared to a high-fat diet increased milk protein yield, utilization of dietary N for milk secretion, and tissue energy deposition at fat. Although diets were formulated to be isoenergetic, the high-starch diet

had a greater measured NE_L (1.83 vs. 1.67 ± 0.036 Mcal/kg of DM) which indicates that energy models need improved. In the final experiment, although increasing FA quadratically increased ME content, ME supply decreased at high FA. Increasing supplemental Lys increased milk protein content at low dietary starch, but not at high dietary starch. Increasing supplemental Lys increased N balance at high supplemental Lys. Increasing starch increased conversion of dietary N into milk N. Results suggest that NE_L supply is interrelated to protein metabolism, energy from fat or starch differentially affect milk production and N utilization, and Lys may be preferentially utilized by muscle tissue.

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PREFACE

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CHAPTER 1: DISSERTATION INTRODUCTION

Maximizing the efficiency of nutrient utilization by lactating dairy cows is vital to maintain a dairy farm's economic and environmental sustainability. From an economic standpoint, energy and metabolizable protein (**MP**) account for 90% of total cost for diets fed to lactating dairy cows (Tebbe, 2020). From an environmental standpoint, excretion of N in manure from dairy cows contributes to environmental pollution and is associated with global warming, poor air quality, and fresh water algal bloom formation (EPA, 2004). It is well established that diets can have a profound impact of how a dairy cow utilizes nutrients. Energy is generally considered the most limiting nutrient for milk production in high producing lactating dairy cows. Accurate representation of energy supply and requirements for milk and maintenance are essential to predict milk production and BW change (NRC, 2001). Increasing energy supply will increase efficiency of energy utilization for milk and tissue deposit via a dilution of the proportion of energy used for maintenance (Vandehaar, 1998). Balancing for the supply of individual amino acids (**AA**) is suggested to increase efficiency of dietary N utilization for milk N (Patton et al., 2014) and may provide opportunities to decrease ration cost by decrease CP inclusion.

Maintenance energy accounts for approximately 25% of the NE_L requirements for a high-producing lactating Jersey cow. However, maintenance requirements are estimated from data generate exclusively from Holsteins and completed over 50 years ago (Flatt et al., 1965b). It is unknown if Jersey will differ in maintenance energy requirements. In 2018, Jerseys account for 7.7% of cows on Dairy Herd Improvement testing which has increased from 4.3% in 2008 (Council on Dairy Cattle Breeding, 2020). Since the 1960s,

milk production has more than doubled and body size has also increased (Hansen, 2000; Britt et al., 2018), which in turn, may affect how an animal utilizes energy.

Current versions of the NRC (2001) and CNCPS v6.5 (Fox et al., 2004) assume that the conversion of DE into ME and ME into NE_L are nearly constant and equal to 0.85 and 0.63, respectively. The difference between DE and ME is gas and urinary energy and equations accounting for sources of variation in gas and urinary energy could improve accuracy in estimating ME. Numerous equations are available to estimate methane production (Appuhamy et al., 2016a), the predominant energy-containing molecule in gas produced from ruminants. However, equations to estimate urinary energy are dated and generally derived from data from experiments using sheep and beef cattle (Blaxter, 1989). Heat production, which represents the difference between ME and NE_L plus maintenance, accounts for about 30% of total energy intake of a lactating dairy cow (Coppock, 1985). Various factors affect heat production and including N intake, feed intake, and dietary forage content (Tyrrell et al., 1970; Reynolds et al., 1991; Reed et al., 2017). Better understanding of these factors can improve our knowledge of energy metabolism and allow for dietary manipulation to maximize a dairy cow's energy efficiency.

Manipulating MP supply and AA profile of MP can influence whole-animal N utilization efficiency. For example, increasing dietary protein increases urinary N excretion (Spek et al., 2013b). However, the addition of individual rumen-protected AA can be an effective tool to meet requirements of specific AA and increase milk protein yield and shift N utilization to increase N efficiency (Lee et al., 2012b; Giallongo et al., 2015b; Giallongo et al., 2016). Compared to the scenario in which MP is feed in excess

of requirements, a diet deficient in MP diet and supplemented with rumen-protected Met, Lys, and His maintained milk protein production and increased the efficiency of dietary N incorporation into milk N (Lee et al., 2012b). Therefore, supplementing rumen-protected AA might be an effective tool to increase energy partitioning towards milk production and increase the efficiency of energy utilization.

Lactating cows require energy to support maintenance functions, produce milk and milk constituents, and gain body weight. This is why increasing energy supply to dairy cows often results in an increase in milk, protein, and fat yield (Brun-Lafleur et al., 2010). Although energy is typically viewed as a single entity, various substrates contribute this to this nutrient. First, energy stores within the body of dairy cow are supplied as such as:

- glucogenic substrates
 - Propionate from rumen fermentation
 - AA except for Lys and Leu
 - Glucose from starch that is digested and absorbed in the small intestine
- lipogenic substrates
 - acetate and butyrate from rumen fermentation
 - fatty acids

Most frequently in the field the supply of glucogenic substrates are increased through starch, and supply of lipogenic substrates are increased by increasing fatty acids or fermentable fiber. Boerman et al. (2015b) reported that increasing supply of starch increased milk protein yield, whereas increasing supply of fatty acids increased milk fat

yield. However, the underlying effects of energy source on energy partitioning and utilization and N partitioning are not well understood.

Current versions of the NRC (2001) and the Cornell Net Carbohydrate and Protein System (CNCPS v6.5; Van Amburgh et al., 2015) account for energy and protein supply as individual entities consequently the user is limited to assume that milk production cannot exceed the more limiting of the two. However, over 40 years ago, Oldham (1984) suggested that an interaction between energy and protein supply exists and this may have a large influence on whole-animal metabolism and milk protein synthesis. Indeed, interactions between energy and protein supply have been observed experimentally (Brun-Lafleur et al., 2010). It is well established that cows possess a requirement for AA to support milk protein synthesis (Patton et al., 2014); therefore, as nutrient modeling systems move away from simplistic representations of the biology there is a need to better understand how supply of energy influences AA requirements.

The overall objective of this dissertation is to increase our understanding of energy requirements and utilization as well as to understand the interrelationship between AA and energy metabolism in lactating Jersey cows. This objective is achieved by pursuing the following aims:

- 1) Explore the maintenance energy requirement of Jersey cows
- 2) Quantify physiological factors affecting heat production
- 3) Explore the relationship between urinary N and energy excretion
- 4) Evaluations of energy and N utilizations in diets i) containing varying supplies of Lys and His; and ii) where sources of energy are shifted from starch to fatty acids

- 5) Evaluations of the main and interaction effects of dietary fatty acid and starch content and lysine supply.

CHAPTER 2: LITERATURE REVIEW: TERMS, CALCULATIONS, MEASUREMENTS FOR QUANTIFYING DIETARY NET ENERGY FOR LACTATION

INTRODUCTION

The net energy system for dairy cows developed by Moe et al. (1972a) has been used to describe the energy content of dairy cow diets for decades. In this system, the base unit for net energy is NE_L which is defined as the energy content of milk. This unit is used to describe the energy requirements for milk production, maintenance, pregnancy, and changes in body reserve with all non-milk energy measurements being adjusted to be equivalent to NE_L . Therefore, although maintenance energy is heat energy, the energy that is used for maintenance must be converted to NE_L . The use of energy for pregnancy, activity, tissue mobilization or accretion must also be converted to NE_L .

The NE_L system as described by NRC (2001) is commonly used to estimate both dietary energy supply and requirements for dairy cows. In this system, digestible energy (**DE**) is estimated via the summative equation via chemical composition of dietary ingredients, enthalpy of nutrient fractions, and estimated nutrient digestibility (NRC, 2001 eq 2-8 pg 16). Supply of NE_L then is estimated from DE through ME using previously estimated efficiencies (NRC, 2001 eq 2-10, 2-11, and 2-12 pg 17). Experimentally, NE_L can be more closely estimated by measurement of metabolizable energy (**ME**) and quantification of heat production using indirect calorimetry techniques, and these measurements serve as the foundation of NE_L system developed by (Moe et al., 1972a). Several factors can affect diet digestibility and efficiency of converting energy into NE_L and these include, diet chemical composition, associative diet effects, and DMI

(Weiss and Tebbe, 2019). In an evaluation of current NRC (2001) estimates for nutrient digestibility, NDF digestibility was found to be under-predicted by 16% and fatty acid and CP digestibility were overpredicted by 26 and 7%, respectively (White et al., 2017c). These errors in predicting nutrient digestibility for a dairy cow with a DMI of 25 kg/d translate to an underestimation of NE_L of about 3 Mcal/d for NDF digestibility and an overestimation of NE_L of 1 to 2 Mcal/d for FA and CP digestibility.

Accurate quantification of dietary manipulation on estimated NE_L , as well as, understanding how dietary manipulation affects milk component yields is essential for formulating accurate diets and feeding dairy cows. Dietary NE_L is estimated to cost about 0.16 \$/Mcal and represents approximately 60% of total dietary nutrient costs (Tebbe, 2020). For 450 kg Jersey cow producing 33 kg of energy-corrected milk (**ECM**), NE_L costs 4.74 \$/d. This clearly represented that NE_L is important financial cost in dairy production. Both carbohydrates and protein are known to affect nutrient digestibility and microbial protein supply (Roman-Garcia et al., 2016; White et al., 2017b; White et al., 2017c). Additionally, supply of fat, glucose, and protein can affect milk fat and protein synthesis in mammary glands (Rius et al., 2010a; Nichols et al., 2019f; Omphalius et al., 2020). Furthermore, the nutrients that contribute energy can affect milk component yield, partitioning of energy between milk and tissue energy (**TE**), thus, potentially altering energetic efficiency (van Knegsel et al., 2007c; Nichols et al., 2018; Morris et al., 2020b). Interactions between energy and MP affects yield of milk protein (Brun-Lafleur et al., 2010); however, the current edition of the NRC (2001) does not account for the effects of these interactions on milk production or energy utilization.

The aim of this review is to describe the components of the NE_L system, to list the calculations and assumptions used to determine energetic fractions in the NE_L system, and discuss the measurements required to experimentally quantify dietary NE_L . Additionally, this review will include a discussion of how dietary manipulation, specifically changes in dietary energy content, energy source, and metabolizable protein (**MP**) content, can affect whole-animal energy utilization and the partition of energy. Most energetic measurements on dairy cows in the United States were collected at least 25 years ago (Moraes et al., 2015). However, at the University of Nebraska, we have completed several energy balance experiments with Jersey cows (Table 2.1). These observations provide a useful comparison of the current NRC (2001) energy system and modern lactating cows.

KEY TERMS IN THE NE_L SYSTEM

Energy flow through the NE_L system is depicted in

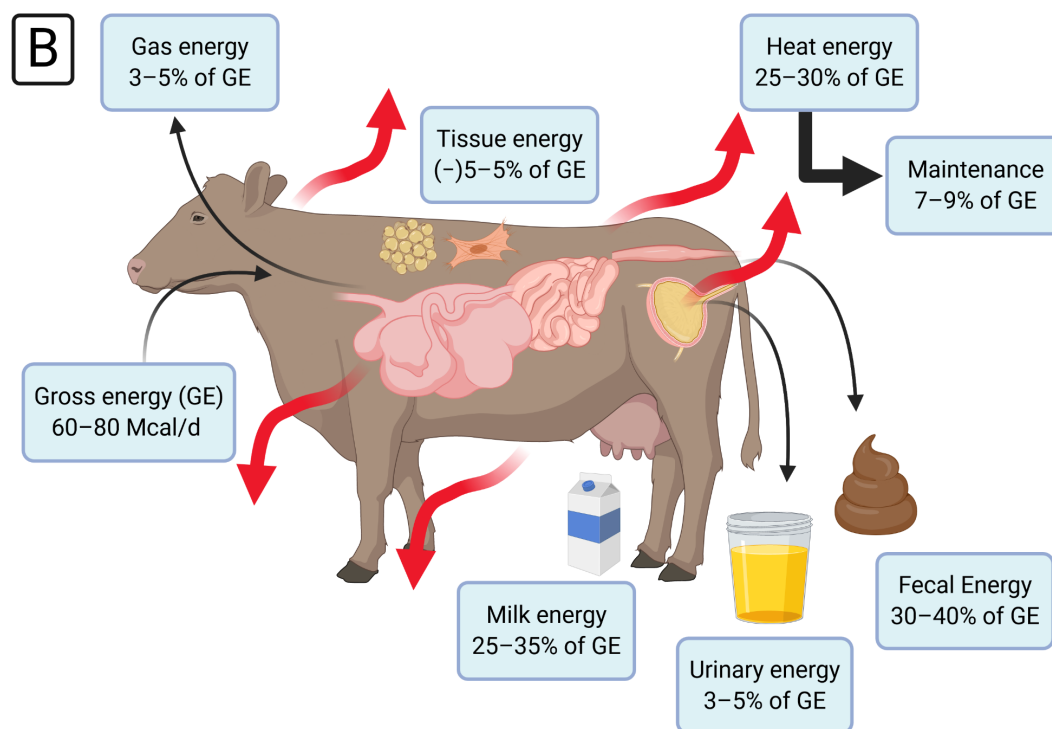


Figure 2.1. Gross energy (GE) is the total amount of chemical energy consumed by an animal. Digestible energy (DE) is $GE - \text{fecal energy (FE)}$. Then, ME is calculated as $DE - \text{gas energy} - \text{urine energy (UE)}$. Total NE_L is the sum of milk energy, tissue energy (TE) on an NE_L basis, net energy for maintenance (NE_M), and net energy for pregnancy. The TE fraction is assumed to be the fraction of energy that is unaccounted for when all other energy fractions are measured, which is calculated as $ME - \text{milk energy} - \text{heat production}$. However, to calculate dietary NE_L , TE must be corrected to be equivalent to milk energy; that is, TE must be corrected to reflect the efficiency of converting TE into milk energy (Reynolds, 2000). Error accumulates for each fraction in the net energy cascade: DE includes error in estimating GE and FE, ME includes error from DE and error in measuring gas energy as well as UE, and TE includes error in

measuring ME, milk energy, and heat production. Heat increment is the difference between ME and NE_L or the difference between total heat production and NE_M . Detailed description for quantifying each fraction of the cascade of net energy are described below.

Net energy consists of multiple fractions, and ME is used with a different efficiency for each of these fractions. The efficiency of converting ME into milk (k_L), and maintenance energy (k_M) are assumed to be equal and are 60–70 (Moe and Tyrrell, 1973; NRC, 2001). The efficiency of converting ME into TE (k_G) is 70–80% (Moe et al., 1971; Moraes et al., 2015). Additionally, TE can be mobilized to support milk production and this efficiency of converting TE into milk energy (k_T) is between 80 and 90% (Moe et al., 1971; Moraes et al., 2015). To calculate TE on an NE_L basis, TE gain is multiplied by k_T and TE loss is multiplied by k_G and divided by k_L .

Although simplicity is created by using a single energy term (NE_L) to describe dietary energy supply and the energy requirements of lactating dairy cows, NE_L cannot be truly measured experimentally and some assumptions must be made to calculate NE_L . These assumptions that are used to calculate NE_L have been a source of debate for many years and their use has continued to evolve (Moe et al., 1971; Moe et al., 1972a; Moraes et al., 2015).

DETERMINING NE_L

Nutritional models such as NRC (2001) are often used to estimate supply of NE_L to a dairy cow from dietary nutrient composition, DMI, and BW. These estimated NE_L values are important for determining if dietary supply of NE_L is adequate to support

lactation, maintenance, and gestation needs and ultimately to determine if animals will be gaining or mobilizing energy reserves. Nutrition models are designed to estimate energy supply as concentration of the diet (e.g., Mcal/kg DM). However, animals require quantities of energy (e.g., Mcal/d) and experiments designed to measure energy supply and requirements collect data on a quantity basis. Thus, energy supply is estimated as a concentration of the diet and energy requirements are measured a quantity, and DMI can be used for interconversion of the two.

Model Estimates of Dietary NE_L

Determining the energy supply from a given feed or diet is expensive and requires specialized facilities; thus, in the field and in most experiments, NE_L is often estimated using chemical composition and a model such as NRC (2001). As discussed by current and former experts, individual feeds cannot be accurately assigned energy values beyond gross energy, and the NE_L systems should be based on diets not individual feeds (Moe et al., 1972a; Kleiber, 1975; Weiss and Tebbe, 2019). This discrepancy primarily occurs because the digestibility of a nutrient for a given feedstuff not only depends on the characteristics of that feed but also associative effects which are dictated by dietary chemical composition and feed intake of the animal (de Souza et al., 2018b).

Additionally, gas energy and UE are a function of diets. For example, the UE excretion associated with a high-protein feedstuff will be greater when included in a diet with excess MP compared to a diet that is deficient in MP which will result in differences in energy availability for the animal. Therefore, because NE_L is a function of diets and composition of possible diets is endless, nutrition models are useful to estimate dietary

NE_L and determine the effect of changes in dietary ingredients or chemical composition of feeds on NE_L.

Estimating DE from Digested Nutrients. Digestible energy is a function of dietary energy content and the digestibility of each nutrient. From 261 observations on Jersey cows at the University of Nebraska-Lincoln, DE averaged 2.88 Mcal/kg of DM (Table 2.1). The efficiency of converting GE into DE averaged 0.662 but was as low as 0.609 (10th percentile) or as high as 0.708 (90th percentile).

In nutrition models, DE is first estimated via a summative equation (Weiss and Tebbe, 2019) which accounts for digestibility and enthalpy of commonly measured feed fraction (Table 2.2). The fractions commonly included to estimate DE included CP, NDF, fatty acids, and non-fiber carbohydrates (NFC). However, NFC includes starch, and since 2001, measurement of starch digestibility and concentration in feeds has increased. Considerable variation in starch digestibility has been observed (see below). Therefore, removing starch from NFC to calculate residual organic matter (**ROM**; 100 – CP – NDF – fatty acids – starch) decreases the nutritional heterogeneity of the NFC fraction and allows for more variation in nutrient digestibility to be accounted for (Tebbe et al., 2017). The ROM fraction consists of predominately water-soluble sugar, as well as lactic acid, acetic acid, glycerol, and soluble fiber, and is assumed to have an enthalpy of 4.0 Mcal/kg of DM. To determine DE, the enthalpy of NDF is equal to cellulose and hemicellulose (4.2 Mcal/kg of DM). Although lignin, as determined by the sulfuric acid method, has a enthalpy of 6.0 Mcal/kg of DM (Voitkevich et al., 2012), because the digestibility and consequently DE of lignin is 0 (Van Soest, 1967). Digestibility of NDF is the most variable of energy supplying nutrients with coefficient of variation that ranges

from 14% up to 26%, whereas the coefficient of variation for CP, fatty acids, and starch are often less than 10% (Morris and Kononoff, unpublished data; White et al., 2017; de Souza et al., 2018). Increasing NDF digestibility by 11% (1 SD) for a cow consuming 25 kg/d of a 35% NDF diet will increase DE by 4.0 Mcal/d (0.16 Mcal/kg DM).

Estimation of energy supply from CP and ROM is relatively straight forward; however, true digestibility must be determined, and metabolic fecal energy discounted. Metabolic fecal matter consists primarily of CP and ROM. This mass is difficult to measure and is often estimated via statistical methods. Removal of the endogenous fractions allows for the calculation of true energy supply. NRC (2001) includes an endogenous fat fraction; however, more recent data estimate that endogenous fat is 0 (Weiss and Tebbe, 2019). For all other energy fractions, no endogenous fraction exists, and thus apparent digestibility is equal to true digestibility. Energy supply from CP is a function of the rumen degradable protein content of a diet and the digestibility of rumen undegradable protein, which is often estimated on individual feedstuffs using in situ or in vitro approaches (Boucher et al., 2009; Paz et al., 2014). Metabolic fecal and endogenous protein consists of undigested microbial protein, slough epithelial cells, mucin, and digestive secretions. Approximately 20% of microbial CP is believed to be undigested (NRC, 2001). Using the diet-level equation of Roman-Garcia et al. (2016) with DMI and dietary starch content as inputs, estimated microbial protein for a cow consuming 25 kg/d of a 25% starch diet is 2075 g/d or 415 g/d of undigested microbial CP. Estimated metabolic fecal protein using the recent equation of Lapierre et al. (2020) with DMI and NDF as inputs for a cow consuming 25 kg/d of a 35% NDF diet is 408 g of CP/d. In terms of energy for a cow consuming 25 kg/d of a 16.5% CP diet, undigested microbial

CP and metabolic fecal protein account for approximately 20% of energy from CP (0.8 kg of undigested microbial CP and endogenous CP/4.1 kg of CP intake). However, undigested microbial protein and metabolic fecal protein are not directly related to dietary CP content, so increased dietary CP will dilute out these fractions. Based on a regression of digestible ROM against dietary ROM, the true digestibility of ROM averaged $96.1 \pm 2.1\%$. From the same regression, the endogenous ROM component was 34.3 ± 2.95 g/kg (Tebbe et al., 2017) or approximately 22% of energy content of ROM for diet with 16% ROM (0.9 kg of endogenous ROM/4.0 kg of ROM intake). Similar to CP, increasing dietary ROM content should also dilute out the endogenous ROM fraction. Because ROM is a residual fraction, it contains the cumulative error associated with measurement of other fractions. For example, in mixed diets NDF insoluble CP and ash generally account for 1.5 to 3.5% of diet DM (Tebbe et al., 2017); therefore, when calculating ROM, neutral detergent insoluble ash and CP is subtracted out twice. Although the removal of neutral detergent insoluble ash and CP is correct, removal of these fractions does not improve accuracy in quantifying DE largely because these errors cancel each other out (i.e., with neutral detergent insoluble ash and CP correction, NDF digestibility increases and ROM digestibility decreases; Tebbe et al., 2017).

Because NDF is a heterogeneous mixture both chemically and nutritionally, predicting its digestibility is difficult because of the complexing of NDF chemical structure and associative effects due to dietary starch and CP content and DMI. Two methods are primarily used to estimate NDF digestibility: the lignin or in vitro NDF digestibility method. The lignin method to estimate NDF digestibility as described by Weiss et al. (1992) accounts for the indigestibility of lignin and the effects of lignification

of the digestibility of cellulose and hemicellulose. Lopes et al. (2015) compared in vivo NDF digestibility estimated using markers to different in vitro time points for 21 diets. In this study, no relationship was observed between 30-h in vitro NDF digestibility and in vivo NDF digestibility; however, 48-h in vitro NDF digestibility was correlated with in vivo NDF digestibility with a root mean squared error of 14.6% of mean. Additionally, in vitro and in vivo NDF digestibility are not synonymous and equations must be applied to convert in vitro estimates to in vivo values (Lopes et al., 2015). Recent work has separated NDF into an indigestible pool which is approximated by 240-h in vitro incubation and 2 pool of potentially digestible NDF which determined using multiple in vitro timepoints (Raffrenato et al., 2019). However, it is unknown if these multi-dimensional NDF models improve our ability to estimate and explain variation in in vivo NDF digestibility. The number of experiments where in vivo and in vitro NDF digestibility of diets is measured is few. Research is needed to further our understanding of the relationship between in vivo NDF digestibility and lab methods that are used to predict NDF digestibility. Additionally, NDF digestibility is negatively related to dietary starch content and DMI (NRC, 2001; Weiss et al., 2009a; de Souza et al., 2018b). In a meta-regression of individual animal data with 1,900 observations, on average, NDF digestibility decreased by 0.59 percentage units with 1 percentage unit increase in dietary starch (de Souza et al., 2018b). Increased starch may suppress NDF digestibility via decreased ruminal pH and negative associated effects on the rumen environment; however, confounding effect of NDF source should be considered. In general, increased dietary starch occurs via the replacement of non-forage fiber sources with cereal grains. Because the digestibility of NDF in non-forage fiber source is often greater than forages

(Firkins, 1997), decreased proportion of NDF from highly digestible source may explain some of the negative relationship between dietary starch content and NDF digestibility. In an individual studies testing multiple dietary starch contents and varying forage and nonforage source of NDF in a nonsystematic manner, NDF digestibility decreased by 0.55 percentage units with each 1 percentage unit increase in dietary starch (Weiss et al., 2009a). In the meta-regression of de Souza et al. (2018b) increasing DMI above 3.5% of BW decreased NDF digestibility by approximately 1.1 percentage units. The negative relationship between NDF digestibility and DMI is primarily due to increased passage rate and subsequently decreased rumen retention time with increased DMI.

Digestibility of fatty acids is dependent on source, the profile of fatty acids reaching the duodenum, and fatty acid intake (Boerman et al., 2015a; Daley et al., 2020). In a regression of digestible fatty acid against dietary fatty acids, fatty acid digestibility averaged 74.5% from 206 observations across 37 diets that ranged in fatty acid content from 1.5 to 7.5% of DM (Weiss and Tebbe, 2019). Multiple meta-analysis suggests that fatty acid digestibility differs by source (Schmidely et al., 2008; Boerman et al., 2015a; White et al., 2017c). Differences in fatty acid digestibility are partially a function of differences in fatty acid profile across sources, which affect the profile of fatty acids that reach the duodenum. In general, fatty acid digestibility decreases with increasing degree of saturation (Pantoja et al., 1996; Harvatine and Allen, 2006) and esterification (Pantoja et al., 1995; Weiss et al., 2011). This is reflected in greater digestibility coefficients for Ca-salts of fatty acids and vegetable oil compared to more saturated fat sources such as hydrogenated tallow (Boerman et al., 2015a). Increasing duodenal fatty acid flow, especially 18:0, decreases fatty acid digestibility (Boerman et al., 2015a). Basal diets

contain up to 3% long-chain fatty acids and dietary fatty acid content is further increased with supplemental fatty acids. If supplemental fatty acids depress digestibility of fatty acids in the basal diet, the challenge is presented on whether to assign this depression in fatty acid digestibility to supplemental source or to the basal diet. Depression in fatty acid digestibility with increasing fatty acid flow to the duodenum appears to be independent on whether the fatty acid molecules is from basal or supplemental sources (Boerman et al., 2015a). Therefore, assuming a constant basal fatty acid digestibility and allowing the depression in digestibility to be reflected in the digestibility coefficient for supplemental fat is the most accurate for nutritional valuation of fat supplements. Increasing proportion of C18:1 fatty acids in supplemental fat (de Souza et al., 2019) and abomasal infusion of emulsifiers (de Souza et al., 2020) increases fatty acid digestibility.

Starch digestibility is affected by grain type, endosperm virtuousness, processing method, DMI, and ensiling time for fermented sources. For similar processing methods, differences in digestibility across grain types are primarily due to differences in the accessibility of starch granules in a feedstuff to rumen microbes. Starch in wheat, barley, and oats is contained in more floury endosperm, which is more accessible to rumen microbes, whereas sorghum grain has more vitreous endosperm, which is less accessible to rumen microbes (Huntington, 1997). The virtuousness of corn grain is intermediate, ranging from 0 to 95% (Hoffman et al., 2010), and increases with increasing maturity (Philippeau and Michalet-Doreau, 1997). In a meta-analysis, ruminal digestibility was less for corn compared to wheat and barley (54 vs. 75%); however, total-tract starch digestibility was similar between corn, wheat, and barley (averaging 93%), which is partially confounded by processing method and harvest maturity. Increased processing to

decrease particle size will increase starch digestibility (Firkins et al., 2001; Ferraretto et al., 2013b), and more vitreous grain sources, such as corn and sorghum, are more likely to be processed prior to feeding and are more responsive to processing method than less vitreous sources, such as wheat and barley (Huntington, 1997). Average digestibility of corn grain ranges from 78% for coarse ground ($> 3500\ \mu\text{m}$) corn up to 92% for fine ground corn ($<1000\ \mu\text{m}$; Ferraretto et al., 2013). Additionally, A greater starch digestibility is observed for high-moisture and steam flaked (94–99%) compared to dry ground corn (78–91%; Firkins et al., 2001; Ferraretto et al., 2013). Starch digestibility may also be affected by dietary starch content and DMI. In a meta-regression, starch digestibility decreased by 0.12 percent units for every percent unit increase in dietary starch content and by 1.0 to 1.1 percent units for every unit increase in DMI as percent of BW (de Souza et al., 2018b). Increasing dietary starch content will increase small intestinal flux of starch via mass action. Additionally, increasing DMI will increase rumen passage rate consequently shifting site of digestion for some starch from the rumen to the small intestine. Increasing passage rate decreases time for starch hydrolysis in the intestine and it may be possible to exceed the capacity of the small intestine to digest starch (Owens et al., 1986), which may explain the depression in starch digestibility with increased dietary starch content and DMI. With increase length of storage for corn silage, in vitro digestibility of starch increases due to increasing breakdown of protein matrix surrounding starch molecules (Der Bedrosian et al., 2012; Ferraretto et al., 2015). However, it is unknown how length of fermentation translates to in vivo starch digestibility.

Estimating ME and NEL from DE. In energetic models, ME is often estimated from DE using an empirical equation that assumes a near constant conversion of DE to ME. In our dataset, the efficiency of converting ME to DE averaged 0.873 but did vary (10th–90th percentile; 0.836 – 0.899; Table 2.1). Empirical equations utilized by NRC (2001) do not account for the effect that different nutrients will have on energy that is lost from DE to ME as CH₄ and urine. Digestible fatty acids will not lead to gas energy or UE production. NRC (2001) assumed that ME = DE for feed that is 100 fat (eq 2-10 pg 17). Additionally, increasing dietary CP will increase urinary N excretion which is correlated with UE (Martin and Blaxter, 1965), and digestible carbohydrate is correlated with CH₄ production (Appuhamy et al., 2016b). Equations are available that account for the effects of dietary factors on CH₄ production (Yan et al., 2000; Nielsen et al., 2013) and urinary N excretion (Kauffman and St-Pierre, 2001; Spek et al., 2013b). Utilization of these equations to estimated gas and UE and subsequently estimating the conversion of DE to ME will account for additional source of variation in gas energy and UE and may improve predictions than published empirical equations.

The conversion of ME to NE_L (i.e., K_L) has historically been assumed to be a near constant value of 0.64 (Moe et al., 1972a; NRC, 2001). In the Moe et al., (1972) database, cows were often fed high forage (>60% of DM) and CP diets (>18%), and diets rarely included supplemental fat. Thus, K_L for dairy cow fed modern diets may differ. From our database, K_L averaged 0.669 and ranged from 0.582 (10th percentile) to 0.738 (90th percentile) when assuming a maintenance energy of 0.08 Mcal/kg of metabolic BW (**MBW**; NRC, 2001). Additionally, we observed that K_L is affected by diet (Morris et al., 2020d). Increased K_L compared to historical measurements may partially be a function of

increasing NE_M estimates, which was observed in our study (Morris et al., 2020d) and others (Moraes et al., 2015). Milk yield is positively correlated with fasting heat production (Holter, 1976), and NE_M may increase as lactation progresses (Ellis et al., 2006). However, increased NE_M or decreased K_L are indistinguishable, and K_L is likely affected by dietary chemical composition and milk component production (see *Effects of Diets on Energy Partitioning and Utilization*).

Experimental Measurements to Determine NE_L .

Models to predict dietary NE_L are based on measurements designed to determine NE_L . Specifically, this includes measurements of intake, fecal, gas, urine, heat, tissue, milk, and maintenance energy. Collecting all of these measurements requires intensive metabolism experiments.

Measurements through the energy cascade down to ME are relatively straightforward. Measurement of GE is conducted by determining DMI and the energy concentration of a diet. The latter is measured in a bomb calorimeter where total combustible energy is measured on a diet sample after complete oxidation. The energy content is dependent on the chemical composition of the diet as different nutrients have different enthalpies (Table 2.2). Increasing dietary fat or CP at the expense of carbohydrate will increase dietary energy content, whereas increasing dietary ash will decrease energy content. To determine DE, fecal energy must be determined by measuring total fecal output and determining energy content of feces. Measurement of ME requires quantification of gas energy and UE loss.

Although ruminants produce trace amounts of H_2 , CO , acetate, ethane, and H_2S , which are ignored when calculating gas energy, CH_4 is the predominant combustible gas produced by ruminants (NRC, 1981). Gas energy is determined by quantification of CH_4 production and multiplying by its enthalpy (9.45 kcal/L). Urinary energy is determined by collection of total urine excretion and determination of combustible energy content of urine. Markers can be used to estimate fecal and urine output; however, accuracy may be an issue (Morris et al., 2018b; Tebbe and Weiss, 2018; Lee et al., 2019). Additionally, variance in estimating fecal output and nutrient digestibility was greater when indigestible NDF or acid-insoluble ash was used as a marker compared to total collection (Morris et al., 2018b). Conversely, when estimating urine output using urinary creatinine concentration, variance is lower compared to total collection, because the variance is ignored in the regression used to estimate total urinary creatine excretion from BW (Tebbe and Weiss, 2018). Therefore, the use of markers to estimate fecal and urine output for determining ME and subsequently TE and NE_L is not recommended.

Quantification of NE_L requires measurement of energy in milk, TE gain or loss, and maintenance. The latter two cannot be directly measured in lactating cows and require assumptions to quantify; thus, NE_L cannot be directly measured.

Milk energy is quantified by determining energy content of milk secretion and can be measured via bomb calorimetry or estimated via multiplying milk fat, protein and lactose yield by their corresponding enthalpies of 9.29, 5.63, and 3.95 Mcal/kg, respectively (NRC, 2001). Protein concentration of colostrum can be up to 15% (Hibbs et al., 1951), thus, energy content of colostrum is likely greater than milk. Estimated values use this method are highly accurate as indicated by a root mean squared error of less 1% of the

mean when compared to measurement via bomb calorimetry for marine milk samples (Ofteidal et al., 2014).

Quantification of TE gain or loss in lactating dairy cow is challenging. By definition, TE gain or loss is the change in total energy content of an animal, and changes in whole-body energy content can never be truly measured, because quantification of animal energy content is a terminal measurement. The comparative slaughter technique has been used to approximate changes in TE in beef animals (Lofgreen and Garrett, 1968). However, this method is costly because of the terminal nature and is generally not used on lactating animals. Changes in BW can be used to approximate changes in TE; however, it is well known that changes in BW do not necessarily reflect changes in body reserves, because energy reserves can be mobilized without observing changes in BW, and changes in gut fill associated with changes in DMI will affect BW but not necessarily body energy reserves (Moe et al., 1971; NRC, 2001). Increase DMI by 1 kg is estimated to increase gut full by 2.5 kg (NRC, 2001). Changes in TE can be estimated by using an ultrasound to measure changes in backfat thickness (Boerman et al., 2015b), or by urea or D₂O dilution method (Andrew et al., 1995). From the urea or D₂O dilution method body water content is quantified which allows for calculation of empty BW. Then, assuming constant empty BW to protein and ash ration, body fat content can be calculated. Measure of change in body energy via change in backfat thickness or by using the D₂O or urea dilution method are indirect methods and still require several assumptions. As described above, if all other energy intake and all other energy loss from an animal are measured, the residual can be assumed to be equal to TE. Therefore, to quantify TE, heat production must be quantified and is often done via measurement of O₂ consumption and CO₂

production using indirect calorimetry techniques (McLean and Tobin, 1987; Reynolds, 2000). The objective of this review is not to describe indirectly calorimetry method and interested readers are referred to comprehensive books on the subject (McLean and Tobin, 1987; Gerrits and Labussière, 2015).

The NE_M fraction represents the heat generated from the use of energy to support basal metabolic functions such as protein turnover, Na^+ transport to maintain membrane potential, heart work, nervous functions, and respiration (Baldwin, 1995). The NE_M of a lactating cow cannot be directly measured and must be quantified by using assumptions regarding maintenance energy or by statistical techniques. NRC (2001) use a NE_M value of 0.080 Mcal/MBW. This value is based off an average measured fasting heat production in dry non-pregnant dairy cows of 0.073 Mcal/MBW (Flatt et al., 1965a) plus a 10 percent activity allowance for normal voluntary activity. Book values for NE_M can be used to estimate NE_L or NE_M can be quantified for a group of observations as the intercept of the regression between milk energy output and ME. In this regression, it is essential that milk energy is corrected for tissue mobilization and that ME is corrected for tissue retention (Kebreab et al., 2003; Morris et al., 2020d), because this isolates milk energy that is derived from ME and ME that is utilized for milk production or maintenance. The effects of correcting milk energy and ME for TE are illustrated in Figure 2.2. When milk and ME are corrected for TE, biologically reasonable estimate of K_L of 0.72 and NE_M of 101 kcal/MBW are generated, whereas, uncorrected data generated biologically unrealistic estimate of 0.08 for k_L and -223 for NE_M . The heat increment associated with TE gain (k_G) and mobilization (k_T) is different from k_L which will bias regression estimates if milk energy and ME are not corrected for tissue gain and

mobilization. Because use of energy for NE_M generates heat, NE_M is measured as fraction of total heat production. The difference between HP and NE_M is heat increment which is the heat produced by the act of consuming, digesting, and absorbing nutrients; heat resulting from the biosynthesis of products such as body tissue or milk; heat of waste formation and excretion; and heat of fermentation (NRC, 1981). Heat increment also represents the difference between ME and NE_L or, in other words, the energy lost in the conversion of ME into NE_L .

Accurate measurement of heat production is essential for accurate calculation of TE and dietary NE_L , and measurement of O_2 consumption and CO_2 production are essential for accurate quantification of heat production. System accuracy can be determined by a number of different methods and included release of a known quantity of CH_4 or CO_2 into the system and measuring recovery or by burning 100% ethanol and measuring accuracy in measurement of O_2 consumption and CO_2 production from quantity of ethanol burned (McLean and Tobin, 1987; Gerrits and Labussière, 2015). Yet, calibration of indirect calorimetry systems is not conducted or at least not reported in the literature. As reported by Gerrits et al. (2018), in the 2016 and 2017 volumes of the *Journal of Dairy Science* only 27% (7 out of 26) of publications where gas production was measured reported quantitative gas recoveries. Full system recoveries can be poor. In a ring test of recovery of respiration chamber in the UK (6 facilities, 22 chambers) recovery of CH_4 ranged from 59 to 115% (Gardiner et al., 2015). Incomplete gas recoveries can result in an underestimation of heat production and overestimation of TE (Table 2.3). This is demonstrated by a theoretical calculation using a reference Jersey cow consuming 58 Mcal of DE, excreting 4.0 Mcal/d of UE, producing 25.1 Mcal/d of

milk, and with a true TE balance of 0 Mcal/d. Additionally, incomplete recovery was assumed to affect O₂, CO₂, and CH₄ equally, and it is unknown if this assumption is valid. For each 5% decrease in gas recovery, calculated ME intake increased by 0.2 Mcal and heat production decreased by 1.3 Mcal due to underestimation of gas fractions. Overestimation of ME intake and underestimation of heat production leads to a decrease in TE of 1.3 Mcal of NE_L (4.0% of true NE_L) per 5% decrease in gas recovery. The increase in the fractional effect of incomplete gas recovery when proceeding along the energy system from ME to TE is due to compounding of errors in estimating gas consumption and production on energy fractions. Use of correction factors to compensate for inaccurate gas recovery is not considered good practice as errors may not be stable in time, volume, and concentration, and requires the assumption that the error is proportional to the correction factor (McLean and Tobin, 1987; Gerrits et al., 2018). To assure accuracy in quantification of energetic fractions, indirect calorimetry system calibrated must be checked and recovery test values should be reported (Gerrits et al., 2018).

Effects of Diets on Energy Partitioning and Utilization

From 261 observations on Jersey cows at the University of Nebraska-Lincoln, energy lost as heat increment accounts for (mean; 10–90th percentile) 18.1 (14.5–22.3) % of GE in a lactating cow and represents the second largest loss of energy after fecal energy excretion (Table 2.1; Morris et al., 2020b). Therefore, improving understanding of factors affecting heat increment is needed to improve our understanding of energy utilization and ability to predict NE_L. Diets can differ in ME-to-NE_L efficiency (Morris et

al., 2020d) and it is well established that the heat increment associated with the utilization of fat is less than carbohydrates or protein (Andrew et al., 1991; NRC, 2001). The current NRC (2001) uses an average efficiency of converting ME from fat into NE_L of 0.80; however, this correction factor may not improve estimation of dietary NE_L (Morris et al., 2020b). Additionally, it is well established that increasing energy supply from starch compared to fat or digestible NDF increases energy partitioning towards TE at the potential expense of milk energy (van Knegsel et al., 2007a; Boerman et al., 2015b; Morris et al., 2020b). The effects of energy source on energy utilization are not accounted for in NRC (2001).

Metabolizable energy is not a homogenous entity, but rather, is composed of various nutrients and metabolites which are used with a different efficiency for each purpose. Post-absorptive energy is supplied as VFA, long-chain fatty acids, glucose, glycerol, AA, and as some other minor fermentation products such as lactate. Some dietary fats are directly transferred to milk fat (Rico et al., 2014; Boerman et al., 2015b; Nichols et al., 2019e), which will increase the efficiency at which ME is converted into NE_L because incorporation of preformed fatty acids into milk fat is more energetically efficient than de novo lipogenesis (94–97% vs. 70–75%; Baldwin et al., 1985). We have found that the heat increment associated with milk protein production is more than 3-fold that of milk fat (6.97 vs 1.61 Mcal/kg; Morris et al., 2020a), which is, in part, due to the high energetic efficiency of converting dietary fat into milk fat and heat increment associated with protein synthesis and catabolism (see later discussion). The effects of specific pools of VFA nor the whole-animal efficiency of synthesis of milk components are not well understood (Baldwin et al., 1985). The efficiency of utilizing ME from VFA

for maintenance in fasting non-lactating Holstein cows were acetate 60%; propionate 82%, butyrate 82%, and a mixture of 52 acetate:31 propionate:17 butyrate 68% (Holter et al., 1970). In theory, these efficiencies represent the efficiency of producing ATP from the given substrate. Dado et al. (1993) generated theoretical estimates for ME requirements for the synthesis of milk constituents by examining ATP use in associated metabolic pathways. Maximum theoretical efficiency for synthesizing milk lactose, fat and protein were 79, 82, and 88%, respectively, and decrease with increasing proportion of glucose derived from AA. However, these calculations only account for energy loss in the conversion of substrates such as glucose or VFA into products and do not account for energy expenditure of digesting, absorbing, and transporting substrates to mammary glands. Clearly, K_L is dependent of composition of ME and the specific product generated from a given substrate. Currently, data are lacking to account for the mechanistic nature of the energetic efficiency of converting ME to NE_L for individual milk components; research and modeling work to improve our understanding is warranted.

Feed intake, dietary nutrient, and physical composition affect energy expenditure. Increased energy expenditure by the gastrointestinal tract and liver has been observed with increased DMI (Reynolds et al., 1991), increased dietary NDF (Cantalapiedra-Hijar et al., 2014a), increased dietary forage (Reynolds et al., 1991), and increased dietary CP (Ferrell et al., 2001). Increased energy expenditure associated with increased DMI and dietary NDF and forage are presumably due to increased digestive tract mass (Reynolds et al., 1991; McLeod and Baldwin, 2000). The cause of the increase in energy expenditure with increased CP intake has been debated. In a historical study with sheep,

Martin and Blaxter (1965) reported that ureagenesis from ammonia increased heat production by 3.8 Mcal/kg of N. However, more recently the idea of a large energetic cost to ureagenesis has been challenged; ruminants are evolutionarily adapted to be dependent on urea recycling, so, a large energetic cost to ureagenesis would be constrictive to the evolutionary adaptability of ruminants (Firkins and Reynolds, 2005). Rather, it has been suggested that increased energy expenditure associated with feeding excess protein is due to heat generated from catabolism of AA in the liver (Reynolds, 2006). In support of this, when infusing ammonium chloride into the mesenteric vein of sheep, liver O₂ consumption was not affected (Lobley et al., 1995), and furthermore, feeding true protein compared to a N equivalent amount of urea increased liver O₂ consumption in sheep (Ferrell et al., 2001). Although increasing dietary CP will increase heat energy loss, CP has a greater energy density than carbohydrates (5.65 vs. 4.2 Mcal/kg), and thus when CP is added to a diet to replace carbohydrates differences in energy supply are likely dictated by digestibility (Morris et al., 2020a).

Energetic substrates can be broadly divided into two categories: glucogenic substrates, which support milk lactose synthesis and include glucose, propionate, lactate, glycerol, and most EAA; and lipogenic substrates which support milk fat synthesis and include fatty acids, acetate, and butyrate. Both groups of nutrients are essential for milk production and metabolism within each class differs. In lactating dairy cows, up to 80% of whole-body glucose production is taken up by the mammary glands with up to 70% of the glucose uptake being used for lactose synthesis (Lemosquet et al., 2009). However, net portal appearance of glucose in lactating ruminants is negative to negligible (Galindo et al., 2011; Cantalapiedra-Hijar et al., 2014a), and thus most glucose is synthesized in

the liver from propionate (60–74% of net hepatic glucose release), lactate (16–26%, AA (16–22%), and glycerol (0.5–3%; Aschenbach et al., 2010). Absorptive supply and splanchnic release of acetate, and fatty acids as well as butyrate, which is released from splanchnic tissue as beta-hydroxy butyrate, is much greater than glucogenic nutrients in lactating cows (Cantalapiedra-Hijar et al., 2014a). Therefore, in high producing dairy cows, lipogenic nutrients are generally in adequate supply while glucogenic nutrient may become limiting or AA may be utilized to meet metabolic demand for glucose.

Changing supply of glucogenic and lipogenic nutrients affects the nature of energy utilization for milk component synthesis and deposition in body reserves. Increased milk protein yield has been observed in diets formulated to be isoenergetic with greater starch content compared to fat and fiber (Boerman et al., 2015b; Morris et al., 2020b). Increased milk protein with increased starch versus fat is likely multifaceted and due to a combination of increased microbial protein supply (Roman-Garcia et al., 2016), decreased energy requirement of the portal drained viscera (Cantalapiedra-Hijar et al., 2014a) which may spare AA, and increased efficiency of AA utilization by mammary glands (Cantalapiedra-Hijar et al., 2015). Additionally, with diets formulated to be isoenergetic with higher starch compared to fat and fiber, TE increases and milk fat yield sometimes decreases (van Knegsel et al., 2007a; van Knegsel et al., 2007c; Boerman et al., 2015b; Morris et al., 2020b). In an experiment where 4.9 Mcal of either glucose, or palm olein were abomasally infused, glucose increased TE by 1.6 Mcal/d and decreased milk energy by 1.6 Mcal/d, whereas palm olein increased milk energy by 1.8 Mcal/d due to increased ME by 2.2 Mcal/d (Nichols et al., 2019e). Differences in milk energy were due to decreased milk fat with glucose infusion and increased milk fat with palm olein

infusion. Energy partitioning towards TE at the expense of milk energy with increased supply of glucogenic nutrients is likely due to increased circulating insulin when feeding cows increased glucogenic precursors (Rius et al., 2010a; Cantalapiedra-Hijar et al., 2014a; Boerman et al., 2015b), which signals for increased lipid synthesis in peripheral tissues. In agreement, increased respiratory quotient, which indicates increased lipid synthesis, is observed with increased supply of glucogenic precursors (Nichols et al., 2019e; Morris et al., 2020b). Therefore, although glucose is likely more limiting for milk production than lipids, increasing dietary glucogenic precursors does not unequivocally increase milk energy output, which may occur because glucose supply to the mammary glands does not increase with increased glucogenic nutrients. When feeding cows diets containing 35% starch and 31% NDF compared to diets containing 16% starch and 46% NDF, the high-starch diet increased net portal appearance of glucose, yet, net splanchnic release of glucose was not different among diets (Cantalapiedra-Hijar et al., 2014a).

Physiological state might influence response to changes in energy sources. For example, increased dietary starch and sugar (25.9 vs. 20.1% of DM) increased milk protein yield by 12% in late-lactation cows (190 days in milk) but not in early-lactation cows (30 days in milk; Piccioli-Cappelli et al., 2014), which might occur due to insulin resistance in early-lactation cows. Effects of energy source on milk component yield and energy partitioning will have an effect energetic efficiency, ability to predict productive performance, and estimate energy requirements. Currently, nutrition models do not account for the effects of energy source on milk protein and fat production or energy partitioning.

Effects of Dietary Energy and Protein on Energy Utilization

Adequate supply of both energy and protein are essential for maximal milk production.

Current nutrition models evaluate energy and protein supply as individual entities and assume that milk production cannot exceed the more limiting of the two (NRC, 2001). However, it has been well known for several years that energy and protein are interrelated and that the dairy cows has the ability to adapt intermediary metabolism to conserve the nutrient that is in shortest supply (Oldham, 1984).

In experiments where supply of both energy and MP were manipulated, response in milk production have been variable. According to the theory of most limiting nutrient that is used by NRC (2001), increased supply of energy or MP will only increase milk production if the other is supplied above requirements. In several individual studies, increased supply of energy and MP individually and additively increased milk protein, fat, and lactose production has been observed (Broderick, 2003; Rius et al., 2010b; Nichols et al., 2018). In these studies, interaction between MP and energy were not observed. However, Brun-Lafleur et al. (2010) observed an interaction between energy and MP in an experiment designed to test interaction between energy and MP by testing multiple levels of each factor. In this experiment, NE_L and MP ranged from estimated balance of -3.4 to 3.4 Mcal and -271 to 271 g. When energy was deficient, increasing MP slightly increased milk protein yield and when MP was deficient, increasing energy had no effect on milk protein yield. Milk protein yield only increased when the most limiting of the two nutrients was supplied. However, in disagreement with the theory of most limiting nutrient, milk fat yield was increased independently and additive as NE_L

and MP increased (Brun-Lafleur et al., 2010). Discrepancy between studies testing the interaction between protein and energy clearly demonstrate the need to better understand this relationship and to design studies that more thoroughly test this interaction. For example, the experiments of (Rius et al., 2010b) and (Nichols et al., 2018) used 2×2 factorial which assuming linearity between two levels of a factors. Whereas, Brun-Lafleur et al. (2010) observed that increasing both energy and MP resulted in a quadratic increase in milk protein yield such that the rate of increase in milk protein decreased with increasing energy or MP.

Interactions between energy and protein supply with milk production occurs in the rumen between digestible carbohydrates (i.e., energy) and degradable protein to affect microbial protein supply and nutrient digestibility and consequently affect energy and protein supply to the animal (NRC, 2001). Microbial N on average accounts for greater than 50% of MP of total supply of MP in a lactating dairy cow, and adequate supplies of both rumen digestible carbohydrate and protein are necessary to maximize microbial protein production (White et al., 2017b). Synchronization between ruminal energy and protein supply is essential to maximize the efficiency of energy utilization in the rumen. With excess glucose, rumen microbes increase energy spilling via futile cycles leading to increased non-productive heat production as demonstrated by mixed rumen cultures (Hackmann and Firkins, 2015). Therefore, feeding excess carbohydrates might increase non-production heat loss and decrease overall energetic efficiency of the animal; however, it is unknown if energy spilling occurs in vivo. Additionally, in diet where RDP was 42 to 192 g/d below NRC (2001) recommendations, NDF digestibility decreased by

4 to 5 percentage units compared to diets that had an RDP balance of -17 to 141 g/d (Lee et al., 2011; Lee et al., 2012a), which will subsequently decrease energy digestibility.

Interaction between energy and protein occur in the mammary gland and throughout the body and demonstrate the metabolic flexibility of cows to adapt to differences in energy and MP to maintain milk production. Increased supply of glucogenic precursors might decrease utilization of AA by splanchnic tissues and increase splanchnic release of AA (Cantalapiedra-Hijar et al., 2014a). These responses are supported by a recent study by Omphalius et al. (2020) where abomasal infusion of glucose decreased hepatic catabolism of non-essential AA and His, Met, and Phe, and increased splanchnic release of His, Met, and Phe. In the mammary glands, increasing protein via dietary manipulation or infusion generally increases mammary gland uptake of essential AA; however, catabolism of AA increases and consequently efficiency of converting AA into milk protein decreases with increased flux of essential AA (Nichols et al., 2019f; Omphalius et al., 2019; Omphalius et al., 2020). Increasing energy will increase milk protein yield if supply of AA is adequate which will, in turn, increase energetic efficiency by sparing AA from catabolism. In mammary cells, regulation of protein synthesis is sensitive to both AA and energy (Rius et al., 2010a; Arriola Apelo et al., 2014; Cant et al., 2018). Additionally, quantity of mammary secretory cells may increase with increased AA and energy as indicated by an upregulation of genes responsible for cellular endoplasmic reticulum biogenesis and mammary secretory cell proliferation (Nichols et al., 2017; Nichols et al., 2019d).

Because catabolism of AA contributes to substantial heat production, the effects of dietary manipulation on utilization of AA in the mammary glands and total milk

protein output should be considered as it relates to energetic efficiency. Infusion of EAA generally increases heat production which might be due to increased AA catabolism as suggested by an overall increase in plasma urea concentration (Nichols et al., 2019a; Nichols et al., 2019e). However, AA catabolism is a balance between total AA supply and utilization of AA for milk protein synthesis. Plasma urea decreased when glucose was infused with AA corresponding to a numerical increase in milk protein yield (Nichols et al., 2019e). Furthermore, with provision of different AA profile in abomasal infusion, treatments that increased milk protein yield had similar plasma urea concentration as control (Nichols et al., 2019a). From these studies, it was not possible to separate the effects of EAA provision on heat generated from AA catabolism or increased milk protein synthesis.

CONCLUSIONS

The NE_L system developed by Moe et al. (1972a) has been successfully used to feed dairy cows for decades. As our understanding of the biology underlying energy utilization of dairy cows continues to improve, incorporation of this knowledge into the NE_L system is needed. Increasing accuracy in estimating ME by accounting for source of variation in nutrient digestibility, CH₄, and UE excretion will improve the system. Additionally, dietary nutrients are known to affect milk component production and partitioning of nutrients between milk output and tissue reserves. These factors should be considered in future energy systems to improve our ability to predict energy utilization and requirements. Currently, a knowledge gap exists regarding the efficiency of converting ME to milk components and experimental and modeling work to improve our

understanding is warranted. The NE_L system has served the dairy industry for many decades and will continue to do so for decades to come.

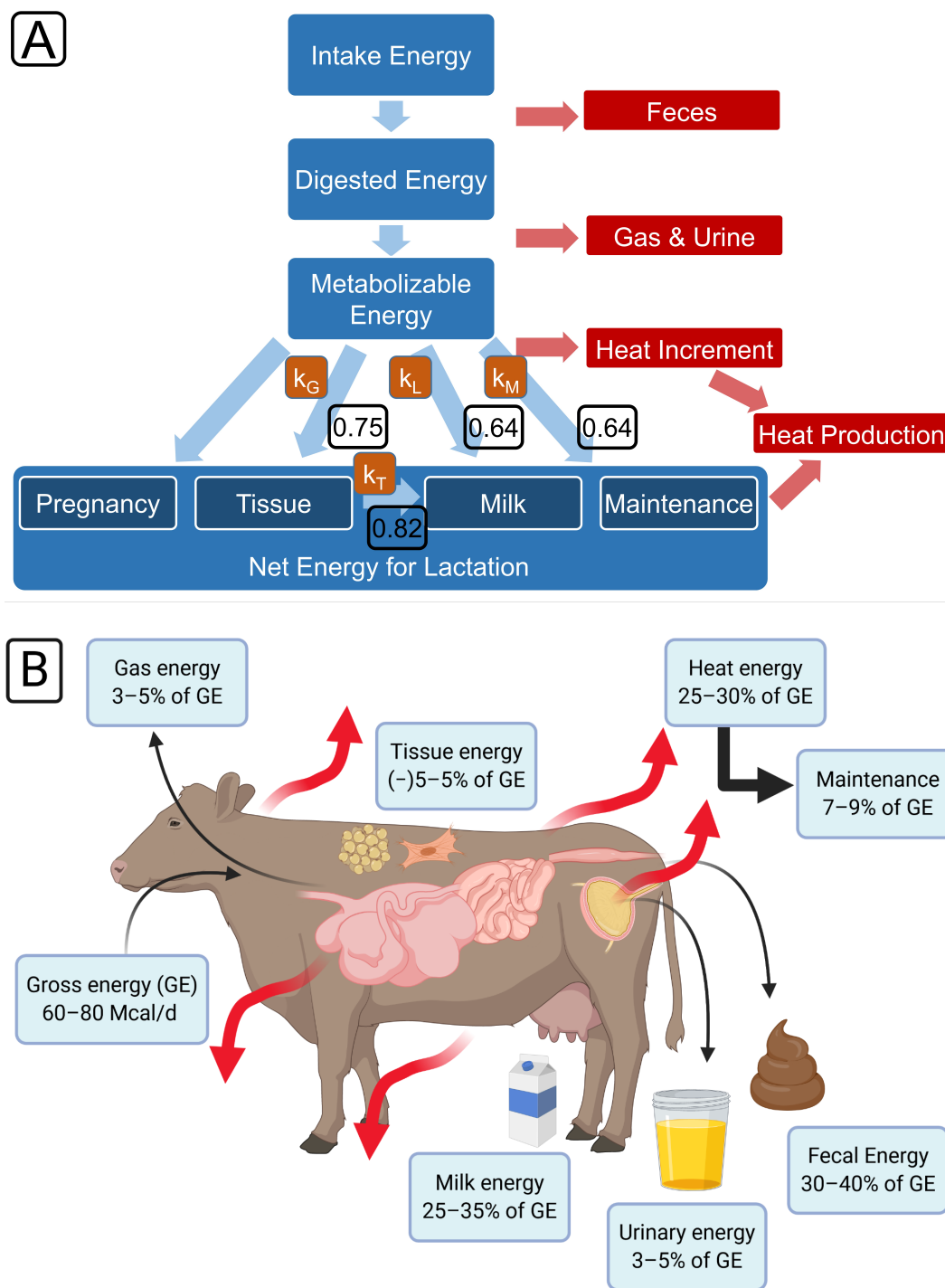


Figure 2.1. The net energy for lactation system (panel A) and the average gross energy intake and energy utilization for a lactating Jersey cow. k_G , k_L , and k_M are the efficiency

of converting metabolizable energy into tissue, milk, and maintenance energy, respectively. k_T is the efficiency of converting tissue energy into milk energy. In panel A, values in black boxes represent NRC, 2001 values for k_G , k_L , k_M , and k_t . See *key terms in the NE_L system* for further description.

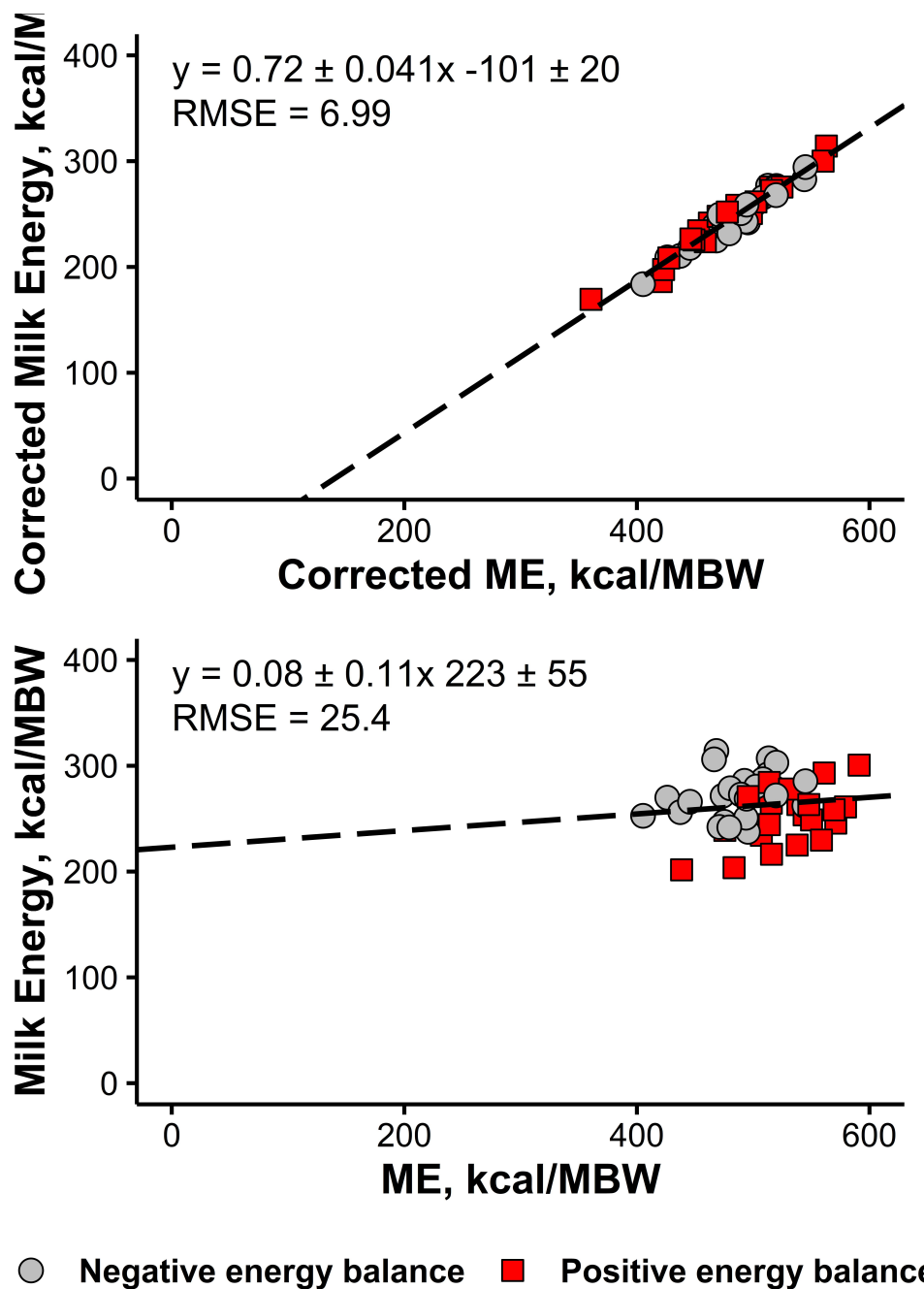


Figure 2.2. Regression of lactation energy on ME intake per unit of metabolic body weight that is either corrected for tissue mobilization or not corrected. The slope represents k_L and the y-intercept represent NE_M . $n = 48$, negative energy balance (mean \pm standard deviation) = -3.2 ± 2.7 Mcal of NE_L/d ($n = 25$), positive energy balance = $2.8 \pm$

2.0 Mcal of NE_L/d ($n = 23$). All data are corrected for the random effect of square, cow within square, and period.

Table 2.1. Descriptive statistics of energy balance data on lactating Jersey cows from the University of Nebraska-Lincoln (n = 261)¹

Item ¹	Mean	SD	10 th percentile	90 th percentile
Animal descriptions				
DIM, d	166	52	95	237
Parity	3.0	0.95	2.0	4.0
BW, kg	451	43	391	509
DMI, kg/d	18.4	2.5	15.2	21.6
ECM, kg/d ¹	33.4	6.1	26.3	41.6
Energy fractions, Mcal/d				
GE	79.8	11.1	66.2	92.8
DE	52.9	8.1	43.0	63.0
ME	46.3	7.9	36.7	55.8
NE _L	31.2	7.0	22.6	39.3
FE	26.9	4.6	20.9	32.3
UE	2.93	0.84	1.97	4.1
Gas energy	3.68	0.66	2.81	4.53
Milk energy	22.1	4.0	17.5	27.3
Heat increment	14.4	2.8	10.8	17.8
Maintenance	7.8	0.6	7.0	8.6
TE, NE _L basis	1.3	6.8	-7.6	9.6
Energy fractions, % GE				
FE	33.8	3.8	29.2	39.1
UE	3.8	1.2	2.3	5.7
Gas energy	4.6	0.8	3.7	5.5
Milk energy	28.0	4.9	22.2	34.4
Heat increment	18.1	3.1	14.5	22.3
Maintenance	10.0	1.6	8.3	11.6
TE, NE _L basis	0.9	8.8	-10.2	10.4
Energy fractions, Mcal/kg				
DM				
GE	4.34	0.17	4.15	4.53
DE	2.88	0.22	2.61	3.15
ME	2.52	0.23	2.22	2.77
NE _L	1.70	0.29	1.33	1.99
Utilization, %				
DE/GE	0.662	0.038	0.609	0.708
ME/DE	0.873	0.026	0.836	0.899
NE _L /ME	0.669	0.070	0.582	0.738

¹All variable are defined and calculated as described in the text. Maintenance was calculated as 0.080 Mcal/MBW and used to calculated heat increment and NE_L.

$ECM = 0.327 \times \text{milk yield (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{true protein (kg)}$ (Tyrrell and Reid, 1965).

Table 2.2. Gross energy value of nutrients and chemical components of diets

Items	Mcal/kg DM
CP (average)	5.65
Fatty acids (average)	9.4
Starch (average)	4.2
NDF (average) ¹	4.2
ROM (average) ²	4.0
Ash	0.0
Leu	6.5
Ala	4.4
Palmitic acid	9.4
Glucose	3.7
Cellulose	4.2
Lignin ³	6.0
Acetic acid	3.5
Glycerol	4.3
Urea	2.5
TDN ⁴	4.4

¹True gross energy is > 4.2 Mcal/kg and depends on % lignin. Because lignin is indigestible it can be ignored for determining energy supply to the animal.

²Residual organic matter (ROM) = 100 – % CP – % fatty acids – % starch – % NDF – % ash; assumed to be predominately sugars, lactic acid, acetic acid, glycerol, and soluble fiber.

³Sufuric lignin extracted from rape straw (Voitkevich et al., 2012)

⁴Total digested nutrients (Swift, 1957)

Table 2.3. Effects of incomplete gas recovery on calculated energy fractions¹

Items	Gas recovery, % ²					
	100	95	90	80	70	60
Gases, L/d						
O ₂ consumption	5000	4750	4500	4000	3500	3000
CO ₂ production	5250	4988	4725	4200	3675	3150
CH ₄ production	400	380	360	320	280	240
Energy fractions, Mcal/d						
ME intake	50.2	50.4	50.6	51.0	51.4	51.7
NE _L intake	32.9	34.2	35.5	38.1	40.7	43.3
Gas	3.78	3.59	3.40	3.02	2.65	2.27
Heat production ³	25.1	23.8	22.6	20.0	17.5	14.9
Tissue ⁴	0.0	1.3	2.6	5.2	7.8	10.4
Tissue/NE _L ⁵ , %	0.0	4.0	7.9	15.8	23.7	31.6

¹Data were calculating under the following assumptions: mature lactating Jersey cow;

BW, kg = 450; digestible energy intake, Mcal/d = 58.0; milk energy, Mcal/d = 25.1;

urinary energy, Mcal/d = 4.0; true tissue energy balance, Mcal/d = 0; maintenance

energy, Mcal/d = $0.08 \times \text{MBW}$ (NRC, 2001).

²Assumed recovery of each gas was effect equally.

³Heat production, kcal/d = $3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 - 0.518 \times \text{CH}_4 - 1.431 \times \text{N}$

(Brouwer 1965).

⁴Tissue energy, Mcal of NE_L = (ME intake – heat production – milk energy) \times 0.89.

⁵NE_L is the NE_L from 100% gas recovery.

CHAPTER 3: DERIVATION OF THE MAINTENANCE ENERGY REQUIREMENTS FOR JERSEY COWS

INTERPRETIVE SUMMARY. Morris and Kononoff (202X). “Derivation of the maintenance energy requirements for Jersey cows,” Maintenance energy requirements of Jersey cows during lactation, maintenance feeding or when fasted were derived. Energy balance was quantified via total collection and indirect calorimeter. Metabolizable energy for maintenance was 0.146 ± 0.0087 Mcal per unit of metabolic body weight (MBW). Net energy for maintenance as determined via fasting heat production was 0.102 ± 0.0071 Mcal/MBW. Net energy for maintenance was not different between lactating and dry cows; however, the efficiency of converting metabolizable energy into net energy was greater for dry compared to lactating cows (0.717 vs. 0.688).

RUNNING HEAD: MAINTENANCE OF JERSEY COWS

Derivation of the maintenance energy requirements for Jersey cows

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ABSTRACT

Maintenance energy is the energy required to conserve the state of an animal when no work is completed. Dietary energy must be supplied to meet maintenance requirements must be met before milk can be produced. The objectives of the current experiment were to quantify maintenance energy requirement of Jersey cows when lactating or dry. Energetic measures were collected on eight Jersey cows and evaluated across three physiological phases and nutritional planes: lactation, dry cows fed at maintenance, and fasted dry cows. Through total collection of fecal and urine as well as using headbox-style indirect calorimeters, energy balance and heat production data were measured across all phases. Lactation data were collected across 4 28-d periods. Data for cows fed at maintenance were collected after 14 d and fasting heat production was measured on the 4th d of a 96 h fast. Net energy for maintenance (NE_M) requirements, and the efficiency of converting metabolizable energy (ME) into net energy were compared between lactating and dry (maintenance or fasting phase) cows. Heat production of dry cows fed at maintenance, which was assumed to represent ME for maintenance, was 0.146 ± 0.0087 Mcal per unit of metabolic body weight (MBW). Fasting HP, which represents NE_M, was 0.102 ± 0.0071 Mcal/MBW. When estimated via regressing energy balance on ME intake, NE_M was not different between dry and lactating cows (0.128 ± 0.033 vs. 0.104 ± 0.0054 Mcal/MBW). However, the slope of the regression of energy balance on ME intake was greater for dry compared to lactating cows (0.717 ± 0.047 vs. 0.688 ± 0.011). This suggests that dry cows were more efficient at converting ME into net energy and that the efficiency of utilizing ME for maintenance may be greater than for lactation. Our measurements of NE_M and the slope of ME on energy balance are

greater than the value used by NRC (2001), which are 0.080 Mcal/MBW for NE_M and approximately 0.64 for the slope. Results of this study suggest that NE_M and the efficiency of converting ME into NE_M of modern lactating cows is greater than previous measurements.

Keywords: fasting, heat production, energy utilization, energetic efficiency

INTRODUCTION

Maintenance energy is the energy required to conserve the state of an animal when no work is performed or no products, such as meat or milk, are formed (Baldwin, 1995). Maintenance energy expenditure can be divided into three categories: 1) work and service functions, such as resorption of ion in the kidney, respiration, heart work, and nervous tissue and liver functions; 2) membrane transport; and 3) synthesis of cellular components (Baldwin et al., 1985). Total portion of these are estimated to be 40 – 50%, 25 – 35% and 15-25% respectively. Reported estimates for net energy required for maintenance (NE_M) range from 0.073 (Flatt et al., 1965a) to 0.124 (Moraes et al., 2015) Mcal per unit of metabolic BW (MBW). For a mature Jersey cow weighing 450 kg, the difference between an NE_M of 0.073 or 0.124 Mcal/MBW would yield a difference of 5.0 Mcal/d. Moraes et al. (2015) suggested that NE_M might have increased overtime in lactating cows and is associated with increased DMI and milk yield. Changes in the mass of tissue with a high metabolic activity per unit of weight such as the heart, liver, kidney and gastrointestinal tract can affect maintenance energy expenditure, and increased mass and energy expenditure of these tissues likely occur with increased DMI and milk yield

and in lactating compared with non-lactating animals (Smith and Baldwin, 1974; Canas et al., 1982; Reynolds et al., 1991).

The point estimate of NE_M of a lactating cow is theoretical because it cannot be directly measured. However, NE_M can be estimated via one of two methods: by measuring heat production (**HP**) at fasting or via regression of energy balance on metabolizable energy (**ME**) with the y-intercept representing NE_M . Published estimates of NE_M (Mcal/MBW) of fasted dairy cows are 0.073 (Flatt et al., 1965a), 0.098 (Birnie et al., 2000), 0.100 (Holter, 1976), and 0.108 (Yan et al., 1997b). Additionally, it is known that NE_M in fasted dairy cows is positively correlated with milk yield (Holter, 1976) and negatively correlated with BCS (Birnie et al., 2000). Estimates for NE_M via regression in lactating cows are 0.085 (Moe et al., 1972a), 0.086 (Moraes et al., 2015), 0.098 (Morris et al., 2020d), 0.104 (Yan et al., 1997a), and 0.105 (Dong et al., 2015). NRC (2001) assumes that NE_M is 0.080 Mcal/MBW which is based off the work of (Flatt et al., 1965a) plus 10% to account for increased activity requirements for animals in the field compared to those data collected on cows in tie-stalls. Compared to non-lactating, maintenance energy requirements in lactating animals are generally thought to be 10 to 24% greater. This difference is thought to be a result of increases in both mass and activity of tissues and organs (Smith and Baldwin, 1974; Canas et al., 1982). Holter (1976) observed that the fasting HP of cows immediately following lactation was 9% greater than 31 d after lactating ceased. Because of the abrupt cessation of lactation, this increase in fasting HP may have been a result of mammary involution. To our knowledge, the most recent study where fasting HP was measured in dairy cows was published 20 years ago (Birnie et al., 2000) and no known measurements have been

reported on Jersey cows. In addition, we are unaware of estimates of NE_M on the same group of dairy cows during lactation and after drying off while fasted. Therefore, the objective of this work was to quantify maintenance energy requirements of Jersey cows that are lactating or dry. Because of genetic improvements in milk production, we hypothesize that our measures of NE_M will be greater than what is used by NRC (2001). Additionally, we expect NE_M to be greater when estimated on lactating cows compared to dry cows.

MATERIALS AND METHODS

Animals and Treatments

The University of Nebraska–Lincoln Animal Care and Use Committee approved animal care and experimental procedures of this study. Eight multiparous Jersey cows sourced from a commercial dairy 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. All cows were confirmed non-pregnant by blood test for pregnancy-specific protein B (Romano and Larson, 2010).

The experiment consisted of three phases: lactation, dry maintenance feeding and dry fasting (Figure 3.1). For the lactation phase, data were used from a study conducted in the same 8 cows in which measurements were collected across 4 periods on each cow ($n = 32$ total; CHAPTER 8:). A full description of the procedures used to evaluate energy utilization can be found elsewhere (CHAPTER 8:). For variables that were measured on both dry and lactating cows, methods were identical. According to the objectives of

CHAPTER 8:, cows were fed diets that varied in carbohydrates (18.9 to 30.8% starch or 35.9 to 25.5% NDF), fatty acids (2.90 to 6.80% fatty acids) or supplemental Lys (0 to 16 g/d of supplemental digestible Lys).

Prior to the start of the maintenance feeding phase, cows were dried off. The maintenance feeding phase was 18 d in length. All cows were fed the same TMR to meet maintenance energy requirement which was assumed to be 0.100 kcal of NE_L /MBW (Moraes et al., 2015). Feed delivery was based on individual cow BW, which was measured twice weekly, and NRC (2001) estimates of dietary NE_L . Feed offering were based upon the most recent BW measure. Dietary ingredients for the base diet (corn silage, alfalfa hay, and concentrate) were placed in a Calan Data Ranger (American Calan, Inc., Northwood, NH), and subsequently used to mix and deliver TMR once daily at 0930 h. During the final 4-d of the maintenance feeding period, energy balance was determined. Following the maintenance feeding phase, cows were fasted for 96-h. Throughout all phases, cows were allowed ad libitum access to water.

Sample Collection and Analysis

Measurement of DMI, fecal, and urine output were completed during the lactation and dry maintenance phase as described previously (Morris and Kononoff, 2020). Briefly, DMI, milk yield (lactation only), as well as fecal and urine output were determined for 4 consecutive d. Catharized inserted into the bladder were used to separate feces and urine and upon storage urine was acidified to maintain a $pH < 5.0$ with 50% HCl. Daily samples of feed ingredients, feed refusals, milk (lactation only), feces, and

urine were collected and composited on a wet-weight basis. All samples were stored at 4°C until further analysis.

Feeds, refusals, and feces were dried at 60°C for 48 h to determine DM and for further analysis. Dry feeds, refusals, and feces were ground to pass a 1-mm screen (Wiley Mill; Arthur A. Thomas Co., Philadelphia, PA), and analyzed by Cumberland Valley Analytical Services, Inc. (Waynesboro, PA) for N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF with sodium sulfite (Van Soest et al., 1991) and α amylase and corrected for ash contamination (**NDF_{OM}**), starch (Hall, 2009), and ash (943.05; AOAC International, 2000). Feed ingredients were also analyzed for acid detergent lignin (Goering and Van Soest, 1970) and crude fat (2003.05; AOAC International, 2000) by Cumberland Valley Analytical Services Inc. Dry ground samples were analyzed for gross energy (**GE**) content (Parr 6400 Calorimeter, Moline, IL) in the nutrition laboratory of the University of Nebraska-Lincoln. The chemical composition of the maintenance diet is listed in Table 3.1. Milk energy was calculated from yield of milk fat, protein and lactose (NRC, 2001).

Headbox-style indirect calorimeters were used to measure O₂ consumption and CO₂ and CH₄ production (Figure 3.2). Total volume of gas flow through the headbox was measured using a mass flow meter (MCW Whisper, Alicat Scientific, Tucson, AZ) and using a data logger (Model XR440, Pace Scientific Inc.) flow rate was recorded every minute. 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 O₂, CO₂ and CH₄ using a gas

analyzer (X-stream; Emerson Process, St. Louis, MO) according to the method of Nienaber and Maddy (1985). To calculate O₂ consumption and CO₂ and CH₄ production, difference between incoming and outgoing gas was multiplied by total air flow. Heat production was calculated using the Brouwer (1965) equation. Urinary N excretion was not measured in dry cows. In cows fed at maintenance, estimated urinary N excretion was calculated from dietary CP (Spek et al., 2013b) and equaled 81 g/d for all cows. In fasting cows urinary N excretion was assumed to be 0.13 g/kg of BW (Birnie et al., 2000). Correcting for urinary N excretion in the Brouwer (1965) equation decreases HP by less than 1%; therefore, the effect of any error in our assumptions is minimal (McLean and Tobin, 1987). While each cow was inside the headbox, free water intake was measured using a water meter (Model DLJSJ75, Daniel L. Jerman Co., Hackensack, NJ).

Prior to the start of the experiment and upon completion, efficiency of gas collection via headboxes was determined by burning 100% ethyl alcohol and measuring O₂ consumption of CO₂ production relative to expected value calculated from alcohol disappearance. Consumption of O₂ and production of CO₂ were (average \pm SD) 101 ± 0.7 and $99 \pm 1.6\%$ of expected.

Gas measurements were collected during each of the three phases. During the lactation phase, cows were placed in headboxes for 1 d. During the maintenance feeding phase, each cow was placed in headboxes for 2 consecutive d. During the fasting phase, cows were placed in headboxes 72 h subsequent to the initiation of feed restriction. While cows were in the headboxes, a collection period of 23-h was used to measure O₂ consumption and CO₂ and CH₄ production for cows on all phases. Gas data were adjusted to a 24-h period. Cows were adapted to headboxes for a minimum of 3 d prior to the start

of the experiment. For the lactation and maintenance feeding phase, feed was placed in the bottom of the headbox. Cows were allowed ad libitum access to water from a water bowl placed inside the headbox, and free water intake was measured as described above

Energy Calculations

Respiratory quotient (**RQ**) was calculated using the ratio of CO₂ produced to O₂ consumed. Energy loss as CH₄ was estimated by multiplying CH₄ production by its enthalpy (9.45 kcal/L). Calculations for digested energy (**DE**) and ME were as follows:

$$\text{DE (Mcal/d)} = \text{GE (Mcal/d)} - \text{fecal energy (Mcal/d)} \quad [1]$$

$$\text{ME (Mcal/d)} = \text{DE (Mcal/d)} - \text{urine energy (Mcal/d)} - \text{methane energy (Mcal/d)} \quad [2]$$

Unaccounted for energy was assumed to represent tissue energy (**TE**):

$$\text{TE (Mcal/d)} = \text{ME (Mcal/d)} - \text{HP (Mcal/d)} - \text{milk energy (Mcal/d)} \quad [3]$$

To compare NE_M and the efficiency of converting ME into NE_L (**k_L**) between lactating cows and cows fed at maintenance or fasting (termed dry cows), regression were conducted as described by Kebreab et al. (2003) and (Morris et al., 2020d), except the term energy balance was used to represent the fact that dry cows were not producing milk. For this, ME was corrected for the ME that was used for tissue retention and energy balance was corrected for heat increment from tissue mobilization. Corrected ME and energy balance were calculated as follows:

$$\text{corrected ME} = \text{ME (kg/d)} - |\text{positive residual energy (Mcal/d)}|/\text{k}_G, \quad [4]$$

$$\text{corrected energy balance} = -\text{HP (Mcal/d) for fasting cows or milk energy (Mcal/d)} - |\text{negative residual energy (Mcal/d)}| \times \text{k}_T, \quad [5]$$

Where k_G and k_T were 0.89, 0.75, respectively (Moraes et al., 2015), and milk energy was 0 for maintenance cows.

Statistical Analysis

Corrected energy balance was regressed against the fixed effect of stage (lactating or dry) and the interaction of stage with corrected ME using the “lmer” package in R (v3.6.3). Differences between regression intercept values (representing NE_M) between stage was tested via the effect of stage. Differences between slope values (representing k_L) between stage were tested via the interaction of stage with corrected ME.

RESULTS AND DISCUSSION

Maintenance Phase

Respiratory quotient is generally considered to be a gross indicator of whole-body substrate oxidation. Production formation from the oxidation of lipids, protein, and carbohydrates result in an RQ of 0.71, 0.81, and 1.00, while lipid synthesis results in an RQ greater than 1 (Blaxter, 1989). In the current experiment during the maintenance phase, RQ was observed to be 0.942 ± 0.014 (Table 3.2) suggesting that cows were utilizing glucose as an energy source and to a lesser degree some fat and/or protein. Additionally, RQ was positively correlated with TE ($r = 0.80$; $P = 0.02$). This likely occurred due to greater lipid oxidation in cows in negative energy balance and greater lipid synthesis in cows in positive energy balance. Previously, increased RQ has been

observed in lactating cows when feeding diets that likely result in increased lipid synthesis (Nichols et al., 2019e; Morris et al., 2020b).

When cows were fed at maintenance, average TE was 0.3 ± 1.1 Mcal/d, and thus we concluded that NE_L supply was near or equal to maintenance requirements. Heat production of cows fed at maintenance represents the maintenance energy requirements on an ME basis (ME_M ; Moe et al., 1972). In the current study, HP of cows when fed at maintenance was observed to be 0.146 ± 0.0087 Mcal/MBW. In an retrospective analysis of 1,038 observations from 284 cows, ME_M on average was reported to be 0.126 Mcal/MBW; however time was observed to have an effect; observations of ME_M increased from 0.121 to 0.177 Mcal/MBW from 1963–1973 to 1984–1995, respectively (Moraes et al., 2015). Increased ME_M from 1963–1973 to 1984–1995 was suggested to have occurred because of increased milk yield. In an analysis of data from 221 lactating Holsteins, ME_M averaged 160 kcal/MBW (Yan et al., 1997a). As described by Moraes et al. (2015), energetic parameters are not exclusively independent and the recursive relationship should be considered. Consequently, examining ME_M without considering NE_M and the relationship between the two (i.e., k_L) can be misleading. For example, increasing dietary forage inclusion is associated with an increase in NE_M (Yan et al., 1997a), which likely occurs because increasing dietary forage increases whole-body HP via an increase in energy expenditure by tissue services by the portal drained viscera (Reynolds et al., 1991).

Fasting Phase

During fasting, cows lost 6.5 ± 1.7 kg/d of BW (Table 3.3). This BW loss is because cows were in negative energy balance and to some extent, loss was due to a decrease in gut fill. Although neither can be directly measured, HP throughout the 4-d fast should be equal to negative energy balance and the difference between BW loss and BW loss due to negative energy balance represents BW loss due to loss of gut fill. On the 4th d of fasting, HP was 9.00 ± 0.49 Mcal/d. Heat production at the start of fasting should be equal to HP during the feeding period and then decrease with increasing duration of fasting (Yan et al., 1997b). Heat production during the maintenance feeding phase was 12.9 ± 1.01 Mcal/d (Table 3.2). Therefore, negative energy balance throughout fasting is computed to be 11.0 Mcal/d, which corresponds to a BW loss 1.9 kg/d for a cow with a BCS of 3.0 (NRC, 2001). We therefore, conclude that the remaining 4.6 kg/d decrease in BW during fasting can be attributed to decrease in gut fill and digestive tract organ mass, both of which are positively correlated with DMI (McLeod and Baldwin, 2000; NRC, 2001).

Because RQ represents whole-body substrate oxidation, it is an indicator of whether or not a physiological state of fasting has been reached, as an observation of 0.71 is believed to represent a true fasting state (Blaxter, 1989). In the current experiment, RQ was 0.726 ± 0.011 during the fasting phase (Table 3.3), which suggests that all animals were at or very near this fasting state. Yan et al. (1997b) measured RQ and HP throughout a 120-h fast and reported that although RQ continued to decrease after 72 h of fasting, HP was constant at 0.108 Mcal/MBW. Additionally, in the current experiment, CH₄ production during the fasting phase was 18 ± 5 L/d which suggests minimal rumen

fermentation and is much lower than CH₄ production for cows fed at maintenance (171 ± 17 L/d; Table 3.2). Similarly, Yan et al. (1997b) reported that CH₄ output approached 0 around 72 h of fasting.

In the NE_L system, fasting HP is generally considered to represent NE_M requirements (Moe and Tyrrell, 1973). Heat production during the 4th d of fasting was observed to be 0.102 ± 0.0071 kcal/MBW (Table 3.3). This value is greater than 0.073 reported by (Flatt et al., 1965a), and 0.080 currently used by the NRC (2001). Our observation is closer to other studies reporting fasting HP (0.098–0.108 kcal/MBW) (Holter, 1976; Yan et al., 1997b; Birnie et al., 2000). For several decades, milk production and DMI have steadily increased (Capper et al., 2009) and has likely led to increased NE_M requirements. However, similarity between our measurements of fasting heat production and those reported by (Yan et al., 1997b; Birnie et al., 2000) might suggest that NE_M requirements have stopped increasing with increase plane of production over the last 20 years.

Comparison of Maintenance and Efficiency in Lactating and Dry Cows

In the current study comparisons between estimates of maintenance and efficiency of converting ME into NE for lactating and dry cows, were made using data from the same 8 cows in the lactation immediately prior to dry-off. These cows were on average 141 ± 32 DIM, with a DMI of 19.2 ± 2.3 kg/d and ECM of 35.5 ± 4.4 kg/d (Table 3.4). Additionally, by treatment design, diets fed to lactating cows included a range in starch (min to max; 18.9 to 30.8% DM), NDF (25.5 to 35.9% DM), and fatty acids (2.90 to 6.80% DM)

When regressing corrected energy balance on corrected ME, the derived estimate of NE_M was not different between lactating and dry cows (0.103 ± 0.0054 vs. 0.103 ± 0.033 kcal/MBW; $P = 0.99$; Figure 3.3A). Variance for estimating NE_M for lactating cow was 6 times that of dry cows (0.033 vs. 0.0054) which was likely a response from the large extrapolation between the minimum ME intake for lactating cows (0.406 Mcal/MBW) and the y-intercept. We previously observed a similar large extent of variance (22% of mean) when estimating NE_M using the same methods (Morris et al., 2020d). Nevertheless, our data suggest that NE_M estimated via regression or as fasting heat production are similar.

A second regression was fit with a common intercept for lactating and dry cows because intercepts did not differ. Because a strong negative correlation ($r < -0.61$; data not shown) was observed between intercept and slope coefficients, fitting a common intercept is more appropriate for comparing slope coefficients. Based on previous work the efficiency of utilizing ME for maintenance (0.62) was nearly identical to NE_L (0.64) (Flatt et al., 1965a; Moe et al., 1972a). Therefore, the current NRC (2001) assumes a common efficiency for utilizing ME for maintenance and lactation. From the current experiment, the regression slope (i.e., k_M or k_L) was greater for dry cows compared to lactating cows (0.721 ± 0.047 vs. 0.683 ± 0.011) which suggests that cows may have been more efficient at utilizing ME for maintenance purposes than for lactation. The efficiency between maintenance and fasting (k_M) is a measure of the relative efficiency of dietary energy compared to body tissue energy to meet the maintenance needs. This relationship is not similar to the efficiency of utilizing energy above maintenance to generate end products such as meat or milk (Moe, 1981). Holter et al. (1970) reported

that the k_M of using a mixture of VFA to meet maintenance energy requirements was 0.68 but was high as 0.82 if only butyrate or propionate were supplied to meet maintenance requirements. In general, these values are similar to the k_M we observed. In lactating cows, k_L included heat increment from meeting maintenance requirement plus the efficiency of converting ME into milk fat, protein, and lactose. We recently reported that heat production associated with milk protein synthesis is 6.2 Mcal/kg which translates to a partial efficiency of 0.48 (Morris et al., 2020a). Therefore, the efficiency of synthesizing milk components may be less than the efficiency of meeting maintenance requirements. As discussed by (Moe and Tyrrell, 1973), as ME intake increases the overall efficiency approaches the k_L due to a dilution of the proportion of ME used for maintenance. This was also observed in the current experiment as k_L for the whole dataset was 0.684 ± 0.0094 (Figure 3.3C) and for lactating cows only was 0.689 ± 0.068 (Figure 3.3A).

Measurements of efficiency of utilizing ME for maintenance compared to lactation are confounded by many factors and our measures are no exception. For example, maintenance and fasting phases were conducted following the lactation measurements. However, measurements were collected in a climate-controlled environment using identical techniques and personnel; thus, potential confounding effects of sampling period should be minimized. Diets differed between lactating and fasting cows, and diets are well established to affect the efficiency of utilizing ME for lactation (Coppock et al., 1964; Moraes et al., 2015; Morris et al., 2020d). Consequently, we concede observed differences in efficiency for lactating and dry cows may be influenced by the diets fed. Specifically dry cows were fed diets with greater forage NDF (39.3 vs.

18.6% DM; data not shown), which could decrease efficiency of utilizing ME (Coppock et al., 1964; Reynolds et al., 1991). Additionally, dry cows were fed a diet with less CP (12.3 vs. 15.7% DM) which should result in a lower heat increment associated with catabolism of excess CP (Tyrrell et al., 1970; Reed et al., 2017; Morris et al., 2020a). In the field, dry and lactating cows are almost always fed vastly different diets with dry cow diets being higher in forage and lower in CP compared to lactation diets. More research with different diets is needed to confirm our finding that the efficiency of utilizing ME for maintenance is greater than lactation.

Overall efficiency of converting ME into NE_L

NRC (2001) assumes that k_L is approximately 0.63. In the current study, k_L determined using both lactating and dry cow data was observed to be 0.684 ± 0.0094 (Figure 3.3C). Moraes et al. (2015) reported that k_L has increased over time from 0.60 to 0.68, and is positively associated with milk yield, heart rate, and dietary ether extract. Therefore, it is plausible that k_L for modern dairy cows is greater when compared to their ancestors (Moe et al., 1972a) that were used as the basis for NRC (2001) recommendations. Additionally, increased NE_M will increase k_L , because these coefficients are inherently positively correlated (Moe, 1981). Therefore, both coefficients must be considered simultaneously. Although NE_M is likely greater than previous estimates, increased k_L will counter this increase in non-productive energy expenditure. For example, in a 450-kg Jersey cow with a ME intake of 45.8 Mcal, NE will be 2.2 Mcal greater when using k_L from the current experiment compared to NRC (2001) and NE_M will also be 2.2 Mcal greater. Therefore, energy available for milk and

tissue ($NE_L - NE_M$) will be the computed to be similar across the current estimates for NE_M and k_L or those used by NRC (2001).

CONCLUSIONS

Net energy required for maintenance in Jersey cows as measured as fasting HP was 0.102 ± 0.0071 Mcal/MBW which is greater than the 0.080 used by NRC (2001). Derivation for NE_M determined using lactating cow data did not differ from dry cows. The efficiency of utilizing ME in dry cows was greater than the efficiency of utilizing ME in lactating cows (0.721 vs. 0.683) which suggests that ME may be used with a greater efficiency for maintenance than for lactation. Although NE_M may be greater for modern lactating dairy cows, increased k_L compared to previous research (0.679 vs. 0.63) annul the effect of increased NE_M on energy available for milk and tissue production.

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Table 3.1. Ingredient and chemical composition of diet fed to Jersey cows during maintenance feeding period (% of DM)

Item ¹	Diet
Ingredient	
Corn silage ²	40.0
Wheat straw, chopped ³	30.0
Soyhulls	16.0
Soybean meal	9.1
Molasses	0.7
Urea	1.0
Mineral-vitamin mix ⁴	3.2
Chemical composition	
DM, as-is	56.0
CP	12.3
NDF	51.0
NDF _{OM}	48.4
Starch	15.8
Crude fat	2.2
GE, Mcal/kg DM	3.9
ME, Mcal/kg DM	2.11
NE _L , Mcal/kg DM ⁵	1.30

¹GE = gross energy; ME = metabolizable energy.

²Corn silage was (% of DM) 8.1 CP, 36.7 NDF, 37.3 NDF_{OM}, 38.7 starch, 4.8 ash, 2.9 lignin, 3.1 crude fat.

³Straw was (% of DM) 3.9 CP, 81.9 NDF, 73.5 NDF_{OM}, 0.1 starch, 16.2 ash, 11.2 lignin, 0.8 crude fat.

⁴Formulated to deliver as % of diet DM 1.33% calcium carbonate, 0.67% salt, 0.67% magnesium oxide, 0.27% trace mineral mix, and 0.23% vitamin mix.

⁵Estimated with NRC (2001) using mean DMI from Table 3.2, and forage chemical composition.

Table 3.2. Descriptive statistics for Jersey cows fed at maintenance

Item ¹	Mean	SD	Minimum	Maximum
Animal descriptions				
BW, kg	396	34	347	433
BCS ²	2.97	0.36	2.38	3.50
DMI, kg/d	6.15	0.38	5.55	6.57
Gases				
O ₂ consumption, L/d	2627	228	2304	2879
CO ₂ production, L/d	2471	190	2206	2686
CH ₄ production, L/d	171	17	146	197
RQ, L/L	0.942	0.014	0.921	0.966
Energy				
GE, Mcal/d	24.0	1.5	21.6	25.6
DE, Mcal/d	16.2	1.0	14.9	17.4
DE, Mcal/kg DM	2.63	0.11	2.44	2.82
ME, Mcal/d	13.3	0.8	12.2	14.3
ME, Mcal/kg DM	2.16	0.10	1.97	2.32
HP, Mcal/d	12.9	1.11	11.4	14.1
HP, Mcal/MBW	0.146	0.0087	0.135	0.159
Urine, Mcal/d	1.30	0.10	1.18	1.43
CH ₄ , Mcal/d	1.62	0.16	1.38	1.86
TE, Mcal/d	0.3	1.1	-1.0	2.4
Digestibility, %				
DM	62.6	3.3	56.9	68.0
OM	69.5	2.6	64.8	74.0
NDF	53.5	4.6	44.4	60.1
NDF _{OM}	61.8	3.8	55.2	68.1
CP	65.0	2.2	61.2	68.6
Starch	99.2	0.5	98.4	99.8
Energy	67.5	2.8	62.5	72.3
Fecal excretion, kg as-is	26.5	4.68	19.7	34.0
Urine excretion, kg as-is	11.7	3.32	9.4	19.5
Free water intake, L/d	24.1	7.0	13.4	37.4

¹GE = gross energy; DE = digestible energy; ME = metabolizable energy; RQ =

respiratory quotient, CO₂ production/O₂ consumption, L/L, MBW = metabolic BW

(kg^{0.75}).

²Scored 1 to 5 by 2 independent observations.

Table 3.3. Descriptive statistics for fasting Jersey cows' dataset

Item ¹	Mean	SD	Minimum	Maximum
Animal descriptions				
BW post fast, kg	370	37	321	411
BW loss, kg/d ²	6.5	1.7	2.8	8.5
Gases				
O ₂ consumption, L/d	1917	105	1772	2060
CO ₂ production, L/d	1392	73	1299	1495
CH ₄ production, L/d	18	5	14	29
RQ	0.726	0.011	0.712	0.747
Energy				
HP, Mcal/d	9.00	0.49	8.36	9.67
HP, Mcal/MBW	0.102	0.0071	0.092	0.113
Free water intake, L/d	5.3	5.6	1.1	16.3

¹RQ = respiratory quotient, CO₂ production/O₂ consumption, L/L, MBW = metabolic

BW (kg^{0.75}).

²Calculated as (BW prior to fasting – BW at the end of fasting)/4

Table 3.4. Descriptive statistics for lactating Jersey cows dataset¹

Item ^{1,2}	Mean	SD	Minimum	Maximum
Animal descriptions				
BW, kg	432	40	378	497
DIM	141	32	91	190
DMI, kg/d	19.2	2.3	14.7	24.0
ECM, kg/d	35.5	4.4	28.4	46.4
Energy				
HP, Mcal/d	24.7	2.2	21.5	28.7
ME, Mcal/d	45.8	5.9	35.0	58.3
TE, Mcal of NEL/d	−3.8	4.1	−14.6	6.1
Diet, % DM				
CP	15.7	0.46	15.1	16.5
NDF	30.7	3.20	25.5	35.9
Starch	25.1	4.41	18.9	30.8
Fatty acids	5.04	1.16	2.90	6.80

¹Subset of data from (CHAPTER 8:).

²ME = metabolizable energy; TE = tissue energy.

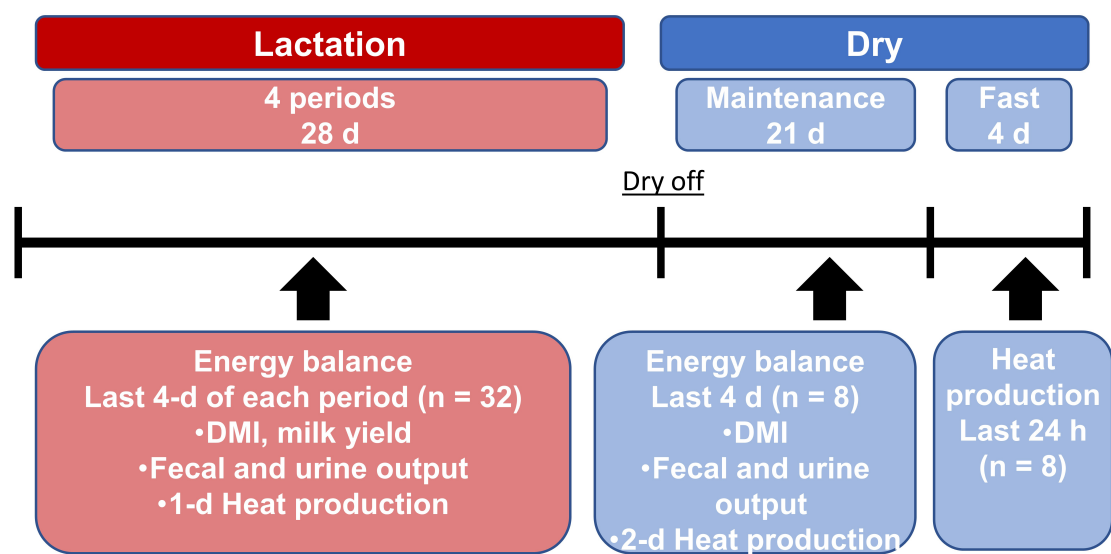


Figure 3.1. Illustration of time course and samples collected during the experiment.

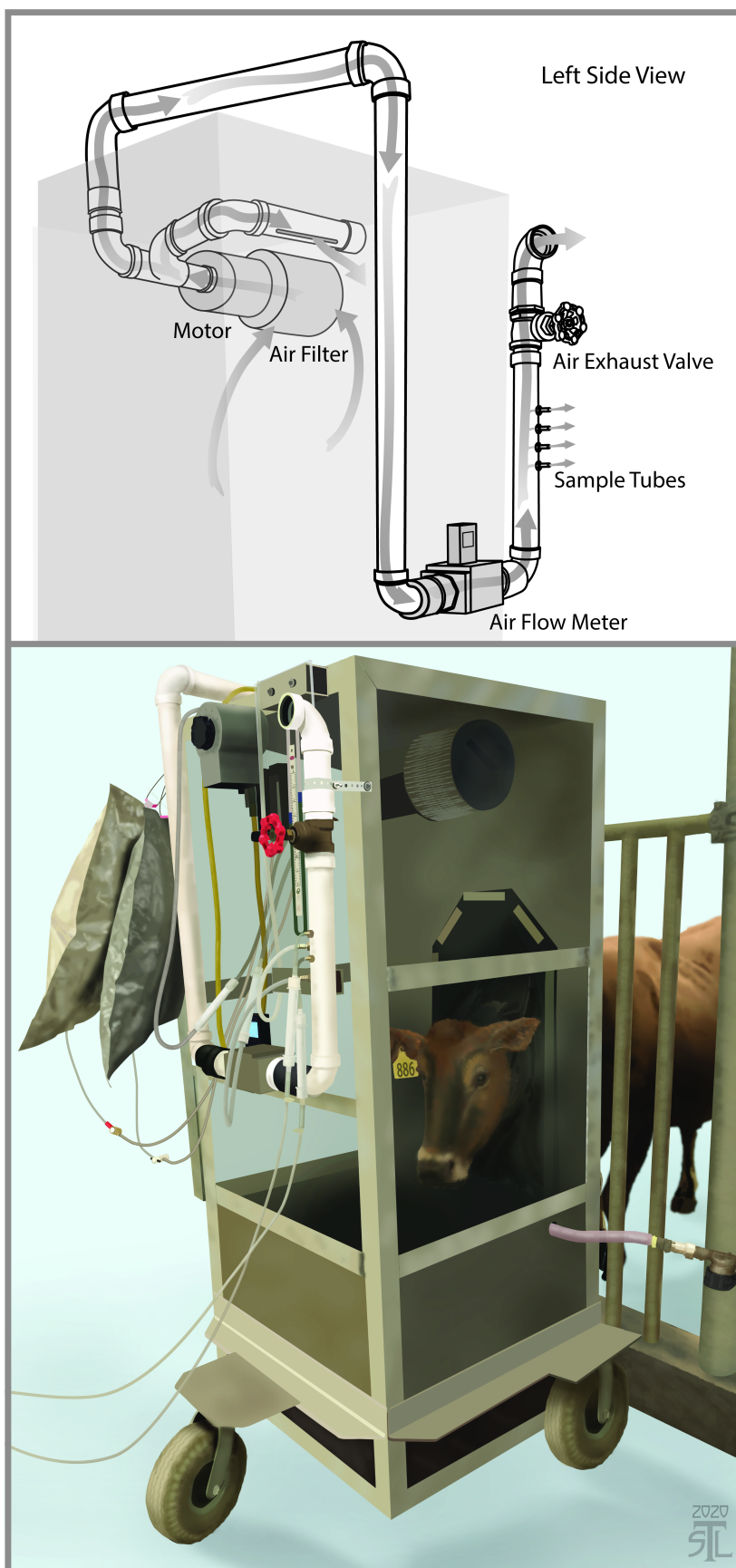


Figure 3.2. Illustration of headbox-style indirect calorimeter. The top panel show air flow through the system. Air is pulled into the headbox and through the exhaust system by a blower motor. Prior to entering the exhaust system, air is filtered. A fraction of the exhaust air is recirculated throughout the headbox which ensure adequate mixing of incoming air and respiratory gases from the animal. The remaining exhaust air exits the headbox where is pass through an air flow meter. Positive pressure is created by the air exhaust valve which allows for diversion of a small fraction of the exhaust air to sample bags. (Illustration by Sara Sara Taliaferro, Happy Beetle Studio, Lawrence Kansas).

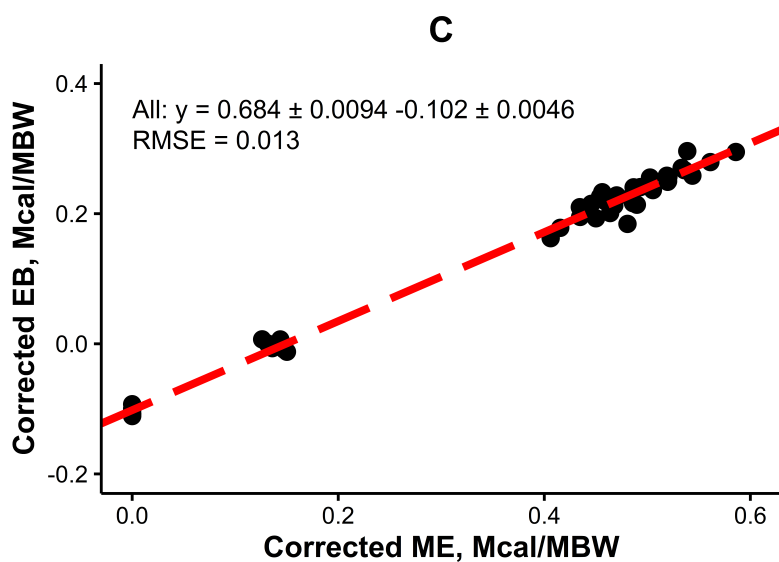
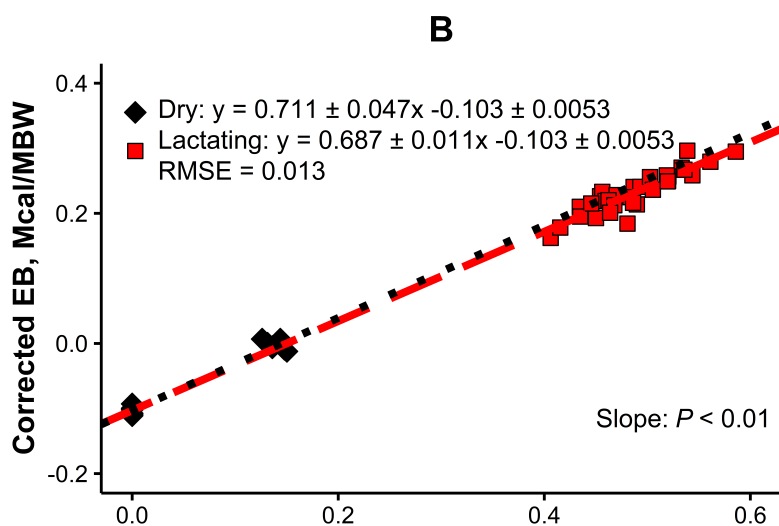
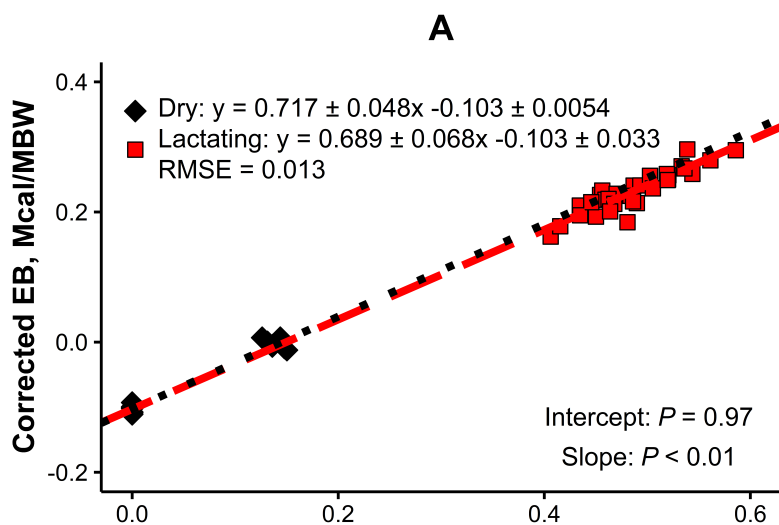


Figure 3.3. Regression of energy balance (EB) corrected for the heat increment of tissue mobilization, and ME intake corrected for tissue retention (see Materials and Methods). Both variables are expressed per unit of metabolic body weight (MBW). The intercept, which represents the inverse of NE_M , was not different ($P = 0.99$) between lactating and dry cows (Panel A) and was thus removed (Panel B). Average NE_M was 0.104 ± 0.0053 Mcal/MBW. The slope of each regression represents k_L (efficiency of utilizing ME intake for milk or maintenance) which was greater for dry cows compared to lactating cows (0.717 ± 0.047 vs 0.688 ± 0.011 ; $P < 0.01$; Panel C). A regression with all data pooled was analyzed (Panel C). From this, k_L was 0.685 ± 0.0094 and NE_M was 0.102 ± 0.0046 Mcal/MBW. Dry = dry cows; Lactating = lactating cows; RMSE = root mean square error.

CHAPTER 4: FACTORS THAT AFFECT HEAT PRODUCTION IN LACTATING JERSEY COWS

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INTERPRETIVE SUMMARY. Morris et al. (20XX). “Factors that affect heat production and heat increment in lactating Jersey cows,” Factors that affect heat production (HP) of lactating Jersey cows from feed intake, milk component yield, digestibility, and urinary N excretion were evaluated. Equations were derived from a database containing 293 cow-period observations of energy balance. Average HP was $28.1 \pm 3.7\%$ of gross energy intake. Variation in HP was adequately explained by metabolic body weight, and dry matter intake. Including milk production and nutrient digestibility variables resulted in similar model performance. Compared to milk fat synthesis, milk protein synthesis was associated with 2-fold more heat.

RUNNING HEAD: HEAT PRODUCTION

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ABSTRACT

Our objectives were to derive models to explain variation in HP of lactating Jersey cows. Individual animal-period data from 9 studies ($n = 293$) were used. The dataset included cows with a wide range (min to max) in d in milk (44 to 410), and milk yield (7.8 to 43.0 kg/d), and diets included corn silage as the predominate forage source, but diets varied (min to max on DM basis) in CP (15.2 to 19.5%), NDF (35.5 to 43.0%), starch (16.2 to 31.1%), and crude fat (2.2 to 6.4%) contents. Average HP was (mean \pm standard deviation) 22.1 ± 2.86 Mcal/d or $28.1 \pm 3.70\%$ of gross energy intake. Eight models were fit to explain variation in HP: 1) dry matter intake (INT); 2) milk fat, protein, and lactose yield (MILKCOMP); 3) INT and milk yield (INT+MY); 4) INT and MILKCOMP/DMI (INT+MILKCOMP); 5) mass of digested NDF, CP, and starch (DIG); 6) INT and digested energy (INT+DE); 7) or INT and NDF, CP, and starch digestibility (INT+DIG); 8) INT+MILKCOMP model plus urinary N excretion (INT+MILKCOMP+UN). For all HP models, metabolic body weight (MBW) was included. All models were derived via a backward elimination approach and included the random effects of study, cow, and period within block within study. The INT models adequately explained variation in HP with a non-random-effect adjusted concordance (uCCC) of 0.84. Similar uCCC (0.79 to 0.85) was observed for other HP models. The HP associated with milk protein yield and supply of digestible protein was greater than other milk production and nutrient digestibility variables. The HP associated with urinary N excretion was 5.32. Overall, HP can be adequately predicted from MBW and dry matter intake. Milk component yield, nutrient digestibility or urinary N excretion explained similar variation as dry matter intake. Coefficients for milk protein, and protein digestion suggest that digestion and

metabolism of protein and synthesis of milk protein contribute substantially to HP of a dairy cow.

Keywords: energy, indirect calorimetry, empirical model

INTRODUCTION

Heat in animals is generated from the catabolism of organic compounds. According to the laws of thermodynamics, heat generated from reactions in the body is equal to the difference in chemical energy between all substrates and products in a reaction (Kleiber, 1975). In lactating Jersey cows, heat production (**HP**) accounts for 25 to 32% of the gross energy (**GE**) intake (Drehmel et al., 2018; Judy et al., 2018; Reynolds et al., 2019). Heat production can be categorized into net energy for maintenance energy (**NEm**)—the basal energy expenditure of an animal—and heat increment (**HI**)—the HP that is associated with digestion and metabolism of food (NRC, 1981). Because HI represents a loss of energy and is the difference between ME and NEL, changes in HI may affect net milk production and tissue energy accretion of a dairy cow. Energetic efficiency, HP, and HI are affected by plane of production (Belyea and Adams, 1990), forage inclusion (Reynolds et al., 1991), and N intake above requirements (Tyrrell et al., 1970; Reed et al., 2017). Various factors are likely to influence HP and HI, and quantification of these may improve our ability to estimate NEL of diets.

We recently observed that the NRC (2001) model underestimated dietary NE_L content compared to measured NE_L, when NEm was assumed to equal 0.080 Mcal per metabolic BW (BW^{0.75}; **MBW**; Morris et al., 2020a). Furthermore, it has been shown that the efficiency of converting ME into NE_L (denoted as **k_L**) is affected by diet (Moraes et

al., 2015; Morris et al., 2020d). Historically, the NE_L system assumed a constant or near constant k_L (NRC, 2001, Moe et al., 1972). A recent analysis of indirect calorimetry data from the 1960s through the 1990s reported that k_L ranged from 0.60 to 0.70 and was associated with heart rate, milk yield, and dietary crude fat (Moraes et al., 2015). Biologically, k_L is likely dependent upon the substrates utilized and products formed (Baldwin, 1995). For example, HI has been reported to be greater for acetate than propionate or butyrate when these substrates were used for maintenance (Holter et al., 1970). Therefore, the use of a static k_L value may misrepresent underlying understanding of energy transformations. Consequently, the estimation of dietary NE_L may improve with models that predict HP and HI by accounting for sources of energy substrates and end products formed. The objective of this study was to derive models that explain variation in HP and HI of lactating Jersey cows. We hypothesized that HP and HI could be estimated from MBW and DMI and that the addition of variables that represent the nature of nutrients contained in feed consumed would further explain variation by better representing the biological basis of energy transformations.

MATERIALS AND METHODS

Data collection

Data from nine research experiments conducted at the University of Nebraska-Lincoln's Dairy Metabolism Unit were used (Foth et al., 2015; Drehmel et al., 2018; Judy et al., 2018, 2019a, b; Knoell et al., 2019; Reynolds et al., 2019; Morris et al., 2020a, b). Across these 9 experiments, data from 293 animal-periods were collected on 54 Jersey cows. However, digestible energy (**DE**), and nutrient digestibility were not measured on

all observations ($n = 261$ to 277 for dataset without DE and nutrient digestibility). In 2 studies (Judy et al., 2019a; Judy et al., 2019b), a covariate period was conducted where DE, ME, and nutrient digestibility were not measured. Additionally, during the experimental period of Judy et al. (2019a), nutrient digestibility was measured, but, of the energy fractions variables, only HP and HI were measured. Descriptive statistics for variables are listed in Table 4.1.

In all 9 experiments, O_2 consumption and CO_2 and CH_4 production were determined using headbox-type indirect calorimeters as described by Foth et al. (2015). Cows were housed in a temperature-controlled room ($20\text{ }^{\circ}C$) in tie-stalls equipped with rubber mats. In all studies except for one, cows were adapted to experimental diets for 21 or 28 d. In Judy et al. (2018), a 10-d adaptation period was used; however, in this study, experimental diets remained the same throughout the experiment and only feeding frequency (once or twice per d) changed. In all experiments except for one, a single group of cows were used. In Foth et al. (2015), cows were divided into two groups (termed block in the statistical model). All studies used a 23-h gas collection period that occurred after adaption. All gas data were adjusted to a 24-h period. Gas data were collected for 1 or 2 d. For the dataset where gas was collected for 2 d ($n = 155$), daily variation in HP was low (coefficient of variation of 4.6%). Therefore, given our main objectives and the conditions of the current experiment in which cattle, where housed a climate-controlled tie-stall facility we believe that 1 d of gas collection was adequate to estimate HP for this study. Gas collection for 1 d may not be adequate when environmental conditions can vary or feed delivery is not help constant. Cows were fed within the headboxes and allowed free access to water via a water bowl. Details for the

specific methods used to quantify gas consumption and production can be found in individual publications. Heat production was estimated through calculation of observed O₂ consumption and CO₂ production and with correction for CH₄ production and urinary N excretion according to Brouwer (1965):

$$\text{Heat production (HP, Mcal)} = 0.003866 \times \text{O}_2 (\text{L}) + 0.001200 \times \text{CO}_2 (\text{L}) - 0.000518 \times \text{CH}_4 (\text{L}) - 0.001431 \times \text{Urinary N (g)} \quad (1)$$

In all 9 experiments, milk production and composition were quantified for 4 consecutive d. Cows were milked twice per d and fed once per d in all experiments, except in (Morris et al., 2020d) where cows were milked 3 times per d and Judy et al. (2018) where cows were fed once or twice per d. In all studies, fecal and urine output was quantified by total collection for 4 consecutive d. Chemical composition of feeds, refusals, and feces was determined by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) except for Foth et al. (2015) and Reynolds et al. (2019) where analyses were completed at the Ruminant Nutrition Laboratory of the University of Nebraska-Lincoln (Lincoln, NE). Methods were similar across location and are described in individual publications. Crossover experimental designs were used in all 9 experiments.

Model Derivation Procedure

Response variables were HP (Mcal/d) and HI (Mcal/d). Models were fit in a hierarchical structure (Figure 4.1). The first level included individual models using the variable categories of DMI, milk, and digestibility. In the second level, milk or digestibility variables were combined with DMI, where milk yield was used as the milk

variable and digested energy (**DE**) was used as the digestibility variable and each variable was expressed as a function of DMI to minimize collinearity. In the third level, the constituents of milk yield (fat, protein and lactose) and DE (digested CP, NDF and starch) were used with all variables expressed as a function of DMI. To calculate DE from digested nutrients, digested CP, NDF, and starch (kg) were multiplied by their corresponding enthalpies of 5.65, 4.2, and 4.23 Mcal/kg, respectively (NRC, 2001). This resulted in 7 models for each response variables. Specifically, models are as follows with reference names for each model is listed parenthetically in bold: 1) DMI (**INT**); 2) milk fat, protein, and lactose yield (**MILKCOMP**); 3) DMI and milk yield (**INT+MY**); 4) DMI and MILKCOMP variables as a function of DMI (**INT+MILKCOMP**); 5) daily intake of digested NDF (**dNDF**), CP (**dCP**), and starch (**dSTA**) (**DIG**); 6) DMI and DE (**INT+DE**); and 7) DMI and NDF, CP, and STA digestibility (**INT+DIG**). An 8th model was fit to determine HP and HI associated with urinary N excretion, when accounting for DMI and milk fat, protein, and lactose yield as a function of DMI (**INT+MILKCOMP+UN**). Metabolic body weight ($BW^{0.75}$; **MBW**) was included in all models to estimate HP but was removed from HI models because it was used to calculate HI. As discussed previously, although forage inclusion, specifically forage NDF, can affect HP (Reynolds et al., 1991), it was not evaluated in the current experiment, because in most studies the forage NDF was similar across treatments consequently differences between treatments was small (mean \pm SD; $22.4 \pm 2.28\%$). Initially, models with DMI included BCS, and DIM; however, neither BCS nor DIM were significant, thus neither were included in derivation of final models. Fatty acid digestibility was only measured in 3 studies (n = 87), and its effects not further explored.

Models were fit in R (3.5.2) with the lmer function (Kuznetsova et al., 2017). To account for the variation associated with using individual animal observations, all models included the random effects of study ($n = 9$), cow ($n = 55$), and period within block within study ($n = 36$). If parameter estimates were close to 0 and non-significant ($P > 0.15$), the corresponding explanatory variables were removed from the model.

Model Evaluation

Simple correlation coefficients among all variables were assessed (Table 4.2). These correlation coefficients were used to assess potential multicollinearity among explanatory variables and used to aid in the explanation of observed parameters. In the final model, multicollinearity among variables were assessed by variance inflation factor (Roman-Garcia et al., 2016) which were less than 5 for all variables in the final models, and in most cases less than 2. This indicates that the inflation in variance due to multicollinearity was minimal in the models presented.

To compare among models with the same response variables, concordance correlation coefficients (Lin, 1989) were calculated from predicted values with (CCC) or without (uCCC) adjustment for study, cow, and period within block within study. Concordance correlation coefficients assess both accuracy and precision of models. Because the CCC value includes the random intercept terms for each random effect, they typically produce more favorable values compared to uCCC (White et al., 2017a). When mixed-effects models are used in an uninformed setting, the random effects will not be known; therefore, removal of the random effects, although not a statistically accurate depiction of model fit, is conducted to provide information on model fit when using the

model for future use in naïve settings (White et al., 2017a). Furthermore for each model, we report the estimated standard deviation for cow ($\hat{\sigma}_c$), study ($\hat{\sigma}_s$), and error ($\hat{\sigma}_e$) which is more appropriate than the root mean squared error for evaluating mixed effect models (Boerman et al., 2015a). The units for $\hat{\sigma}$ all values were the same as the response variable (Mcal/d).

The objective of the current study was to explain variation in HP and not to develop a model that would definitively predict HP. We explored generating prediction error of models using a Monte Carlo cross-validation as described by White et al. (2017b); however, the current dataset only contained 9 studies that differ greatly by DIM, DMI, milk production and dietary chemical composition. This resulted in prediction errors, slope, and intercept biases that were affected by which studies were selected by the Monte Carlo simulation. Therefore, results from the cross-validation may be misleading and are not reported herein.

RESULTS AND DISCUSSION

The number of observations for each variable tested were between 261 and 293 (Table 4.1). Average HP was 22.1 ± 2.86 Mcal/d. The dataset included cows with a wide range in DIM (44 to 410), DMI (9.6 to 25.0 kg/d), and milk yield (7.8 to 43.0 kg/d). Residual vs. predicted plots for all HP models are reported in Figure 4.2. Although a slope bias was observed ($P < 0.10$) for some models, the magnitude was less than 2% of error variance in all cases and was thus not a concern.

Factors that Affect Heat Production

Heat arises from the inefficiency in the conversion of a substrate into a product and from the complete oxidation of a substrate (Baldwin, 1995) and is commonly estimated in ruminants using the Brouwer (1965) equation. Negative coefficients are included in the Brouwer equation for urinary N excretion because protein oxidation leads to urinary urea excretion and thus protein oxidation is not complete. Therefore, O₂ and CO₂ associated with this incomplete oxidation must be accounted for when estimating HP (McLean and Tobin, 1987). Total HP is an aggregate of heat for basal metabolism, activity, digestion and absorption, fermentation, product formation, thermoregulation, and waste formation and excretion (NRC, 1981). We hypothesized that MBW and DMI could predict HP, and the addition of milk component yield, digestibility of nutrients, and urinary N excretion would explain more variation in HP by decreasing $\hat{\sigma}_e$ and increasing CCC. Specifically, we expected the following: 1) MBW to account for the NEm component of HP; 2) DMI to indirectly account for the digestion and absorption, fermentation, and product formation component of HP; 3) milk fat, protein, and lactose yield to account for heat of production formation; 4) digested NDF, CP, and starch to account for heat of digestion and absorption, and fermentation; and 5) urinary N excretion to account for heat associated with waste formation and excretion and protein catabolism.

In agreement with our hypothesis, INT explained most of the variation in HP (uCCC = 0.84, and $\hat{\sigma}_e$ = 1.38; Table 4.3). However, counter to our hypothesis, the addition of nutrient digestibility and milk component yield did not improve our ability to explain variation in HP based on similar uCCC and $\hat{\sigma}_e$. A lack of improvement in model

performance with inclusion of nutrient digestibility and milk component yield may have occurred because most of the variation in HP in the current study was explained by MBW and DMI. Furthermore, no difference in model performance would suggest that similar variation was explained by milk component yield or digested nutrients compared to DMI, which is not surprising given the correlation between most of these variables ($r > 0.55$; $P < 0.05$; Table 4.2). The relationship between HP and other response variables may improve our understanding of biological sources of variation in HP.

Given the other variables included in the models, increasing MBW increased HP on average by 0.111 to 0.165 Mcal per unit of MBW (Table 4.3). Because basal metabolism is a function of MBW (Brody, 1945; Kleiber, 1975), MBW was expected to account for a large fraction of the variation in HP. The MILKCOMP and DIG models, which did not include DMI, resulted in MBW coefficients of 0.165 and 0.147. These values are likely larger than actual NEm and thus demonstrate the importance of accounting for DMI when explaining variation in HP. The MILKCOMP and DIG models aside, our average MBW coefficient was 0.122 Mcal, which is 53% greater than the current NEm value used by dairy NRC (2001). However, Moraes et al. (2015) recently reported that NEm has increased over time from 0.074 to 0.124 for measurements taken during 1963 to 1973, and 1984 to 1995, respectively. From 935 observations from European calorimetry studies published between 1992 to 2010, NEm averaged 0.105 Mcal/MBW (Dong et al., 2015). Most of the historical energetics data was collected on Holsteins cows; however, energy utilization and maintenance energy requirements did not differ between Holsteins cows and Norwegian, Jersey \times Holstein, or Norwegian \times Holstein (Dong et al., 2015).

Increasing DMI on average increased HP by 0.560 to 0.600 Mcal per kg. Increased DMI typically corresponded with increased HP, and this has been observed previously (Purwanto et al., 1990; Reynolds et al., 1991). Up to 50% of total body O₂ consumption in ruminants can be attributed to metabolism in the gastrointestinal tract and liver (Seal and Reynolds, 1993). In sheep, increasing DMI increased digestive tract and organ weight as a proportion of BW (McLeod and Baldwin, 2000), and in growing heifers, increased DMI increased total O₂ consumption by the portal-drained viscera and liver (Reynolds et al., 1991). Additionally, DMI is correlated with milk production ($r = 0.63$; Table 4.2), thus a strong relationship between DMI and HP was not surprising. Synthesis of milk constituents is a major contributor to the HI of lactating cows. Although empirical models do not directly measure the underlying mechanistic nature of biology (Baldwin, 1995), interpretation of parameter estimates may be useful in determining the relationship between response and explanatory variables. When accounting for MBW and DMI, HP increased on average by 1.54 ± 0.57 Mcal per unit increase in milk yield over DMI (kg/kg DMI); therefore, we were interested in partitioning the HP associated with MY into HP due to fat, protein, or lactose synthesis. For the MILKCOMP and INT+MILKCOMP models to estimate HP, the parameter estimate for milk lactose yield was not different from 0 ($P > 0.27$) and was therefore removed from the final model. Although synthesis of milk lactose results in HP, milk lactose was highly correlated with milk fat ($r = 0.75$) and milk protein ($r = 0.83$; Table 4.2); thus, changes in milk fat and protein yield likely explain the variation in HP that arises from changes in milk lactose yield. The parameter estimate for milk protein was 2-fold greater than that of milk fat when expressed on a kg/d (6.17 ± 1.13 vs. 1.83 ± 0.656)

or kg/kg of DMI basis (40.2 ± 23.0 vs. 16.4 ± 12.0 ; Table 4.3). This difference may be explained by a greater energetic efficiency for synthesizing milk fat compared to milk protein. De novo synthesis of milk fat is about 0.70 efficient (Dado et al., 1993), whereas, conversion of dietary fat or tissue fat into milk fat has an energetic efficiency of 0.94 to 0.97 (Baldwin et al., 1985). Given that milk fat is 50% dietary or tissue in origin (Bauman and Griinari, 2003), efficiency of milk fat synthesis is 0.83. Synthesis of milk protein is estimated to require approximately 2 Mcal of ME/kg (Dado et al., 1993), thus the theoretical maximum efficiency of milk protein synthesis is 0.74. However, because of protein turnover, actual protein synthesis in mammary glands will always exceed milk protein yield. Rates of protein turnover range from 140% of milk protein yield in lactating dairy cows (Lemosquet et al., 2010) to 300% of milk protein yield in lactating dairy goats (Hanigan et al., 2009) with the prediction depending which labeled AA used to quantify. When accounting for actual protein synthesis being 40 to 200% greater than milk protein yield, energetic efficiency of milk protein synthesis ranges from 0.48 to 0.67. Consequently, milk fat production will lead to a lower HI compared to milk protein synthesis.

Increasing supply of digested nutrients was expected to increase HP because of the underlying correlation with DMI. However, digestion of NDF and starch gives rise to different VFA profiles, HI associated with intermediary metabolism differs by VFA (Holter et al., 1970), and catabolism of absorbed AA contributes to HI (Reynolds, 2006). Therefore, we expected to see differences HP associated with digested NDF, CP, and starch. The parameter estimates for dNDF (kg/d) in the DIG HP model was not different from 0 (0.146 ± 0.242 Mcal per kg; $P = 0.55$) and was therefore removed from the final

model. Because heat of fermentation is a component of HP (NRC, 1981) and NDF is primarily digested via ruminal fermentation, we were surprised that the effect of dNDF on HP was small. However, dNDF was correlated with dCP ($r = 0.49$) and dSTA ($r = -0.19$; Table 4.2); therefore, variation in HP associated with dNDF was likely explained by dCP and dSTA. Furthermore, in the dataset used in the current study change in dietary NDF content were primarily drive by changes in byproduct inclusion ($r = 0.83$ for dietary NDF and nonforage NDF). It is likely that nonforage NDF does not stimulate a similar increase in HP as increasing forage inclusion (Reynolds et al., 1991). Dietary NDF content rather than digestible NDF may be a better variable for explaining variation in HP. Therefore, we tested the effects of dietary NDF content in place of NDF digestibility. When dietary NDF content replaced dNDF in the DIG model, HP on average increased by 0.221 ± 0.0587 Mcal per % unit increase in dietary NDF (data not shown).

Additionally, for the DIG HP model, both dCP and dSTA were associated with HP when MBW was included (Table 4.3). For the DIG HP model, the coefficient for dCP was nearly two-fold that of dSTA (1.34 ± 0.372 vs. 0.762 ± 0.167 Mcal per kg; Table 4.3). An increase in HP with increased dCP was expected. Tyrrell et al. (1970) reported that feeding CP in excess of requirements resulted in decreased NE_L which may be attributable to an increase in HP associated with metabolizing excess CP and transforming digestible energy into urinary energy (Reed et al., 2017). The HP associated with excess N may originate from ureagenesis and (or) AA catabolism (see discussion on the effects of urinary N excretion on HP and HI). Most dietary starch is digested to VFA in the rumen and will subsequently be used as an energy source for the body or be converted into products. Our results suggest that a greater quantity of HP is associated

with each kg of dCP compared to dSTA, which agrees with work from over a 100 years ago by Rubner that showed that the heat production associated with oxidation of protein is greater than fat or carbohydrates (Kleiber, 1975).

Additional digestibility models were fit that included DE and digestibility of CP, NDF and starch. We expected INT+DE and INT+DIG to improve model performance compared to INT; however, as previously discussed these models resulted in similar uCCC and $\hat{\sigma}_e$. For the INT+DE model to estimate HP, increasing DE (Mcal/kg DM) decreased HP (-1.72 ± 0.592 Mcal). In the current database, DE content was positively correlated ($r = 0.25$) with dietary crude fat and negatively correlated ($r = -0.29$) with dietary NDF content (data not shown). As discussed previously, increased dietary fat and decreased NDF should result in a diet that will lead to less HP. For the INT+DIG HP model, decreased HP was observed with increased digestibility of CP and NDF (Mcal/%; -0.0432 ± 0.27 and -0.0666 ± 0.018 Mcal/%), and as starch digestibility increased HP increased on average by 0.0910 ± 0.046 Mcal/%. Starch digestibility was positively correlated with milk and lactose yield (Table 4.2) which will lead to increase heat production. Negative coefficients for CP and NDF digestibility may have occurred because CP digestibility were negatively correlated ($r < -0.25$) with DMI and milk component yields and NDF digestibility was negatively correlated ($r = -0.13$) with MBW. As NDF digestibility increased, energy expenditure associated with digested and HP from mass of ruminal tissue may have decreased because changes in source of NDF (Reynolds et al., 1991; Cantalapiedra-Hijar et al., 2014a). Additionally, at a constant DMI, increasing NDF and CP digestibility may increase the partitioning of energy toward milk and away from heat leading to decreased HP.

The Effects of Urinary N Excretion on Heat Production and Increment

Feeding excess protein to dairy cows has increased HP and decreased energy balance (Tyrrell et al., 1970; Reed et al., 2017). Urinary N excretion can serve as an indicator of amino acid oxidation and therefore excess protein, so we hypothesized that the inclusion of urinary N excretion could improve our ability to explain variation in HP and HI. Compared to other models for HP and HI, the models that included urinary N excretion had similar uCCC (0.83) and $\hat{\sigma}_e$ (1.40 Mca; Table 4.4). Overall, partitioning of the variation in HP was not improved with the inclusion of urinary N excretion.

A secondary objective of determining the relationship between HP and urinary N excretion was to quantify the contribution of protein catabolism to heat energy. On average when accounting for MBW, DMI, milk fat yield, and milk protein yield, HP increased by 5.32 ± 2.42 Mcal/kg of urinary N excretion. In an analysis of a historical indirect calorimetry dataset, Reed et al. (2017) determined that HP associated with excess N intake, which was defined as the digested N supply minus N requirements for maintenance, milk production, and pregnancy as defined by NRC, (2001). In the Reed et al. (2017) analysis, HP increased by 6.5 to 7.6 Mcal/kg of excess N when accounting for energy intake, MBW, and milk energy output. Coefficients from the Reed et al. (2017) analysis are larger than ours, which is likely because some urinary N excretion is a function of maintenance and milk production (NRC, 2001) and metabolic fecal N was subtract out as a component of fecal N excretion and maintenance N requirements thus underestimating excess N. Both will lead to a greater coefficient than the method used in the current study. In lactating dairy cows, the primary form of urinary N is urea (Spek et

al., 2013b), and the energetic cost of ureagenesis from ammonia is estimated to be 3.8 Mcal/kg of N (Martin and Blaxter, 1965). Ureagenesis occurs as a result of catabolism of AA by rumen microbes, catabolism of AA that are supplied in excess of requirements, and turnover of body protein stores (Lobley, 1992; Firkins and Reynolds, 2005; Reynolds, 2006). Reynolds (2006) suggested that ureagenesis may not be the primary contributor to increased HP with excess protein intake, rather, catabolism of AA leads to increased HP with increase CP intake. As described by Firkins and Reynolds (2005), because ruminants are evolutionarily adapted to be dependent on urea recycling, a large energetic cost to ureagenesis would be constrictive to urea recycling. Supporting evidence includes that increasing ammonia supply or feeding urea does not reduce milk yield (Moorby and Theobald, 1999) or increase liver O₂ consumption (Lobley et al., 1995; Firkins and Reynolds, 2005). The HP that was associated with UN in the current experiment on average was 1.0 Mcal/d (1.3% of GE intake). In the current study, urinary N excretion explained variation in HP that is associated with the catabolism of protein.

Due to the amount of heat associated with urinary N excretion, the question arises: will increasing dietary CP above requirements to replace carbohydrates decrease NE_L supply. Assuming a true digestibility of dietary CP of 85% (NRC, 2001) and that all dietary CP is excreted as urinary N, the HP associated with urinary N excretion is 0.72 Mcal/kg CP. Additionally, N from excess CP is primarily excreted as urea in urinary N (Spek et al., 2013b) which has an enthalpy of 5.4 Mcal/kg N or 0.86 Mcal/kg CP. Thus, the sum of energy associated with increase HP and urinary energy excretion from feeding CP as an energy source is 1.6 Mcal/kg. This is similar to the difference in enthalpy between CP and carbohydrates (5.6 vs. 4.2 Mcal/kg; NRC, 2001); therefore, differences

in energy supply between feeding CP or carbohydrates is dependent of digestibility of the CP or carbohydrate source. Average true digestibility of CP is slightly less than starch (85 vs. 92%), but true digestibility of CP is much greater than NDF (NRC, 2001; Ferraretto et al., 2013b) and thus replacing dietary NDF with CP may improve NE_L supply.

CONCLUSIONS

From 293 individual animal observations on lactating Jersey cows, HP averaged 22.1 ± 2.86 Mcal/d or $28.1 \pm 3.70\%$ of GE intake. Our results suggest that variation in HP is explained by MBW and DMI. Counter to our hypothesis, the inclusion of milk component yield, and nutrient digestibility did not improve our ability to explain variation in HP and HI. Parameter estimates from these models agree with other biological and biochemical estimates of energy transaction in lactating cows. For example, HP associated with milk protein synthesis was approximately 2-fold that of milk fat synthesis. Additionally, for every kg of urinary N excreted, HP increased on average by 5.32 Mcal.

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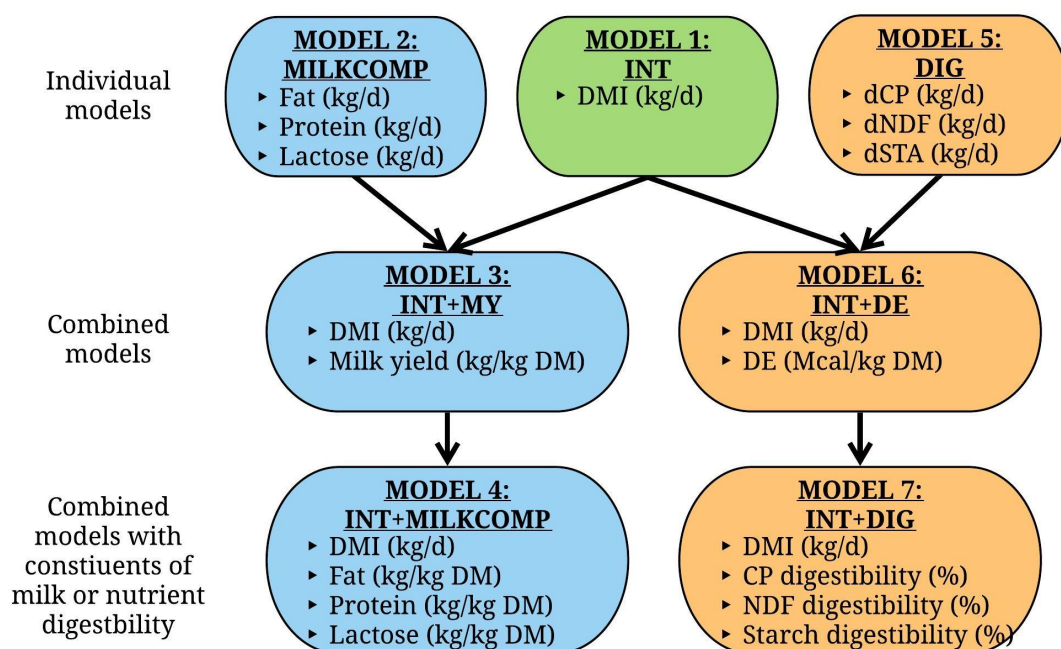


Figure 4.1. Illustration of seven models that were fit for heat production. All models included metabolic BW. dCP = digested CP, dNDF = digested NDF, dSTA = digested starch, DE = digestible energy.

Table 4.1. Descriptive statistics of the data used for the analysis to estimate heat production and heat increment of lactating Jersey cows

Item ¹	n	Mean	SD	Minimum	Median	Maximum
GE intake, Mcal/d	293	79.8	11.1	41.6	79.8	106.4
HP, Mcal/d	293	22.1	2.86	15.3	22.1	29.2
HP, % of GE intake	293	28.1	3.70	18.8	27.6	42.2
NEm, Mcal/d	293	9.8	0.72	8.0	9.8	11.6
Animal descriptions						
DIM, d	293	182	72.3	44	174	410
Parity	293	3.0	0.95	2.0	3.0	5.0
BW, kg	293	453	44	342	452	568
Metabolic BW, kg ^{0.75}	293	98.2	7.2	79.5	98.0	116.4
BCS ²	272	3.27	0.35	2.00	3.25	4.25
DMI, kg/d	293	18.2	2.53	9.6	18.2	25.0
Milk and components yield						
Milk yield, kg/d	293	25.0	5.49	7.8	24.6	43.0
Milk yield, kg/kg DMI	293	1.38	0.242	0.72	1.37	2.15
Fat, kg/d	293	1.40	0.292	0.48	1.39	2.19
Fat, kg/kg DMI	293	0.0768	0.0127	0.0485	0.0766	0.116
Protein, kg/d	293	0.90	0.165	0.33	0.89	1.29
Protein, kg/kg DMI	293	0.0493	0.0058	0.0332	0.0490	0.0701
Lactose, kg/d	290	1.20	0.285	0.33	1.19	2.10
Lactose, kg/kg DMI	290	0.0658	0.0130	0.0339	0.0656	0.110
Digested energy and nutrients						
DE, Mcal/kg DM	261	2.88	0.219	2.30	2.89	3.77
dCP, kg/d	277	2.25	0.404	1.32	2.21	3.39
CP digestibility, %	277	68.2	6.3	49.4	68.3	82.8
dNDF, kg/d	277	2.88	0.722	0.81	2.83	5.06
NDF digestibility, %	277	47.3	6.6	24.5	46.9	65.8
dSTA, kg/d	277	4.02	1.10	1.52	4.19	6.62
Starch digestibility, %	277	95.0	2.7	93.5	95.2	100.0
Urinary N excretion, g/d	261	188	57.4	56.9	182	394
Dietary composition, % DM						
CP	285	17.7	0.95	15.2	17.7	19.5
NDF	285	31.9	3.51	25.5	32.0	43.0
Starch	285	25.0	3.48	16.2	25.8	31.1
Crude fat	281	4.2	0.74	2.2	4.2	6.4

¹HP = heat production; GE = gross energy; NEm = net energy for maintenance [$0.100 \times$ metabolic BW (Moraes et al., 2015)]; DE = digestible energy; dCP = apparent digested crude protein; DE CP = digested energy from CP; dNDF = digested NDF; DE NDF =

digested energy from NDF; dSTA = digested starch; DE STA = digested energy from starch.

²On a 1 to 5 scale

Table 4.2. Simple correlation coefficients among heat production, animal description, milk yield and components, and digestibility variables included in model development¹

Item	Variables																	
	MBW	DMI	MY	MY/DMI	Fat	Fat/DMI	Pro	Pro/DMI	Lact	Lact/DMI	DE/DM	dCP	CP digestibility	dNDF	NDF digestibility	dSTA	Starch digestibility	UN
HP	0.26*	0.68*	0.50*	0.09	0.40*	-0.08	0.59*	0.12*	0.49*	0.10	-0.14*	0.43*	-0.21*	0.38*	0.07	0.32*	0.15*	0.01
MBW		0.20*	-0.05	-0.26*	0.03	-0.17*	0.07	-0.16*	-0.09	-0.28*	0.06	0.13*	0.04	-0.03	-0.13*	0.30*	0.00	0.16*
DMI			0.63*	0.01	0.63*	-0.03	0.78*	0.08	0.60*	0.03	-0.05	0.58*	-0.30*	0.55*	0.09	0.54*	-0.02	-0.02
MY				0.77*	0.78*	0.48*	0.86*	0.64*	0.99*	0.78*	-0.05	0.25*	-0.39*	0.21*	-0.01	0.47*	0.12*	-0.18*
MY/DMI					0.50*	0.66*	0.48*	0.78*	0.79*	0.99*	-0.03	-0.13*	-0.25*	-0.16*	-0.09	0.16*	0.17*	-0.18*
Fat						0.76*	0.77*	0.50*	0.75*	0.49*	-0.03	0.22*	-0.40*	0.16*	-0.11	0.53*	0.01	-0.03
Fat/DMI							0.34*	0.60*	0.47*	0.63*	0.01	-0.18*	-0.25*	-0.24*	-0.21*	0.21*	0.02	0.00
Pro								0.67*	0.83*	0.47*	-0.06	0.40*	-0.33*	0.28*	-0.01	0.57*	0.03	-0.09
Pro/DMI									0.63*	0.75*	-0.04	-0.04	-0.17*	-0.18*	-0.13*	0.26*	0.07	-0.10
Lact										0.81*	-0.05	0.25*	-0.36*	0.22*	0.02	0.42*	0.13*	-0.18*
Lact/DMI											-0.03	-0.08	-0.22*	-0.10	-0.04	0.12	0.16*	-0.18*
DE/DM												0.37*	0.55*	0.18*	0.34*	-0.12	0.08	0.08
dCP													0.54*	0.49*	0.27*	0.06	-0.04	0.41*
CP														0.04	0.30*	-0.44*	-0.02	0.50*
digestibility																		
dNDF															0.73*	-0.19*	-0.07	-0.06
NDF																-0.55*	0.03	-0.08
digestibility																		
dSTA																	0.14*	-0.12*
Starch																		-0.14*
digestibility																		

* $P < 0.05$.

¹Items are repeated horizontally and vertically in the same order and the same units. HP = heat production (Mcal), MBW = metabolic BW ($\text{kg}^{0.75}$), DMI (kg/d), MY = milk yield (kg/d), MY/DMI = milk yield/DMI (kg/kg), Fat = milk fat yield (kg/d), Fat/DMI = milk fat yield/DMI (g/kg), Pro = milk protein yield (kg/d), Pro/DMI = milk protein yield/DMI (g/kg), lactose = milk lactose yield (kg/d), lactose = milk lactose yield/DMI (g/kg), DE/DM = digested energy (Mcal/kg DM), dCP = apparent digested crude protein (kg/d), CP

digestibility (%), dNDF = digested NDF (kg/d), NDF digestibility (%), dSTA = digested starch (kg/d), Starch digestibility (%), UN = urinary N excretion (g/d).

Table 4.3. Parameter estimates and fit statistics for models to predict heat production (Mcal/d) of Jersey cows from metabolic BW (MBW) plus DMI (INT); milk fat, protein, and lactose yield (MILKCOMP); INT plus milk yield (INT+MY); INT plus MILKCOMP variables as a function of DMI (INT+MILKCOMP); digested NDF, CP, and starch (DIG); INT plus digested energy (INT+DE); or INT plus NDF, CP, and starch digestibility (INT+DIG)

Item	INT		MILKCOMP		INT+MY		INT +MILKCOMP		DIG		INT+DE		INT+DIG	
	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE
Model	1		2		3		4		5		6		7	
Variable ¹														
Intercept	-0.198 ^a	1.80	-2.30 ^a	1.90	-3.52	2.16	-4.78	2.25	0.719 ^a	2.02	5.02	2.58	-2.34	4.84
MBW, kg ^{0.75}	0.122	0.018	0.165	0.018	0.129	0.018	0.132	0.018	0.147	0.020	0.114	0.020	0.111	0.019
DMI, kg/d	0.560	0.048			0.591	0.48	0.577	0.047			0.598	0.051	0.600	0.048
Milk fat, kg/d			1.83	0.66										
Milk protein, kg/d			6.17	1.13										
Milk yield, kg/kg DMI					1.54	0.57								
Milk fat, kg/kg DMI							16.4	12.0						
Milk protein, kg/kg DMI							40.2	23.0						
dCP, kg/d									1.63	0.37				
dSTA, kg/d									0.762	0.167				
DE, Mcal/kg DM											-1.72	0.59		
CP digestibility, %													-0.0432	0.027
NDF digestibility, %													-0.0666	0.018
Starch digestibility, %													0.0910	0.046
Fit statistics ²														
n	293		293		293		293		277		261		277	
BIC	1161		1178		1159		1148		1157		1045		1118	
CCC	0.89		0.88		0.89		0.89		0.86		0.89		0.90	
uCCC	0.84		0.81		0.85		0.84		0.79		0.85		0.84	
$\hat{\sigma}_e$	0.61		0.60		0.59		0.56		0.68		0.62		0.64	

$\hat{\sigma}_s$	1.59	1.82	1.51	1.62	2.02	1.47	1.63
$\hat{\sigma}_e$	1.38	1.44	1.36	1.37	1.52	1.38	1.33

^aNot different from 0 ($P > 0.20$).

¹MBW = $BW^{0.75}$, dCP = apparent digested crude protein, dSTA = digested starch, dNDF = digested NDF.

²n = number of observation; CCC = concordance correlation coefficient, uCCC = concordance correlation coefficient without random effects adjustment, $\hat{\sigma}_c$ = square root of the estimated variance associated with cow (Mcal), $\hat{\sigma}_s$ = square root of the estimated variance associated with study (Mcal), $\hat{\sigma}_e$ = square root of the estimated variance associated with error (Mcal).

Table 4.4. Parameter estimates and fit statistics for models to predict heat production and heat increment (Mcal/d) of Jersey cows from metabolic BW (MBW; heat production only); DMI; milk fat, protein, and lactose yield; and urinary N excretion

Item	Heat production	
	Value	SE
Variable ¹		
Intercept	−5.30	2.28
MBW, kg ^{0.75}	0.120	0.19
DMI, kg/d	0.602	0.52
Milk fat, kg/kg DMI	18.6	12.5
Milk protein, kg/kg DMI	46.4	24.1
Urinary N excretion, kg/d	5.32	2.43
Fit statistics ²		
n	261	
BIC	1030	
CCC	0.89	
uCCC	0.84	
$\hat{\sigma}_c$	0.52	
$\hat{\sigma}_s$	1.65	
$\hat{\sigma}_e$	1.39	

^aNot different from 0 ($P > 0.20$).

¹MBW = BW^{0.75}.

²n = number of observation; CCC = concordance correlation coefficient, uCCC = concordance correlation coefficient without random effects adjustment, $\hat{\sigma}_c$ = square root of the estimated variance associated with cow (Mcal), $\hat{\sigma}_s$ = square root of the estimated variance associated with study (Mcal), $\hat{\sigma}_e$ = square root of the estimated variance associated with error (Mcal).

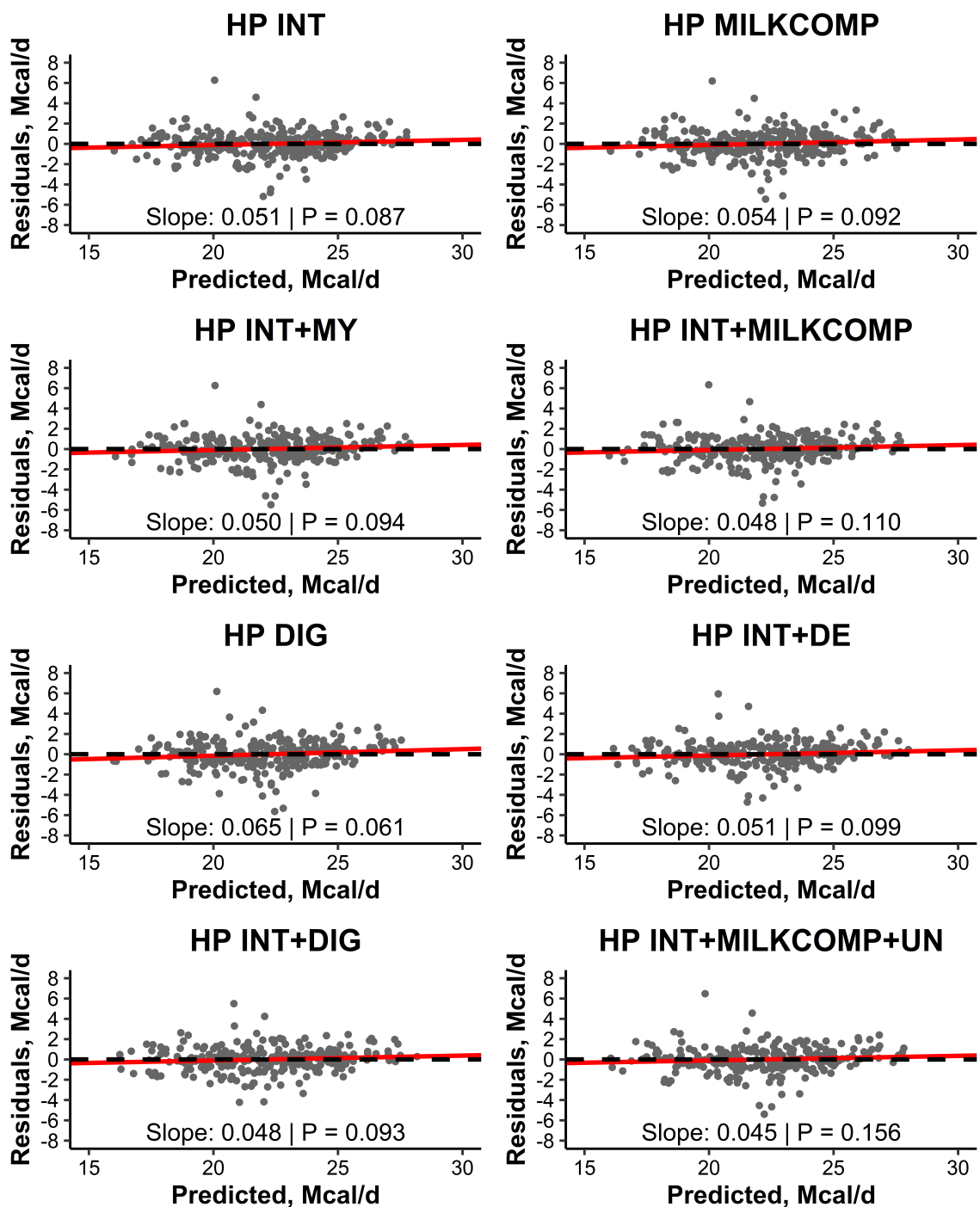


Figure 4.2. Residuals (observed – predicted values) versus predicted values for equations to explain variation in heat production (HP). The *P*-value and coefficient are shown along the x-axis.

CHAPTER 5: RELATIONSHIP BETWEEN URINARY ENERGY AND URINARY N OR C EXCRETION IN LACTATING JERSEY COWS

INTERPRETIVE SUMMARY. Morris et al. (20XX). “Relationship between urinary energy and urinary N or C excretion in lactating Jersey cows,” Equations were developed to estimate urinary energy (UE) from urinary N (UN) or C (UC). A database of 134 individual observations were assembled. Average UE, UN, and UC were 2381 ± 314 kcal/d, 158 ± 24 g/d, and 199 ± 24.5 g/d, respectively. With increasing UN and UC, UE increased. As UN increased, UE (kcal/g N) decreased and was estimated to be 17.7, 15.6, and 13.6 at 100, 150, and 200 g of UN, respectively. Error variance was greater for UC than UE. Using UN equations to estimate UE improved our ability to predict ME as dietary CP changed.

RUNNING HEAD: URINARY ENERGY, N and C

Relationship between urinary energy and urinary N or C excretion in lactating Jersey cows

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ABSTRACT

Measurement of urinary energy (UE) excretion is essential to determine metabolizable energy supply. Our objectives were to evaluate the precision of using urinary N (UN) or C (UC) to estimate UE. Individual animal data ($n = 134$) were used from 4 studies with Jersey cows at the University of Nebraska-Lincoln where UE and UN were measured on an as-is basis using the same methodology. The dataset included a range (min to max) in d in milk (88 to 346), dry matter intake (11.6 to 24.6 kg/d), N intake (282 to 642 g/d), energy-corrected milk yield (14.8 to 48.2 kg/d), UE excretion (1390 to 3160 kcal/d), UN excretion (85 to 220 g/d or 20.6 to 59.5% of N intake), and UC excretion (130 to 273 g/d). As indicated by a bias in residuals between observed and predicted ME as dietary CP increased, the NRC, 2001 does a poor job of predicting ME of diets as CP varies. In the initial models between UE and UN excretion (kcal/d), the intercept was 880 ± 140 kcal for the linear model. Because an intercept of 880 is biologically unlikely, the intercept was forced through 0. The regressions of UE (kcal/d) on UN (g/d) were $UE = 14.6 \pm 0.32 \times UN$ and $UE = 20.9 \pm 1.0 \times UN - 0.0357 \pm 0.0056 \times UN^2$. In the quadratic regression, UE increased but at a diminishing rate as UN excretion increased. The first derivative estimate from the quadratic equation at 100, 150, and 200 g of UN was 13.8, 10.2, and 6.6 kcal/g N, respectively. This decrease was likely because of an increase in the proportion of UN that is from urea, which has a lower enthalpy per g of N compared to the non-urea components of urine (5.4 vs. > 24.8 kcal/g N). When fitted without an intercept, a linear increase in UE was observed as UC increased with a regression of UE (kcal/d) = $11.9 \pm 0.22 \times UC$ (g/d). However, error variance was greater for regression with UC compared to UN as explanatory variables (8.42 vs. 7.42 % of mean UE).

Predicting ME using UN equations to estimate UE removed slope bias between residuals and diet CP. Therefore, using equations to predict UE from UN should improve our ability to predict diet ME compared to the equations used by NRC, 2001.

Keywords: metabolizable energy, bomb calorimetry, regression

INTRODUCTION

Accurate estimation of dietary energy supply is essential to predict performance of lactating dairy cows. Considerable work has been completed regarding estimation of digestible energy (**DE**) of diets by estimating the digestibility of individual nutrients (Weiss and Tebbe, 2019) as well as to estimate CH₄ production (Appuhamy et al., 2016a). However, minimal work has been completed to estimate urinary energy (**UE**) loss in modern dairy cows, which is needed to calculate ME. Historically, empirical equations have been used to estimate ME from DE. NRC (2001) calculates dietary ME content (Mcal/kg DM) as $1.01 \times \text{DE (Mcal/kg DM)} - 0.45$ with a slight correction for fat to account for a 100% efficiency in conversion of DE to ME for fat. For typical dairy diets, the efficiency of converting DE to ME averages about 85%. Urinary energy (**UE**) represents 5 to 7% of DE supply or up to 50% of the energy difference between DE and ME (Drehmel et al., 2018; Judy et al., 2018; Reynolds et al., 2019). Increasing dietary CP increases UE excretion (Hynes et al., 2016). Therefore, the efficiency of converting DE to ME for a diet with excess CP may be lower than a diet in which CP is closer to requirements. In current nutrition models, variation in dietary CP does not contribute to variability in DE to ME efficiency.

Both urinary N (UN) and urinary C (UC) concentration are correlated with UE concentration (Blaxter, 1989). The relationship between UN or UC and UE is a function of the relationship between the enthalpy of the energy containing molecules and the respective N or C contents. Variation in the heat of combustion per unit of N is considerably greater than that of C and thus UC is thought to be a better predictor of UE (Blaxter et al., 1966). The heat of combustion (kcal/g N) of major energy containing molecules in urine are: urea 5.4, allantoin 7.3, hippuric acid 71.7, creatinine 13.2, creatine 13.2, uric acid 8.2, xanthine 9.2, hypoxanthine 10.3, and AA (average) 35.3 (Calculated from NIST, 2020). Historical estimates suggest that on average UE equaled 14.3 kcal/g of N (Blaxter, 1989). Because urea and hippuric acid have the lowest and largest UE-to-UN ratio, respectively, the UE-to-UN ratio of urine is sensitive to their excretion. Urinary excretion of hippuric acid, which is formed from the conjugation of dietary derived benzoic acid with glycine (Martin, 1982), is correlated with DMI (Blaxter et al., 1966). Additionally, the proportion of UN that is excreted as urea increases with increasing UN excretion by dairy cattle (Spek et al., 2013b); consequently, increasing proportion of UN as urea should decrease the enthalpy of urine/g N. For the same molecules listed above, UE-to-UC ratio are between 7 and 13 kcal/g C and averaged 10 kcal/g C in cattle and sheep (Blaxter et al., 1966). Additionally, in cattle, average mean squared error of regressions between UE and UN or UC were 16.4 and 5.3% of mean UE, respectively (Blaxter et al., 1966). Therefore, the objectives of the current work were to develop an equation to describe the relationship between UN or UC and UE concentration and excretion. We hypothesized that residual variation would be lower when UC compared to UN was used to explain UE.

MATERIALS AND METHODS

Data Collection

Individual Jersey cow data were sought, because urinary excretion and N are measured on an individual animal basis and UE is predicted on an individual animal basis not on a treatment basis. We identified 11 experiments conducted at the University of Nebraska-Lincoln's Dairy Metabolism Unit (Lincoln, NE) from 2013 to 2019. Because individual animal data were not available from other literature data, they were not considered. Additionally, animal were sources from numerous commercial dairies and therefore should be representative of US Jersey cow population. In total, 433 observations (cow-periods) were assembled across all 11 experiments.

Across all 11 experiments, urine output was determined for 4 d via total collection using a size 30 French Foley bladder catheter. Two distinct methods were used to quantify UE and UN. For experiments 1 to 7 ($n = 299$), urine samples were boiled in a hot water bath to remove moisture, and the resulting paste was freeze dried to remove most of the remaining moisture (Drehmel et al., 2018). Dried samples were then analyzed for gross energy content using an isoperibol bomb calorimeter (Parr 6400 Calorimeter, Moline, IL), and N content (FlashSmart N/Protein Analyzer, CE Elantech Inc., Lakewood, NJ; AOAC International, 2000, method 990.03). For experiments 8 to 11 ($n = 134$), urine samples were not boiled and freeze dried prior to analysis. Gross energy content was determined after drying at 60°C approximately 4 g of sample in a bomb capsule until dry (~4 h). This method was similar to the method used by Jacobs et al.

(2011), who determined UE of swine urine on undried or dried samples. To determine N concentration of urine in experiments 8 to 11, samples were submitted to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis on an as-is basis using a combustion method (Leco FP-528 N Combustion Analyzer; Leco Corp., St. Joseph, MI). Samples for experiments 8 to 11 were also analyzed for UC using a combustion method (Flash 2000; Thermo Fisher Scientific, Waltham, MA) following the procedure of urine processing described in Morris et al. (2019). Acidification method differed between experiments 1 to 7 and 8 to 11. In experiments 1 to 7, 50 to 100 mL of 12N HCl was added to urine collection containers 1 to 2 times per day. For experiments 8 to 11, 6N HCl was added to the urine collection container at the beginning of the collection d. Urine pH was measured at the end of each d and quantity of acid used was adjusted to maintain a $\text{pH} < 5.0$. The sample collection and handling method (dried for 7 or as is for 4 experiments) was coded into the dataset as a categorical variable.

Model Derivation and Evaluation

To evaluate the NRC, 2001 calculation of ME, treatment means were derived from the full dataset ($n = 45$ treatment means). Then, residuals between observed ME and ME calculated from DE using NRC, 2001 equation 2-10 were calculated and regressed against dietary CP. Intercept, slope biases and residual mean squared error (RMSE) as a percent of mean were evaluated.

Models were fit using R (3.6.2) with the lmer function (Kuznetsova et al., 2017). To account for the variation associated with using individual animal observations, all models included the random effects of cow, period within study, and study. Study effect was generally equal to 0 because period likely accounted for similar variation as study.

Therefore, the random effect of study was removed from final models. The fixed effect of method (dried or as-is) and interaction of method with continuous variables was included in initial models.

Rather than reporting root mean squared error, we chose to report the square root of the estimated variance associated with cow, period within study, and error. These values are suggested to be more appropriate than root mean squared error for evaluating mixed effect models (Boerman et al., 2015a). The units for all variance estimates are in the same units as the response variable or expressed as a percent of the mean of the response variable. Slope bias was evaluation by regressing residual against predicted values and comparing the slope coefficient to 0.

To evaluate application of the derived models to improve our ability to estimate ME, the same method described above was used except ME was predicted by subtracting from DE urinary energy estimated using equation we derived and CH₄ energy estimated as $0.294 \times \text{DMI (kg/d)} - 0.347 \times \text{dietary crude fat (\% DM)} + 0.0409 \times \text{NDF digestibility (\%)};$ Nielsen et al., 2013).

RESULTS AND DISCUSSION

Evaluation of NRC, 2001 Equation to Predict ME

Dietary ME content (Mcal/kg DM) in NRC (2001) calculates from DE with a slight correction for dietary fat to account for 100% efficiency in conversion of DE to ME for fat energy. This approach does not account for the CP intake which is known to be positively correlated with UE (Hynes et al., 2016). NRC, 2001 under-predicted ($P < 0.01$) ME content by 0.070 Mcal/kg DM on average (Figure 5.1). As dietary CP

increased, residual ME decreased (slope < 0.01), which occurred because CP was positively correlated with UE ($r = 0.57$; $P < 0.01$; data not shown). Therefore, when predicting ME, the positive relationship between dietary CP and UE should be accounted for.

Effect of Method of Urine Collection and Processing

Intercepts and slope for regressions between UE and UN differed by method ($P < 0.02$; data not shown). Additionally, variance estimates, in general, were at least 2-fold greater for the full dataset compared to when the same regressions were generated using the as-is dataset (i.e., samples were acidified to $\text{pH} < 5.0$ during collection and analysis was completed on samples that were not dried). Boiling and drying might have increased N and/or energy loss from samples prior to analysis and the additional step in sample processing for the dried method might have induced additional variation into UE and UN measurements. Adequate acidification is essential to prevent N loss (Spanghero and Kowalski, 1997). Samples collected and analyzed via the dried method may not have been adequately acidified to prevent N loss; pH was not measured on these samples. Therefore, data collected and analyzed via the as-is method, where samples were acidified to a $\text{pH} < 5.0$ during collection, were used. The dataset used to derive equations included 134 observations (cow-periods) from 4 experiments, totaling 14 periods, and 32 cows. Descriptive statistics for this dataset are reported in Table 5.1.

Relationship between Urinary Energy and N or C

Urinary N and C are associated with energy-containing compounds and are correlated with UE (Street et al., 1964; Blaxter et al., 1966). Our objective was to evaluate the relationship between UE and UN or UC in lactating Jersey cows and generate equations to estimate the error associated with estimating UE from UN or UC. Additionally, we were interested in deriving equations that could predict UE with adequate precision and accuracy. In a regression of 402 data points from growing and lactating cattle and sheep, Street et al. (1964) reported that $UE \text{ (kcal/g)} = 0.117 UN \text{ (g/100 g)} + 0.026$. The same regression from our dataset (UN equation 1) and illustrated in Figure 5.2 was $UE \text{ (kcal/g)} = 0.127 \pm 0.0074 UN \text{ (g/100g)} + 0.0165 \pm 0.0054$. Across the range of our dataset, these two equations closely agree in terms of estimating urine from UN.

Estimating UE from UN is useful because UN is more commonly measured than UE, and UE is needed to estimate ME. When regressing UE (kcal/d) on UN (g/d) with an intercept (UN equation 2), the quadratic term was not different from 0 ($P = 0.27$) and was thus removed, the intercept was $880 \pm 139 \text{ g/d}$ (Figure 5.2). The observed intercept in UN equation 2 of 880 g/d is biologically unlikely because there is a very small quantity of organic compounds in urine that do not contain N. The large intercept value for UN equation 2 is likely due to the wide range between the minimum UN of 80 g/d and the y axis and by fitting a linear regression to a relationship that is inherently curvilinear. Some UE can originate from N-free molecules such as hydrogen sulfide, and ketones; however, in general, quantity of S and ketones excreted in urine is small (Huhtanen et al., 1993; Morris et al., 2018a). Further regressions were fit by assuming an intercept of 0. In a

linear regression without an intercept term (UN equation 3), the relationship between UE and UN was 14.6 ± 0.35 kcal/g N. A similar value of 14.3 kcal/g N was reported for ruminants, although species was not specified (Blaxter, 1989). In residual analysis of UN equation 3, UE was under-predicted below the mean UN and over-predicted above the mean ($P < 0.01$; Figure 5.3), which likely occurred because the regression was forced through 0. When the intercept was forced through 0, the quadratic term was different from 0 (UN equation 4; $P < 0.01$; Figure 5.2). With increasing UN in equation 4, UE increased but at a diminishing rate. The first derivative of UN equation 4 at 100, 150, and 200 g of UN was 13.8, 10.2, and 6.6 kcal/g N, respectively. Slope bias was not different ($P = 0.12$) from 0 in UN equation 4 (Figure 5.3).

The quadratic relationship between UE and UN excretion suggests the UE per unit of UN decreases with increasing UN excretion. To better understand this relationship, we then fit additional regressions with UE expressed as kcal/g N as the response variable (Figure 5.4). As UN excretion (g/d) increased, UE per g N decreased (UN equation 5). The ratio of UE to UN at 100, 150, and 200 g of UN excretion was 17.7, 15.6, and 13.6 kcal/g N, respectively. A decreasing UE-to-UN ratio with increasing UN excretion supports the notion that, as UN excretion increases, compounds with high enthalpies per N (i.e., hippuric acid, free AA, creatinine and creatine) are diluted by urea, which has an enthalpy of 5.4 kcal/g N. In a meta-regression from which dairy breed was not specified, when urea-N excretion was regressed on UN excretion, the intercept term, which represents the quantity of daily non-urea N excretion, was 51.9 ± 4.42 g/d (Spek et al., 2013a). However, non-urea N excretion is likely related to DMI, because increased DMI is correlated to increased urinary excretion of purine derivatives and hippuric acid

via increased microbial protein synthesis and thus absorption of hippuric acid precursors, respectively (Spek et al., 2013a). Furthermore, UN and urea N excretion were linearly related (Spek et al., 2013b) and urea N excretion increases with dietary CP supply. This means that at similar DMI, as UN excretion increases, the proportion of UN that is urea, in theory, increases asymptotically towards 100%. Bristow et al. (1992) measured the major constituents of urine from 10 Holstein dairy cows and reported that the non-urea N portion was on average $24.4 \pm 6.3\%$ hippuric acid, $28.7 \pm 14.5\%$ allantoin, $5.1 \pm 1.5\%$ uric acid, $2.0 \pm 0.7\%$ xanthine/hypoxanthine, $15.1 \pm 4.5\%$ creatinine, $10.7 \pm 4.5\%$ creatine, $3.5 \pm 5.0\%$ AA, and $10.4 \pm 13.3\%$ ammonia. The non-urea N proportion has an average enthalpy of combustion of 24.8 ± 5.4 kcal/g N, which is much larger than urea's enthalpy of 5.4 kcal/g (Calculated from Britow et al., 1992 and NIST, 2020).

Excretion of some non-urea N compounds in urine is likely a function of BW and DMI. The total mass of urinary excretion of creatinine is derived from muscle turnover and is linearly associated with BW (Brody, 1945). However, the enthalpy of creatinine per g N is 13.2, which is similar to the average of our data (14.6 from UN equation 3). Thus, changes in excretion of creatinine likely has little effect on enthalpy of urine per g of N. Urinary purine derivatives (e.g., allantoin, uric acid, xanthine, and hypoxanthine) are derived from absorbed microbial protein (Gonzalez-Ronquillo et al., 2003), which along with dietary chemical composition is driven by DMI (Roman-Garcia et al., 2016). As energy retention and DMI increased, % of UN present as hippuric acid and kcal/g N for urine increased in sheep (Blaxter et al., 1966). We tested the effects of DMI and BW on UE (kcal/g N) by adding DMI (% of BW) to UN equation 5. In this equation (UN equation 6), increasing DMI by 1% of BW increased UE by 0.64 ± 0.28 kcal/g N (Figure

5.4). However, in general, the effect of DMI was small. At the average UN excretion (158 g/d), increasing DMI from 4 to 5% of BW increased UE by 101 kcal/d or 0.2% of average DE intake for the dataset.

Historically, UE has been accurately estimated from UC (Blaxter et al., 1966). In the current dataset, UE and UC concentration were linearly associated with a regression that was $UE \text{ (kcal/g)} = 0.0959 \pm 0.0050 UC \text{ (g/100g)} + 0.0211 \pm 0.0046$ (Figure 5.5; UC equation 1). When regressing UE (kcal/d) on UC (g/d) with an intercept (UC equation 2), the quadratic term was not different from 0 ($P = 0.17$) and was thus removed and the intercept was 1180 ± 189 g/d. Similar to UN equation 2, a large positive intercept is not biologically founded, and thus further models were fit by assuming an intercept of 0. When regressing UE (kcal/d) on UC (g/d) without an intercept term (UC equation 3), the relationship between UE and UC was 11.9 ± 0.22 kcal/g C. This value is in the range (7 to 13 kcal/g C) of UE-to-UC ratio of urinary compounds, but slightly greater than the value of 10 kcal/g C reported for cattle and sheep (Blaxter et al., 1966). When using the urinary compound concentration reported by Bristow et al. (1992), the calculated ratio between UE and UC was 10.5 kcal/g C. A quadratic relationship was also observed when regressing UE on UC without an intercept (UC equation 4); however, unlike when regressing UE on UN, this quadratic relationship for UE vs UC is not biologically founded. As discussed for equations based on UN, excretion of urinary urea is more variable than the non-urea fraction (Bristow et al., 1992; Spek et al., 2013b) and the enthalpy of urea is greater than the enthalpy of the non-urea fractions of urine (12.7 vs. 9.5 kcal/g C; calculated from Britow et al., 1992 and NIST, 2020). Therefore, as UC and urea excretion increase, UE should increase at an increasing rate and a positive quadratic

term would be expected. However, urea account for approximately 33% of urinary C excretion on average (Calculated from Britow et al., 1992), and because urea only contains one C, increasing urea excretion will only have a small effect on the proportion of UC that is from urea. A linear relationship between UE and UC is most logical.

Based on the work of Blaxter et al. (1966), we expected $\hat{\sigma}_e$ to be less when regressing UE on UC compared to UN. However, $\hat{\sigma}_e$ for UN equation 2 to equation 4 was 7.14 to 7.93% of the mean; in contrast, predicted variance for UC equation 2 to equation 4 was 7.73 to 9.73 % of the mean and was on average 1.0% units greater compared the corresponding UN model (Table 5.2). Blaxter et al. (1966) reported that predicted variance for estimating UE with UC for cattle and sheep was 3.9% of mean and did not predict variance for UN because the relationship was non-significant. The precision in estimating UE from UN is likely much improved compared to the work of Blaxter et al. (1966) because methods of determining N have been improved considerably since the 1960's. In the dataset used by Blaxter et al. (1966), UN was determined by the Kjeldahl method, which has been recently shown to have a more than 3-fold greater analytical variation resulting from incomplete recovery of some N-containing compounds compared to modern combustion methods that were similar to those used in the current dataset (Bremner and Mulvaney, 1982; Morris et al., 2019). Additionally, slope bias was observed for UC equation 2 to equation 4 (Figure 5.6), which further supports the use of UN rather than UC to estimate UE.

Evaluation of Using Estimate UE to Predict ME

By using the regression generated in the current experiment to estimate UE and previously published equations to estimate CH₄ energy, ME can be predicted. Numerous

equations are available to estimate CH₄ energy; however, the objectives of this analysis were not to evaluate these equations. An independent evaluation of CH₄ equations was recently conducted by (Appuhamy et al., 2016a) and the equation deemed best from this was used; $0.294 \times \text{DMI (kg/d)} - 0.347 \times \text{dietary crude fat (\% DM)} + 0.0409 \times \text{NDF digestibility}$ (Nielsen et al., 2013). When regressing residual ME (observed – predicted from DE, estimated UE, and estimated CH₄ energy) on dietary CP, an under-prediction of 0.10 Mcal/kg DM was observed ($P < 0.01$; Figure 5.7). This under-prediction of ME occurred because CH₄ energy was over-predicted by 0.11 Mcal/kg DM (data not shown). When UN equation 3 was used to estimate UE, slope bias was not observed ($P = 0.41$) between residual ME and diet CP. However, when UN equation 4 was used to estimate UE, a slope bias was observed ($P = 0.05$) because UE was under-predicted at high dietary CP. This bias with UN equation 4 likely occurred because 6 treatment means in (13.3%) from the full dataset had greater UN excretion than the maximum value in the derivation dataset (220 g/d).

CONCLUSIONS

Current equations used by NRC, 2001 to predict ME do not account for the positive correlation between diet CP and UE. As UN excretion (g/d) increased, UE excretion (kcal/d) increased quadratically such that, as N excretion increased, the rate of increase in energy excretion diminished. This quadratic relationship occurred probably because the enthalpy per g of N for urea is lower than non-urea N in urine (5.4 vs. 23.9 kcal/g N), and the proportion of urinary N from urea likely increases as UN increased. A linear increase in UE was observed as UC increased. However, error variance was greater

for regression with UC compared to UN as explanatory variables. Predicting ME using derived equation to estimate UE improved our ability to account for the negative relationship between ME and diet CP that is not currently accounted for in NRC, 2001. Although the curvilinear relationship between UE and UN derived in the current experiment is biologically based, the curvilinear equation underestimated UE when datapoints were greater than those used for derivation.

ACKNOWLEDGMENTS

The authors thank the University of Nebraska Dairy Metabolism (Lincoln, NE) staff and students for care of the experimental animals and assistance with collections. The authors state no conflicts of interest.

Table 5.1. Descriptive statistics of the data used for determining the relationship between urinary energy and N excretion in lactating Jersey cows when urine was analyzed as-is

Item ¹	N ¹	Mean	SD	Minimum	Maximum
Animal descriptions					
Colostrum intake	134	100	0	100	100
DIM, d	134	209	63	88	346
Parity	134	3.04	1.14	2.00	6.00
BW, kg	134	461	54	363	606
BCS ²	134	3.24	0.40	2.00	4.13
DMI, kg/d	134	18.3	2.4	11.6	24.6
Milk yield, kg/d	134	21.5	4.8	10.8	34.3
ECM ³ , kg/d	134	29.4	5.6	14.8	48.2
Fat yield, kg/d	134	1.26	0.25	0.61	2.28
Protein yield, kd/d	134	0.80	0.15	0.46	1.21
Urine					
Excretion, kg/d	134	23.8	5.3	15.3	46.1
N, %	134	0.686	0.136	0.203	0.977
C, %	134	0.869	0.187	0.334	1.25
Energy, kcal/g	134	0.104	0.021	0.035	0.155
Energy, kcal/d	134	2381	314	1390	3160
N utilization, g/d					
Intake	134	486	66	285	642
Feces	134	176	29	111	249
Milk	134	141	27	80	217
Urine	134	158	24	85	220
Urine, % of N intake	134	32.7	4.61	20.6	59.5
Balance	134	12	30	-98	88
Urine C excretion, g/d	134	199	24.5	130	273
Dietary composition					
DE, Mcal/kg DM	134	2.91	0.14	2.49	3.29
ME, Mcal/kg DM	131	2.57	0.14	2.17	2.95
CP, % DM	134	16.6	0.7	14.8	17.6
NDF, % DM	134	32.1	3.8	21.4	43.0
Starch, % DM	134	26.4	3.7	16.2	35.7
Crude fat, % DM	113	4.3	1.0	2.2	5.9
S, % DM	113	0.28	0.01	0.26	0.30

¹N = number of observations.

²On a 1 to 5 scale.

³ECM = $0.327 \times \text{milk yield (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{true protein (kg)}$ (Tyrrell and Reid, 1965).

Table 5.2. Fit statistics for models to explain the relationship between urinary energy and (UN) or urinary C (UC) concentration or excretion¹

Item ²	AICc	$\hat{\sigma}_c$	$\hat{\sigma}_c$, % of mean	$\hat{\sigma}_{p(s)}$	$\hat{\sigma}_{p(s)}$, % of mean	$\hat{\sigma}_e$	$\hat{\sigma}_e$, % of mean
Urinary N equation							
1	-835	0.0061	5.84	0.0054	5.21	0.0078	7.50
2	1806	119	5.01	95	3.98	171	7.21
3	1848	121	5.10	161	6.75	189	7.93
4	1826	124	5.22	92	3.88	170	7.14
5	499	0.954	6.27	0.497	3.27	1.21	7.98
6	497	0.912	5.99	0.569	3.74	1.19	7.81
Urinary C equation							
1	-806	0.0032	3.04	0.0052	4.99	0.0089	8.52
2	1819	149	6.23	100	4.21	186	7.80
3	1859	30.9	1.30	150	6.28	232	9.73
4	1855	157	6.57	100	4.19	184	7.73

¹ Equation 1 = linear regression between UE (kcal/g) and UN or UC(g/100 g); Equation 2

= linear regression between UE (kcal/d) and UN or UC (kcal/d); Equation 3 = linear

regression between UE (kcal/d) and UN or UC (kcal/d) with a 0 intercept; Equation 4 =

quadratic regression between UE (kcal/d) and UN or UC (kcal/d) with a 0 intercept;

Equation 5 = linear regression between UE (kcal/g of N) and UN (kcal/d); Equation 6 =

linear regression between UE (kcal/g of N) and UN (kcal/d) plus DMI (% BW).

²Number of observation = 134; AICc = corrected Akaike information criterion, $\hat{\sigma}_c$ =

square root of the estimated variance associated with cow, $\hat{\sigma}_{p(s)}$ = square root of the

estimated variance associated with period within study, $\hat{\sigma}_e$ = square root of the estimated

variance associated with error.

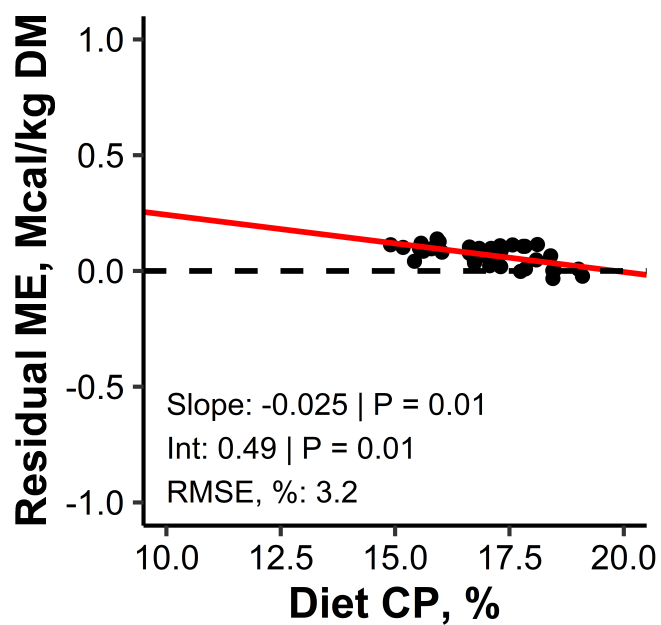


Figure 5.1. Residual (observed – predicted) ME (Mcal/kg DM) versus dietary CP (%). Predicted ME was calculated from observed DE using NRC (2001) equation 2-10. Each data point represents a treatment mean from the full dataset ($n = 45$). Slope and intercept estimate as well as corresponding P -values are listed at the bottom of the figure. RMSE = residual mean squared error expressed as a percent of mean observed ME.

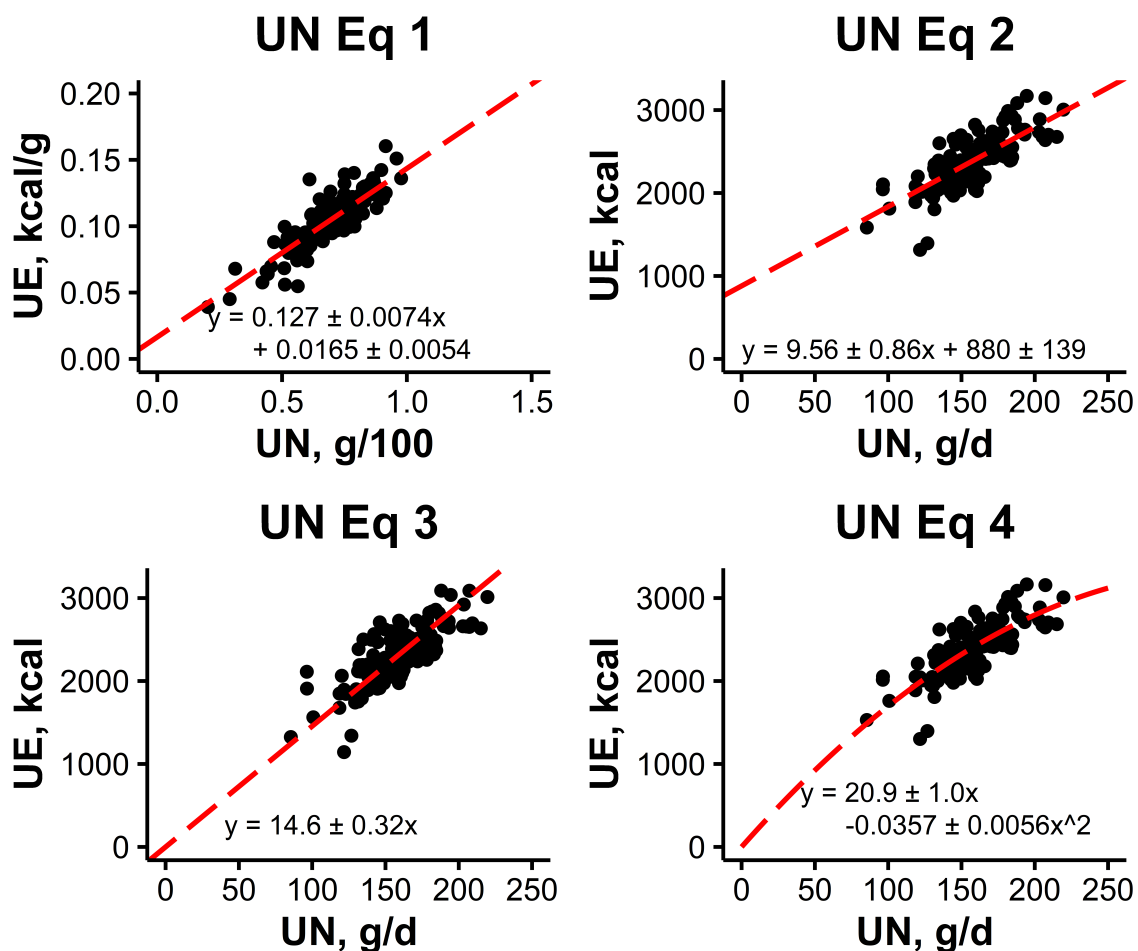


Figure 5.2. Relationship between urinary energy (UE) concentration or excretion and urinary N (UN) concentration or excretion for lactating Jersey cows for equation 1, 2, 3, and 4. Intercept was forced through zero in equation 3 and equation 4. All linear and quadratic coefficients were different from zero ($P < 0.01$). Refer to Table 5.2 for equation fit statistics. All data are adjusted for the random effects of cow and period within experiment.

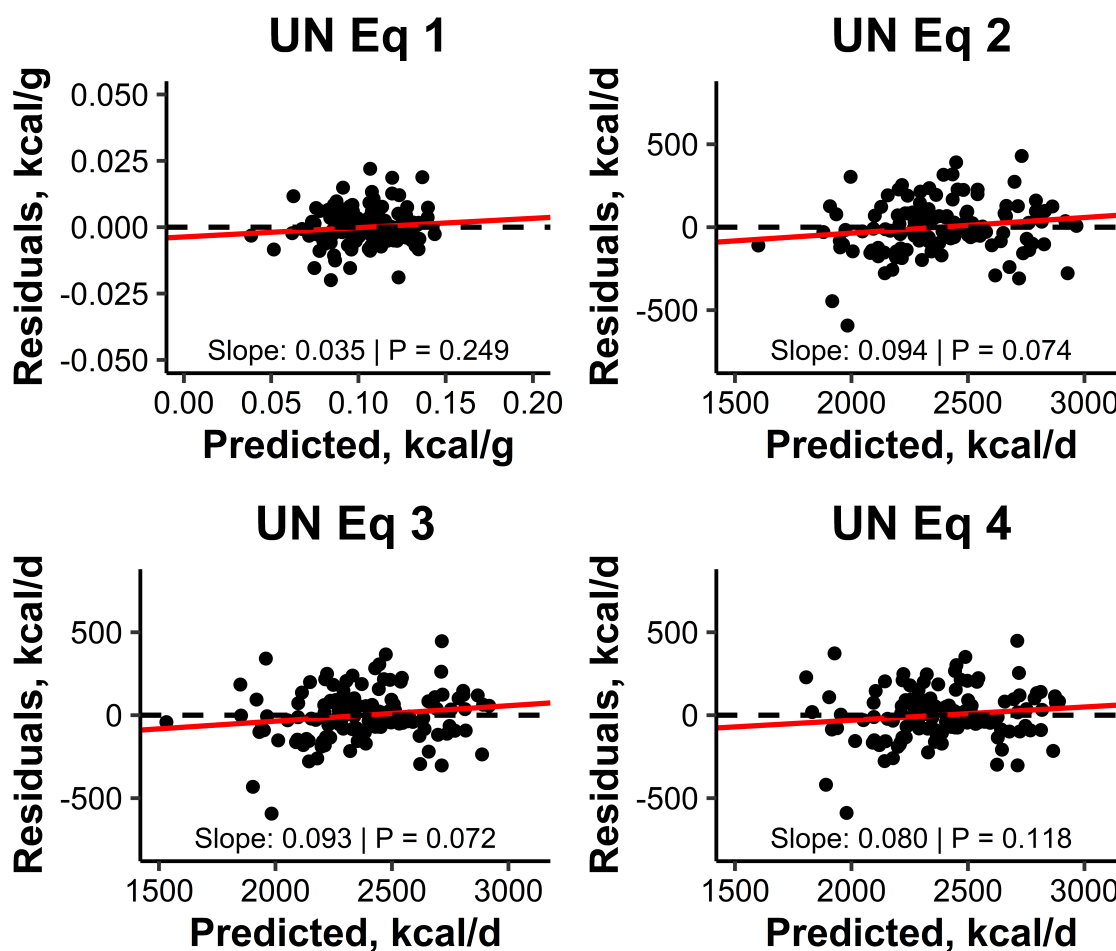


Figure 5.3. Residuals (observed – predicted) versus predicted urinary energy concentration or excretion for relationship with urinary N (UN) concentration or excretion for equation 1, 2, 3, and 4 (Figure 5.2). Slope estimate and P-values for the slope of the linear relationship are listed at the bottom of the figure. All data are adjusted for the random effect of cow and period within experiment.

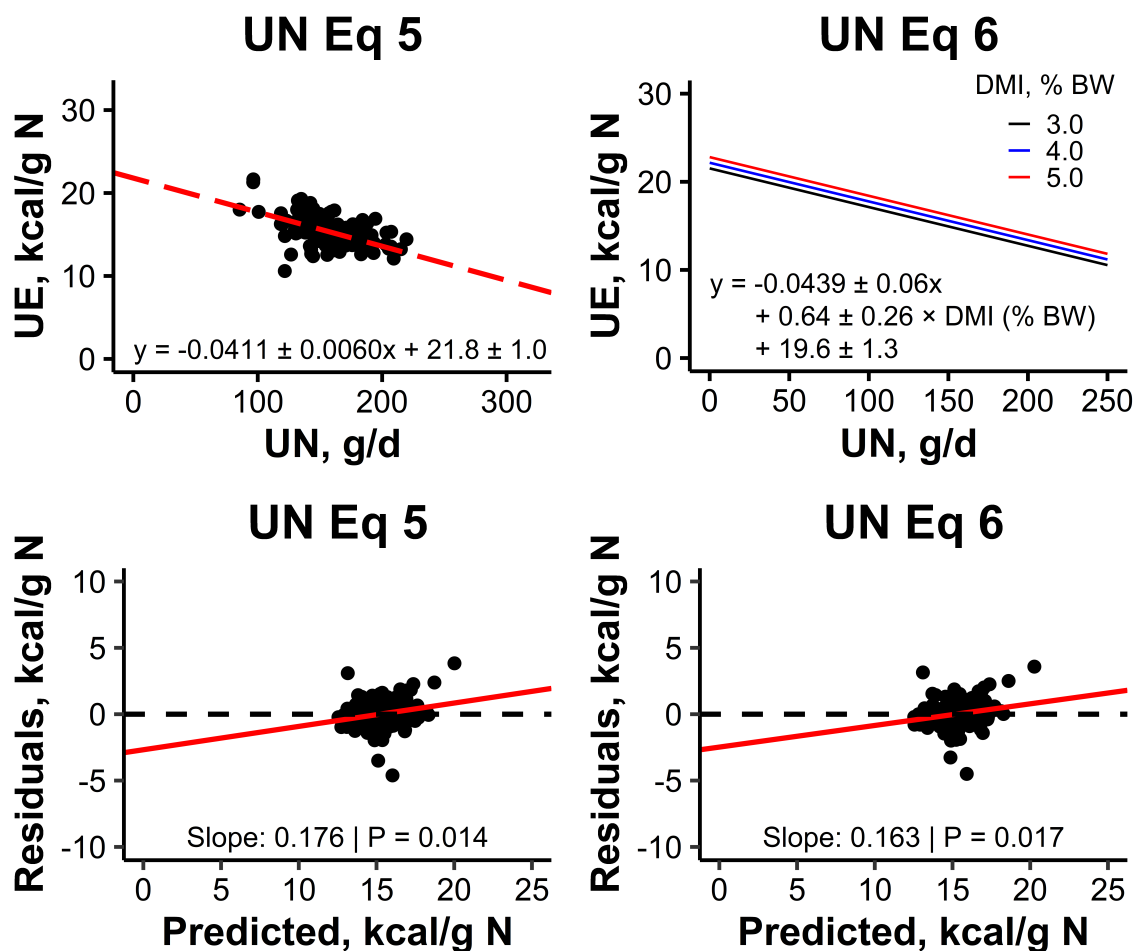


Figure 5.4. Relationship between urinary energy (UE) per g N and urinary (UN) excretion or concentration for lactating Jersey cows for equation 5 and 6, and residual (observed – predicted) versus predicted values. Slope estimate and P-values for the slope of the linear relationship for residuals vs. predicted plots are listed at the bottom of the figure. All data are adjusted for the random effect of cow and period within experiment.

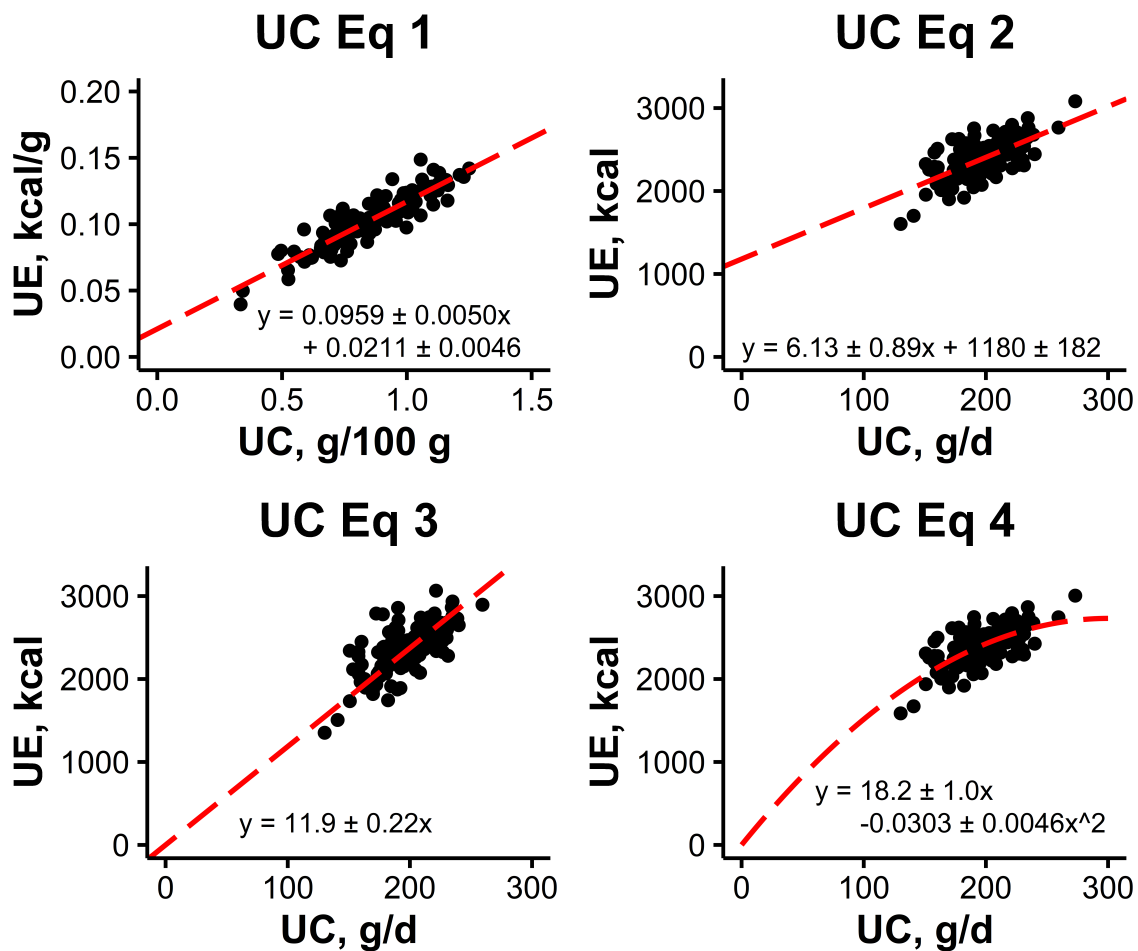


Figure 5.5. Relationship between urinary energy (UE) concentration or excretion and urinary C (UC) concentration or excretion for lactating Jersey cows for model 1, 2, 3, and 4. Intercept was forced through zero in equation 3 and equation 4. All linear and quadratic coefficients were different from zero ($P < 0.01$). All data are adjusted for the random effect of cow and period within experiment.

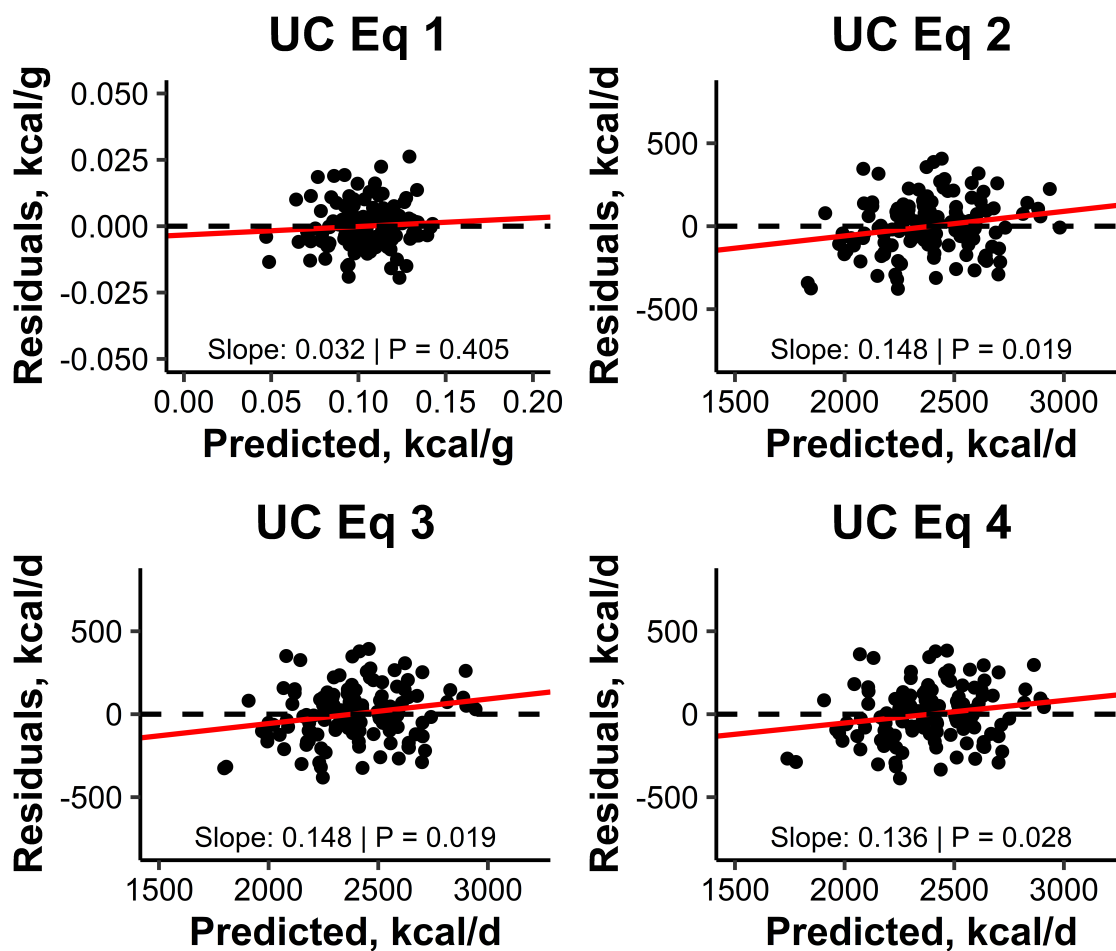


Figure 5.6. Residuals (observed – predicted) versus predicted urinary energy concentration or excretion for relationship with urinary C (UC) concentration or excretion for model 1, 2, 3, and 4 (Figure 4). Slope estimate and P-values for the slope of the linear relationship are listed at the bottom of the figure. All data are adjusted for the random effect of cow and period within experiment.

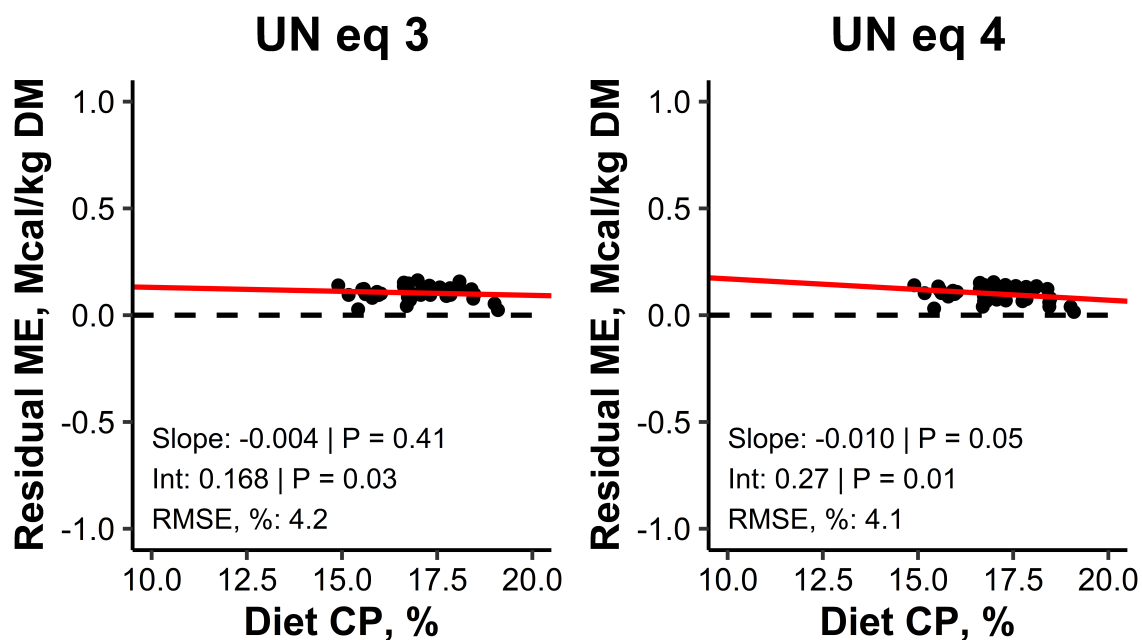


Figure 5.7. Residual (observed – predicted) ME (Mcal/kg DM) versus dietary CP (%).

Predicted ME was calculated from observed DE using UN equation (eq) 3 or 4 (Figure 2) to estimate UE and CH₄ energy estimated as $0.294 \times \text{DMI (kg/d)} - 0.347 \times \text{dietary crude fat (\% DM)} + 0.0409 \times \text{NDF digestibility (\%)}$; Nielsen et al., 2013). Each data point represents a treatment mean from the full dataset (n =45). Slope and intercept estimate as well as corresponding *P*-values are listed at the bottom of each figure. RMSE = residual mean squared error expressed as a percent of mean observed ME.

**CHAPTER 6: EFFECTS OF RUMEN-PROTECTED LYSINE AND HISTIDINE
ON MILK PRODUCTION AND ENERGY AND NITROGEN
UTILIZATION IN DIETS CONTAINING HYDROLYZED FEATHER
MEAL AND FED TO LACTATING JERSEY COWS**

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Morris, D. L. and P. J. Kononoff. 2020. Effects of rumen-protected lysine and histidine on milk production and energy and nitrogen utilization in diets containing hydrolyzed feather meal fed to lactating Jersey cows. J. Dairy Sci. 103:7110-7123. <https://doi.org/10.3168/jds.2020-18368>

INTERPRETIVE SUMMARY. Morris and Kononoff. (2020). “Effects of rumen-protected lysine and histidine on milk production and energy and nitrogen utilization in diets containing hydrolyzed feather meal and fed to lactating Jersey cows.” Rumen-protected lysine (**Lys**) and (**His**) were supplemented to a diet contained 5% hydrolyzed feather meal. Supplementing rumen-protected Lys increased plasma Lys only when rumen-protected His was not supplemented, and supplementing rumen-protected His increased plasma His. Rumen-protected Lys did not affect milk production and components; whereas, increased milk and milk protein yield were observed with supplemental rumen-protected His. Utilization of energy was not affected by treatment, but N balance increased with rumen-protected Lys. Results of this study suggest that lysine was not limiting in diets containing 5% hydrolyzed feather meal diets, however, histidine may have been limiting.

RUNNING HEAD: LYSINE AND HISTIDINE IN DIETS WITH FEATHER MEAL

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ABSTRACT

Hydrolyzed feather meal (**HFM**) is high in crude protein, most of which bypasses rumen degradation when fed to lactating dairy cows, allowing direct supply of AA to the small intestine. In comparison to other feeds that are high in bypass protein such as blood meal or heat-treated soybean meal, HFM is low in His and Lys. The objectives of this study were to determine the effects of supplementing rumen-protected (**RP**) Lys and His individually or in combination in a diet containing 5% HFM on milk production and composition as well as energy and N partitioning. Twelve multiparous Jersey cows (mean \pm SD; 91 ± 18 d in milk) were used in a triplicated 4×4 Latin square with 4 periods of 28 d (24-d adaptation and 4-d collection). Throughout the experiment, all cows were fed the same TMR with HFM included at 5% of diet DM. Cows were grouped by dry matter intake and milk yield and cows within a group were randomly assigned to one of 4 treatments: no RP Lys or RP His; RP Lys only [70 g/d of Ajipro-L (24 g/d of digestible Lys); Ajinomoto Co., Inc., Tokyo, Japan]; RP His only [32 g/d of experimental product (7 g/d of digestible His); Balchem Corp., New Hampton, NY]; or both RP Lys and His. Plasma Lys concentration increased when RP Lys was supplemented without RP His (77.7 vs. 66.0 ± 4.69 μ M) but decreased when RP Lys was supplemented with RP His (71.4 vs. 75.0 ± 4.69 μ M). Plasma concentration of 3-methylhistidine decreased with RP Lys (3.19 vs. 3.40 ± 0.31 μ M). With RP His, plasma concentration of His increased (21.8 vs. 18.7 ± 2.95 μ M). For milk production and milk composition, no effects of Lys were observed. Supplementing RP His increased milk yield (22.5 vs. 21.6 ± 2.04 kg/d) and tended to increase milk protein yield (0.801 vs. 0.772 ± 0.051 kg/d). Across treatments, dry matter intake (18.5 ± 0.83 kg/d) and energy supply (32.2 ± 2.24 Mcal of net energy

for lactation) were not different. Supplementing RP His did not affect N utilization; however, supplementing RP Lys increased N balance (25 vs. 16 ± 9 g/d). The lack of response to RP Lys suggests that Lys was not limiting or that the increase in Lys supply was not large enough to cause an increase in milk protein yield. However, increased N balance and decreased 3-methylhistidine with RP Lys suggest that increased Lys supply increased protein accretion and decreased protein mobilization. Furthermore, His may be a limiting AA in diets containing HFM.

Key Words: rumen-protected lysine, rumen-protected histidine, hydrolyzed feather meal

INTRODUCTION

Hydrolyzed feather meal (**HFM**) is a widely available high-CP (92%) feedstuff that may be used as a source of protein in lactating dairy cow rations. Although HFM is high in CP, of which approximately 65% is RUP, Lys (2.6% of CP) and His (1.2% of CP) content are low compared to other commonly used feeds such as blood meal (6.0 and 6.4% of CP for Lys and His, respectively; NRC, 2001). Because of the Lys and His content of HFM is low, feeding HFM may result in deficiencies in AA (Stahel et al., 2014). We recently fed HFM at 0, 3.3, 6.7, and 10% of diet DM as a replacement for blood meal and nonenzymatically-browned soybean meal (Morris et al., 2020d). In this study, DMI was maintained when HFM was fed up to 6.7% of diet DM and ECM was similar across treatments, but milk protein linearly decreased with increasing HFM inclusion. Given the low Lys and His content of HFM compared to blood meal, these AA may have limited milk protein synthesis. Therefore, research is needed to understand how Lys and His supply may influence performance of lactating cows fed high-HFM diets.

Feeding diets with HFM as the primary protein source compared to blood meal or soybean meal, is an excellent scenario from which to evaluate potentially limiting AA. Along with Met, Lys (NRC, 2001; Giallongo et al., 2016) and His (Lee et al., 2012b; Giallongo et al., 2015a; Giallongo et al., 2016) are commonly studied as potentially limiting AA in lactating dairy cows. NRC (2001) suggested that maximal milk protein concentration and yield are achieved when Lys was 7.1 and 7.2 % of MP, respectively. In corn-based diets, these concentrations of Lys may be difficult to reach leading to a more practical targets of 6.6 for Lys as a % of MP (Schwab et al., 2005). In several recent studies, increasing Lys supply via rumen-protected (**RP**) Lys increased milk protein concentration in some (Paz and Kononoff, 2014; Giallongo et al., 2016; Fleming et al., 2019a), but not in others (Paz et al., 2013; Apelo et al., 2014) and generally did not affect milk protein yield. Therefore, the effects of increasing Lys supply on milk protein production remain unclear. In diets low in MP and His, increased milk protein yield has been observed with abomasal infusion of His (Vanhatalo et al., 1999; Huhtanen et al., 2002) or by feeding RP His (Giallongo et al., 2015a; Zang et al., 2019). The effects of RP Lys and His on milk protein appears to, at least in part, be influenced by another co-limiting AA. For example, Giallongo et al. (2016) reported that when individually supplementing Met, Lys, and His to a MP deficient diet milk protein yield was not affected, however, when all three AA were supplemented simultaneously, milk protein yield increased by 0.10 kg/d. The results of the study by Giallongo et al. (2016) would suggest that response to increased Lys supply may, at least in part, be influenced by the supply of His; however, the interactive nature between supply of Lys and His has not been studied. Therefore, the objectives of the current study were to determine the effects

of supplementing RP Lys and His individually or in combination in a diet containing HFM on milk production and energy and N partitioning in lactating dairy cows. We hypothesized that the addition of Lys and His would individually and additively increase milk protein production, and thus increase partitioning of energy and N toward milk.

MATERIALS AND METHODS

Animals and Treatments

The University of Nebraska–Lincoln Animal Care and Use Committee approved animal care and experimental procedures. Twelve multiparous Jersey cows [(average \pm SD) 91 ± 18 DIM at the beginning of the experiment) sourced from a commercial dairy were used. Sample size was based on previous work at the University of Nebraska–Lincoln (Reynolds et al., 2019). 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 and milked at 0700 and 1800 h. All cows were less than 40 d pregnant at the end of the last experimental period, thus, fetal energy was assumed to be zero (NRC, 2001).

The experimental design was a triplicated 4×4 Latin square balanced for carryover effects with 4 periods of 28 d periods. Cows were fed the same TMR with HFM included at 5% of diet DM throughout the experiment. Cows were grouped by DMI and milk yield and cows within a group were randomly assigned to treatment sequence (randomization and enrolment was completed by D. L. Morris). Treatment sequence was based on Kononoff and Hanford (2006). Treatments were as follows: 1) no supplemental Lys and no supplemental His (**LYS0HIS0**), 2) supplemental RP Lys only (**LYS+HIS0**;

70 g/d of Ajipro; Ajinomoto Co., Inc., Tokyo, Japan), 3) supplemental RP His only (**LYS0HIS+**; 32 g/d of experimental product; Balchem Corp., New Hampton, NY), or 4) both supplemental RP Lys and His (**LYS+HIS+**; 70 g/d of Ajipro; Ajinomoto Co., Inc., Tokyo, Japan and 32 g/d of experimental product; Balchem Corp., New Hampton, NY). Treatments were applied at time of feeding by top dressing evenly over the TMR. In general, treatments were known by personnel; however, because most measurements were objective, outcomes likely were not biased. Three sources of HFM (American Proteins Inc., Cumming, GA; Pilgrim's, Greeley, Colorado; Simmons Foods, Siloam Springs, AR) were used to minimize effects of individual source and represent commercially available HFM. To accommodate the amount of HFM received from each source, these sources of HFM were blended in the following proportions: 33.3, 18.6 and 48.1% of DM for American Protein Inc., Pilgrim's, and Simmons Foods, respectively. Concentrate mixes for each treatment that included all dietary ingredients except for forages and cottonseed hulls (Table 6.1) were mixed at the University of Nebraska-Lincoln feed mill. Dietary ingredients for the base diet (corn silage, alfalfa hay, and concentrate) were placed in 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. To limit refusal during collection, cows were fed at 100% of the prior week's intake.

The ruminal and intestinal digestibility of HFM and RP AA used in the current study were estimated using the mobile bag technique (Paz et al., 2014). Briefly, each product was placed in the rumen (16 h), 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 dairy cows. Samples incubated in the rumen and passed through the duodenal were analyzed for N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI). Residue from bags that were incubated in the rumen was assumed to bypass rumen degradation and the disappearance in sample from the duodenally incubated bags was assumed to be represent intestinal digestibility of the bypass fraction. The AA content of RP Lys and RP His was determined as the N content of the product divided by the N content of the corresponding AA (19.2% for Lys and 27.1% for His).

Sample Collection and Analysis

Individual feed ingredients were sampled daily during collection periods and frozen at -20°C . Corn silage was dried at 60°C for 48 h, and then all feeds 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 of N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF with sodium sulfite (Van Soest et al., 1991) and α amylase and corrected for ash contamination (**NDF_{OM}**), 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), ash (943.05; AOAC International, 2000) and minerals (985.01; AOAC International, 2000). Additionally, feed ingredients were analyzed for gross energy (**GE**) content (Parr 6400 Calorimeter, Moline, IL) in the nutrition laboratory of the University of Nebraska-Lincoln. The chemical composition of diets and individual feed ingredients are listed in Table 6.1 and Table 6.2, respectively. For complete AA profile of feedstuffs, samples were analyzed by the Missouri–Columbia Agricultural Experiment Station Chemical Laboratory by cation

exchange chromatography coupled with post column ninhydrin derivatization with norleucine as internal standard (method 982.30; AOAC International, 2000). Tryptophan was determined after alkaline hydrolysis and sulfur AA were analyzed after performic acid oxidation (method 988.15; AOAC International, 2016). The AA profile for corn silage, alfalfa hay, concentrate mix and HFM and calculated for the basal TMR are listed in Table 6.3. 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 (60°C 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, NDF_{OM}, 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. A 137 × 76 cm rubber mat was placed behind the cow to aid in fecal collection. Feces were manually collected by personnel 24-h per d during defecation or occasionally were picked up from the rubber mat and deposited into a trash can (Rubbermaid, Wooster, OH). Daily feces were subsampled (~500 g 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 fecal 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. Urine pH was

measured at the end of each d and quantity of acid used was adjusted 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 and N as described above. Urine GE was determined by drying (60°C) approximately 4 mL of sample in a bomb capsule until dry (4 h) and then combusting the sample (Parr 6400 Calorimeter, Moline, IL). Urine N of liquid sample was determined by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) using a 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 was 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 milk samples were analyzed for GE and N as described previously for urine.

Blood samples were collected into evacuated K_2EDTA tubes from the tail vessel approximately 3 h after feeding during 2 d within collection wk when cows were not in a headbox. Day of blood sampling was randomized with respect to treatment. One blood sample was analyzed for hemoglobin concentration using a Siemens Advia 2120 (Siemens Healthineers; Erlangen, Germany). For the second blood sample, plasma was immediately separated by centrifugation at $1,500 \times g$ at 4°C for 20 min. 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 $1,500 \times g$ at 4°C for 20 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 . Blood samples collected on the second d were processed the same way and added to CryoTube vials. Plasma samples were submitted to the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratory for analysis of free AA, carnosine, and 3-methylhistidine (Deyl et al., 1986; Fekkes, 1996). Plasma AA concentrations 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. Gas data were adjusted to a 24-h period. Four headboxes were used and data were collected across 3-d during the 4-d collection period. Cows were adapted to headboxes for a minimum for 3 d prior to the start of the experiment. 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., Mooresville, 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 gas meter (Model AL425, American Meter, Horsham, PA) and corrected to standard temperature and pressure (0°C, 101.3 kPa) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). 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 O₂, CO₂ and CH₄ using an Emerson X-stream 3-channel analyzer (Solon, OH) according to the method of Nienaber and Maddy (1985). System efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O₂ and CO₂ were (average ± SD) 90 ± 4.2 and 89 ± 4.5%, respectively. Gas measurements were adjusted to 100% using recoveries for individual headboxes.

Energy Calculations

The respiratory quotient (**RQ**) was calculated using the ratio of CO₂ produced to O₂ consumed (L/L). Methane energy was estimated by multiplying CH₄ production by its enthalpy (9.45 kcal/L). Calculations for digested energy (**DE**) and ME were as follows:

$$\text{DE (Mcal/d)} = \text{GE (Mcal/d)} - \text{fecal energy (Mcal/d)} \quad [1]$$

$$\text{ME (Mcal/d)} = \text{DE (Mcal/d)} - \text{urine energy (Mcal/d)} - \text{methane energy (Mcal/d)} \quad [2]$$

Unaccounted for energy was assumed to represent tissue energy retention or mobilization which was corrected to an NEL basis as follows:

$$\text{Residual energy (Mcal/d)} = \text{ME (Mcal/d)} - \text{heat production (Mcal/d)} - \text{milk energy (Mcal/d)}$$

[3]

Tissue energy (TE; Mcal of NEL/d) = positive residual energy $\times k_T$ or negative residual energy $\times k_G/k_L$ [4]

Where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain, and k_L is the efficiency of utilizing ME intake for milk production. Values of 0.89, 0.75, and 0.66 were used for k_T , k_G , and k_L , respectively (Moraes et al., 2015).

Statistical Analysis

Data were analyzed in R (v3.5.1) using the lmer (Kuznetsova et al., 2017) package. Models were developed apriori and included the fixed effects of Lys and His level and interaction of Lys and His 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 anova function of R. All data are presented as least-squares means \pm largest standard error. Significance and trends were declared at $P \leq 0.05$ and $P \leq 0.10$, respectively.

RESULTS AND DISCUSSION

This experiment was designed to test the effects of RP Lys and RP His alone or in combination on milk production and composition, N and energy utilization and AA status when feeding a base diet containing HFM. In our previous work, we observed a linear decrease in milk protein with increasing inclusion of HFM (Morris et al., 2020d). Because HFM is low in Lys and His (NRC, 2001), the current experiment was designed to determine if Lys and/or His are limiting in diets containing HFM diets. Most previous studies designed to test RP Lys and RP His have looked at individual effects of these AA

(Giallongo et al., 2016; Zang et al., 2019) or in combination with Met (Lee et al., 2012b; Giallongo et al., 2016). The current experiment differs because it was designed to examine the interaction between supply of Lys and His.

Upon initiation of the study, it was assumed that no cows had been previously bred, but breeding synchronization during the third period caused a late-term iatrogenic abortion in one cow. All data from this cow were removed prior to statistical analysis. During period 1, one cow (on treatment LYS0HIS0) refused to eat normally while in the headbox resulting in lower DMI compared to when she was out of the headbox (11.0 vs. 19.6 kg/d). This led to gas consumption and production measurements that were 22–32% below the mean of other cows during this period. However, this was on d 4 of collection period, thus, data from the first 3 d of collection were used, and only gas-related variables were not calculated. Another cow became ill during period 4 (pneumonia, on treatment LYS0HIS+) and data for this period were removed prior to statistical analysis. Therefore, 42 out of a 48 possible data points were used for gas-related energy calculations and 43 out of 48 were used for all other variables.

The HFM used in the current study is indicative of that available commercially. The HFM was $89.8 \pm 2.2\%$ CP, $67.8 \pm 9.2\%$ RUP, and digestibility of RUP was $64.7 \pm 9.0\%$. In a recent study using 10 HFM from different sources, the CP, RUP, and RUP digestibility were 90.7 ± 2.86 , $77.8 \pm 7.8\%$ and $61.1 \pm 9.1\%$, respectively (Buse et al., 2019). The RUP content of the blend of HFM used in the current study is similar to other bypass protein sources such as blood meal and expellers soybean meal (63–70% RUP); however, the digestibility of the RUP is much lower compared to the same bypass protein sources (88–99%; Paz et al., 2014). The Lys and His content of the HFM was 2.37 and

1.13% of CP, respectively (Table 6.3). The Lys content of HFM is similar to corn and corn byproducts (2.2–2.8% of CP) but lower than soybean products (5.8–6.3% of CP) and blood meal (9.0% of CP; NRC, 2001). The His content of HFM is lower than soybean products (2.4–2.8% of CP), corn and corn byproducts (2.5–3.1% of CP), and blood meal (6.8% of CP; NRC, 2001). Additionally, compared to target AA content of MP, microbial protein has a high Lys content (8.1% of total AA) and low His content (1.9% of total AA; Sok et al., 2017). Because of the low His content, HFM may be a poor complementary protein source for microbial protein.

In previous studies where the effects of RP AA were tested, diets are commonly formulated to be deficient in MP and with a low supply of the AA of interest, and RP AA are supplemented to bring AA supply to target contents (Lee et al., 2012b; Giallongo et al., 2016; Zang et al., 2019). To prevent inadequate supply of AA besides Lys or His, we initially formulated a diet (NRC 2001) to be adequate in MP (+16 g/d) under the following assumptions: 19.5 kg/d DMI, 27.0 kg/d milk yield, 5.00% milk fat and 3.35% milk true protein. However, predicting animal performance a priori is challenging, and throughout our study, MP balance was on average 123 g/d due to a lower than expected milk and milk protein yield (Table 6.4). We targeted Lys and His supplies of 6.6 (Schwab et al., 2005) and 2.2 (Lee et al., 2012b) % of MP, respectively. The increased supply of MP resulted in estimated digestible AA supplies that were only slightly below targets (–4 to –2 g/d) in the non-RP-AA-supplemented treatments. The RP Lys used in the current study was 44.5% Lys, $80.3 \pm 5.31\%$ RUP with a RUP digestibility of $95.8 \pm 1.23\%$. Therefore, 70 g of RP Lys was estimated to supply 24 g of digestible Lys. The RP His was 43.2% His, $57.4 \pm 3.02\%$ RUP with a RUP digestibility of $92.4 \pm 3.67\%$. Therefore,

32 g of RP His was estimated to supply 7 g of digestible His. When RP Lys and His were supplemented, supply exceeded targets by 20–22 and 4–5 g/d for Lys and His, respectively. Because the supply of Lys and His in the basal diets were only marginally below targets, it is plausible that supply of Lys and His may have been adequate without the inclusion of either RP Lys or His. Variance in model estimated supply of individual AA can be large (Fleming et al., 2019b); therefore, the true supply of Lys and His in the current study is unknown. Admittedly, the target for Lys and His supply are not well established, and suppling increasing amounts of Lys (Vyas and Erdman, 2009) or His (Zang et al., 2019) may increase milk production and milk component yield.

Plasma Amino Acids, Hemoglobin, Carnosine, and 3-Methylhistidine

Plasma AA concentration may serve as a gross indicator of the metabolic status of these AA. In the current study an interaction between RP Lys and RP His was observed. Specifically, supplementing RP Lys increased plasma concentration of Lys without RP His (77.7 vs. 66.0 ± 4.69 μ M), however, when RP His was supplemented, RP Lys decreased plasma concentration of Lys (71.4 vs. 75.0 ± 4.69 μ M; interaction $P = 0.04$; Table 6.5). It is generally understood that plasma AA concentration are affected by plane of nutrition, stage of lactation, proportion of MP from microbial protein, and milk protein yield; and thus, plasma AA concentration may not always reflect the changes in duodenal supply of AA as it is (Patton et al., 2015; Martineau et al., 2017). Others have observed increased plasma Lys and His when supplementing the same RP AA products used in this study (Lee et al., 2012b; Giallongo et al., 2016). In a meta-regression, plasma Lys concentration was quadratically related to supply of metabolizable Lys such that with increasing metabolizable Lys supply, plasma Lys concentration increased and then

plateaued (Martineau et al., 2019). In the current study, decreased plasma Lys with RP Lys when RP His was supplemented may be due to increased milk protein yield and increased protein accretion as indicated by increased N balance (see later discussion). Milk protein yield is negatively related to plasma Lys (Patton et al., 2015). Increased plasma Lys when RP Lys was supplemented and RP His was not supplemented and increased plasma His when RP His was supplemented indicates adequate rumen protection and intestinal digestibility for the RP AA products used in the current experiment.

Histidine is unique among essential AA (**EAA**) in that a labile pool exists in carnosine (β -alanyl-l-His), anserine (β -alanyl-N-methyl-His), and blood hemoglobin, and this pool may be mobilized to cover deficiencies in the short term (Lapierre et al., 2008). In the current experiment, RP His did not affect ($P > 0.58$) plasma carnosine and hemoglobin concentration. In a His deficient diet, plasma carnosine and hemoglobin concentration were decreased; however, plasma hemoglobin was not decreased by a low-His diet until the 5th week on experimental diets (Giallongo et al., 2017). Thus, the duration of the current experiment, 4 wk, may not have been long enough for changes in carnosine and hemoglobin status to occur. Increased plasma carnosine with increased His supply has been observed (Zang et al., 2019), but not in all studies (Lee et al., 2012b; Giallongo et al., 2015a). The reason for this discrepancy is not clear and we suggest that future research seek to further describe the labile pools of His and may need to test the effects of His supply on His status in continuous experiments that are at least 5-wks in duration.

Supplementing RP Lys decreased ($P = 0.04$) 3-methylhistidine (3.19 vs. $3.40 \pm 0.31 \mu\text{M}$). In the current experiment, the cause for decreased 3-methylhistidine with increased Lys supply is unknown and may suggest that muscle breakdown was decreased with increased Lys supply. 3-methylhistidine is formed from the methylation of His and muscle breakdown is the primary source of circulating 3-methylhistidine (Harris, 1981). Thus, 3-methylhistidine may serve as a biomarker for muscle breakdown (Akamatsu et al., 2007). Appuhamy et al. (2011) observed an increase in 3-methylhistidine with jugular infusion of Met, Lys, and branched-chain AA. These authors suggested that increased milk protein synthesis with supplemental AA treatments may have been supported by AA mobilization from muscle.

Similar to plasma Lys, interactions ($P < 0.07$) were observed for plasma concentration of EAA, Arg, Leu, Met, and Thr. The cause of this interaction is unknown, but may be due to an increase in utilization of these AA by mammary glands and peripheral tissue when both RP Lys and His were supplemented. Supplementing RP His tended to increase milk protein yield and supplementing RP Lys tended to increase N balance (see later discussion) and decreased 3-methylhistidine which suggests an increase in protein accretion and decrease in protein mobilization from muscle. In other experiments where His or Lys were supplemented individually or in combinations, in general, plasma concentration of non-supplemented EAA were not affected (Lee et al., 2012b; Paz and Kononoff, 2014; Zang et al., 2019). However, plasma Leu concentration decreased with jugular infusion of Met and Lys (Appuhamy et al., 2011), and with jugular infusion of Met, Lys, and His, plasma concentration of nonsupplemented EAA,

Leu, and Thr decreased (Yoder et al., 2020). A third EAA may have become limiting when both RP Lys and His were supplemented.

DMI, Milk Yield, and Milk Composition

Quantification of the effects of individual AA supply on milk protein yield is needed to advance our understanding of AA requirements and to allow nutritionist to formulate diets that minimize CP while maximizing the efficiency of AA utilization for milk protein synthesis. In the current study, supplementation of RP Lys had no effect ($P > 0.14$) on DMI, milk yield, or milk composition (Table 6.6). Milk protein synthesis is one of the primary roles of plasma Lys (Schwab et al., 2005; Manjarin et al., 2014). Additionally, uptake of Lys in the mammary glands exceeds secretion in milk protein due to the utilization of Lys in the mammary glands for NEAA synthesis which appears to be obligate (Lapierre et al., 2009). In support of this notion, even when Lys was removed from an abomasal infusion, uptake of Lys in the mammary glands still exceeded Lys output in milk due to the utilization of Lys for NEAA synthesis (Lapierre et al., 2009). This highlights the importance of adequate Lys supply to support milk protein synthesis. We hypothesized that increasing supply of Lys would increase milk protein yield. Although our results contradict this hypothesis, others have observed a lack of response to increased supply of Lys. In a meta-analysis, Robinson (2010) reported that supplementing RP Lys compared to control diets had no effect of milk composition. In an individual study, supplementing RP Lys in an MP deficient diet increased milk protein composition by 0.09 percentage units, but milk protein yield was similar (Giallongo et al., 2016). We speculate that the observed lack of response to Lys with or without increased His supply may have occurred due to one or a combination of the following: 1)

Lys supply may not have been limiting in the basal diet; 2) increased supply of Lys from the RP Lys in the current study may not have been enough to elicit a response; 3) His supply in the RP His treatment may have still been limiting; 4) a third EAA may have been limiting; or 5) increasing supply of Lys has minimal to no effect on milk production and composition. Further investigation is warranted where RP Lys is incrementally supplemented to diets where Lys supply is low and tested in conjunction with other potentially limiting AA such as His.

Histidine status may be involved in the regulation of feed intake via the central nervous system, and increased His status may have a positive effect on feed intake (Giallongo et al., 2015a). Several studies have observed increased DMI with RP His or abomasal His infusion (Vanhatalo et al., 1999; Giallongo et al., 2015a; Giallongo et al., 2016). However, in the current feeding study, RP His did not affect ($P = 0.44$) DMI. Because endogenous pools of His can be mobilized to increase His status in the short-term (Lapierre et al., 2012), decreased DMI due to a His deficiency may have been prevented in the short term in cows fed diets without RP His. Giallongo et al. (2017) observed that the low-His diet did not affect DMI during the first 2 weeks of the experiment, but decreased DMI from week 5 to 10 of the experiment.

Supplementing RP His increased ($P = 0.05$) milk yield (22.5 vs. 21.6 ± 2.04 kg/d) and tended ($P < 0.10$) to increase milk protein yield (0.801 vs. 0.772 ± 0.051 kg/d) and milk lactose yield (1.09 vs. 1.04 ± 0.106 kg/d), whereas the concentration of milk fat tended ($P = 0.09$) to decrease (5.75 vs. $5.99 \pm 0.359\%$). Because milk fat yield was not affected ($P = 0.91$) by RP His (averaging 1.27 ± 0.094 kg/d), the decreased milk fat concentration was likely a dilution effect caused by increased milk yield. Consistent with the increased

milk protein production in the current study, several individual studies have observed similar responses with abomasal infusion of His (Vanhatalo et al., 1999; Huhtanen et al., 2002) or by feeding RP His (Giallongo et al., 2015a; Zang et al., 2019). Furthermore, RP His decreased ($P = 0.02$) MUN (16.3 vs. 17.2 ± 0.76 mg/dL) which suggests that decreased whole body catabolism of AA may have occurred in response to increased incorporation of AA into milk protein. In the study by Giallongo et al. (2016), the addition of RP His increased milk protein concentration, but did not affect milk protein yield; however, when RP His was fed in combination with RP Met and RP Lys, milk protein concentration and yield increased. This suggests that supply of other EAA may have limited the response to individual addition of AA, thus, demonstrating the importance of adequate supply of individual AA to support milk protein production.

Gas Consumption and Production, Energy Utilization, Nutrient Digestibility, and Nitrogen Utilization

To assess the effects of the use of the headboxes on feeding behavior, we compared DMI when cows were in the headboxes to when cows were out of the headboxes. When cows were in the headboxes, DMI decreased ($P < 0.01$) compared to when cows were not in the headboxes (17.6 vs. 18.6 ± 0.65 kg/d; data not shown). This effect was consistent across treatments, thus, differences between treatment effects for energy partitioning may be interpreted with confidence. Although we strive to make animals comfortable and familiar with headboxes, a slight reduction in DMI was not unexpected (Morris et al., 2020d). However, DMI was still within normal ranges, and we are unaware of alternative techniques that would allow us to make the measures described herein.

We expected to observe differences in energy utilization due to increased milk protein yield with supplemental Lys and His. Specifically, we expected to see increased milk energy output with supplemental AA. Supplementing RP Lys increased ($P < 0.01$) efficiency of converting DE into ME (0.885 vs. 0.878 ± 0.006) which was due to a tendency for decreased ($P = 0.06$) CH₄ energy (3.95 vs. 4.24 ± 0.27 Mcal/d; Table 6.7). Decreased CH₄ with RP Lys was not expected and the cause is unknown. The RP Lys was lipid coated and fat is often associated with decreased CH₄ production (Appuhamy et al., 2016b). However, the RP Lys only increased fat intake by 35 g/d (approximately 0.2% of diet DM) which should not have a large effect on CH₄ production. Treatment did not affect ($P > 0.14$) any other measures of energy utilization.

Although tendencies for differences ($P < 0.10$) in nutrient digestibility were observed when supplementing RP Lys (Table 6.8), the magnitude of responses were small and did not result in a difference in energy digestibility or milk production and composition, and thus differences in nutrient digestibility with RP Lys may not be biologically important or may not be large enough to cause differences in production. Several studies observed increased DMI with RP His or with abomasal His infusion (Vanhatalo et al., 1999; Giallongo et al., 2015a; Giallongo et al., 2016) which will increase energy intake. Because energy and protein supply interact for milk protein synthesis (Oldham, 1984; Brun-Lafleur et al., 2010), increased milk protein yield in these studies may be a function of increased energy intake. In the current study, because no differences were observed for energy intake and utilization when supplementing RP His, the increase in milk protein yield with His may have not been large enough to affect energy utilization.

We hypothesized that increasing Lys and His supply would decrease N excretion, an effect supported by an increase in milk N secretion. However, the addition of neither RP Lys nor RP His affected ($P > 0.16$) fecal or urinary N excretion (Table 6.9). Although RP His increased milk protein production, RP His did not affect ($P > 0.20$) total milk N secretion. Variation for milk N secretion was about 16% greater (adjusted for units) compared to milk protein yield. Therefore, although secretion of milk N was directionally increased by a similar quantity as N output from increased milk protein (~ 4 g/d), increased variation for milk N secretion may have limited our ability to observe differences in milk N secretion. Similar to our observations, N utilization was not affected when RP AA were supplemented compared to unsupplemented treatments (Lee et al., 2012b; Lee et al., 2015). Supplementing RP Lys tended ($P = 0.08$) to increase retained N (25 vs. 16 ± 9 g/d). With abomasal infusion of EAA to increase MP and improve AA profile, Nichols et al. (2019b) observed an increase in N balance. Increased N balance with RP Lys suggests that increasing Lys supply improved AA profile and allowed for increased protein synthesis in non-mammary tissues. This response agrees with decreased protein mobilization suggested by decreased 3-methylhistidine with supplemental RP Lys.

CONCLUSIONS

Supplementing RP Lys increased plasma Lys only when RP His was not supplemented, whereas supplementing RP His increased plasma His. Supplementing RP His to a diet containing HFM increased milk and milk protein yield, whereas supplementing RP Lys did not affect milk production, nor did it affect milk components. Supplemental RP Lys or His did not affect utilization of energy, except for an unexpected

decrease in methane with RP Lys. With increased Lys, N balance increased and plasma 3-methylhistidine decreased which suggests an increase in protein accretion and decrease in protein mobilization. A lack of production response to added Lys suggest that the EAA may not have been limiting for milk protein synthesis, or the increase in Lys supply may not have been large enough to increase a milk protein. Increased milk yield and milk protein yield suggest that His may be a potentially limiting AA in diets containing hydrolyzed feather meal and fed to lactating dairy cows.

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Table 6.1. Ingredient, chemical composition, and particle size distribution of the basal

TMR feed to all experimental cows (% of DM)

Item	Basal TMR
Ingredient	
Corn silage	39.0
Alfalfa hay	16.0
Hydrolyzed feather meal	5.0
Corn grain, ground	17.5
Soyhulls	9.3
Soybean meal	1.7
Molasses	3.0
Whey	2.5
Fat ¹	2.5
Urea	0.55
Rumen-protected Met ²	0.09
Mineral-vitamin mix ³	2.86
Chemical composition	
DM	57.6 (2.47)
CP	17.1 (0.42)
ADF	22.2 (1.34)
NDF	32.4 (0.72)
NDF _{OM} ⁴	31.7 (0.82)
Starch	26.3 (1.20)
Crude fat	5.3 (0.63)
Ash	7.9 (0.28)
Particle size	
>19.0 mm, % as-is	6.0 (2.27)
8.0–19.0 mm, % as-is	34.9 (4.74)
1.18–8.0 mm, % as-is	37.1 (4.99)
<1.18 mm, % as-is	22.0 (0.72)
>19.0 mm, % of DM	5.3 (1.84)
8.0–19.0 mm, % of DM	29.4 (3.27)
1.18–8.0 mm, % of DM	40.7 (3.01)
<1.18 mm, % of DM	24.6 (1.28)

¹Energy Booster 100 (Milk Specialties, Eden Prairie, MN).²Smartamine M (Adisseo, Alpharetta, GA).

³Contained per kg of premix: 319 g of CaCO₃, 269g of NaHCO₃, 175g of Ca₂PO₄, 112 g of MgO, 94 g of salt, 17 g of vitamin premix (14,850 IU/g Vitamin A, 3,850 IU/g Vitamin D, and 90 IU/g of Vitamin E), and 14 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).

${}^4\text{NDF}_{\text{OM}} = \text{NDF corrected for ash.}$

Table 6.2. Chemical composition of corn silage, alfalfa hay, concentrate mixes, and hydrolyzed feather meal used in the current experiment (% of DM)¹

Item ⁴	Corn silage		Alfalfa hay		Concentrate		HFM ^{2,3}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, % as-is	35.3	4.28	87.5	3.13	92.2	0.64	91.5	0.26
CP	8.8	0.17	19.8	0.98	23.4	0.60	89.8	2.22
ADF	23.3	4.38	36.1	2.34	16.2	0.85	— ⁵	—
NDF	36.9	4.11	43.4	3.35	24.5	1.83	—	—
NDF _{OM}	36.1	4.21	42.7	3.46	24.0	1.71	—	—
ADICP	0.80	0.22	1.28	0.12	2.23	0.09	—	—
NDICP	1.03	0.32	2.18	0.88	3.25	0.38	—	—
Lignin	3.05	0.53	8.09	0.29	2.98	0.83	—	—
Sugar	1.15	0.75	5.23	0.96	8.38	0.33	—	—
Starch	37.4	4.83	1.8	0.25	25.4	2.86	—	—
Crude fat	3.08	0.49	1.80	0.29	8.36	1.10	8.95	1.71
Ash	5.21	0.14	10.6	0.18	9.17	0.56	2.40	0.63
Ca	0.26	0.05	1.16	0.10	1.56	0.12	0.52	0.22
P	0.24	0.06	0.34	0.03	0.44	0.05	0.28	0.13
Mg	0.14	0.03	0.24	0.01	0.58	0.03	—	—
K	1.03	0.07	3.45	0.05	1.17	0.01	—	—
S	0.14	0.01	0.25	0.02	0.44	0.01	2.38	0.27

¹n = 4 for corn silage, alfalfa hay and concentrate, n = 3 for hydrolyzed feather meal.

²Hydrolyzed feather meal blend from three sources (33.3%, American Proteins Inc., Cumming, GA; 18.6%, Pilgrim's, Greeley, Colorado; 48.1%, Simmons Foods, Siloam Springs, AR); $67.8 \pm 9.18\%$ RUP (16-h rumen in situ incubation) with RUP digestibility of $64.7 \pm 8.97\%$ (mobile bag technique).

³Mean and SD weighted based on the inclusion of each source.

⁴ADICP = Acid detergent insoluble CP, ADICP = Acid detergent insoluble CP, NDF_{OM} = NDF corrected for ash.

⁵Not determined.

Table 6.3. Concentration of AA in the basal diet, corn silage, alfalfa hay, concentratemix, and hydrolyzed feather meal used in the current experiment (% of CP)¹

Item	Basal TMR		Corn silage		Alfalfa hay		Concentrate		HFM ^{2,3}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EAA										
Arg	3.78	0.34	1.75	0.15	3.54	0.14	4.99	0.08	6.91	0.16
His	1.52	0.07	1.49	0.11	1.71	0.11	1.45	0.07	1.13	0.20
Ile	3.59	0.11	3.33	0.09	3.90	0.05	3.66	0.09	4.84	0.02
Leu	6.93	0.23	8.12	0.10	6.39	0.13	6.80	0.24	7.98	0.08
Lys	3.31	0.19	2.78	0.22	4.59	0.30	3.01	0.08	2.37	0.36
Met	1.42	0.12	1.43	0.13	1.26	0.11	1.66	0.20	0.70	0.07
Phe	3.88	0.19	3.50	0.31	4.28	0.08	3.91	0.10	4.92	0.18
Thr	3.30	0.06	3.07	0.16	3.73	0.06	3.36	0.07	4.57	0.02
Trp	0.50	0.05	0.34	0.01	0.80	0.04	0.48	0.03	0.50	0.06
Val	4.91	0.17	4.47	0.09	4.99	0.08	5.27	0.13	7.60	0.39
NEAA										
Ala	5.20	0.50	8.52	0.63	4.66	0.10	4.07	0.10	4.54	0.08
Asp	6.86	0.55	4.45	0.43	10.4	0.49	6.44	0.12	6.72	0.10
Cys	2.30	0.21	1.29	0.13	1.14	0.07	3.45	0.07	5.91	0.25
Glu	9.84	0.40	10.5	0.68	7.97	0.17	10.2	0.31	10.4	0.09
Gly	4.68	0.17	3.70	0.07	4.14	0.15	5.52	0.11	7.60	0.28
Pro	6.37	0.39	5.54	0.38	7.51	1.23	6.80	0.09	9.60	0.59
Ser	4.66	0.35	2.47	0.20	3.59	0.10	6.34	0.13	10.5	0.70
Tau	1.31	0.11	2.21	0.10	1.01	0.11	1.02	0.06	0.03	0.01
Tyr	2.14	0.07	1.49	0.15	1.95	0.03	2.51	0.02	2.65	0.30

¹n = 4 for basal TMR, corn silage, alfalfa hay and concentrate, n = 3 for hydrolyzed

feather meal.

²Hydrolyzed feather meal blend from three sources (33.3%, American Proteins Inc.,

Cumming, GA; 18.6%, Pilgrim's, Greeley, Colorado; 48.1%, Simmons Foods, Siloam

Springs, AR).

³Mean and SD weighted based on the inclusion of each source.

Table 6.4. Estimated dietary net energy and MP and AA balance in lactating Jersey cows fed a diet containing 5% hydrolyzed feather meal and supplemented with rumen-protected Lys and His¹

Item	Treatment ²			
	LYS0		LYS+	
	HIS0	HIS+	HIS0	HIS+
NEL, Mcal/kg	1.64	1.63	1.64	1.63
MP, g/d				
Required	1822	1856	1809	1885
Supply	1958	1958	1946	2000
Balance	136	102	137	115
dLys, g/d				
Target ³	120	122	119	124
Supply from dietary and microbial protein	118	118	117	120
Supply from RP Lys ⁴	0	0	24	24
Balance	-2	-4	22	20
dHis, g/d				
Target ³	40	41	40	41
Supply from dietary and microbial protein	38	38	38	39
Supply from RP His ⁵	0	7	0	7
Balance	-2	4	-2	5

¹All values estimated with NRC, 2001 using mean production and measured feed

composition.

²LYS0 = 0 g/d of RP Lys, LYS+ = 70 g/d RP Lys, HIS0 = 0 g/d of RP His, HIS+ = 32 g/d of RP His.

³Targets for digestible Lys (dLys) and digestible His (dHis) were calculated as 6.6 (Schwab et al., 2005) and 2.2 (Lee et al., 2012b), respectively, % of MP requirements.

⁴80.3 ± 5.31% rumen bypass with a 95.8 ± 1.23 intestinal digestibility as determined using the mobile bag technique (Paz et al., 2014).

⁵57.4 ± 3.02% rumen bypass with a 92.4 ± 3.67 intestinal digestibility as determined using the mobile bag technique (Paz et al., 2014).

Table 6.5. Plasma concentration (μM) of AA, urea, hemoglobin, carnosine, and 3-methylhistidine of lactating Jersey cows fed a diet containing 5% hydrolyzed feather meal and supplemented with rumen-protected Lys and His

Item	Treatment ^{1,2}				SEM	P-value ³		
	LYS0		LYS+			L	H	L×H
	HIS0	HIS+	HIS0	HIS+				
EAA	892	971	972	941	46.8	0.39	0.40	0.07
Arg	76.2	85.1	82.4	78.7	5.61	0.98	0.38	0.04
His	18.2	23.3	19.2	20.3	2.95	0.52	0.06	0.22
Ile	116	126	127	122	7.59	0.45	0.64	0.14
Leu	137	150	153	146	8.89	0.27	0.53	0.07
Lys	66.0	75.0	77.7	71.4	4.69	0.26	0.70	0.04
Met	27.5	29.6	29.3	27.2	1.72	0.77	0.97	0.06
Phe	52.4	54.8	56.0	53.9	2.40	0.31	0.91	0.12
Thr	89.0	100.2	98.1	95.7	6.63	0.40	0.11	0.02
Trp	23.8	25.1	25.4	24.6	2.27	0.51	0.82	0.22
Val	284	305	305	302	20.5	0.41	0.41	0.27
NEAA	1307	1346	1329	1316	89.5	0.87	0.61	0.31
Ala	264	258	267	266	19.3	0.49	0.70	0.75
Asn	39.7	43.7	43.1	41.9	3.05	0.63	0.38	0.12
Asp	1.80	2.12	1.73	1.47	0.27	0.18	0.91	0.29
Gln	232	241	222	230	21.1	0.30	0.43	0.98
Glu	49.9	48.5	48.0	46.9	1.87	0.31	0.46	0.94
Gly	371	386	386	379	31.0	0.68	0.69	0.22
Pro	120	125	125	123	6.74	0.62	0.64	0.32
Ser	133	141	140	133	11.4	0.88	0.85	0.09
Tau	45.3	47.3	43.4	42.5	3.98	0.13	0.81	0.52
Tyr	48.6	52.1	51.3	50.0	4.11	0.89	0.63	0.30
Urea, mg/dL	4.29	4.23	4.21	4.08	0.33	0.37	0.43	0.79
Hemoglobin, g/dL	11.6	10.7	11.3	11.5	0.80	0.65	0.58	0.37
Carnosine	8.75	8.92	8.72	8.26	0.78	0.38	0.70	0.42
3-methylhistidine	3.40	3.40	3.27	3.11	0.31	0.04	0.46	0.42

¹LYS0 = 0 g/d of RP Lys, LYS+ = 70 g/d RP Lys, HIS0 = 0 g/d of RP His, HIS+ = 32

g/d of RP His; n = 43 for all variables.

²Least squares means; largest standard error of treatment mean is listed.

³L = main effect of Lys supply, H = main effect of His supply, L × H = interaction

between Lys and His.

Table 6.6. Dry mater intake, milk production and milk composition, free water intake, BW, and BCS of lactating Jersey cows fed a diet containing 5% hydrolyzed feather meal and supplemented with rumen-protected Lys and His

Item	Treatment ^{1,2}				SEM	P-value ³		
	LYS0		LYS+			L	H	L×H
	HIS0	HIS+	HIS0	HIS+				
DMI, kg/d	18.4	18.4	18.3	18.8	0.83	0.64	0.44	0.39
Milk yield, kg/d	21.4	22.2	21.7	22.7	2.04	0.36	0.05	0.76
ECM, kg/d ⁴	29.5	29.4	29.2	30.5	2.16	0.55	0.36	0.29
ECM/DMI, kg/kg	1.61	1.61	1.60	1.62	0.067	0.93	0.56	0.73
Fat, %	6.13	5.75	5.85	5.75	0.359	0.31	0.09	0.33
Fat, kg/d	1.28	1.25	1.26	1.30	0.094	0.64	0.91	0.24
Protein, %	3.65	3.62	3.57	3.59	0.135	0.14	0.86	0.47
Protein, kg/d	0.775	0.789	0.768	0.812	0.051	0.66	0.10	0.40
Lactose, %	4.79	4.81	4.82	4.82	0.060	0.57	0.73	0.87
Lactose, kg/d	1.03	1.07	1.05	1.10	0.106	0.43	0.07	0.87
MUN, mg/dL	17.2	16.1	17.1	16.5	0.76	0.59	0.02	0.44
Free water intake, L/d	77.0	77.4	75.7	79.7	4.88	0.87	0.43	0.53
BW, kg ⁵	458	459	461	459	15.5	0.56	0.91	0.63
BCS ⁶	3.20	3.21	3.12	3.22	0.11	0.43	0.14	0.21

¹LYS0 = 0 g/d of RP Lys, LYS+ = 70 g/d RP Lys, HIS0 = 0 g/d of RP His, HIS+ = 32

g/d of RP His; n = 43 for all variables.

²Least squares means; largest standard error of treatment mean is listed.

³L = main effect of Lys supply, H = main effect of His supply, L × H = interaction

between Lys and His.

⁴ECM = $0.327 \times \text{milk yield (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{true protein (kg)}$ (Tyrrell and Reid, 1965).

⁵Average from 2 measurements during last 4 d of each period.

⁶Scored 1–5 by 2 independent observations.

Table 6.7. O₂ consumption and CO₂ and CH₄ production, respiratory quotient, and energy utilization of lactating Jersey cows fed a diet containing 5% hydrolyzed feather meal and supplemented with rumen-protected Lys and His

Item ⁴	Treatment ^{1,2}				SEM	P-value ³		
	LYS0		LYS+			L	H	L×H
	HIS0	HIS+	HIS0	HIS+				
Gases, L/d								
O ₂ consumption, L/d	4708	4749	4568	4769	181	0.61	0.30	0.49
CO ₂ production, L/d	5061	5064	4874	5083	204	0.54	0.44	0.45
CH ₄ production, L/d	453	443	408	428	28	0.06	0.75	0.33
RQ	1.07	1.07	1.07	1.07	0.006	0.36	0.19	0.32
Components, Mcal/d								
Feces	30.3	30.7	29.5	30.9	2.01	0.63	0.19	0.44
CH ₄	4.28	4.19	3.86	4.04	0.27	0.06	0.75	0.33
Urine	2.40	2.40	2.42	2.43	0.11	0.39	0.89	0.88
HP ⁵	23.8	24.0	23.1	24.1	0.93	0.61	0.34	0.49
Milk	19.4	19.1	19.6	20.2	1.32	0.23	0.84	0.42
TE	4.84	4.04	5.43	4.66	2.31	0.57	0.46	0.99
Fractions, Mcal/d								
GE	85.1	84.9	84.6	86.9	3.93	0.62	0.46	0.41
DE	54.7	54.2	55.0	56.1	2.40	0.35	0.82	0.51
ME	48.4	47.8	48.8	49.6	2.39	0.34	0.94	0.52
NEL ⁶	32.0	30.9	33.0	32.8	2.24	0.18	0.58	0.66
Fractions, Mcal/kg of DM								
GE	4.62	4.62	4.62	4.62	0.024	0.24	0.67	0.06
DE	2.97	2.96	3.01	2.98	0.061	0.20	0.38	0.87
ME	2.62	2.60	2.66	2.64	0.063	0.14	0.37	0.94
NEL ⁶	1.73	1.68	1.79	1.74	0.066	0.14	0.26	0.91
Efficiencies								
ME/DE	0.877	0.878	0.885	0.884	0.006	0.04	0.90	0.88
Milk/ME	0.402	0.405	0.405	0.410	0.031	0.76	0.78	0.95
HP/ME	0.496	0.505	0.477	0.489	0.027	0.24	0.47	0.91
NEL/ME	0.657	0.646	0.675	0.661	0.021	0.21	0.30	0.91

¹LYS0 = 0 g/d of RP Lys, LYS+ = 70 g/d RP Lys, HIS0 = 0 g/d of RP His, HIS+ = 32

g/d of RP His; n = 43 for feces, urine, milk, GE, and DE, TE_P, n = 42 for all other variables.

²Least squares means; largest standard error of treatment mean is listed.

³L = main effect of Lys supply, H = main effect of His supply, L × H = interaction between Lys and His.

⁴RQ = respiratory quotient, CO₂ production/O₂ consumption, (L/L), HP = heat production, GE = gross energy, DE = digestible energy, TE = tissue energy (NEL basis), TE_P = tissue energy as body protein, TE_f = tissue energy as body fat.

⁵HP = $3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 - 0.518 \times \text{CH}_4 - 1.431 \times \text{N}$ (Brouwer, 1965).

⁶NEL = $0.080 \times \text{BW}^{0.75} + \text{milk energy} + \text{tissue energy}$ (NRC, 2001)

Table 6.8. Apparent total-tract digestibility of nutrient of lactating Jersey cows fed a diet containing 5% hydrolyzed feather meal and supplemented with rumen-protected Lys and His

Item	Treatment ^{1,2}				SEM	<i>P</i> -value ³		
	LYS0		LYS+			L	H	L×H
	HIS0	HIS+	HIS0	HIS+				
DM	65.7	65.2	66.6	66.1	1.20	0.07	0.36	0.99
OM	67.1	66.7	68.0	67.5	1.22	0.09	0.38	0.93
NDF	47.3	47.7	49.7	47.2	1.69	0.29	0.23	0.11
NDF _{OM} ⁴	48.2	48.5	50.4	47.5	1.71	0.45	0.12	0.07
CP	62.2	62.0	63.5	63.4	1.37	0.04	0.86	0.97
Starch	95.6	94.5	95.9	95.7	0.66	0.06	0.09	0.25
Energy	64.4	64.0	65.2	64.6	1.22	0.20	0.39	0.91

¹LYS0 = 0 g/d of RP Lys, LYS+ = 70 g/d RP Lys, HIS0 = 0 g/d of RP His, HIS+ = 32

g/d of RP His; n = 43 for all variables.

²Least squares means; largest standard error of treatment mean is listed.

³L = main effect of Lys supply, H = main effect of His supply, L × H = interaction

between Lys and His.

⁴NDF_{OM} = NDF corrected for ash.

Table 6.9. Nitrogen utilization of lactating Jersey cows fed a diet containing 5% feather meal and supplemented with rumen-protected Lys and His

Item	Treatment ^{1,2}				SEM	<i>P</i> -value ³		
	LYS0		LYS+			L	H	L×H
	HIS0	HIS+	HIS0	HIS+				
Output, kg/d (as is)								
Feces	88.3	88.0	86.2	89.3	5.54	0.86	0.53	0.45
Urine	23.1	23.3	23.5	23.2	1.37	0.83	0.92	0.77
Mass, g/d								
N intake	504	509	508	525	23	0.29	0.23	0.48
Fecal N	191	193	185	193	13	0.49	0.31	0.55
Urinary N	162	165	161	168	7	0.83	0.16	0.62
Milk N	136	138	136	142	9	0.46	0.20	0.52
Retained N	17	14	26	23	9	0.08	0.56	0.91
As proportion of N intake, %								
Fecal N	37.8	38.0	36.5	36.6	1.37	0.04	0.86	0.97
Urinary N	31.9	32.5	31.7	32.0	1.02	0.58	0.49	0.84
Milk N	27.0	27.2	26.8	27.1	1.04	0.76	0.64	0.96
Retained N	3.3	2.7	5.1	4.4	1.78	0.08	0.50	0.97

¹LYS0 = 0 g/d of RP Lys, LYS+ = 70 g/d RP Lys, HIS0 = 0 g/d of RP His, HIS+ = 32

g/d of RP His; n = 43 for all variables.

²Least squares means; largest standard error of treatment mean is listed.

³L = main effect of Lys supply, H = main effect of His supply, L × H = interaction

between Lys and His.

CHAPTER 7: EFFECTS OF HIGH-STARCH OR HIGH-FAT DIETS

FORMULATED TO BE ISOENERGETIC ON ENERGY AND NITROGEN PARTITIONING AND UTILIZATION IN LACTATING JERSEY COWS

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INTERPRETIVE SUMMARY. Morris et al. (2020). “Effects of high-starch or high-fat diets formulated to be isoenergetic on energy and nitrogen partitioning and utilization in lactating Jersey cows,” This article describes an experiment in which feeding a high-starch diet compared to a high-fat diet increased milk protein yield, net energy content of the diet, and tissue energy deposition as fat. Furthermore, the high-starch diet increased nitrogen partitioning towards milk nitrogen secretion and away from urinary nitrogen excretion. Results of this study suggest that feeding a high-starch diet compared to a high-fat diet can increase nitrogen utilization efficiency which will decrease nitrogen excretion, thus offering an avenue to potentially decrease environmental impact.

RUNNING HEAD: HIGH-STARCH OR HIGH-FAT DIETS IN DAIRY COWS

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ABSTRACT

The objective of this study was to determine the effects of high-starch or high-fat diets formulated to be isoenergetic on energy and N partitioning and utilization of energy. Twelve multiparous Jersey cows (mean \pm SD; 192 ± 11 d in milk; 467 ± 47 kg) in a crossover design with 28-d periods (24-d adaptation and 4-d collection) were used to compare two treatment diets. Treatments were high-starch (HS; 30.8% starch, 31.8% NDF, and 1.9% fatty acids) or high-fat (HF; 16.8% starch, 41.7 % NDF, and 4.1% fatty acids). Diets were formulated to have net energy for lactation (NEL) content of 1.55 Mcal/ kg of dry matter according to the National Research Council 2001 dairy model. Nutrient composition was varied primarily by replacing corn grain in HS with a rumen-inert fat source and cottonseed hulls in HF. Gross energy content was lower for HS (4.43 vs. 4.54 ± 0.01 Mcal/kg of DM); while, digestible (2.93 vs. 2.74 ± 0.035 Mcal/kg of DM), metabolizable energy (ME; 2.60 vs. 2.41 ± 0.030 Mcal/kg of DM) and NEL (1.83 vs. 1.67 ± 0.036 Mcal/kg of DM) content were all greater than HF. Tissue energy deposit as body fat tended to be greater for HS (4.70 vs. 2.14 ± 1.01 Mcal/d). For N partitioning, HS increased milk N secretion (141 vs. 131 ± 10.5 g/d) and decreased urinary N excretion (123 vs. 150 ± 6.4 g/d). Compared to HF, HS increased apparent total-tract digestibility of DM (66.7 vs. $61.7 \pm 1.06\%$), OM (68.5 vs. $63.2 \pm 0.98\%$), energy (66.0 vs. $60.4 \pm 0.92\%$), and 18-carbon fatty acids (67.9 vs. $61.2 \pm 1.60\%$). However, apparent total-tract digestibility of starch decreased for HS from 97.0 to $94.5 \pm 0.48\%$. Compared to HF, HS tended to increase milk yield (19.7 vs. 18.9 ± 1.38 kg/d), milk protein content (4.03 vs. $3.93 \pm 0.10\%$), milk protein yield (0.791 vs. 0.740 ± 0.050 kg/d), and milk lactose yield (0.897 vs. 0.864 ± 0.067 kg/d). In addition, HS decreased milk fat content

(5.93 vs $6.37 \pm 0.15\%$) but did not affect milk fat yield (average of 1.19 ± 0.09 kg/d) or energy-corrected milk yield (average of 27.2 ± 1.99 kg/d). Results of the current study suggest that the HS diet had a greater ME and NEL content, increased partitioning of N towards milk secretion and away from urinary excretion and may have increased partitioning of energy towards tissue energy deposit as fat.

Key Words: starch, fat, energy partitioning, nitrogen partitioning

INTRODUCTION

In diets formulated for lactating dairy cows, both starch (from grains and forages) and supplemental fat are often included as a source of energy. Although the enthalpy for starch (4.2 Mcal/kg of DM) is lower than that of fatty acids (9.3 Mcal/kg of DM; NRC, 2001), the concentrations of these nutrients may be manipulated to produce isoenergetic diets (van Kneegsel et al., 2007a,b; Boerman et al., 2015b). In addition to differing in the concentration of energy, energy from starch and fat may be assimilated by ruminants via different routes. Increasing dietary starch increases ruminal energy supply and supports microbial metabolism and rumen outflow of microbial protein (Roman-Garcia et al., 2016); however, approximately 35 to 45% of starch from corn bypass rumen digested and can directly supply energy. Fats are primarily utilized as a post-absorptive energy source and may be directly transferred to milk fat (Rico et al., 2014; Boerman et al., 2015b; Nichols et al., 2019e). In low-starch high-fat diets, dietary NDF is also often increased (van Kneegsel et al., 2007a,b; Boerman et al., 2015b). Replacing a portion of starch with NDF and fatty acids will decrease production of propionate and increase production of acetate (Dijkstra, 1994; Sutton et al., 2003), thus, decreasing supply of glucogenic

substrates and increasing supply of lipogenic substrates. Given the differential effects of energy source on microbial protein synthesis, VFA production, and post-absorptive metabolism, the source of energy in a diet will affect both milk production and composition. However, the effects of energy source on whole-animal energy use is not well understood or completely characterized and as a result difficult to predict (NRC, 2001). Additionally, models, such as the NRC 2001, are often used to formulate diets on an NEL basis and evaluation of the predictive ability of these models is useful.

Studies that have evaluated the effects of starch or fat on milk production, composition, and metabolism often do so by feeding anisoenergetic diets, but several experiments have tested diets that were formulated to be isoenergetic (van Knegsel et al., 2005). In general, feeding isoenergetic diets with higher starch content (26–33% of diet DM) compared to fat (5.0–5.4% of diet DM) decreased milk fat concentration and yield and increased tissue energy in Holsteins (van Knegsel et al., 2007a,b; Boerman et al., 2015b). Milk protein yield increased when cows were fed an isoenergetic high-starch diet (Boerman et al., 2015b). However, In some studies where the inclusion of starch and fat were manipulated in isoenergetic diets, a response in milk protein was not observed (van Knegsel et al., 2007a,b). Consequently, the effects of increasing dietary starch in isoenergetic diets on animal N use and milk protein are not clear. Furthermore, increasing energy supply from fat may increase the efficiency at which ME is converted into NEL (NRC, 2001), because incorporation of preformed fatty acids into milk fat is more energetically efficient than *de novo* lipogenesis (Kronfeld, 1982). Therefore, our objective was to determine the effect of an isoenergetic high-starch or high-fat diet on energy and N partitioning and efficiency of energy utilization in Jersey cows. We

hypothesized that a high-starch diet would increase efficiency of converting dietary N into milk N, and a high-fat diet would increase the utilization of energy for milk production by increasing efficiency of conversion of ME into NEL.

MATERIALS AND METHODS

Animals and Treatments

The University of Nebraska–Lincoln Animal Care and Use Committee Animal approved animal care and experimental procedures. The experiment used twelve multiparous Jersey cows averaging 192 ± 11 DIM and 467 ± 47 kg at the beginning of the experiment. 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 and milked at 0700 and 1800 h. All cows were less than 135 d pregnant at the end of the last experimental period, thus, fetal energy was assumed to be zero (NRC, 2001).

The experimental design was a cross-over with 2 periods of 28 d. In period 1, cows were randomly assigned to 1 of 2 treatment diets (6 cows per treatment): 1) a high-starch (**HS**) or 2) high-fat (**HF**). For period 2, the alternative diet was fed. Concentrate mixes for each treatment that included all dietary ingredients except for forages and cottonseed hulls (Table 7.1) were mixed at the University of Nebraska-Lincoln feed mill. Corn silage, alfalfa hay, concentrate, and cottonseed hulls 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 where diets were fed at 100% of the prior week's intake to limit refusals.

Experimental diets were formulated using NRC (2001) to contain differing amounts of starch, fat, and NDF while being isonitrogenous with a similar predicted NEL content (Using measured DMI and dietary chemical composition; Table 7.1). Energy source was manipulated by including 22.5% of the DM as ground corn grain or supplementing at 2.6% of diet DM with a commercial rumen-inert fat [Energy Booster 100 (Milk Specialties, Eden Prairie, MN)]. Isonitrogenous and isoenergetic diets were maintained by manipulating inclusion rate of soyhulls, soybean meal, non-enzymatically browned soybean meal, cottonseed hulls, and dried distillers grains with solubles. Additionally, diets were estimated to supply similar amounts of Lys, Met, and His (NRC, 2001).

Sample Collection and Analysis

Individual feed ingredients, which included corn silage, alfalfa hay, concentrate mixes and cottonseed hulls, were sampled daily during collection periods and frozen at -20°C . Corn silage was dried at 60°C for 48 h, and then all other feeds 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 of N (method 990.03; AOAC International, 2000), NDF with sodium sulfite (Van Soest et al., 1991) and α -amylase and corrected for ash contamination (**NDF_{OM}**), ADF (method 973.18; AOAC International, 2000), acid detergent lignin (Goering and Van Soest, 1970), sugar (Dubois et al., 1956), starch (Hall, 2009), ash (943.05; AOAC International, 2000), and minerals (985.01; AOAC International, 2000).

Additionally, feed ingredients were analyzed for gross energy (**GE**) content (Parr 6400 Calorimeter, Moline, IL) in the nutrition laboratory of the University of Nebraska-Lincoln and for long-chain fatty acids (**LCFA**) content and profile in the nutrition laboratory of the Penn State University as described by Rico et al. (2014). The chemical composition of diets and individual feed ingredients are listed in Table 7.1 and Table 7.2, respectively. During each d of the collection period, refusals were sampled and composited on a wet-weight basis. Refusals were analyzed for N, NDF, NDF_{OM}, starch, ash, GE, and LCFA 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. 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 (~500 g as-is), composited on a wet-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. Urine pH was measured at the end of each d and quantity of acid used was adjusted to maintain a pH < 5. Urine was subsampled daily and

composited on a wet-weight basis. Urine samples were frozen (-20°C) until later analysis. Urine GE was determined by drying approximately 4 mL of sample in a bomb capsule until just dry (2 h) and then combusting the sample (Parr 6400 Calorimeter, Moline, IL). Urine N on liquid sample was determined by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) using a Leco FP-528 N Combustion Analyzer (Leco Corp., St. Joseph, MI)

Cows were weighed, before feeding, on first and last day of collection periods. Milk production was measured daily, and milk samples were collected during both the morning and evening milking of collection periods. Milk from individual milking events was 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 by and N as described previously for urine except 1 mL of milk was used to determine GE.

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. Gas data were adjusted to a 24-h period. Four headboxes were used and data were collected across 3-d during the 4-d collection period. 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., Mooreville, 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 air flow through the headbox was measured using a gas meter (Model AL425, American Meter, Horsham, PA) and corrected to standard temperature and pressure (0 °C, 760 mmHg) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). From the headbox, continuous samples of incoming and outgoing air were collected into separate aluminum mylar 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 O₂, CO₂ and CH₄ 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):

$$\text{Heat production (HP, kcal/d)} = 3.866 \times \text{O}_2 \text{ (L/d)} + 1.200 \times \text{CO}_2 \text{ (L/d)} - 0.518 \times \text{CH}_4 \text{ (L/d)} - 1.431 \times \text{Urinary N excretion (g/d)} \quad [1]$$

The respiratory quotient (**RQ**) was calculated using the ratio of CO₂ produced to O₂ consumed (L/L). Methane energy was estimated by multiplying CH₄ production by its enthalpy (9.45 kcal/L). Unaccounted for energy was assumed to represent mobilization or accretion of whole-animal tissue energy which was expressed as NEL (NRC, 2001) and partitioned into body protein and fat energy as follows (NRC, 2001; Freetly et al., 2006; van Knegsel et al., 2007a):

$$\text{DE (Mcal/d)} = \text{GE (Mcal/d)} - \text{fecal energy (Mcal/d)} \quad [2]$$

$$\text{ME (Mcal/d)} = \text{DE (Mcal/d)} - \text{urine energy (Mcal/d)} - \text{CH}_4 \text{ energy (Mcal/d)} \quad [3]$$

$$\text{Residual energy (Mcal/d)} = \text{ME (Mcal/d)} - \text{HP (Mcal/d)} - \text{Milk energy (Mcal/d)} \quad [4]$$

$$\text{Tissue energy (TE; Mcal of NEL/d)} = \text{positive residual energy} \times 0.82 \text{ or negative residual energy}/1.12 \quad [5]$$

$$\text{Tissue energy as body protein (TE}_P\text{; Mcal/d)} = \text{retained N (g/d)} \times 5.88 \text{ (g of protein/g of N)} \times 0.0057 \text{ (Mcal/g of protein)} \quad [6]$$

$$\text{Tissue energy as body fat (TE}_F\text{; Mcal/d)} = \text{TE (Mcal/d)} - \text{TE}_P\text{(Mcal/d)} \quad [7]$$

$$\text{NEL (Mcal/d)} = \text{milk energy (Mcal of NEL/d)} + \text{TE (Mcal of NEL/d)} + 0.080 \times \text{BW (kg)}^{0.75} \quad [8]$$

Prior to the start of the experiment, system efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O₂ and CO₂ were (average \pm SD) 93.5 ± 1.57 and $91.4 \pm 3.44\%$, respectively.

Statistical Analysis

Data were analyzed in R (v3.4.1) using the lmer (Kuznetsova et al., 2017) package. The model included the fixed effect of treatment and the random effect of period, cow, and error. Denominator degrees of freedom were calculated using the Kenward-Rodger option of the anova function. All data are presented as least-squares means \pm largest standard error. Significance and trends were declared at $P \leq 0.05$ and $P \leq 0.15$, respectively.

RESULTS AND DISCUSSION

This experiment was designed to test the effects of a feeding an isoenergetic high-starch or high-fat diet on energy and N partitioning and efficiency of energy utilization. Previous research with Holsteins suggests that a high-starch diet compared to isoenergetic high-fat diets will decrease milk fat yield and increase energy utilization for body reserves (van Knegsel et al., 2007a,b; Boerman et al., 2015b). Furthermore, increasing energy supply from fat is thought to increase the efficiency at which ME is converted into NEL (NRC, 2001). Feeding starch as an energy source compared to fat in isoenergetic diets increased milk protein yield in some (Boerman et al., 2015b) but not in other studies (van Knegsel et al., 2007a,b). The reason for this discrepancy is unclear, but if increased starch increases milk protein yield, N excretion may decrease. Therefore, we expected that HS would result in the diversion of N towards milk and away from urine, whereas HF would increase the utilization of energy for milk production by increasing efficiency of conversion of ME into milk energy.

Gas Consumption and Production and Energy Partitioning and Utilization

One cow refused to eat while in the headboxes during training prior to the experiment. Thus, fecal and urine output, DMI, milk production and composition, BW, BCS were collected, but this cow was not placed in a headbox and thus gas measures were not made. We tested the effects of placing animals in the headboxes on DMI by comparing DMI on the day cows were placed inside the headboxes to the days they were not. Dry matter intake did not differ ($P = 0.47$) when cows were either in or out of the headboxes (17.7 vs. 18.0 ± 0.58 kg/d; data not shown). This observation is similar to a previous experiment at the University of Nebraska where DMI was not affected by placing cows in headboxes (Judy et al., 2018).

In general, the oxidation of lipids, protein, and carbohydrates result 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). In the current study, the respiratory quotient was greater ($P < 0.01$) for cows fed HS (1.09 vs. 1.05 ± 0.012 ; **Table 7.3**) suggesting that lipid synthesis was greater for cows fed HS. Previously, abomasal infusion of glucose increased RQ while an isoenergetic abomasal infusion of palm olein (primarily palmitic, oleic, and linoleic acid) decreased RQ in Holsteins (Nichols et al., 2019e). In support of increased lipid synthesis for cows consuming HS compared to HF, estimated whole-animal TE_F tended ($P = 0.11$) to be higher for HS (4.33 vs. 1.93 ± 0.94 Mcal/d). When feeding isoenergetic diets over week 1-7 of lactation, van Knegsel et al. (2007a) observed that whole-animal TE_F was greater in high-starch (27% starch, 3.4% crude fat, and 32% NDF) than a high-fat diet (10% starch, 5.4% crude fat, and 40% NDF). Boerman et al. (2015b) reported increased yield of milk fatty acids of de novo origin and increased body fat thickness when feeding mid-lactation dairy cows a high-starch diet (32% starch, 3.2% fatty acids, and 25% NDF) compared to an isoenergetic high-fat diet (16% starch, 5.4% fatty acids, and 33% NDF). Increasing supply of glucogenic precursors typically increases circulating insulin (Rius et al., 2010a; Cantalapiedra-Hijar et al., 2014a; Boerman et al., 2015b), which would signal for increased lipid synthesis in peripheral tissue. It is worth noting that RQ data from ruminants should be interpreted with caution, because a small fraction of CO_2 originates from rumen fermentation and thus is not a direct product of substrate metabolism in the animal, and CO_2 may be liberated or sequestered from the bicarbonate pool to maintain metabolic pH (Brody, 1945). However, the consistency between RQ and whole-animal TE_F data suggest that HS

compared to HF increased energy partitioning towards lipid stores which is supported by similar observations from the literature (van Knegsel et al., 2007a; Boerman et al., 2015b). Additionally, because dietary NEL was greater for HS (see later discussion), increased TE_F may not have occurred due to starch, but due to increased energy content.

Although differences between diets were not detected for daily consumption of any energy fractions, when energy fractions were expressed per kg of DMI differences were observed (Table 7.3). This discrepancy occurred because DMI is an important contributor to the variation in supply of each energy fraction and accounting for DMI resulted in a decrease in SEM (4.4 vs. 1.2% of mean). The GE content was lower ($P < 0.01$) for HS (4.43 vs. 4.54 ± 0.012 Mcal/kg of DM) due to a lower LCFA content (1.88 ± 0.02 vs. $4.06 \pm 0.14\%$ of DM). Because cottonseed hulls are poorly digested, digestible energy (DE) content was expected to be similar across diets (NRC, 2001); however, DE content was greater ($P < 0.01$) for HS (2.93 vs. 2.74 ± 0.035 Mcal/d) which suggests that digestibility of corn grain in HS was greater than expected or digestibility of cottonseed hulls in HF was lower than expected. Fecal energy output was greater ($P < 0.01$) for HF (31.7 vs. 26.0 ± 1.16 Mcal/d). Consistent with DE, ME and NEL content were greater ($P \leq 0.01$) for HS (2.60 vs. 2.41 ± 0.03 Mcal of ME/d and 1.83 vs. 1.67 ± 0.036 Mcal of NEL/d). The efficiency of converting DE to ME was greater ($P = 0.05$) for HS (0.888 vs. 0.876 ± 0.003) due to decreased ($P = 0.04$) urinary energy loss (2.07 vs. 2.44 ± 0.11 Mcal/d). The decreased urinary energy excretion for cows fed HS was due to decreased urinary N excretion (see later discussion).

Diets were formulated to contain similar NEL contents (averaging 1.55 Mcal of NEL/kg of DM; NRC, 2001); however, as discussed previously, observed NEL was

greater in the HS treatment and both diets exceeded NRC (2001) estimated NEL (average measured NEL of 1.77 Mcal/kg of DM), which was due to cumulative error in the estimated energy lost with the conversion of DE to ME, and also ME to NEL. To better understand the discrepancy between observed NEL and NRC, 2001 estimated NEL, DE and ME were calculated using NRC, 2001 equations. Dietary fat is included as a variable in NRC, 2001 to estimated transformation of DE to ME and ME to NEL; however, the inclusion of fat as a variable did not appear to improve accuracy in estimating DE, ME, and NEL. Because accuracy in estimating DE, ME, and NEL was similar across diets, only HS will be discussed. For HS, observed DE was similar to NRC (2001) estimated DE, 2.93 vs. 2.91 Mcal/kg of DM, respectively. The efficiency of energy transfer from DE to ME for HS when using NRC estimates was 0.877, whereas we observed an DE to ME efficiency of 0.888 ± 0.003 (Table 7.3). The use of a fixed conversion factor ($ME = 1.01 \times DE - 0.45$ for diets with less than 3.0% crude fat; NRC, 2001) does not account for the effect that diet, milk production, and intake have on gas and urinary energy losses. The NRC (2001) estimated ME-to-NEL efficiency of 0.621 for HS, whereas the observed efficiency was 0.703 ± 0.008 . Similar to the conversion of DE to ME, NRC (2001) uses a fixed conversion of $0.703 \times ME - 0.19$ for the conversion of ME to NEL for diets with less than 3.0% crude fat. In addition, because whole-animal TE in the current study was calculated by difference, this fraction may accumulate error associated with determining other fractions and consequently lead to an overestimation of TE. Nonetheless, on average, the TE values for the current study correspond to a reasonable BW gain of about 0.6 kg/d (assuming 5.84 Mcal NEL/kg of BW gain when BCS equals 3.5; NRC, 2001). Therefore, an overestimation of TE may explain some but not all of the discrepancy

between NRC (2001) estimated and observed NEL, and thus the equations for calculating NEL may need to be revisited. Heat increment, which is the difference in ME and NEL, is the aggregate of the heat associated with digestion and absorption, fermentation, production formation, waste synthesis and excretion (NRC, 1981). The use of an equation that incorporates animal variation, nutrient flux, and product formation to estimate heat increment may be more accurate than assuming a constant conversion of ME to NEL.

We hypothesized that HF would increase the efficiency of ME utilization for milk production, because incorporation of dietary fat into milk is more energetically efficient than de novo lipogenesis (Baldwin et al., 1985). However, the efficiency of converting ME into milk energy was not different ($P = 0.26$) between diets, averaging 0.441 ± 0.17 . Similarly, the efficiency of converting ME into NEL was not different ($P = 0.39$) between diets, averaging 0.699 ± 0.008 . In an energy balance experiment, the estimated efficiency of ME from supplemental fat as Ca-salts was 0.774 (Andrew et al., 1991). Although this is greater than the average ME-to-NEL efficiency that we observed, increased fatty acid supply from the HF diet only account for approximately 5% of ME supply assuming a 100% conversion of DE from fatty acids into ME. Thus, the effect of supplemental fat on ME-to-NEL efficiency may have been negligible or too small to observe.

Nitrogen Partitioning and Utilization

Evaluation of how N is partitioned can be useful in determining how dietary N is utilized for productive, maintenance, or metabolic functions. Urinary N excretion decreased ($P < 0.04$) for HS compared to HF on a mass (123 vs. 150 ± 6.4 g/d; Table 7.4), or when expressed as a function of N intake (28.7 vs. $33.3 \pm 1.38\%$). A decrease in urinary N excretion in cows consuming HS, occurred as a result of N being diverted

toward milk secretion and away from urinary excretion. In the current study, feeding HS compared to HF increased ($P < 0.03$) milk N secretion on a mass basis (141 vs. 131 ± 10.5 g/d) and when expressed as a function of N intake (32.3 vs. $28.8 \pm 1.05\%$). With abomasal infusion of wheat starch to Holsteins, Reynolds et al. (2001) observed decreased urinary N excretion; yet, inconsistent with our results milk N excretion was not affected and retained N increased with wheat starch infusion. In the current study, retained N did not differ ($P = 0.98$). The discrepancy between the current study and Reynolds et al. (2001) may have occurred because our treatments were applied through dietary manipulation, whereas Reynolds et al. (2001) directly infused wheat starch into the abomasum. Compared to HF, HS may have stimulated microbial protein synthesis and thus increased intestinal absorption of EAA which may have caused the increased milk N secretion. Similar to our results, an increase in milk N efficiency was observed with isoenergetic abomasal infusions of glucose compared to palm olein (Nichols et al., 2019e). However, these investigators attributed the increase in milk N efficiency to a decrease in N intake with the glucose infusion. The partitioning of N toward milk and away from urine in cows fed HS may be due to increased AA availability to the mammary gland and increased utilization efficiency of AA by the mammary gland (Cantalapiedra-Hijar et al., 2014, 2015) which may be mediated by an upregulation of protein synthesis via insulin and energy substrates (Rius et al., 2010a). Increased utilization of AA for milk protein synthesis would explain why a decrease in urinary N excretion was observed.

Nutrient Digestibility

Feeding HS increased ($P < 0.01$) apparent DM and OM digestibility (66.7 vs. 61.7 ± 1.06 and 68.5 vs. $63.2 \pm 0.95\%$, respectively, Table 7.5). Boerman et al. (2015b) observed a greater DM digestibility in their high-starch diet. Increased DM and OM digestibility for HS can mostly be attributed to the replacement of a less digestible carbohydrate (NDF_{OM} , average digestibility of $45.5 \pm 1.38\%$) with a highly digestible carbohydrate (starch, average digestibility of $95.9 \pm 0.48\%$). Similarly, when replacing starch with NDF, DM and OM digestibility decreased while NDF digestibility was unchanged in Holsteins (Beckman and Weiss, 2005). Consistent with DM and OM digestibility, energy digestibility was greater ($P < 0.01$) for HS (66.0 vs. $60.4 \pm 0.92\%$). However, starch digestibility increased ($P < 0.01$) from 94.5 to $97.0 \pm 0.48\%$ for HS and HF, respectively. The effects of dietary manipulation on starch digestibility in the literature are variable. Starch digestibility has been shown to increase with decreasing dietary NDF (Firkins et al., 2001) and increasing dietary starch content (Weiss et al., 2009a). However, this would suggest increased starch digestibility for HS in the current experiment. Source of starch can also influence starch digestibility. For example, in a meta-analysis, the digestibility of starch from high-moisture corn was on average about 2 percentage units greater than dry-ground or rolled corn (Ferraretto et al., 2013a). In the current study, starch from corn silage provided about 46% of the dietary starch for HS and about 88% of the dietary starch for HF. Therefore, a lower starch digestibility for the ground corn compared to corn silage could result in a lower overall starch digestibility for HS. Although total LCFA and 16-carbon fatty acid digestibility was not different ($P > 0.18$) between diets, digestibility of 18-carbon fatty acids decreased ($P < 0.01$) for HF

compared HS (61.2 vs. $67.9 \pm 1.60\%$). The HF diet increased 16-carbon fatty acids in the diet by 0.67 percentage units and 18-carbon fatty acids by 1.38 percentage units.

Increasing duodenal fatty acid flow, especially 18:0, is known to decrease fatty acid digestibility (Boerman et al., 2015a). Decreased digestibility of 18-carbon fatty acids with the same supplement used in HF were recently reported in Holsteins (de Souza et al., 2018a). Stearic acid is the predominant fatty acid escaping the rumen and additional supplementation may have decreased intestinal digestibility of this fatty acid.

DMI, Milk Yield, and Milk Composition

Although our objectives included measurement of energy partitioning towards body reserves, BW changes was not reported in the current experiment, because changes in BW may not necessarily reflect changes in TE (Moe et al., 1971; NRC, 2001). This is particularly a problem in cross-over experimental designs where any change in DMI that may occur with dietary change is measured in BW. Milk yield tended ($P = 0.12$) to increase from 18.9 to 19.7 ± 1.38 with HS in the current experiment (Table 7.6).

Boerman et al. (2015b) observed increased milk yield when feeding mid-lactation cows a high-starch diet compared to an isoenergetic high-fat diet. Milk lactose production also tended ($P = 0.15$) to increase from 0.86 to 0.90 ± 0.07 kg/d for HS. Boerman et al. (2015b) observed increased milk lactose production when feeding a high-starch diet to Holsteins. Because milk lactose is a major osmotic driver of milk yield (Linzell and Peaker, 1971), increased lactose synthesis typically increases milk yield. Although milk yield increased with HS, ECM was not different ($P = 0.84$), averaging 27.2 ± 1.99 .

Decreased milk fat production when feeding an isoenergetic high-starch diet compared to a high-fat and/or high-fiber diet to Holsteins has been observed in a number

of studies (van Knegsel et al., 2007a,b; Boerman et al., 2015b). This response is usually attributed to the partitioning of energy away from milk at the expense of body tissue energy. Although milk fat concentration decreased ($P < 0.01$) from 6.37 to $5.93 \pm 0.15\%$ with HS, milk fat yield was not different ($P = 0.29$) between diets. Thus, the decreased milk fat concentration for HS may be due to a dilution effect caused by increased milk yield. Additionally, the profile of FA in fat supplements are known to affect milk production; de Souza et al. (2018a) observed that milk fat yield increased when feeding a predominantly palmitic acid fat source compared to the same palmitic and stearic blend as used in the current study.

Compared to HF, concentration of milk protein tended ($P = 0.13$) to increase from 3.93 to $4.03 \pm 0.10\%$ and milk protein yield tended ($P = 0.07$) to increase from 0.74 to 0.79 ± 0.05 kg/d. When feeding isoenergetic high-starch compared to a high-fat diet, Boerman et al. (2015b) observed increased milk protein yield by 0.10 kg/d, whereas van Knegsel et al., (2007a,b) observed no response. However, the use of early-lactation cows in the latter study may explain the difference. Piccioli-Cappelli et al. (2014) observed increased milk protein yield with increased dietary starch content in late-lactation cows (190 DIM) but not in early-lactation cows (30 DIM). Increasing supply of glucogenic precursors typically increases plasma insulin (Rius et al., 2010a; Cantalapiedra-Hijar et al., 2014a; Boerman et al., 2015b) which may increase milk protein synthesis in the mammary gland (Rius et al., 2010a). However, during early lactation insulin resistance may increase (De Koster and Opsomer, 2013). Nonetheless, the current study was not designed to test the effect of stage of lactation. The increased milk protein yield with HS in the current study may be multifaceted. First, increased starch supply may have

increased microbial protein synthesis—an enriched source of Lys and Met (Sok et al., 2017). Using the diet-level equation (i.e., DMI, and dietary starch content as parameters) of Roman-Garcia et al. (2016), estimated microbial N flow was 252 and 237 g/d for HS and HF, respectively. Secondly, increased whole-body supply of glucogenic precursors may spare utilization of AA as an energy source and increase utilization of AA for milk protein synthesis. Specially, feeding isoenergetic diets with starch compared to NDF as an energy source to Jerseys increased net splanchnic release of AA, post-hepatic availability of AA, and mammary utilization of EAA for milk protein synthesis (Cantalapiedra-Hijar et al., 2014, 2015). However, these authors concluded that the increased utilization of dietary N for milk is not from an AA sparing effect per se, but rather decreased energy requirement of the portal-drained viscera and increase duodenal flow of microbial N. Additionally, the energy content (NEL and ME) was greater for HS which may have caused the increased milk protein production and confounded the interpretation of the effects of energy source in isoenergetic diets on milk protein production. Our results suggest that not only energy supply, but composition of energy supply may influence milk protein synthesis and should be considered in future nutritional models. For example, increased supply of starch as an energy source tended to increase milk protein production.

CONCLUSIONS

Feeding a high-starch versus a high-fat diet did not affect total metabolizable energy intake, but increased overall dietary metabolizable energy content, increased milk N secretion, and decreased urinary N excretion. Feeding starch as an energy source also

tended to increase estimated whole-animal deposition of tissue energy as fat. Compared to the NRC (2001) model, observed dietary NEL content was greater primarily due to increased conversion of ME into NEL. As hypothesized, feeding a high-starch compared to a high-fat diet tended to increase milk protein; however, efficiency of energy utilization for milk was not different. Milk fat content but not yield increased when feeding a high-fat diet. Results from the current study suggest that source of energy supply may influence milk protein production and N partitioning.

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Table 7.1. Ingredient and chemical composition of a high-starch or high-fat diet (% of DM)¹

Items	HS	HF
Ingredient		
Corn silage	38.1	38.1
Alfalfa hay	21.0	21.0
Corn grain, dry ground	22.5	2.5
Soyhulls	4.1	6.5
Soybean meal	11.5	10.9
Non-enzymatically browned soybean meal ²	—	0.6
Cottonseed hulls	—	12.5
Dried distillers grains and solubles	—	2.5
Fat ³	—	2.6
Rumen-protected Lys ⁴	0.20	0.19
Rumen-protected Met ⁵	0.16	0.17
Mineral-vitamin mix ⁶	2.37	2.37
Chemical composition ⁷		
DM	61.1 (0.09)	61.8 (0.21)
CP	15.5 (0.52)	16.0 (0.35)
ADF	20.7 (1.26)	29.8 (1.80)
NDF	31.8 (3.19)	41.7 (1.90)
NDF _{OM}	30.8 (2.20)	40.5 (1.27)
Starch	30.8 (0.42)	16.8 (0.85)
Sugar	2.34 (0.47)	2.27 (0.19)
LCFA	1.88 (0.02)	4.06 (0.14)
16 carbon	0.35 (0.01)	1.02 (0.03)
18 carbon	1.48 (0.01)	2.86 (0.10)
ROM	12.8 (1.85)	13.9 (1.24)
Ash	7.3 (0.12)	7.5 (0.13)
Ca	0.73 (0.03)	0.76 (0.02)
P	0.40 (0.01)	0.40 (0.01)
Mg	0.37 (0.03)	0.34 (0.01)
K	1.62 (0.03)	1.79 (0.05)
S	0.28 (0.01)	0.29 (0.01)
Na	0.42 (0.11)	0.32 (0.03)
Cl	0.28 (0.01)	0.32 (0.03)
NEL, Mcal/kg ⁸	1.56	1.54
Lys, g/d ⁸	132	131
Met, g/d ⁸	53	53
His, g/d ⁸	41	40
MP balance g/d ⁸	52	110

¹HS = high-starch diet; HF = high-fat diet; values in parenthesis indicate SD (n = 2).

²Soypass (LignoTech, Overland Park, KS).

³Energy Booster 100 (Milk Specialties, Eden Prairie, MN). According to manufacturer specifications contained 99% fatty acids (31.2% 16:0, 47.7% 18:0, 9.9% 18:1).

⁴Ajipro (Ajinomoto, Chicago, IL).

⁵Smartamine M (Adisseo, Alpharetta, GA).

⁶Contained per kg of premix: 245g of NaHCO₃, 224 g of CaCO₃, 148g of CaHPO₄, 127 g of salt, 127 g of MgO, 97g of NaSO₄, 21g of vitamin premix (14,850 IU/g Vitamin A, 3,850 IU/g Vitamin D, and 90 IU/g of Vitamin E), and 13g 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).

⁷NDF_{OM} = NDF – NDF ash, LCFA = long-chain fatty acids, ROM = 100 – %CP – %LCFA – %Ash – %Starch – %NDF.

⁸Calculated with NRC, 2001 using measured dietary nutrient composition for forage and cottonseed hulls from Table 7.2 and mean DMI, and milk production and composition from Table 7.6.

Table 7.2. Chemical composition of corn silage, alfalfa hay, concentrate mixes and cottonseed hulls (% of DM)¹

Items ²	Corn silage		Alfalfa hay		HS concentrate		HF concentrate		Cottonseed hulls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, % as-is	39.8	2.03	88.1	0.75	91.7	1.39	91.5	1.09	91.2	1.1
CP	8.5	0.35	18.6	1.06	20.6	1.06	28.2	1.56	7.1	0.07
ADF	24.5	3.68	36.0	0.78	9.5	0.07	17.7	2.26	63.3	0.64
NDF	40.0	3.04	44.8	3.18	17.7	3.32	24.5	0.64	81.2	0.85
NDF _{OM}	39.2	2.69	43.6	1.91	16.5	1.91	23.7	0.57	77.4	2.47
ADICP	1.00	0.39	1.97	0.40	0.81	0.25	1.43	0.76	3.06	0.45
NDICP	1.11	0.37	3.04	0.65	1.09	0.23	1.91	0.50	3.54	0.44
Lignin	3.7	0.55	7.9	0.13	1.0	0.06	1.3	0.04	19.7	0.23
Sugar	0.4	0.14	3.3	0.14	3.7	1.20	4.9	0.35	0.4	0.49
Starch	36.0	2.26	2.6	0.01	40.5	1.06	8.8	0.07	0.3	0.07
LCFA	2.15	0.03	0.94	0.14	2.10	0.05	9.81	0.34	1.96	0.01
ROM	8.7	1.55	22.4	1.75	11.7	2.20	17.7	1.36	6.4	0.87
Ash	4.8	0.38	10.2	0.20	8.2	0.16	11.1	1.17	3.1	0.13
Ca	0.21	0.02	1.33	0.07	0.91	0.06	1.33	0.11	0.21	0.05
P	0.30	0.03	0.37	0.00	0.51	0.01	0.67	0.04	0.16	0.02
Mg	0.14	0.01	0.24	0.00	0.65	0.05	0.73	0.02	0.21	0.01
K	1.14	0.07	3.46	0.05	1.13	0.01	1.69	0.03	1.20	0.03
S	0.14	0.01	0.22	0.00	0.44	0.03	0.63	0.02	0.13	0.04

¹n = 2.

²ADICP = Acid detergent insoluble CP, ADICP = Acid detergent insoluble CP, NDF_{OM} =

NDF – NDF ash, LCFA = long-chain fatty acids, ROM = 100 – %CP – % LCFA –

%Ash – %Starch – %NDF.

Table 7.3. Effects of a high-starch or high-fat diet fed to lactating Jersey cows on energy partitioning and utilization

Items ^{3,4}	Treatment ^{1,2}		SEM	<i>P</i> -value
	HS	HF		
Gases				
O ₂ consumption, L/d	4048	4120	136	0.59
CO ₂ production, L/d	4410	4345	193	0.67
CH ₄ production, L/d	391	388	27	0.83
RQ	1.09	1.05	0.012	<0.01
Components, Mcal/d				
Feces	26.0	31.7	1.16	<0.01
CH ₄	3.70	3.66	0.26	0.83
Urine	2.07	2.44	0.11	0.04
HP ⁵	20.6	20.7	0.74	0.81
Milk	19.1	18.9	1.48	0.82
TE	4.89	2.51	1.08	0.16
TE _P	0.53	0.50	0.25	0.92
TE _F	4.33	1.93	0.94	0.11
Fractions, Mcal/d				
GE	76.7	80.0	3.23	0.20
DE	50.7	48.3	2.56	0.23
ME	46.4	43.5	1.88	0.18
NEL ⁵	32.8	30.1	1.32	0.19
Fractions, Mcal/kg of DM				
GE	4.43	4.54	0.012	<0.01
DE	2.93	2.74	0.035	<0.01
ME	2.60	2.41	0.030	<0.01
NEL	1.83	1.67	0.036	0.01
Efficiencies				
ME/DE	0.888	0.876	0.003	0.05
Milk/ME	0.428	0.454	0.017	0.26
HP/ME	0.449	0.478	0.014	0.19
TE/ME	0.100	0.055	0.020	0.16
TE _P /ME	0.010	0.013	0.005	0.70
TE _F /ME	0.090	0.042	0.018	0.10
NEL/ME	0.703	0.694	0.008	0.47

¹HS = high-starch diet; HF = high-fat diet; n = 24 for feces, urine, milk, GE, and DE, n =

22 for all other variables.

²Least squares means; largest standard error of treatment mean is listed.

³RQ = respiratory quotient (CO₂ production/O₂ consumption, L/L), HP = heat production
 $[3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 - 0.518 \times \text{CH}_4 - 1.431 \times \text{N}]$ (Brouwer, 1965)], GE = gross
 energy, DE = digestible energy, TE = tissue energy, TE_P = tissue energy as body protein,
 TE_f = tissue energy as body fat.

⁴Milk yield, DMI and BW measured during collection period (Table 7.6) was used to
 calculated corresponding energy components and fractions.

⁵NEL = $0.080 \times \text{BW}^{0.75}$ + milk energy + tissue energy corrected for efficiency of
 conversion to milk energy (NRC, 2001)

Table 7.4. Effects of a high-starch or high-fat diet fed to lactating Jersey cows on fecal and urinary output and N excretion, secretion, and utilization

Items ³	Treatment ^{1,2}		SEM	P-value
	HS	HF		
Output				
Feces, as-is	79.7	86.5	3.56	<0.01
Feces, DM	5.73	6.76	0.24	<0.01
Urine, as-is	23.0	25.5	2.46	<0.01
Mass, g/d				
N Intake	434	455	21.4	0.16
Fecal N	154	158	6.7	0.52
Urinary N	123	150	6.4	<0.01
Milk N	141	131	10.5	0.03
Retained N	16	15	7.3	0.92
As proportion of N intake, %				
Fecal N	35.6	34.8	1.02	0.23
Urinary N	28.7	33.3	1.38	0.04
Milk N	32.3	28.8	1.05	<0.01
Retained N	3.3	3.1	1.52	0.94

¹HS = high-starch diet; HF = high-fat diet.

²Least squares means; largest standard error of treatment mean is listed.

³Milk yield, and DMI measured during collection period (Table 7.6) was used to calculated corresponding N fractions.

Table 7.5. Effects of a high-starch or high-fat diet fed to lactating Jersey cows on total-tract digestibility (%)

Items ^{3,4}	Treatment ^{1,2}		SEM	P-value
	HS	HF		
DM	66.7	61.7	1.06	<0.01
OM	68.5	63.2	0.98	<0.01
NDF	43.7	43.8	1.09	0.93
NDF _{OM}	45.3	45.5	1.38	0.87
CP	64.4	65.2	1.02	0.23
Starch	94.5	97.0	0.48	<0.01
LCFA	63.7	60.5	1.57	0.18
16 carbon	63.1	63.8	1.32	0.72
18 carbon	67.9	61.2	1.60	<0.01
ROM	72.5	79.9	2.96	<0.01
Energy	66.0	60.4	0.92	<0.01

¹HS = high-starch diet; HF = high-fat diet.

²Least squares means; largest standard error of treatment mean is listed.

³NDF_{OM} = NDF – NDF ash, LCFA = long-chain fatty acids, ROM (residual organic matter) = 100 – %CP – % LCFA – %Ash – %Starch – %NDF.

⁴Nutrient digestibilities were calculated using DMI measured during collection period (Table 7.6).

Table 7.6. Effects of a high-starch or high-fat diet fed to lactating Jersey cows on intake, milk production and composition, free water intake, BW, and BCS

Items	Treatment ^{1,2}		SEM	P-value
	HS	HF		
DMI, kg/d	17.3	17.6	0.75	0.55
Milk yield, kg/d	19.7	18.9	1.38	0.12
ECM, kg/d ³	27.2	27.1	1.99	0.84
Fat, %	5.93	6.37	0.15	<0.01
Fat, kg/d	1.17	1.20	0.094	0.29
Protein, %	4.03	3.93	0.10	0.13
Protein, kg/d	0.791	0.740	0.050	0.07
Lactose, %	4.55	4.57	0.042	0.60
Lactose, kg/d	0.897	0.864	0.067	0.15
MUN, mg/dL	9.78	9.70	0.16	0.50
Free water intake, L/d	73.0	79.2	5.60	0.03
BW, kg ⁴	467	467	13	0.92
BCS ⁵	3.38	3.42	0.071	0.21

¹HS = high-starch diet; HF = high-fat diet; n = 22 for free water intake, n = 24 for all

other variables.

²Least squares means; largest standard error of treatment mean is listed.

³ECM = $0.327 \times \text{milk yield (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{true protein (kg)}$ (Tyrrell and Reid, 1965).

⁴Average from 2 measurements during last 4 d of each period.

⁵Scored 1–5 by 2 independent observations.

**CHAPTER 8: VARYING DIETARY FAT AND STARCH CONTENT AND
SUPPLEMENTAL LYS SUPPLY AFFECTS ENERGY AND N
METABOLISM**

INTERPRETIVE SUMMARY. Morris and Kononoff. (20XX). “Varying dietary fatty acids and starch content and supplemental Lys supply affects energy and N utilization in lactating Jersey cows.” Fifteen diets that varied in fatty acid (**FA**) and starch content and supplemental digestible Lys (**sdLys**) were fed. Increasing FA decreased DMI, quadratically increased metabolizable energy (**ME**) content, and decreased supply of ME supply at high FA. Increasing sdLys increased milk protein content as low dietary starch, but not at high dietary starch. Increasing sdLys initially decreased N balance but increased N balance at high sdLys. Starch increased conversion of dietary N into milk N. These results indicate that excess FA can decrease energy supply and Lys may be utilized by muscle instead of mammary glands.

(100-word limit)

RUNNING HEAD: FAT, STARCH, AND LYS AFFECTS ENERGY AND N
UTILIZATION

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ABSTRACT

The effects of dietary fatty acid (FA) and starch content as well as supplemental digestible Lys (sdLys) on production, energy utilization, and N utilization were evaluated using a central composite design. Each factor was feed at five different amounts, and factor limits were as follows: 3.0 to 6.2% of dry matter (DM) for FA; 20.2 to 31.3% of DM for starch, and 0 to 17.8 g of sdLys. Dietary FA and starch were increased by replacing soyhulls and sdLys was increase via rumen-protected Lys. Twenty-five Jersey cows (mean \pm standard deviation; 80 ± 14 d in milk) were used across 3 blocks. Each block consisted of 4 28-d periods, where the final 4-d were used to determine milk production and composition, feed intake, N utilization (via total collection), and energy utilization (via total collection and headbox-style indirect calorimetry). Increasing dietary FA decreased dry matter intake and milk protein yield. A starch by sdLys interaction occurred for milk protein concentration. When dietary starch was less than 24%, milk protein concentration increased with increasing sdLys, but when dietary starch was greater than 26% milk protein concentration decreased with increasing sdLys. Increasing dietary FA decreased neutral detergent fiber (NDF) digestibility. Digestibility of FA increased when dietary FA increased from 3.0 to 4.2% and decreased as FA increased beyond 4.2%. Although NDF digestibility decreased as dietary starch increased, energy digestibility increased. As dietary FA increased, metabolizable energy (ME) content quadratically increased; however, ME supply increased as dietary FA increased from 3.0 to 4.2% and decrease as FA increased beyond 4.2%. Increasing dietary FA and starch decreased CH₄ and urinary energy corresponding with an increase in the conversion of DE into ME. Increasing dietary starch increased the efficiency of utilizing dietary N for

milk N. Increasing sdLys quadratically decrease N balance as of sdLys increased from 0 to 8 g/d and increased N balance as sdLys increased from 8 to 18 g/d. Increasing dietary FA can increase ME content, however, at high dietary FA, decreased DMI, NDF, and FA digestibility resulted in a plateau in ME content and a decrease in ME supply. Our results demonstrate that sdLys supply is important for milk protein at low dietary starch, and some Lys may be preferentially used for muscle protein synthesis at the expense of milk protein at high sdLys.

Key Words: indirect calorimetry, energy metabolism, net energy system, digestibility

INTRODUCTION

Dietary fatty acids (FA), starch, and Lys are known to affect energy and N utilization in lactating dairy cows (Nichols et al., 2019e; Morris et al., 2020b; Morris and Kononoff, 2020). Dietary FA and starch generally supply 4–10 % and 25–40%, respectively, of the total digested energy (DE) supply of diets fed to a lactating dairy cow (calculated from Weiss et al., 2009a; de Souza et al., 2019; Morris et al., 2020b). Dietary FA are energy dense and can be used to increase dietary energy content. This energy from fat is primarily used postabsorptively and may be directly transferred to milk fat (Boerman et al., 2015b; Nichols et al., 2019e). Dietary starch supplies both ruminal and post-ruminal energy to lactating dairy cows and can support microbial protein synthesis (Roman-Garcia et al., 2016) as well as insulin-dependent anabolic signaling (Cantalapiedra-Hijar et al., 2014a; Boerman et al., 2015b). In diets formulated to be isoenergetic, high-starch compared to high-fat diets have been observed to increase milk protein yield but also decreased milk fat yield (Boerman et al., 2015b; Morris et al.,

2020b). It is well established that energy and MP interact with each other to affect milk protein synthesis (Oldham, 1984; Brun-Lafleur et al., 2010). Therefore, interactions likely exist between energy and individual essential AA, but these interactions are not well understood. Lysine is commonly thought to be a potentially limiting AA for milk protein synthesis in lactating dairy cows, particularly in corn-based diets (NRC, 2001). However, response to supplemental rumen-protected (**RP**) Lys is not always consistent as increased milk protein concentration has been observed, but often no effect of RP Lys on milk protein yield is observed (Giallongo et al., 2016; Fleming et al., 2019a; Morris and Kononoff, 2020). This variability in response may, in part, occur because the basal diet may interact with Lys supply to affect the response to increased Lys supply.

Understanding how diets affect energy utilization and efficiency are important for predicting milk production and tissue energy (**TE**). Generally, high-starch diets or abomasal infusion of glucose increases energy utilization for TE; whereas, high-fat diets may increase milk energy (Boerman et al., 2015b; Nichols et al., 2019e; Morris et al., 2020b). This difference in response to increasing glucose supply compared to FA likely occurs because increased glucose will increase insulin (Boerman et al., 2015b; Nichols et al., 2019e) and thus signal for an increase in energy utilization for tissue deposition. Additionally, increasing dietary FA should increase the efficiency of converting DE into ME and ME into NE_L. This occurs because CH₄ production is decreased and the heat increment of FA is lower than other nutrients (Andrew et al., 1991; Romo et al., 1996). Because milk protein synthesis is an energy dependent process, diets that differ in energy source and affect production of milk components may affect both the energetic efficiency of milk synthesis and utilization of energy for milk and TE.

Manipulating supply of FA, starch, and Lys has affected N utilization in previous experiments. Specifically, increasing dietary starch or infusing glucose in the abomasum increased the efficiency of converting dietary N into milk N (Nichols et al., 2019e; Morris et al., 2020b). Additionally, increasing dietary NE_L density by increasing supply of FA or starch and FA, increased conversion of dietary N into milk N (Rius et al., 2010b; Nichols et al., 2018). Supplementing RP Lys has also increased N balance (Morris and Kononoff, 2020). Therefore, the objective of the proposed research is to explore the interaction between dietary FA, starch, and supply of lysine on milk production and energy and N utilization.

MATERIALS AND METHODS

Experimental Treatments

To determine the response to a set group of factors, at least 3 levels are needed for each factor; therefore, to test responses to 3 factors, a minimum of 27 dietary treatments are needed (St-Pierre and Weiss, 2009). To improve experimental efficiency, a central composition design can be utilized to decrease treatment numbers and maintain or increase efficiency in generating linear and quadratic parameter estimates compared to a factorial experiment with 3 levels of each factor (St-Pierre and Weiss, 2009). In this study fifteen unique diets were fed that differed in 3 factors (Table 8.1). Factors tested were 1) FA content (% of DM), 2) type of carbohydrate (designated as starch, % of DM) and 3) supply of supplemental Lys (expressed in g of digestible Lys (**dLys**)). Each factor was fed at five different amounts, and factor limits were as follows: 3.0 to 6.2% of DM for FA; 20.2 to 31.3% of DM for starch, and 0 to 17.8 g of supplemental dLys. Intermediate

levels of each factor were set to create orthogonality in parameter estimates as described by St-Pierre and Weiss (2009). Dietary content of FA was manipulated by replacing soyhulls with a commercially available supplemental FA source (Energy Booster Merge; Milk Specialties, Eden Prairie, MN). Starch was manipulated by replacing soyhulls with dry ground corn. Supplemental dLys was manipulated by topdressing RP Lys (EB Lys; Milk Specialties, Eden Prairie, MN) evenly over the TMR. The RP Lys product was coated in a FA shell and contained 53% FA. To control for the difference in FA supply from RP Lys between the low and high supplemental dLys treatments, a FA supplement that was identical to the shell of the RP Lys product was included in topdress. Therefore, all cows received 104 g/d of supplemental FA via top dressing and this was also accounted for in dietary chemical composition listed in Table 8.1. Concentration of intermediate levels of the main effects are a function of the experimental design (St-Pierre and Weiss, 2009).

Cows and Experimental Design

The University of Nebraska–Lincoln Animal Care and Use Committee approved animal care and experimental procedures. Twenty-five multiparous Jersey cows [(average \pm SD) 80 ± 14 DIM at the beginning of the experiment] which originated from a commercial dairy were used. Sample size was based on Weiss et al. (2009a) where 6 observations per treatment was adequate to detect treatment differences in milk production and nutrient digestibility. 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 and milked at 0700 and 1800 h.

The experimental design consisted of 3 blocks (groups of cows) with each block representing an incomplete Latin square with 4 consecutive 28-d periods (24-d adaptation followed by 4-d collection). The experiment was initially designed such that 8 cows would be utilized in each block; however, a dietary calculation error occurred in block 1 and resulted in the loss of 10 observations. Therefore, to increase observations, 9 cows were used in block 2. The experiment was designed to ensure near orthogonality and balanced for possible carryover effects as described by St-Pierre and Weiss (2009). A genetic algorithm from the “rgenoud” package in R (version 3.6.3) was used to optimize the experimental design.

The treatment design was a 3-factor central composite design with 2 replications of the center point (4.6% FA, 25.7% starch, and 8.9 g/d of supplemental dLys). Levels of each treatment factor were chosen to allow for orthogonality in parameter estimates, which enables all nonsignificant effects to be dropped simultaneously from statistical models (St-Pierre and Weiss, 2009). The loss of some observations resulted in an experimental design that was not perfectly orthogonal. However, correlation between parameter estimates was generally $< |0.10|$ and thus the experiment was analyzed as if orthogonal. Treatments were not blinded to personnel; however, due to the complexity in treatment structure, treatments were not generally known by personnel and because most measurements were objective outcomes likely were not biased.

Sample Collection and Analysis

Methods used to estimate nutrient digestibility and energy and N utilization have been described previously by Morris and Kononoff (2020). Briefly, during the 4-d

collection, cows were fed at 100% of the prior 7 d intake to minimize the effects of sorting on nutrient intake, and feed intake and fecal and urine output were determined. Urinary catheters were used to separate feces and urine and urine was acidified to maintain a pH < 5.0. Daily samples of feed ingredients, refusals, milk, feces, and urine were collected and composited on a wet weight basis. Headbox-style indirect calorimeters equipped with a mass flow meter (MCW Whisper, Alicat Scientific, Tucson, AZ) were used to measure O₂ consumption and CO₂ and CH₄ production. While each cow was placed in the headbox, free water intake was measured using a water meter (Model DLJSJ75, Daniel L. Jerman Co., Hackensack, NJ).

Feeds, refusals, and feces were dried at 60°C for 48 h to determine DM. A second subsample of corn silage, refusals, and feces were lyophilized for nutrient analysis. Dry feeds, refusals, and feces were ground to pass a 1-mm screen (Wiley Mill; Arthur A. Thomas Co., Philadelphia, PA), and analyzed at Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF with sodium sulfite (Van Soest et al., 1991), α amylase and corrected for ash contamination (**NDF_{OM}**), starch (Hall, 2009), FA with a 30-m column (Sukhija and Palmquist, 1988), and ash (943.05; AOAC International, 2000). Additionally, dry ground samples were analyzed for gross energy (**GE**) content (Parr 6400 Calorimeter, Moline, IL) in the nutrition laboratory of the University of Nebraska-Lincoln. The chemical composition of diets and forages are listed in Table 8.1.

Milk production was measured daily, and milk samples were collected during both the morning and evening milking of the collection periods. Milk from each individual milking was 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). Milk and urinary N content of liquid samples were determined by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) using a Leco FP-528 N Combustion Analyzer (Leco Corp., St. Joseph, MI). Urinary energy content was determined by drying (60°C) approximately 4 mL of sample in a bomb capsule until dry (4 h) and then combusting the sample (Parr 6400 Calorimeter, Moline, IL). Milk energy content was calculated from milk component yield (NRC, 2001).

Blood samples were collected into evacuated K₂EDTA tubes from the tail vessel approximately 3 h after feeding during 2 d within collection. Plasma was processed as described previously (Morris and Kononoff, 2020). Briefly, plasma was immediately separated by centrifugation, deproteinized with 15% sulfosalicylic acid, and stored at -80°C until analysis. Plasma samples were submitted to the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratory for analysis of free AA (Deyl et al., 1986; Fekkes, 1996). Plasma AA concentrations were adjusted for dilution with sulfosalicylic acid.

Prior to the start of the experiment, efficiency of gas collection via headboxes was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O₂ and CO₂ were (average ± SD) 101 ± 2.1 and 99 ± 1.9%, respectively.

The ruminal and intestinal digestibility of RP Lys used in the current experiment was estimated using the mobile bag technique (Paz et al., 2014). Using this technique, rumen bypass was determined as the residue remaining after a 16-h rumen incubation and intestinal digestibility was determined as the residue from samples incubated in a pepsin-

HCl bath for 3-h and then inserted into the duodenum and allowed to pass with digesta. Samples were replicated across two cows (average \pm SD; 190 ± 15 DIM; 23.9 ± 1.3 kg of milk yield; 22.0 ± 0.72 kg of DMI). Initial sample, and residue from ruminal and intestinal incubation were analyzed for N using the same method described above. The AA content of RP Lys and was determined as the N content of the product divided by the N content of Lys (19.2%).

Energy Calculations

The respiratory quotient (**RQ**) was calculated using the ratio of CO₂ produced to O₂ consumed (L/L). Methane energy was estimated by multiplying CH₄ production by its enthalpy (9.45 kcal/L). Heat production was calculated using the (Brouwer, 1965) equation. All cows were less than 143 d pregnant at the end of the last experimental period, thus, fetal energy was assumed to be zero (NRC, 2001).

Tissue energy was calculated as ME minus heat production and milk energy. For calculation of NE_L, TE was corrected to an NE_L basis as follows:

$$\text{TE (Mcal of NE}_L\text{/d)} = \text{positive TE} \times k_L/k_G \text{ or negative TE} \times k_T \quad [1]$$

Where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain, and k_L is the efficiency of utilizing ME intake for milk production. Values of 0.89, 0.75, and 0.66 were used for k_T , k_G , and k_L , respectively (Moraes et al., 2015).

Statistical Analysis

Data were analyzed with a mixed model using the “lmer” package in R (v3.6.3) as described by St-Pierre and Weiss (2009). All models included the linear, quadratic, and all two-way interactions of FA, starch, and Lys. Random effects were block, period within block and cow within block. Because the experiment was designed to be orthogonal, test of each fixed effects is independent, thus non-significant ($P > 0.10$) fixed effects can be removed simultaneously (St-Pierre and Weiss, 2009). Because of missing observations, the design was not perfectly orthogonal, but correlations between fixed parameter estimates was small and the effects on statistical test were negligible. All model parameter estimates are presented in terms of dietary chemical composition for FA and starch, and in g of supplemental dLys for Lys.

RESULTS AND DISCUSSION

One cow refused to eat while in headboxes during training before the experiment and gas data were not collected on this cow. However, heat production was estimated from MBW, DMI, milk fat yield, and milk protein yield using an equation (model 4) that we recently developed (Morris et al., 2020a) and CH₄ energy was estimated from gross energy intake, dietary NDF and FA (Moraes et al., 2014). Two observations were also lost: one due to poor adaptation to headboxes during period 1 of block 2 and one due to pneumonia. In total, 88 out of 96 observations were collected for all variables except for gas consumption and production where 86 observations were collected.

Descriptive statistics for the data collected are shown in Table 8.2 and Table 8.3. NRC (2001) estimated NE_L supply and balance, and Lys supply and balance are reported

in Supplementary Table 8.1. Least squares means for individual treatments are listed in Supplemental Table 8.2 to 8.6. Because the objective of this experiment was to derive response surfaces, equations and associated responses will be discussed rather than observed treatment means (Weiss et al., 2009a).

DMI, Milk Yield, and Milk Composition

Fatty acid supplements are commonly fed to increase dietary energy density and total energy supply. However, decreased DMI in diets with supplemental FA may result in a decrease in energy supply compared to diets that are not supplemented with FA (Weld and Armentano, 2017). In the current experiment, DMI averaged 19.7 ± 2.1 kg/d (Table 8.2), and linearly decreased ($P < 0.10$) with increasing dietary fat (Table 8.4). Saturated FA supplements did not affect DMI in mid-lactation cows when fed at 1.5 or 3% of diet DM (Weiss et al., 2011; de Souza et al., 2018a; Western et al., 2020). Rico et al. (2014) observed that DMI decreased when feeding a high C16:0 FA at 1.9% of the diet DM to late-lactation cows. Regulation of DMI is believed to be affected primarily by two factors: ruminal fill and energy intake with a shift from the former to the latter as diet DM digestibility increases above 66.7% (Conrad et al., 1964). In the current experiment, increases in dietary energy density with increasing FA likely was an influencing factor that explain the decreased DMI (see later discussion).

Yield of ECM averaged 35.1 ± 4.0 kg/d (Table 8.2) and was not affected by treatment (Table 8.4; $P > 0.10$). Additionally, milk fat yield or concentration was not affected by treatment ($P > 0.10$). Responses in milk fat yield from supplemental FA source is variable, and may, at least in part, be dependent on the profile of the FA

supplement (de Souza et al., 2018a). For example, when feed a FA supplement that was approximately 40% C16:0 and 40% C18:0, milk fat yield increased in one experiment (Weiss et al., 2011), but not two other studies (de Souza et al., 2018a; Western et al., 2020). However, in the studies of de Souza et al. (2018a) and Western et al. (2020), feeding FA supplements that were greater than 80% 16:0, milk fat yield increased compared to the control. The FA profile for the supplement used in the current experiment was intermediate and contained 57.7% of FA as C16:0 and 35.7% of FA as C18:0. To our knowledge, there are no published studies testing similar fatty acid profiles.

We expected milk protein yield to increase with increasing dietary starch, because starch supports microbial protein synthesis (Roman-Garcia et al., 2016) and increases absorptive supply of essential AA (Cantalapiedra-Hijar et al., 2014a). However, this was not observed. Reported responses in milk protein to supplemental Lys are variable (Giallongo et al., 2016; Fleming et al., 2019a; Morris and Kononoff, 2020), and generally not affected or are decreased with increasing dietary FA content (Weiss et al., 2011; Rabiee et al., 2012; de Souza et al., 2018a). In the current experiment, milk protein yield and concentration averaged 981 ± 126 g/d and $3.46 \pm 0.29\%$, respectively (Table 8.2). Milk protein yield linearly decreased ($P < 0.10$) with increasing FA (Table 8.4). Published effects of supplemental FA on milk protein yield are variable. In a meta-analysis of 77 treatment means, Rabiee et al. (2012) reported that on average milk protein yield was not affected by fat supplementation, but variation in response was considerable with heterogeneity accounting for 73% of the variation in response. This large heterogeneity suggest that a number of underlying factors may be responsible for

variation in the response between milk protein and dietary FA. These include individual animal, fats used, study design, and other dietary factors (Rabiee et al., 2012). For example, milk protein yield has increased with increased FA; however, this may only occur if energy is limiting in the control diet (Nichols et al., 2018). Decreased DMI with increasing FA likely contributed to the observed effects of FA on milk protein yield. Milk protein yield was not affected ($P > 0.10$) by dietary starch; however, limited effects of dietary starch on energy supply (see later discussion) may have limited response in milk protein by increased dietary starch in the current experiment. Milk protein concentration linearly decreased ($P < 0.10$) with increasing FA and an interaction was observed between starch and supplemental Lys. The nature of this interaction was such that milk protein concentration increased with supplemental dLys at low dietary starch ($< 24\%$) but decreased with high dietary starch ($> 28\%$; Figure 8.1). Although milk protein yield is considered to be a better indicator of protein synthesis in the mammary glands, when milk yield is unaffected milk protein concentration is also considered to be more sensitive to changes in Lys supply with RP Lys (Paz and Kononoff, 2014; Giallongo et al., 2016; Fleming et al., 2019a). We hypothesize that Lys supply limited milk protein synthesis at low starch and that as supplemental dLys increased at high starch an AA imbalance occurred and limited milk protein synthesis. Increasing dietary starch also increases microbial protein supply (Roman-Garcia et al., 2016) which is high in Lys as well as several other essential AA (Sok et al., 2017). Therefore, we speculate that at low dietary starch, microbial protein and thus Lys supply were limited. To our knowledge, no work has been done with AA imbalance associated with overfeeding Lys in lactating dairy cows. However, in lactating sows estimated milk yield and piglet growth quadratically

decreased at high dietary Lys (Hojgaard et al., 2019). Admittedly, an explanation for this effect may not be simple but may, in part, be due to the competition among AA for transport into mammary cells, increasing supply of a single AA may lead to inefficient synthesis of milk protein (Maas et al., 1998).

Digestibility

Digestibility of individual nutrient affects DE supply (NRC, 2001). Apparent digestibility of CP averaged $66.4 \pm 2.6\%$ (Table 8.2) and increased ($P < 0.10$) linearly as FA and supply of supplemental dLys increased (Table 8.5). The reason for the effects of diets on CP digestibility is unknown, but the effect of each factor on CP digestibility was small ($< 2.4\%$ increase from least to greatest FA or supplemental dLys). Increased CP digestibility with increased dietary fat when feeding tallow, cottonseed oil, or prilled FA has been previously reported (Simas et al., 1997; Drehm et al., 2018). This may occur because metabolic fecal protein, which will decrease apparent CP digestibility, increases as dietary NDF content increases (Lapierre et al., 2020). In the current experiment, dietary NDF decreased with increasing FA which might have increased NDF supply to the large intestine leading to decreased microbial protein synthesis, and thus, decreased metabolic fecal protein.

Summed together, digestibility of both NDF and starch accounts for approximately 55% of DE supply (calculated from Weiss et al., 2009a). In the current experiment, digestibility of NDF_{OM} and starch averaged 46.5 ± 6.0 and $94.9 \pm 2.2\%$, respectively (Table 8.2). Digestibility of NDF_{OM} decreased ($P < 0.10$) with increasing dietary FA and starch (Table 8.5). Digestibility of fiber generally decreases as dietary

starch increases, which likely occurs because of the effect of starch on the rumen environment and microbial populations (Weiss et al., 2009a; de Souza et al., 2018b). However, in the current experiment, as starch increased, source of NDF shifted toward a greater inclusion of forage NDF, which compared to soyhulls is less digestible (Firkins, 1997). Similar to starch, as dietary FA increased, a shift in fiber source occurred which likely contributed to the observed decrease in NDF_{OM} digestibility with increased FA. However, the rate of decrease in NDF_{OM} digestibility ($-0.989 \pm 0.35\%$ per % unit increase in FA) is greater than can be explained by difference in average digestibility of soyhulls compared to corn silage and alfalfa (91 vs. 36%; Firkins, 1997). Decreased NDF_{OM} digestibility has been shown to occur with increased supplemental FA, however, this generally only occurs with unsaturated or medium-chain FA sources (Weld and Armentano, 2017). The FA supplement used in the current experiment contained 85% saturated FA and thus was not expected to affect NDF digestibility. de Souza et al. (2018a) observed a decrease in NDF digestibility when supplementing 1.5% of diet DM with a FA blend that was 40% 16:0, 40% 18:0, and 10% *cis*-9 18:1, and increased NDF digestibility when supplementing a FA blend that was (% of FA) 80% 16:0, 6% 18:0, and 9% *cis*-9 18:1. Our FA supplement was intermediate between these two FA blends as it contained (% of FA) 57.2% 16:0, 20.8% 18:0, and 11.2% *cis*-9 18:1. Digestibility of starch was similar between 20 and 24% dietary starch and quadratically decreased ($P < 0.10$) with increasing dietary starch above 24% of DM. Decreased starch digestibility with high starch diets were likely due to a decrease in the proportion of dietary starch that was from corn silage as corn grain inclusion increased. Similar decreases in starch

digestibility when dietary starch was increased via increasing corn grain inclusion have been reported (Beckman and Weiss, 2005; Morris et al., 2020b).

In practice, supplemental FA are generally fed to increase energy concentration of the diet and ultimately energy intake; however, supplemental sources must be digestible to do so (NRC, 2001; Weiss et al., 2011). In the current study, digestibility of total, 16 carbon, and 18 carbon FA averaged 69.7 ± 6.4 , 70.2 ± 6.0 , and $69.2 \pm 8.3\%$, respectively (Table 8.2). Digestibility of these three FA variables were affected similarly by diet (Table 8.5) and thus total FA digestibility will be discussed. Digestibility of total FA quadratically increased ($P < 0.10$) by 2.3% units as dietary FA increased from 3.0 to 4.2% of DM and decreased thereafter by 6.3 percent units (

Figure 8.2A). Increasing supply of 18:1 increases FA digestibility (de Souza et al., 2019); whereas increasing duodenal flow of 18:0 decreases FA digestibility (Boerman et al., 2015a). Therefore, in the current experiment, increasing supply of 18:1 as dietary FA increased up to 4.2% might have led to an increase in FA digestibility, but increased supply of 18:0 FA at higher dietary FA may have limited FA digestibility.

Digestibility of energy average $65.5 \pm 2.4\%$ (Table 8.2) and linearly increased ($P < 0.10$) with increasing starch and quadratically increased ($P < 0.10$) by 0.8% units as FA increased from 3.0 to 4.2% of DM and decreased by 1.4% units thereafter (Table 8.5;

Figure 8.2B). The effect of dietary FA on energy digestibility followed the same trend as total FA digestibility. When FA was $> 4.2\%$, the magnitude of decrease in energy digestibility was much smaller compared to total FA (regression range of 1.4, and 6.2% units for energy and total FA, respectively). Additionally, NDF_{OM} digestibility decreased by 3.2% units from least to greatest dietary FA, which will contribute to

decreased energy digestibility. We speculate that increased diet energy density due to increased contribution of energy from FA likely diminished the rate of decrease in energy digestibility at high dietary FA. As dietary FA increased least to greatest the proportion of DE that was from digestible FA increased from 7.4 to 13.8% (data not shown). Energy digestibility increased as starch replaced NDF in the diet because average starch digestibility was 95.1%, whereas average NDF_{OM} digestibility was 46.1% (Table 8.2). Energy digestibility also generally increases with increasing dietary starch (Weiss et al., 2009a; Morris et al., 2020b).

Energy Supply and Utilization

Dietary GE content and supply averaged 4.21 ± 0.10 Mcal/kg of DM and 82.9 ± 9.1 Mcal/d (Table 8.2), and as expected GE content increased ($P < 0.10$) as dietary FA increased and decreased as dietary starch increased (Table 8.6). Decreased dietary GE content with increasing starch likely occurred because of lignin has a greater enthalpy compared to carbohydrates (> 6.0 vs. 4.2 Mcal/kg; Voitkevich et al., 2012; NRC, 2001).

Fatty acids are often included in dairy rations to increase energy density and consequently energy supply is expected to increase (Andrew et al., 1991; Weiss et al., 2011; Drehrmel et al., 2018). This response is also a function of diet digestibility and DMI (NRC, 2001). Observed dietary DE, ME, and NE_L content averaged 2.76 ± 0.13 , 2.45 ± 0.14 , and 1.65 ± 0.16 Mcal/kg of DM, respectively, while observed DE, ME, and NE_L supply averaged 54.3 ± 6.6 , 48.3 ± 6.3 , and 32.5 ± 5.2 Mcal/d, respectively (Table 8.2). Similar responses from treatments on for DE, ME, and NE_L were observed, thus, for brevity, only ME will be discussed as it is the smallest unit of dietary energy that can be

measured; NE_L is not directly measured but requires an assumption for maintenance energy and the conversion of TE into NE_L . Dietary ME content quadratically increased ($P < 0.10$) by 0.14 Mcal/kg DM as dietary FA increased from 3.0 to 5.2% of DM and was similar as dietary FA increased thereafter (Table 8.6; Figure 8.3A). Dietary ME content increased from 3.0 to 5.2% FA content due to increased contribution of FA to energy supply and increased FA digestibility, whereas the plateau in ME content above 5.2% FA was likely a result of decreased FA and energy digestibility. Supply of ME quadratically increased by 1.4 Mcal/d from 3.0 to 4.2% FA and decreased by 2.4 Mcal/d thereafter (Figure 8.3B). Additionally, starch and supplemental dLys supply did not affect ME supply ($P > 0.10$) which likely contribute to their limited effect on milk yield and composition observed in the current experiment.

Urine, CH_4 , and heat energy averaged 2.34 ± 0.26 , 3.74 ± 0.61 , and 24.7 ± 2.4 Mcal/d, respectively (Table 8.2). Both urine, and CH_4 energy linearly decreased ($P < 0.10$) with increasing dietary FA and starch (Table 8.6). Although a quadratic relationship between urinary energy and supplemental dLys was observed ($P < 0.10$), this response was small (< 0.1 Mcal/d change from least to greatest). Urinary energy is positively correlated with urinary N excretion (Morris et al., 2020c), which followed the same pattern as urinary energy excretion in the current experiment (see later discussion). Increasing dietary FA content is generally negatively associated with CH_4 energy (Nielsen et al., 2013). Additionally, increased DMI and dietary NDF content contribute to increased CH_4 energy (Nielsen et al., 2013), both of which decreased with increased FA in the current experiment. Decreased CH_4 with increasing starch was likely due to decreased dietary NDF. The rate of increase in CH_4 energy in the Nielsen et al., (2013)

equation with increasing dietary NDF was very similar to the rate of decrease with increasing starch that we observed (0.0406 vs. 0.0398 Mcal/%). Increasing supply of supplemental dLys linearly decreased ($P < 0.10$) heat production by about 1 Mcal/d from least to greatest supply, and cause for this is unknown. Heat production in lactating cows is positively associated with BW, DMI, milk fat and protein yield (Morris et al., 2020a), which were not affected by increasing supplemental dLys in the current experiment.

Milk energy and TE averaged 24.1 ± 2.82 and -0.57 ± 3.80 Mcal/d, respectively (Table 8.2). Because TE was near 0, we conclude that on average, cows were supplied energy approximately equal to energy requirements for maintenance and milk production. Although energy supply was quadratically affected by dietary FA, milk energy was unchanged ($P > 0.10$; Table 8.6). For TE, quadratic responses to increase dietary FA and supplemental dLys as well as an interaction were observed ($P < 0.10$). With increasing dietary FA, TE followed a similar response as ME supply. With increasing dietary FA, TE quadratically increased ($P < 0.10$) from 3.0 to 4.8% FA and decreased thereafter (Figure 8.3C). Feeding high C16 FA supplements (> 80%) has increased negative energy balance in early lactation cows compared to a control diet (de Souza and Lock, 2019), and decreased BW and BCS gain in mid-lactation cows compared to FA supplements with greater 18:1 contents (de Souza et al., 2019). Although the FA supplement used in the current experiment contained less C16:0 FA (57% of FA) compared to the aforementioned studies, dietary inclusion was greater (up to 4% of DM vs. 1.5% of DM). Decreasing energy supply generally decreases milk output (Brun-Lafleur et al., 2010); however, in the current experiment, cows were able to maintain milk energy output by mobilizing body tissue at high dietary FA. As supplemental dLys increased from 0 to 8

g/d, TE decreased and as supplemental dLys increased from 8 to 16 g/d, TE increased (Figure 8.3C). This response is similar to N balance which represents the protein fraction of TE and will be discussed below. We expected TE to increase with increasing dietary starch as has been observed previously (Boerman et al., 2015b; Morris et al., 2020b); however, increased TE in previous experiments may have occurred because of increased NE_L .

Dietary FA should not lead to either urinary or CH_4 energy, and FA are thought to have a lower heat increment compared to carbohydrates or protein; therefore, feeding FA is thought to increase the efficiency of converting DE into ME and ME into NE_L (NRC, 2001). With increasing fat and starch in the current experiment, the efficiency of converting DE into ME increased ($P < 0.10$) which was due to similar decreases in CH_4 and urine energy. We recently observed increased ME/DE when feeding a high starch diet compared to a high fat and fiber diet, which was due to decrease urinary energy (Morris et al., 2020b). Andrew et al. (1991) derived the efficiency of converting DE into ME for calcium salts of FA, which averaged 0.96. In the current experiment, the efficiency of converting ME into NE_L quadratically increased as dietary FA increased from 3.0 to 4.6% and decreased thereafter (Figure 8.3D). Andrew et al. (1991) estimated that the NE_L/ME for ca-salts of FA was 0.77. The decrease in NE_L/ME at high dietary FA was unexpected and was likely caused by the negative TE which is subtracted from NE_L . Therefore, decreased energy efficiency may occur when feeding diets high in FA or when negative energy balance occurs.

N Utilization

On average, N intake was 503 ± 58 g/d and of that, $33.6 \pm 2.6\%$ was excreted in feces, $30.2 \pm 4.2\%$ excreted in urine, $34.1 \pm 3.0\%$ secreted in milk, and $2.1 \pm 6.2\%$ was measured as N balance (Table 8.2). Average N balance was 12 ± 31 g/d which is equivalent to a body protein gain of 75 g/d ($12 \text{ g N balance} \times 6.25 \text{ g protein/g N}$). This suggests that N balance measurements were reasonably indicative of normal biology (Spanghero and Kowalski, 1997).

Consumption of N is one of the most impactful drivers of N excretion in manure (Weiss et al., 2009b). In the current experiment, N intake linearly decreased ($P < 0.10$) as the concentration of dietary FA and starch increased. This corresponds with similar linear or quadratic decreases ($P < 0.10$) in fecal and urinary N excretion (Table 8.7). With increasing starch, N intake decreased, and this response is simply because the CP of corn grain is greater than in soyhulls (8.1 vs. 13.9%; NRC, 2001). Increasing dietary FA resulted in a linear decrease ($P < 0.10$) in MUN and plasma urea likely due to decreased N intake. Increasing dietary starch also linearly decreased ($P < 0.10$) MUN and plasma urea. Replacing dietary NDF with starch usually decreases MUN (Weiss et al., 2009a) which likely occurs due to a decrease in whole-body protein catabolism.

Increasing efficiency of converting dietary N into milk N is an essential means to improve the environmental and economic sustainability of the dairy industry (Arriola Apelo et al., 2014). Increasing dietary starch or abomasal infusion of glucose generally increases the efficiency of converting dietary N into milk N presumable via an upregulation of milk protein synthesis via an insulin-dependent signaling cascade (Rius et al., 2010b; Nichols et al., 2019e; Morris et al., 2020b). Whereas, unless energy supply is

limiting (Nichols et al., 2018) increasing FA does not usually increase milk N secretion (Andrew et al., 1991; Nichols et al., 2019e). In the current experiment, milk N secretion decreased ($P < 0.10$) by 8 g/d as dietary FA increased from least to greatest and increased by 7 g/d as dietary starch increased from least to greatest. Additionally, efficiency of converting dietary N into milk N increased from 32.1 to 36.2% as dietary starch increased from least to greatest. Decreased milk N with increasing FA corresponded with decreased milk protein yield (see previous discussion). Increased milk N secretion, when expressed as g/d or as a proportion of total of N intake, has been observed previously with increasing dietary starch (Beckman and Weiss, 2005; Cantalapiedra-Hijar et al., 2014b; Morris et al., 2020b).

Nitrogen balance expressed as g/d and a proportion of N intake was affected by all three factors tested ($P < 0.10$). With increasing dietary FA, N balance linearly decreased. In the case of increasing starch, N balance quadratically increased by 16 g/d as starch increased from 20 to 25% dietary starch and then decreased by 23 g/d thereafter (Figure 8.4A). Increased N balance was recently observed when feeding diets with approximately 1.5% tallow (Drehmel et al., 2018), but no effect was observed when dietary starch content increased (Morris et al., 2020b). Nichols et al. (2016) suggested that skeletal muscle protein accretion via insulin signaling when infusing glucose. We are unsure why N balance decreased when dietary starch increased from 25 to 32%. Measuring N balance is challenging as errors in estimating all routes of N loss are cumulated (Spanghero and Kowalski, 1997); therefore, differences in N balance due to treatments may not be a result of analytical error and observations may not indicative of biological function.

Circulating Lys pools are primarily regulated via catabolism in mammary glands and other peripheral tissue (Lapierre et al., 2005). As supplemental dLys increased in the current experiment, quadratic responses were observed for urinary N and N balance (when expressed in g/d and as a proportion of N intake) as well as for MUN. Urinary N excretion quadratically increased ($P < 0.10$) by 14 g/d as supplemental dLys increased from 0 to 9 g/d and decreased thereafter (Figure 8.4B). A similar response was observed ($P < 0.10$) with MUN as supplemental dLys increased (Figure 8.4C); however, plasma urea was not affected ($P > 0.10$) by supplemental dLys. As supplemental dLys increased from 0 to 8 g/d, N balance quadratically decreased by 13 g/d, and as supplemental dLys increased from 8 to 18 g/d, N balance increased by 31 g/d (Figure 8.4D). From least to greatest supply of supplemental dLys, N balance increased by 13 g/d. In the current experiment, as supplemental dLys increased up to 8 g/d the increasing urinary N excretion and MUN corresponding with a decrease in N balance and TE, suggesting an increase in body tissue mobilization and whole-body protein catabolism. We recently observed an increase in N balance of 11g/d when supplementing 24 g/d of dLys (Morris and Kononoff, 2020). Feeding RP Lys to supply 13 g of dLys/d to early lactation cows provided evidence for decreased negative energy balance as indicated by decreased plasma concentration of β -hydroxybutyrate and free FA (Girma et al., 2019). Most experiments testing RP Lys in mid-lactation cows, feed a zero control and a treatment designed to supply greater than 20 g of supplemental dLys/d (Lee et al., 2012a; Giallongo et al., 2016; Morris and Kononoff, 2020); therefore, responses within the range between 0 and 20 g/d are not well understood. The mechanism causing increased mobilization and catabolism with initial increases in supplemental dLys or vice versa with further increases

in supplemental dLys is unknown. However, it is known that Lys is preferentially catabolized in peripheral tissues including those in the mammary gland (Lapierre et al., 2005) consequently, increasing Lys supply decreases the efficiency of converting metabolizable Lys into milk Lys (Lee et al., 2015). Lapierre et al. (2009) reported that there is an obligate requirement for using N from Lys to synthesize non-essential AA in mammary glands. Therefore, when diets are deficient in Lys, body tissue may be mobilized to provide Lys to the mammary glands to support milk protein synthesis. We speculate that as the supply of Lys increased the mobilization of tissue was likely suppressed and when the supply of Lys was high, the increased Lys pool may have supported synthesis of muscle protein. This suggestion is supported by observations in other species such as the increased in N balance and decreased body protein loss when the supply of Lys is increased in diets fed to lactating sows (Chen et al., 1978; Hojgaard et al., 2019). Additionally, the Lys supply required to maintain body protein stores in these animals is greater than needed to maximize milk yield and litter growth rate (Hojgaard et al., 2019). Therefore, given that response in milk protein to increasing Lys was minimal, but N balance and TE increased at high supplemental dLys, cows in the current experiment may have a preferentially utilized Lys in skeletal muscle rather than for milk protein synthesis.

Plasma AA

The concentration of AA in blood plasma can serve as a gross indicator of AA metabolism. Only plasma concentration of essential AA and Lys will be discussed because most individual AA followed the same pattern (Supplemental Table 8.7) and dietary Lys supply was manipulated with treatments. Plasma concentration of essential

AA and Lys were affected ($P < 0.10$) by main factors tested namely supplementary FA, starch, and supplemental dLys; an interaction between dietary fatty acids and supplemental dLys and between dietary starch and supplemental dLys was also observed (Table 8.8). Response patterns were similar between plasma essential AA and Lys (Figure 8.5) thus only plasma Lys will be discussed. Plasma Lys increased with increasing supplemental dLys at low dietary FA and starch content and decreased with increasing supplemental dLys at high dietary FA and starch content (Figure 8.5 C, D). Plasma concentration of AA are affected by a number of factors including microbial protein supply, milk protein yield, plane of nutrition, and stage of lactation; therefore, plasma AA concentration may not always reflect changes in metabolizable supply (Patton et al., 2015; Martineau et al., 2017). In the current experiment, plasma Lys concentration was likely affected by the variability of flux of rumen bypass protein and microbial protein, as well as the utilization of AA for milk and body protein synthesis and whole-body catabolism. For these reasons, interpretation of effects of treatments on concentration of plasma Lys is challenging and results should be interpreted with caution. Nonetheless, an interaction between dietary FA and supplemental dLys on plasma Lys was observed, increasing FA increased N balance but decreased milk protein yield. Therefore, at low dietary FA, increasing supplemental dLys likely increased plasma Lys because less AA were needed for muscle protein synthesis; whereas, at high dietary FA, increasing supplemental dLys may have resulted in a decrease in plasma Lys because more Lys was being used for muscle protein accretion. Estimated N balance was greatest for the highest dietary FA and supplemental dLys (48 g/d). The interaction between dietary starch and supplemental dLys in general mirrored the response in milk protein

concentration from these same two factors. As discussed previously, at low dietary starch, microbial protein supply was likely lowest (Roman-Garcia et al., 2016).

Therefore, supply of metabolizable Lys was also likely lower at low dietary starch and increasing supplemental dLys results in a large relative increase in dLys supply. Whereas at high dietary starch, Lys may have been catabolized by peripheral tissues. These suggestions are supported by the observations of Nichols et al. (2019c) where abomasal infusion of glucose decreased plasma Lys concentration and increased catabolism of Lys in mammary glands when MP supply was also increased.

CONCLUSIONS

Lactating dairy cows were fed diets that varied in dietary FA, and starch content as well as in supplemental dLys. Increasing dLys supply increased milk protein concentration at low dietary starch, but not at high dietary starch. As dietary FA increased, DMI and NDF digestibility decreased, and dietary ME increased to a plateau around 5.2% dietary FA. Additionally, at a dietary FA content of 4.2%, FA digestibility, ME supply and TE were maximized. Increasing dietary FA and starch increased the conversion of DE into ME due to decreased CH₄ and urinary energy loss. Increasing dietary starch resulted in minimal effects on milk production, nutrient digestibility, and ME supply. Increasing dietary starch increased the efficiency of utilizing dietary N for milk N. A quadratic response was observed for N balance as dLys supply varied such that N balance was least at 8 g/d and greatest at 18 g/d of supplemental dLys. Plasma concentration of Lys was affected by both a FA by Lys and a starch by Lys interaction which reflected the responses that were observed in DMI, milk protein yield and N

balance. Our results suggest that increasing dietary FA can increase ME supply, however, high supplementation of FA can decrease ME supply due to depressions in DMI, NDF_{OM}, and FA digestibility. Results demonstrated that the supply of Lys is important for milk protein synthesis as well as for supporting muscle protein synthesis and we speculate that Lys may be preferentially diverted towards muscle protein at the expense of milk protein.

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1 **Table 8.1.** Ingredient and chemical composition of diets with differing dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM) and starch
2 contents (20.2, 21.4, 25.7, 30.0, and 31.3% of DM)¹

Dietary FA, %	3.0	3.4	3.4	4.6	5.9	6.2	6.2	6.2	6.2
Dietary starch, %	25.7	21.4	30.0	20.2	25.7	31.3	21.4	30.0	23.5
Item, % of DM									
Ingredient									
Corn silage ²	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0
Alfalfa hay ³	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Corn grain, ground	11.0	4.8	17.2	3.0	11.0	19.0	4.8	17.2	11.0
Soyhulls	13.0	18.8	6.3	19.0	11.0	3.0	15.7	3.2	9.0
Fat ⁴	0.00	0.45	0.45	2.00	2.00	2.00	3.55	3.55	4.00
Other ⁵	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Chemical composition ^{6,7}									
DM, as-is	59.9 (1.76)	58.5 (1.22)	59.2 (1.49)	59.3 (5.93)	59.8 (1.26)	59.1 (2.00)	59.5 (1.39)	58.0 (1.01)	59.7 (1.47)
CP	15.9 (0.80)	16.3 (0.56)	15.4 (0.55)	16.3 (0.36)	15.9 (0.34)	15.5 (0.30)	16.4 (0.26)	15.4 (0.29)	15.9 (0.24)
NDF	30.2 (2.83)	35.8 (1.54)	27.9 (2.29)	35.1 (2.15)	30.2 (2.35)	26.6 (3.13)	32.4 (2.38)	27.0 (1.21)	28.7 (2.49)
NDF _{OM}	29.6 (2.78)	35.0 (1.54)	27.3 (2.19)	34.3 (2.17)	29.6 (2.28)	26.0 (3.02)	31.6 (2.37)	26.3 (1.21)	28.1 (2.44)
Starch	26.4 (1.63)	20.2 (0.88)	30.3 (1.70)	20.1 (1.38)	25.8 (1.41)	30.4 (1.78)	21.1 (2.03)	29.4 (1.10)	26.0 (0.97)
FA	2.88 (0.15)	3.57 (0.26)	3.25 (0.20)	4.78 (0.32)	4.64 (0.26)	4.71 (0.38)	6.00 (0.33)	6.17 (0.27)	6.44 (0.31)

3 ¹Rumen-protected Lys (EB Lys; Milk Specialties, Eden Prairie, MN) was supplemented to supply 0, 2.0, 8.9, 15.8, and 17.8 g/d g of

4 digestible Lys.

5 ²Corn silage chemical composition was (mean \pm SD; n = 12) 37.9 \pm 1.68% DM; 8.35 \pm 0.38% CP; 33.9 \pm 3.92% NDF_{OM}; 40.0 \pm

6 4.11% starch; 2.2 \pm 0.11% FA, 4.8 \pm 0.66% ash.

³Alfalfa hay chemical composition was (mean \pm SD; n = 12) 88.9 \pm 2.08% DM; 19.9 \pm 2.31% CP; 40.5 \pm 4.21% NDF_{OM}; 2.3 \pm 0.97% starch; 1.05 \pm 0.16% FA, 11.5 \pm 0.88% ash.

⁴Energy Booster Merge (Milk Specialties, Eden Prairie, MN; contained 84.1% FA, 57.2 g/100 FA C16:0, 20.8 g/100g FA 18:0, 11.2 g/100g FA *cis*-9 18:1).

⁵All diets contained 11.0% 48% CP soybean meal, 10.0% corn gluten feed, 1.48% beet molasses, 1.00% calcium carbonate, 0.85% sodium bicarbonate, 0.57% dicalcium phosphate 0.40 salt, 0.39% magnesium oxide, 0.24% RP Met (EB Met; Milk Specialties, Eden Prairie, MN), 0.05% vitamin mix, and 0.04% trace mineral mix.

⁶All dietary chemical composition corrected for the addition of 124 g/d of FA via topdress assuming DMI equal to mean experimental DMI (Table 8.4).

⁷Values in parentheses indicate SD (n = 12).

Table 8.2. Simple statistics for DMI, BW, milk yield and composition, digestibility, energy, and N variables (n = 88 for all variables except O₂ consumption, CO₂ production, CH₄ production and RQ where n = 86).

Variable	Mean	SD	Min	Max
DMI, kg/d	19.7	2.1	14.9	24.7
BW, kg/d	424	38	365	498
Milk				
Yield, kg/d	28.4	4.0	20.3	38.3
ECM, kg/d ¹	35.1	4.0	27.7	48.3
Fat, %	5.03	0.68	3.62	6.52
Fat, g/d	1415	186	1078	2167
Protein, %	3.46	0.29	2.79	4.36
Protein, g/d	981	126	758	1324
Lactose, %	4.82	0.13	4.48	5.17
Lactose, g/d	1373	207	921	1932
Digestibility, %				
DM	65.8	1.6	60.5	69.6
OM	67.0	1.9	61.0	71.4
CP	66.4	2.6	61.1	73.7
NDF	45.2	6.2	30.5	59.3
NDF _{OM}	46.5	6.0	32.7	60.4
Starch	94.9	2.2	88.4	98.9
Total FA	70.1	6.2	50.7	84.4
16-Carbon FA	71.1	5.9	54.4	87.3
18-Carbon FA	69.3	7.2	44.1	82.7
Energy	65.5	2.4	58.6	72.2
Energy				
GE, Mcal/kg DM	4.21	0.10	3.93	4.46
GE, Mcal/d	82.9	9.1	62.4	107.5
DE, Mcal/kg DM	2.76	0.13	2.45	3.11
DE, Mcal/d	54.3	6.6	41.9	76.0
ME, Mcal/kg DM	2.45	0.13	2.10	2.83
ME, Mcal/d	48.3	6.3	36.2	68.3
NE _L , Mcal/kg DM	1.67	0.14	1.32	2.03
NE _L , Mcal/d	32.9	4.8	23.3	46.7
Urine, Mcal/d	2.34	0.26	1.93	2.99
CH ₄ , Mcal/d	3.74	0.61	2.48	5.39
Heat, Mcal/d ²	24.7	2.4	19.5	31.3
Milk, Mcal/d	24.1	2.82	18.7	33.5
TE, Mcal/d	-0.57	3.80	-13.47	10.02
ME/DE	0.887	0.015	0.831	0.917
NE _L /ME	0.681	0.029	0.577	0.751
O ₂ consumption, L	4925	479	3831	6207

CO ₂ production, L	5163	500	4120	6542
CH ₄ production, L	395	65	263	571
RQ ³	1.050	0.026	0.995	1.130
Nitrogen				
Intake, g/d	503	58	372	643
Fecal, g/d	169	22	119	232
Urinary, g/d	151	25	100	209
Milk, g/d	171	22	128	224
Balance, g/d	12	31	-65	112
Fecal, % N intake	33.6	2.6	26.3	38.9
Urinary, % N intake	30.2	4.2	20.8	41.6
Milk, % N intake	34.1	3.0	27.6	41.0
Balance, % N intake	2.1	6.2	-15.4	17.6
MUN, mg/dL	13.9	2.0	10.0	19.8
Urea, mg/dL	32.2	5.3	22.8	44.0

20 ¹ECM = 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × true protein (kg) (Tyrrell and

21 Reid, 1965).

22 ²Heat, Mcal/d = 3.866 × O₂ consumption + 1.200 × CO₂ production – 0.518 × CH₄

23 production – 1.431 × urinary N excretion (Brouwer, 1965).

24 ³RQ = respiratory quotient, CO₂ production/O₂ consumption, (L/L)

Table 8.3. Simple statistics for plasma AA concentration (μM) and plasma 3-methyl-His and urea concentration (n = 88)

Variable	Mean	SD	Min	Max
EAA	962	164	583	1267
Arg	74.8	15.0	42.6	112
His	45.0	13.1	18.1	74.4
Ile	129	26	65	180
Leu	148	33	79	211
Lys	88.6	16.3	49.3	138
Met	23.5	3.8	14.7	34.6
Phe	49.1	8.1	28.5	67.3
Thr	112	21	63	158
Trp	38.3	8.5	23.4	59.6
Val	254	52	140	390
NEAA	1207	142	850	1557
Ala	270	50	161	403
Asn	49.7	9.7	28.4	72.7
Asp	2.3	1.7	0.4	8.1
Gln	194	30	102	286
Glu	45.3	7.8	29.7	65.5
Gly	376	71	186	590
Pro	78.9	14.9	44.4	124
Ser	93.1	15.0	54.6	122
Tau	46.8	11.9	15.5	78.8
Tyr	50.9	10.6	26.6	69.6

28 **Table 8.4.** Effects of varying dietary concentration of FA (F) and starch (S) and Lys (L) supply on DMI, milk production and
 29 composition of lactating Jersey cows¹

Variable	Intercept	F	S	L	S×S	L×L	S×L	Error ²	
								RMSE	Cow
DMI, kg/d	21.9 (0.63)	−0.457 (0.099)						0.84	1.83
Protein, %	3.08 (0.20)	−0.0454 (0.012)	0.0241 (0.0064)	0.0451 (0.016)			−0.00177 (0.00060)	0.102	0.170
Protein, g/d	1067 (40)	−181 (6.8)						59.0	107

30 ¹The model included the linear and quadratic effects of FA, starch, and Lys and all 2-way interaction. Final models included only
 31 effects that were significant ($P > 0.10$). Values in parentheses are SE of parameters estimates.

32 ²RMSE = root mean square error; Cow = square root of the variance due to cows. When applied to a group of cows, the prediction
 33 error = [RMSE + (Cow error/ \sqrt{n})], where n = number of cows in the group to which the equations are applied (Weiss et al., 2009a).

34 **Table 8.5.** Effects of varying dietary concentration of FA (F) and starch (S) and Lys (L) supply on nutrient digestibility of lactating
35 Jersey cows¹

Variable	Intercept	F	S	L	F×F	S×S	Error ²	
							RMSE	Cow
DM	57.1 (3.3)	2.37 (01.4)	0.156 (0.037)		-0.280 (0.15)		1.13	0.80
CP	63.9 (1.3)	0.753 (0.21)		0.0846 (0.038)			1.98	0.40
NDF _{OM}	65.9 (4.1)	-0.989 (0.35)	-0.593 (0.10)				3.19	1.47
Starch	73.8 (11)		1.80 (0.89)			-0.0368 (0.017)	1.54	0.99
Total FA	45.0 (12)	12.8 (5.2)			-1.53 (0.57)		3.99	3.82
16-Carbon FA	34.9 (11)	16.4 (4.9)			-1.77 (0.53)		3.70	3.50
18-Carbon FA	50.6 (14)	11.7 (6.1)			-1.56 (0.66)		4.74	4.19
Energy	55.8 (4.7)	3.64 (2.0)	0.0920 (0.051)		-0.415 (0.21)		1.58	1.02

36 ¹The model included the linear and quadratic effects of FA, starch, and Lys and all 2-way interaction. Final models included only
37 effects that were significant ($P > 0.10$). Values in parentheses are SE of parameters estimates.

38 ²RMSE = root mean square error; Cow = square root of the variance due to cows. When applied to a group of cows, the prediction
39 error = [RMSE + (Cow error/ \sqrt{n})], where n = number of cows in the group to which the equations are applied (Weiss et al., 2009a).

40 **Table 8.6.** Effects of varying dietary concentration of FA (F) and stach (S) and Lys (L) supply on energy utilization of lactating Jersey
41 cows¹

Variable	Intercept	F	S	L	F×F	S×S	L×L	F×L	Error ²	
									RMSE	Cow
GE, Mcal/kg DM	3.95 (0.048)	0.0669 (0.0036)	-0.00223 (0.0010)						0.034	0.00018
DE, Mcal/kg DM	2.15 (0.21)	0.241 (0.090)			-0.0223 (0.0097)				0.071	0.047
DE, Mcal/d	41.5 (8.5)	6.68 (3.80)			-0.786 (0.41)				2.76	5.40
ME, Mcal/kg DM	1.82 (0.21)	0.242 (0.095)			-0.0219 (0.010)				0.0745	0.0503
ME, Mcal/d	35.3 (8.0)	6.44 (3.6)			-0.734 (0.39)				2.59	5.18
NE _L , Mcal/kg DM	1.00 (0.23)	0.271 (0.10)			-0.0260 (0.011)				0.0803	0.0703
NE _L , Mcal/d	19.7 (6.4)	6.28 (2.9)			-0.697 (0.31)				2.08	3.83
Urine, Mcal/d	2.99 (0.15)	-0.0632 (0.013)	-0.0152 (0.0038)	0.0193 (0.0099)			-0.000987 (0.00054)		0.112	0.194
CH ₄ , Mcal/d	5.73 (0.33)	-0.203 (0.033)	-0.0398 (0.0096)						0.282	0.481
Heat, Mcal/d	25.3 (0.61)			-0.0540 (0.026)					1.15	2.01
TE, Mcal/d	-17.2 (6.6)	6.6 (2.9)		0.0790 (0.26)	-0.640 (0.31)		0.0185 (0.10)	-0.0769 (0.041)	2.13	2.40
ME/DE	0.845 (0.0075)	0.00377 (0.00075)	0.000953 (0.00021)						0.00623	0.0119
NE _L /ME	0.584 (0.055)	0.0430 (0.025)			-0.00451 (0.0027)				0.0188	0.0172
RQ	1.26 (0.10)	-0.00836 (0.0016)	-0.0138 (0.0080)			0.000270 (0.00016)			0.0129	0.0132

42 ¹The model included the linear and quadratic effects of FA, starch, and Lys and all 2-way interaction. Final models included only
43 effects that were significant ($P > 0.10$). Values in parentheses are SE of parameters estimates.
44 ²RMSE = root mean square error; Cow = square root of the variance due to cows. When applied to a group of cows, the prediction
45 error = $[RMSE + (Cow\ error/\sqrt{n})]$, where n = number of cows in the group to which the equations are applied (Weiss et al., 2009a).

46 **Table 8.7.** Effects of varying dietary concentration of FA (F) and starch (S) and Lys (L) supply on N utilization of lactating Jersey
47 cows^{1,2}

Variable	Intercept	F	S	L	S×S	L×L	Error ³	
							RMSE	Cow
Intake, g/d	634 (32)	-9.58 (3.3)	-3.37 (0.94)				27.6	46.1
Fecal, g/d	224 (15)	-6.70 (1.4)	-0.905 (0.41)				12.6	14.0
Urinary, g/d	443 (93)	-8.44 (1.4)	-17.6 (7.3)	3.13 (1.1)	0.285 (0.14)	-0.169 (0.060)	12.5	13.6
Milk, g/d	166 (11)	-2.40 (1.2)	0.652 (0.33)				9.82	18.2
Balance, g/d	-368 (158)	9.04 (2.4)	27.4 (12)	-4.67 (1.7)	-0.539 (0.24)	0.300 (0.095)	21.4	11.7
Urinary, % N intake	88.0 (19)	-1.25 (0.29)	-3.82 (1.51)	0.619 (0.21)	0.0668 (0.029)	-0.0356 (0.011)	2.63	1.20
Milk, % N intake	24.6 (1.5)		0.373 (0.058)				1.77	1.74
Balance, % N intake	-66.9 (30)	1.99 (0.46)	4.83 (2.4)	-0.829 (0.33)	-0.0943 (0.046)	0.0538 (0.018)	4.08	2.39
MUN, mg/dL	19.5 (1.36)	-0.233 (0.10)	-0.192 (0.029)	0.196 (0.075)		-0.00921 (0.0041)	0.89	0.72
Plasma urea, mg/dL	49.0 (3.1)	-0.809 (0.33)	-0.506 (0.093)				2.76	3.56

48 ¹The model included the linear and quadratic effects of FA, starch, and Lys and all 2-way interaction. Final models included only
49 effects that were significant ($P > 0.10$). Values in parentheses are SE of parameters estimates.

50 ²Regression for fecal N as a % of N intake was identical to CP digestibility except the intercept was 36.8 ± 14 and all other
51 coefficients have the opposite sign (Table 8.5).

52 ³RMSE = root mean square error; Cow = square root of the variance due to cows. When applied to a group of cows, the prediction
53 error = $[\text{RMSE} + (\text{Cow error}/\sqrt{n})]$, where n = number of cows in the group to which the equations are applied (Weiss et al., 2009a).

54 **Table 8.8.** Effects of varying dietary concentration of FA (F) and starch (S) and Lys (L) supply on plasma essential AA and Lys
55 concentration¹

Variable	Intercept	F	S	L	F×L	S×L	Error ²	
							RMSE	Cow
Essential AA, µM	761 (179)	46.7 (17)	-0.579 (5.2)	52.2 (16.7)	-5.90 (1.6)	-0.949 (0.49)	81.8	115
Lys, µM	48.6 (22)	7.86 (2.2)	0.0641 (0.65)	7.13 (2.1)	-0.837 (0.21)	-0.118 (0.0625)	10.9	9.47

56 ¹The model included the linear and quadratic effects of FA, starch, and Lys and all 2-way interaction. Final models included only
57 effects that were significant ($P > 0.10$). Values in parentheses are SE of parameters estimates.

58 ²RMSE = root mean square error; Cow = square root of the variance due to cows. When applied to a group of cows, the prediction
59 error = [RMSE + (Cow error/ \sqrt{n})], where n = number of cows in the group to which the equations are applied (Weiss et al., 2009a).

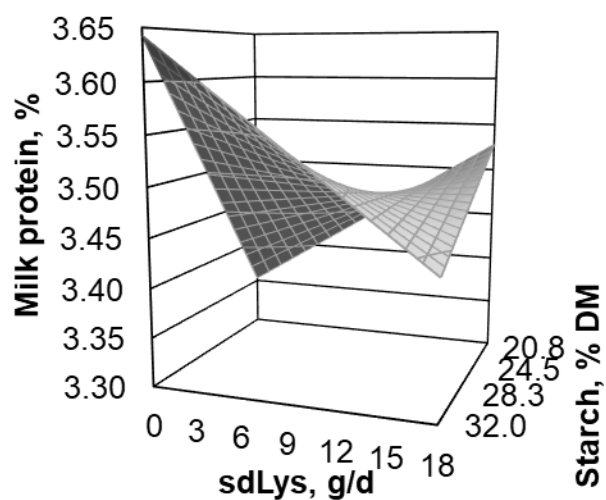


Figure 8.1. Response in milk protein concentration as dietary starch content and supply of supplemental digestible Lys (sdLys) change. Equation for the response surface are reported in Table 8.4. If a factor was significant but not shown in the graphs, then the response is for the mean for that factor.

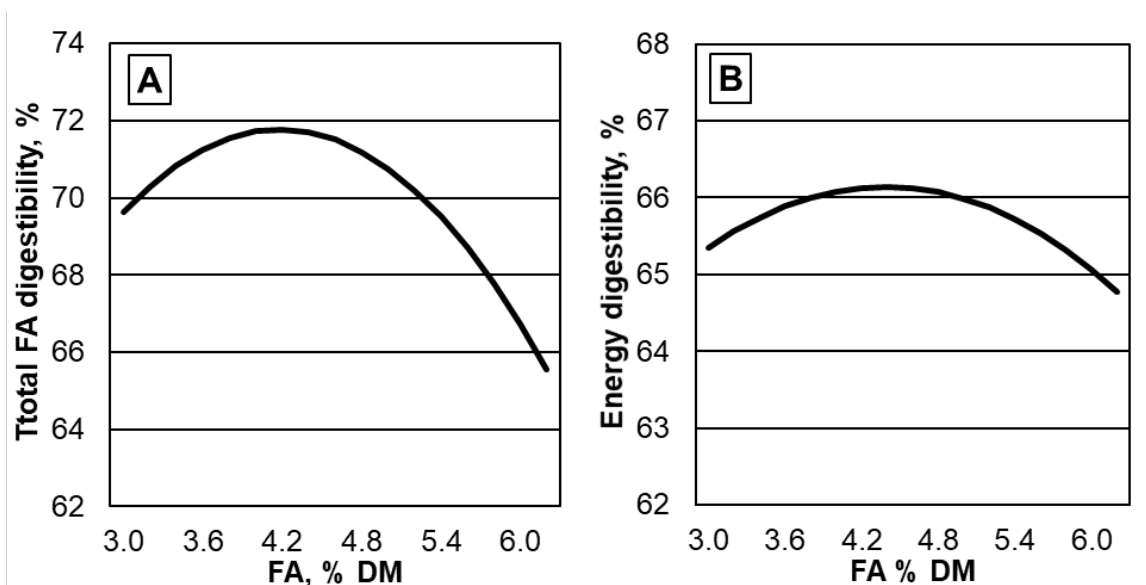


Figure 8.2. Response in total fatty acid (FA) and energy digestibility as dietary FA content changes. Equation for the response surface are reported in Table 8.5. If a factor was significant but not shown in the graphs, then the response is for the mean for that factor.

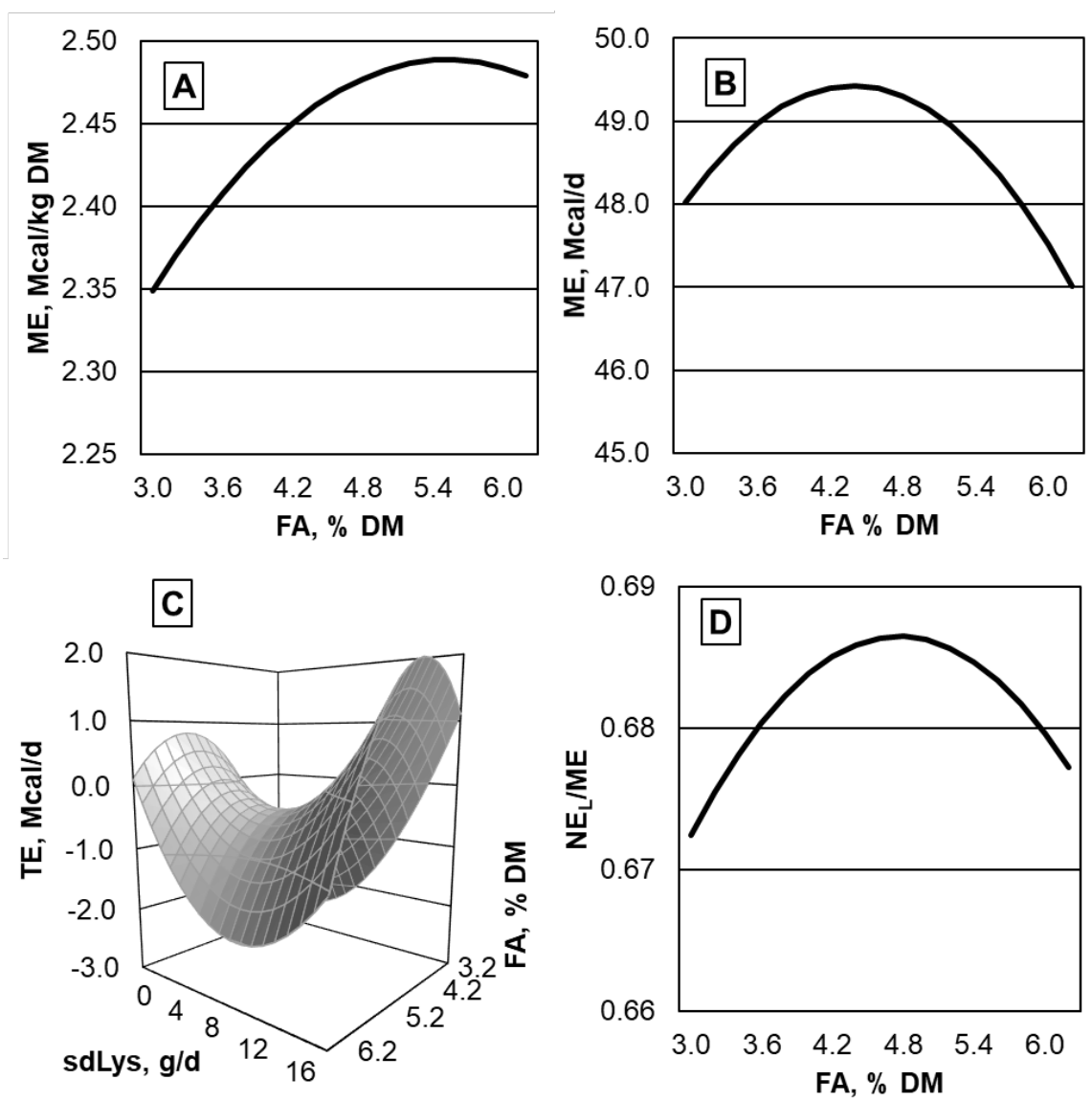


Figure 8.3. Response in metabolizable energy (ME), tissue energy (TE), or NE_L/ME as dietary fatty acids (FA) content or supplemental digestible Lys (sdLys) change. Equation for the response surface are reported in Table 8.6. If a factor was significant but not shown in the graphs, then the response is for the mean for that factor.

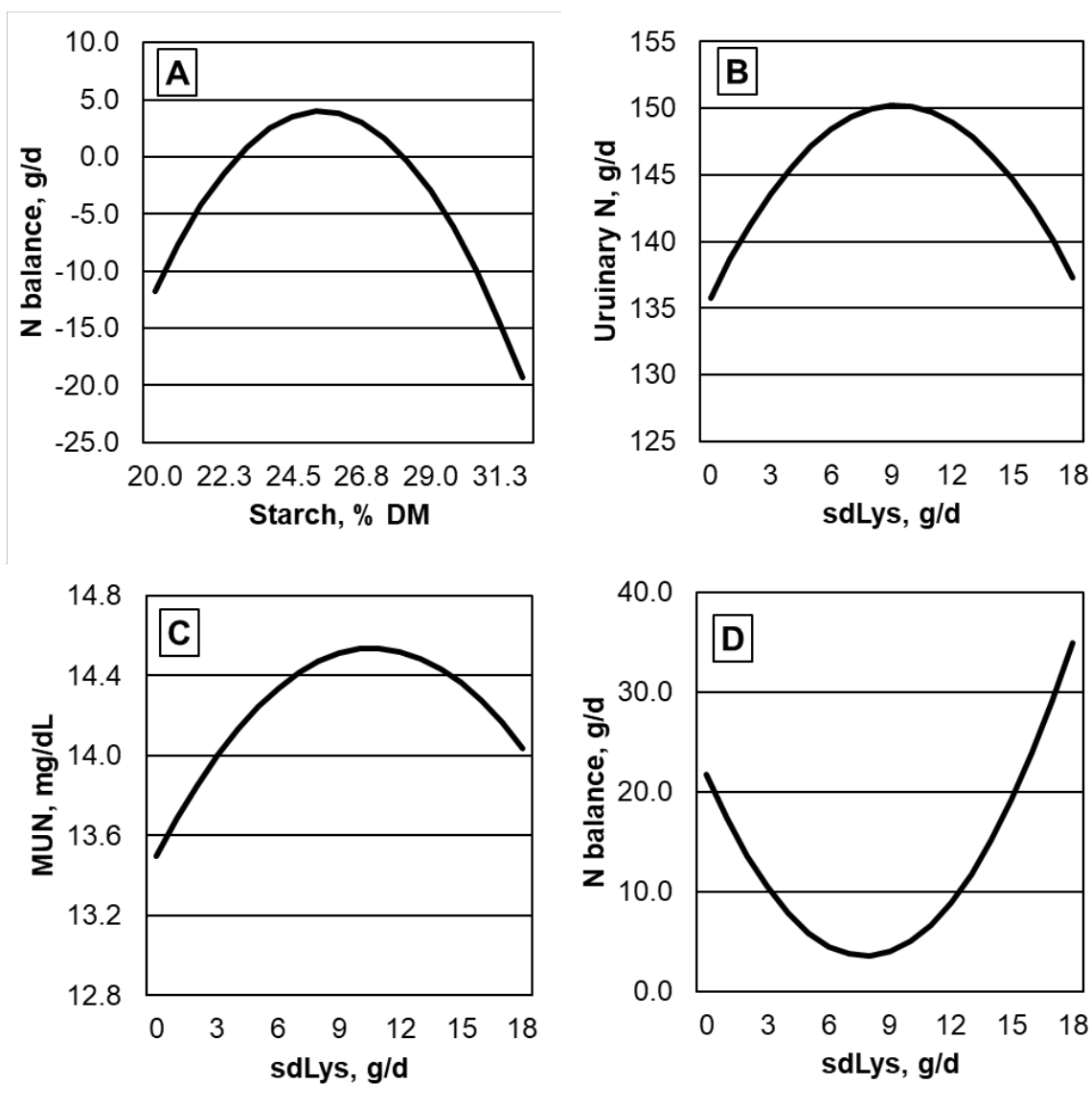


Figure 8.4. Response in N balance, urinary N excretion and MUN as dietary starch content or supplemental digestible Lys (sdLys) change. Equation for the response surface are reported in Table 8.7. If a factor was significant but not shown in the graphs, then the response is for the mean for that factor

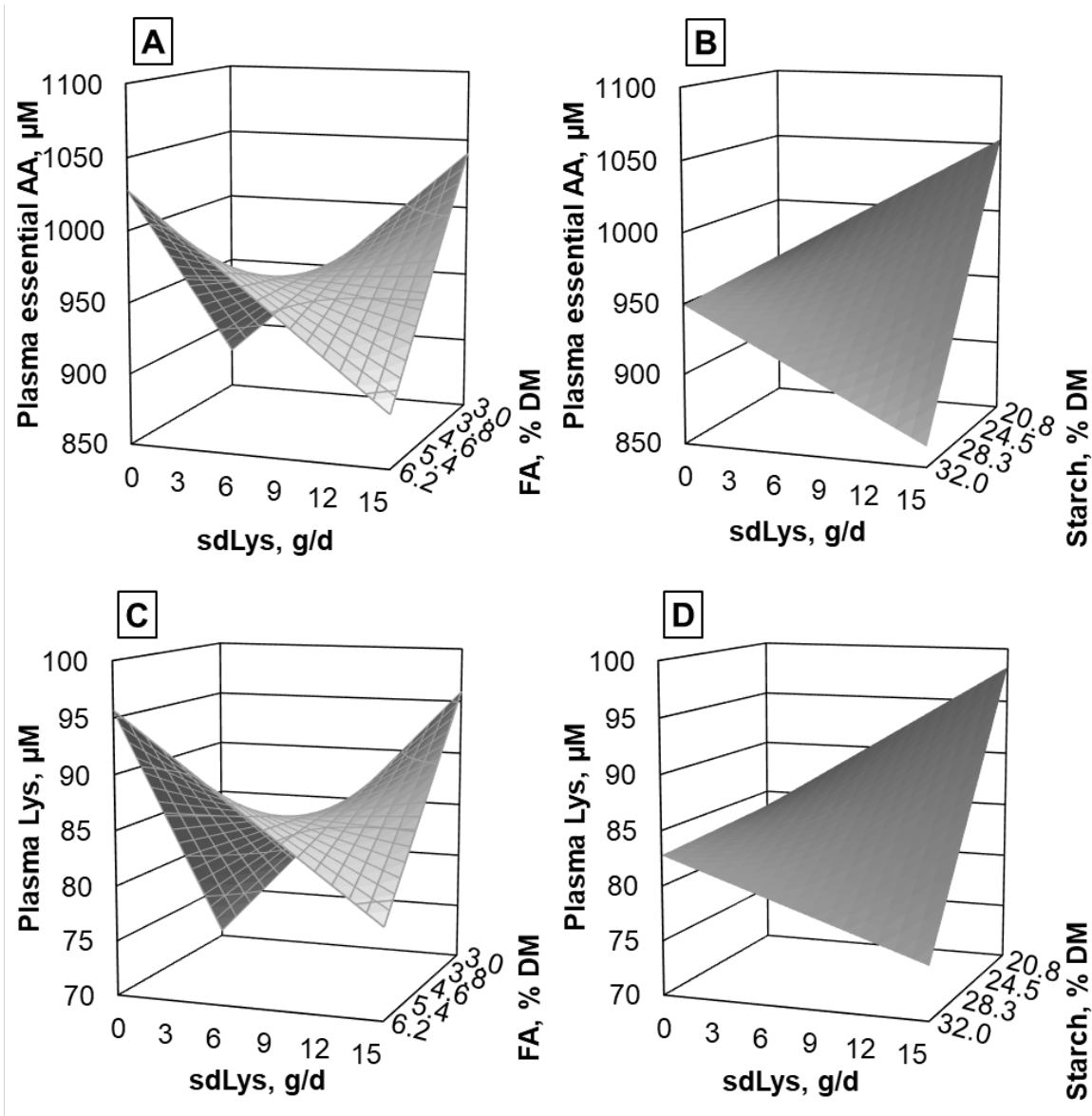


Figure 8.5. Response in plasma concentration of essential AA and Lys as dietary fatty acids (FA) and starch content and supply of supplemental digestible Lys (sdLys) change. Equation for the response surface are reported in Table 8.8. If a factor was significant but not shown in the graphs, then the response is for the mean for that factor.

APPENDIX

Supplemental Table 8.1. Estimated NEL and Lys supply and balance for diets differing dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM) and starch contents (20.2, 21.4, 25.7, 30.0, and 31.3% of DM) and supplemental digestible Lys (sdLYS; 0, 2.0, 8.9, 15.8, and 17.8 g/d)¹

FA, %	3.0		3.4				4.6				5.9		6.2	
Starch, %	25.7	21.4		30.0	25.7	20.2	25.7	31.3	25.7	21.4	30.0	21.4	30.0	25.7
sdLys, g/d	8.9	2.0	15.8	2.0	15.8	0.00	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8
Item														
NE _L														
Mcal/kg DM	1.51	1.51	1.53	1.52	1.53	1.57	1.55	1.57	1.59	1.57	1.62	1.63	1.63	1.64
Supply, Mcal/d	30.1	30.6	31.0	31.1	31.0	31.3	31.8	31.3	30.9	31.3	31.3	31.9	30.6	21.2
Requirements, Mcal/d	31.6	31.4	31.7	32.9	32.1	33.1	32.5	32.7	31.4	32.2	31.3	31.6	31.2	32.4
Balance, Mcal/d	-1.6	-0.9	-0.8	-1.8	-1.1	-1.9	-0.7	-1.4	-0.6	-1.0	0.0	0.3	-0.6	-1.1
MP supply, g/d	2082	2118	2137	2148	2137	2091	2145	2091	2047	2091	2024	2052	1969	1998
MP requirements, g/d	2198	2159	2180	2317	2195	2233	2176	2194	2124	2196	2083	2138	2088	2140
Lys														
% of MP supply	6.86	6.56	7.10	6.47	7.10	6.46	6.94	6.88	6.88	7.31	6.67	6.53	7.40	7.25
Diet supply, g/d	134	137	136	137	136	135	140	135	132	135	133	132	130	129
RP Lys supply, g/d ²	8.9	2.0	15.8	2.0	15.8	0.0	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8
Target, g/d ³	145	142	144	153	145	147	144	145	140	145	137	141	138	141
Balance, g/d	-2	-3	8	-14	7	-12	5	-1	1	8	-2	-7	8	4

¹Values estimated from NRC (2001) using treatment lsmeans for DMI, milk yield and composition, and BW (Table 8.6) and average forage chemical composition (Table 8.1).

²The RP Lys (EB Lys; Milk Specialties, Eden Prairie, MN) contained 23.3% Lys, with a rumen bypass of (mean \pm SD) $80 \pm 5.0\%$ and intestinal digestibility of $47 \pm 12.4\%$ (Paz et al., 2014).

³Targets for digestible Lys (dLys) was calculated as 6.6% of MP requirements (Schwab et al., 2005).

Supplemental Table 8.2. Least squares means for DMI, milk production and composition, BW during 4-d collection period for cows fed diets with differing dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM), starch contents (20.2, 21.4, 25.7, 30.0, and 31.3% of DM), and supplemental digestible Lys (sdLYS; 0, 2.0, 8.9, 15.8, and 17.8 g/d)

FA, %	3.0		3.4			4.6					5.9				6.2		SEM
Starch, %	25.7	21.4		30.0			25.7	20.2	25.7	31.3	25.7	21.4	30.0	21.4	30.0	25.7	
sdLys, g/d	8.9	2.0	15.8	2.0	15.8	0.00	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8	8.9		
Item																	
DMI, kg/d	20.5	20.1	20.1	20.4	20.4	20.2	20.6	19.8	19.4	20.1	19.2	18.8	19.0	18.7	19.4	0.68	
Milk, kg/d	27.9	28.3	28.2	29.1	28.3	30.3	28.6	28.9	27.3	28.8	27.6	27.0	27.2	28.9	29.0	1.41	
ECM, kg/d	34.4	34.5	34.5	36.5	35.1	36.4	36.1	36.1	34.1	35.7	33.7	33.7	33.4	36.4	36.5	1.50	
Fat, %	4.95	4.88	4.95	5.01	5.05	4.81	5.24	5.14	5.11	5.05	5.02	5.12	5.07	5.22	5.19	0.352	
Fat, g/d	1372	1371	1376	1464	1417	1436	1489	1474	1379	1447	1359	1360	1351	1497	1502	85.6	
Protein, %	3.61	3.46	3.52	3.67	3.49	3.42	3.43	3.44	3.54	3.51	3.37	3.51	3.48	3.42	3.39	0.107	
Protein, g/d	1005	992	984	1064	972	1020	979	991	974	1003	931	948	932	988	980	41.9	
Lactose, %	4.96	4.85	4.80	4.80	4.78	4.82	4.90	4.81	4.86	4.86	4.75	4.81	4.75	4.83	4.88	0.050	
Lactose, g/d	1384	1368	1355	1392	1357	1468	1404	1389	1320	1405	1311	1297	1297	1396	1421	74.6	
BW, kg	431	430	430	427	422	422	426	425	426	426	420	429	423	425	430	8.9	

Supplemental Table 8.3. Least squares means for nutrient digestibility during 4-d collection period for cows fed diets with differing amounts of dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM) and starch content (20.2, 21.4, 25.7, 30.0, and 31.3% of DM) and supplemental digestible Lys (sdLYS; 0, 2.0, 8.9, 15.8, and 17.8 g/d)

FA, %	3.0		3.4			4.6					5.9				6.2		SEM
Starch, %	25.7	21.4		30.0			25.7	20.2	25.7	31.3	25.7	21.4	30.0	21.4	30.0	25.7	
sdLys, g/d	8.9	2.0	15.8	2.0	15.8	0.00	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8	8.9		
Item																	
DM	65.0	65.4	66.3	66.6	66.7	65.5	64.7	66.3	67.0	66.4	65.1	66.3	64.4	66.3	64.8	0.71	
OM	65.9	66.3	67.5	67.8	68.1	67.0	65.0	67.6	68.1	68.2	66.5	67.8	65.6	67.7	66.1	0.85	
CP	65.8	65.0	66.7	64.9	63.8	64.6	64.1	66.7	66.9	69.1	67.2	66.6	67.7	67.4	66.1	1.14	
NDF	43.9	48.8	49.6	43.6	43.5	44.8	48.4	45.0	42.3	46.1	47.0	40.9	45.3	40.8	41.8	3.00	
NDF _{OM}	44.9	49.9	50.7	45.2	45.0	45.8	49.8	45.9	43.5	47.4	48.2	42.8	46.5	42.2	42.6	2.95	
Starch	95.7	95.1	96.6	95.9	93.9	94.2	94.6	95.2	92.7	95.8	95.4	94.5	95.0	94.9	95.3	1.12	
Total FA	69.0	75.1	70.1	69.9	69.9	73.8	71.8	72.1	70.8	71.2	68.8	64.4	66.1	68.8	66.8	2.77	
16-Carbon FA	66.9	72.4	67.5	70.5	70.0	75.7	71.5	72.8	73.4	72.4	70.5	68.8	67.3	71.7	69.5	2.60	
18-Carbon FA	71.2	77.0	71.9	70.2	70.6	73.2	71.6	72.0	69.6	70.4	67.0	59.9	64.3	65.9	64.0	3.13	
Energy	65.1	64.9	66.4	65.8	66.4	66.3	65.5	66.2	66.1	66.3	65.0	66.4	64.3	65.7	64.4	1.24	

Supplemental Table 8.4. Least squares means for energy utilization during 4-d collection period for cows fed diets with differing dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM) and starch contents (20.2, 21.4, 25.7, 30.0, and 31.3% of DM) and supplemental digestible Lys (sdLYS; 0, 2.0, 8.9, 15.8, and 17.8 g/d)

FA, %	3.0		3.4			4.6			5.9			6.2			SEM	
Starch, %	25.7	21.4	30.0		25.7	20.2	25.7	31.3	25.7	21.4	30.0	21.4	30.0	25.7		
sdLys, g/d	8.9	2.0	15.8	2.0	15.8	0.00	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8		8.9
Item ¹																
GE, Mcal/kg DM	4.07	4.12	4.13	4.11	4.12	4.21	4.25	4.20	4.20	4.20	4.29	4.28	4.29	4.28	4.32	0.038
GE, Mcal/d	83.4	82.8	83.3	83.5	84.0	85.0	87.5	83.5	81.5	84.2	82.5	80.7	81.7	79.9	83.7	2.86
DE, Mcal/kg DM	2.64	2.68	2.74	2.70	2.74	2.80	2.79	2.78	2.78	2.78	2.79	2.84	2.76	2.81	2.78	0.070
DE, Mcal/d	54.6	53.4	55.3	55.0	55.7	56.1	57.7	55.2	53.3	56.4	53.7	54.0	52.7	52.5	53.2	2.44
ME, Mcal/kg DM	2.32	2.33	2.41	2.39	2.44	2.50	2.47	2.47	2.46	2.49	2.47	2.53	2.45	2.52	2.49	0.067
ME, Mcal/d	47.9	46.5	48.7	48.8	49.7	50.1	50.9	49.1	47.1	50.2	47.7	48.3	46.7	47.1	47.4	2.27
NE _L , Mcal/kg DM	1.50	1.57	1.63	1.64	1.69	1.71	1.66	1.70	1.65	1.74	1.67	1.69	1.67	1.74	1.68	0.058
NE _L , Mcal/d	31.2	31.4	32.9	33.3	34.6	34.4	34.4	33.8	31.6	35.1	32.2	32.4	32.0	32.6	32.0	1.67
Urine, Mcal/d	2.46	2.50	2.53	2.40	2.38	2.27	2.47	2.39	2.39	2.30	2.34	2.21	2.42	2.20	2.24	0.109
CH ₄ , Mcal/d	4.17	4.26	4.06	3.81	3.73	3.67	4.24	3.72	3.72	3.78	3.67	3.50	3.56	3.21	3.48	0.207
Heat, Mcal/d	26.4	24.6	25.1	24.9	24.3	25.0	25.7	24.6	24.9	24.2	24.6	25.2	24.0	24.0	25.3	0.89
Milk, Mcal/d	23.8	23.6	23.6	25.0	24.0	25.0	24.8	24.8	23.4	24.5	23.0	23.1	22.9	24.9	25.1	1.05
TE, Mcal/d	-2.20	-1.77	-0.45	-1.33	1.14	0.10	0.28	-0.52	-1.20	1.62	0.05	-0.22	-0.16	-1.91	-2.22	1.68
ME/DE	0.876	0.871	0.880	0.887	0.891	0.895	0.881	0.889	0.884	0.889	0.887	0.892	0.886	0.896	0.891	0.005
NE _L /ME	0.648	0.676	0.676	0.685	0.694	0.683	0.674	0.690	0.674	0.698	0.678	0.666	0.683	0.692	0.676	0.012
O ₂ consumption, L	5263	4893	4981	4917	4826	4991	5130	4920	4937	4812	4913	5027	4780	4783	5053	168
CO ₂ production, L	5565	5238	5268	5248	5134	5212	5374	5153	5217	5048	5105	5207	5019	4963	5195	185
CH ₄ production, L	440	450	428	402	393	386	447	391	392	398	387	369	376	338	367	22
RQ, L/L	1.055	1.075	1.059	1.069	1.060	1.040	1.046	1.048	1.058	1.046	1.042	1.050	1.048	1.039	1.030	0.011

¹GE = gross energy, DE = digestible energy, ME = metabolizable energy, TE = tissue energy, RQ = respiratory quotient (CO_2 production/ O_2 consumption).

Supplemental Table 8.5. Least squares means for N utilization during 4-d collection period for cows fed diets with differing dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM) and starch contents (20.2, 21.4, 25.7, 30.0, and 31.3% of DM) and supplemental digestible Lys (sdLYS; 0, 2.0, 8.9, 15.8, and 17.8 g/d)

FA, %	3.0		3.4				4.6				5.9				6.2		SEM
Starch, %	25.7	21.4		30.0		25.7	20.2	25.7	31.3	25.7	21.4	30.0	21.4	30.0	25.7		
sdLys, g/d	8.9	2.0	15.8	2.0	15.8	0.00	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8	8.9		
Item																	
g/d																	
Intake	516	516	525	498	506	513	525	505	479	533	501	465	504	467	489	21.2	
Fecal	173	180	174	175	185	183	188	167	161	163	163	155	162	154	167	8.5	
Urinary	180	174	168	145	154	137	182	154	141	143	156	136	157	133	135	11.4	
Milk	176	170	175	184	170	172	169	173	176	175	162	165	164	173	176	7.3	
Balance	-11	-1	6	-9	-9	14	-16	4	10	53	25	11	23	13	22	15	
% of N intake																	
Fecal	34.2	35.0	33.3	35.1	36.2	35.4	35.9	33.3	33.1	30.9	32.8	33.4	32.3	32.6	33.9	1.14	
Urinary	34.5	34.0	32.5	29.2	31.5	27.3	34.7	31.2	29.2	26.4	30.9	29.1	31.1	27.5	26.9	2.07	
Milk	34.0	33.0	33.3	37.2	34.1	33.8	32.4	34.6	36.3	33.0	31.7	35.4	32.7	37.1	35.5	1.13	
Balance	-2.4	-1.6	0.5	-1.9	-2.1	2.7	-2.9	0.6	1.8	9.2	5.0	2.4	4.4	2.5	4.3	2.9	
MUN, mg/dL	14.7	14.8	15.4	12.8	14.0	13.9	16.1	14.4	13.3	14.1	13.9	13.3	14.7	12.8	14.1	1.08	
Urea, mg/dL	31.9	35.9	34.9	29.9	33.1	34.1	34.0	33.1	28.8	31.7	33.3	27.1	33.7	29.8	30.7	1.93	

Supplemental Table 8.6. Least squares means for plasma concentration of AA and 3-methyl-His for cows fed diets with differing dietary FA (3.0, 3.4, 4.6, 5.9, and 6.2% of DM) and starch contents (20.2, 21.4, 25.7, 30.0, and 31.3% of DM) and supplemental digestible Lys (sdLYS; 0, 2.0, 8.9, 15.8, and 17.8 g/d))

FA, %	3.0		3.4				4.6				5.9		6.2		SEM	
Starch, %	25.7	21.4	30.0		25.7	20.2	25.7	31.3	25.7	21.4	30.0	21.4	30.0	25.7		
sdLys, g/d	8.9	2.0	15.8	2.0	15.8	0.00	8.9	8.9	8.9	17.8	2.0	2.0	15.8	15.8		8.9
Item																
EAA, µM	944	957	1098	915	977	958	1046	930	911	992	1004	1032	979	853	969	75.9
Arg	71.6	65.8	85.7	68.5	75.7	73.1	78.0	71.0	71.1	78.9	81.3	81.0	80.8	64.4	79.8	7.19
His	45.0	46.1	56.3	47.1	47.4	44.0	47.5	46.7	44.6	42.9	43.9	41.6	49.2	41.5	42.9	5.43
Ile	117	127	152	118	129	128	144	124	116	137	143	132	131	109	122	11.5
Leu	145	151	170	142	155	149	170	143	145	151	151	160	139	130	144	14.2
Lys	89.7	83.1	104.5	77.9	92.1	88.5	92.2	82.2	80.5	90.1	95.5	98.4	93.8	76.2	93.2	7.80
Met	23.1	21.4	24.2	22.2	23.6	23.4	21.6	22.3	22.3	25.7	26.0	27.0	23.2	21.6	25.9	1.89
Phe	50.7	50.4	53.1	45.0	48.5	50.0	52.9	46.9	49.5	53.1	49.7	52.4	46.9	44.8	49.8	3.93
Thr	114	106	116	115	114	115	107	110	103	120	110	132	118	107	119	10.8
Trp	42.1	38.0	40.7	35.8	37.3	36.4	38.2	37.0	37.3	38.8	39.1	40.9	38.6	36.1	37.6	5.07
Val	239	267	293	245	258	255	293	248	235	253	263	266	256	219	249	19.6
NEAA, µM	1194	1104	1165	1227	1188	1225	1156	1189	1183	1284	1205	1302	1227	1218	1268	74.3
Ala	288	246	275	255	249	269	258	262	250	277	276	320	292	264	315	24.1
Asn	48.7	46.4	54.8	50.1	48.7	49.9	49.1	48.6	46.4	56.4	52.0	54.4	50.7	44.4	50.8	5.14
Asp	4.0	0.8	2.6	2.9	1.2	1.4	2.9	2.2	2.3	1.6	2.8	2.1	2.4	3.6	0.7	0.89
Gln	202	213	200	208	208	201	189	195	196	199	183	182	193	175	185	14.7
Glu	40.7	41.6	39.8	46.0	48.9	41.4	42.6	43.7	44.3	44.2	49.5	48.8	46.5	44.0	41.9	4.01
Gly	347	304	324	388	361	379	343	372	378	412	369	413	390	429	400	32.5
Pro	80.2	73.5	80.9	83.1	80.0	76.5	78.0	77.0	72.1	77.7	79.4	90.2	83.5	76.0	90.5	8.20
Ser	94.8	85.7	90.1	99.7	96.5	86.0	90.8	91.4	96.2	97.6	91.3	101.1	89.1	88.9	98.5	7.45
Tau	47.2	43.9	40.1	51.9	44.1	49.8	44.1	45.9	47.2	50.2	45.8	52.2	43.0	45.3	50.1	4.97

Tyr	51.4	50.8	57.6	44.7	47.5	53.3	52.1	46.2	49.9	56.3	55.0	54.9	49.8	44.8	50.5	4.48
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Supplemental Table 8.7. Effects of varying dietary concentration of FA (F) and starch (S) and Lys (L) supply on plasma essential AA and Lys concentration¹

Variable	Intercept	F	S	L	S×S	F×L	S×L	Error ²	
								RMSE	Cow
Arg, µM	31.1 (19.1)	6.96 (1.9)	0.373 (0.59)	6.14 (1.8)		-0.621 (0.18)	-0.118 (0.053)	9.14	9.32
His, µM	64.7 (8.0)	-1.56 (0.80)	-0.450 (0.23)					6.81	10.6
Ile, µM	143 (20.6)	8.25 (2.96)	-2.07 (0.48)	4.52 (1.33)		-0.965 (0.28)		14.4	16.5
Leu, µM	153 (24)	6.01 (3.40)	-1.20 (0.55)	4.47 (1.5)		-1.01 (0.32)		16.3	25.0
Met, µM	13.5 (3.0)	2.19 (0.59)		0.771 (0.27)		-0.170 (0.055)		3.08	1.77
Phe, µM	47.4 (6.5)	1.79 (0.89)	-0.254 (0.14)	1.10 (0.41)		-0.241 (0.084)		4.33	5.00
Thr, µM	73.9 (23)	1.55 (0.87)		3.90 (2.2)			-0.152 (0.082)	14.8	12.7
Val, µM	610 (180)	6.83 (5.0)	-26.8 (14)	5.11 (2.25)	0.455 (0.28)	-1.16 (0.46)		23.8	41.3

¹The model included the linear and quadratic effects of FA, starch, and Lys and all 2-way interaction. Final models included only effects that were significant ($P > 0.10$). Values in parentheses are SE of parameters estimates.

²RMSE = root mean square error; Cow = square root of the variance due to cows. When applied to a group of cows, the prediction error = [RMSE + (Cow error/ \sqrt{n})], where n = number of cows in the group to which the equations are applied (Weiss et al., 2009a).

CHAPTER 9: GENERAL SUMMARY AND CONCLUSIONS

The overarching objectives of this dissertation was to improve our understanding of energy metabolism in lactating Jersey cows as well as to understand interrelated factors affecting energy and N utilization. This is important because economic sustainability of the dairy industry is dependent on the efficiency at which dietary energy and N are converted in milk energy and N. Understanding the effects of dietary factors on energy and N utilization in dairy cows should allow for dietary formulation to improve economic and environmental sustainability of the dairy industry.

Energy is the most limiting nutrient for milk production of cows in early lactation. Most current dairy nutrition models assume a near constant efficiency of converting ME into NE_L and DE to ME, although these efficiencies are reflective of the loss of energy heat increment and gas and urine, respectively. These models can be improved by accounting for known source of variation in the conversion of ME into NE_L and DE into ME. Maintenance energy represents 7–10% of total energy intake by a lactating dairy cow and evidence exists that maintenance energy requirements have increased in lactating cows. However, maintenance energy requirements are not well understood in Jersey cows. Therefore, the maintenance energy requirements of Jersey cows were derived on lactating cows, dry cows fed at maintenance, dry fasted cows. Currently, the factors affecting whole-animal heat production and urinary energy loss are also not well understood. Therefore, objectives regarding factors affecting energy loss by dairy cows were explored by quantifying how heat production is affected by dry matter intake, yield of milk components, nutrient digestibility and urinary N excretion. Additionally, to improve our understanding of urinary energy loss, we studied the relationship between

urinary N excretion and urinary energy. Finally, it is well established that dietary manipulation can affect energy and N utilization. Dietary NEL density is often increased by increasing dietary starch or fatty acids and rumen-protected AA are often fed to increase supply of potentially limiting AA. The objectives of this dissertation were additionally met by first looking at how energy and N utilization is affected by: supply of two key essential amino acids (AA), Lys and His; diets formulated to be isoenergetic and high in starch or fat; and varying dietary fat and starch as well as supply of digestible lysine.

In the first experiment of this dissertation, maintenance energy requirements (NE_M) and efficiency of converting ME into NE_L (i.e., k_L) in Jersey cows was evaluated. From this dataset, fasting heat production and NE_M estimated via regression for both lactating and dry cows were similar and averaged 0.102 Mcal/unit of metabolic body weight, which is greater than the value of 0.080 used by NRC, 2001. The k_L when using data from lactating and dry cows averaged 0.679. However, when analyzed as separate regressions, k_L was greater for dry cows compared to lactating cows (0.721 vs. 0.683) and compared to NRC, 2001 (~ 0.63), which suggests that dry cows may be more efficient than lactating cows at converting ME into net energy. Although NE_M may be greater for modern lactating dairy cows, increased k_L compared to previous research (0.679 vs. 0.63) mitigates the effect of increased NE_M on energy available for milk and tissue production.

Factors affecting heat production were explored in the second experiment. Heat production averaged $28.1 \pm 3.7\%$ of gross energy intake. Variation in heat production was most effectively and simply explained by accounting for metabolic BW and DMI. Including variables for milk component production, nutrient digestibility and urinary N

excretion instead of DMI did not improve model performance and this suggests that these variables explained similar variation in heat production compared to DMI. Interestingly, heat production associated with milk protein yield or protein digestibility was nearly two-fold that associated with milk fat yield or starch digestibility, respectively. Additionally, heat production associated with urinary N excretion was 5.32 ± 2.42 Mcal/kg or 1.0 Mcal/d. These results clearly show that heat production associated with protein digestion, metabolism and protein synthesis is greater than fat or carbohydrate digestion and metabolism or milk fat synthesis.

Next, the relationship between urinary N and energy excretion was examined. Urinary energy excretion increased quadratically as urinary N excretion increased. The quadratic relationship occurred such that rate of increase in urinary energy diminished with increasing urinary N excretion. This relationship likely occurs because the non-urea N compounds in urine have a greater enthalpy compared to urea (24.8 vs. 5.4 Mcal/kg N), which increases in proportion as urinary N excretion increases. Because dietary CP is positively correlated with urinary N excretion, overfeeding dietary CP will lead to increased urinary energy, which can affect NE_L supply.

In the fourth experiment, cows were supplemented with no AA, rumen-protected Lys (24 g/d of digestible Lys), rumen-protected His (7 g/d of digestible His) or both. Milk protein yield was only increased when His supply was increased. When Lys supply was increased, N balance increased and 3-methylhistidine, a biomarker for protein turnover, decreased, which suggests that cows supplemented with rumen-protected Lys used more N for body protein synthesis. However, energy utilization was not affected by increasing supply of Lys or His.

Cows were fed diets formulated to be isoenergetic and either high in starch (30.8% starch and 1.9% fatty acids % of DM) or high in fat (16.8% starch and 4.1% fatty acids). Feeding the high-starch diet increased milk protein yield, milk N secretion, dietary NE_L content, and tissue energy deposition as fat, and decreased urinary N secretion. These results suggest that diets with increased starch content may increase efficiency of utilizing dietary N for milk N and dietary energy for milk and tissue energy. The NE_L content of the high starch diet was greater than predicted by the NRC, 2001 model which occurred primarily due to an underestimation of efficiency of converting ME into NE_L. Additionally, measured NE_L content was greater for the high-starch diet compared to the high-fat diet which explain observed differences in milk and tissue energy.

Finally, dietary fatty acid and starch content and supply of supplemental rumen-protected Lys were varied independently. Five amounts of each factor were fed, and a central composition arrangement of treatments was used. An interaction was observed between dietary starch and supplemental dLys for milk protein concentration. This occurred such that with increasing supplemental dLys milk protein concentration increased when dietary starch was less than 24% of DM and decreased when dietary starch was greater than 28%. Increasing dietary starch linearly increased the efficiency of converting dietary N into milk N. As supplemental dLys increased from 0 to 8 g/d, N balance quadratically decreased by 13 g/d and increased thereafter by 31 g/d. Increasing dietary fatty acid content linearly decreased DMI and increased dietary ME when fatty acids were increased up to 5.2% after which a plateau was reached. As dietary fatty acid increased, ME supply increased up to 4.2% dietary fatty acids and decreased thereafter. Results from this study demonstrate that milk protein synthesis is both an energy and AA

dependent process. However, increased energy from starch may allow for increased response to supplemental Lys compared to increased energy from fatty acids. Additionally, with increasing supplemental dLys, some Lys and other AA may be preferentially diverted towards muscle protein at the expense of milk protein.

Future work should aim to better understand the efficiency of converting ME into NE_L by estimating the partial efficiency for milk fat, protein, and lactose synthesis as well as for maintenance. Emerging work suggest that milk protein synthesis is responsive to supply of AA beside the commonly study potentially limiting AA; Met, Lys, and His. These AA included Arg, Ile, Leu, and Thr. Additional research is needed to quantify the interactions between energy source and supply and other essential AA as well as MP supply. In the current work, because dietary starch was fed to cows, it is impossible to know if response in milk protein were due to rumen or post-absorptive metabolism. Because high producing dairy cows have large metabolic demand for glucose, it is plausible that glucose is limiting and increasing glucose supply may increase the marginal efficiency of utilizing AA for milk protein synthesis. Future work could utilize emerging rumen-protected glucose sources to better understand pre- and post-absorptive responses to manipulating dietary starch content.

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APPENDIX A: ENERGY BALANCE EXPERIMENT STANDARD OPERATING PROCEDURES AND SYSTEM MAINTENANCE

GAS ANALYSIS

1. Calibration
 - a. Allow 2 minutes on all tanks before calibration, ensure pressure = 15 PSI
 - i. N₂ tank
 - ii. Zero CO₂ and CH₄
 - b. Mixed tank
 - i. Zero O₂
 - ii. Span CO₂ and CH₄
 - c. Air tank
 - i. Span O₂
2. Analysis
 - a. Open new gas analysis sheet from templates folder
 - b. Open dairy lab program called "Gas bag Analysis"
 - c. Ensure pressure = 15 PSI throughout
 - d. Read tanks in order (N₂, Mixed, and Air) recording values at the end of each measurement period
 - e. Read gas bag starting with Cal bag followed by Air bag
 - f. Read gas bag in the reverse order
 - g. Read tanks in reverse order

GAS ANALYSIS SYSTEM—NEW CALIBRATION TANK

1. Calibration tanks
 - a. **Always make sure to have a backup of each tank on hand with calibration values**
 - b. N₂ tank
 - i. Matheson Eshop part # NI80
 - c. Mixed tank
 - i. Order from airgas (matheson can only get fat tanks)
 - ii. Order primary standard with 850PPM CH₄, 8500PPM CO₂, 20.05% O₂, balance N₂, price ~ \$362.00
 - iii. Will take several weeks to make
 - iv. Measure gas values (see #2)
 - d. High O₂ tank
 - i. Matheson eshop part # CA80
 - ii. Measure O₂ values (see #2)
2. Calibration tank values—**MUST DO THIS BEFORE THE TANK IS NEEDED**
 - a. Not needed for N₂ tank-assume values of 0.
 - b. Label tanks with type (Mixed, O₂) and a id #
 - c. Run a full gas analysis (calibration, standard values), but instead of bags run standard tanks
 - i. Values will have to be moved from current standard tanks to new tanks and back
 - d. Record corrected average value (column E in the gas analysis spreadsheet) in an excel sheet labeled “standard tank values”
 - e. Repeat at least 3 times and ensure that SD is low. Run more if necessary and remove outliers
3. Using new tanks
 - a. Update standard values in gas analyzer
 - i. **Setup → Calibration → Gases → Select desired component and update zero and span value**
 - b. Update calibration values in both the gas analysis and lamb run spreadsheet template
 - i. In the gas analysis tab, change values in column E to match new mixed tank and/or new high O₂ tank value.
 - c. Check the daisylab correction factors
 - i. Use the program labeled “Equation Calibration”
 - ii. Check to make the values in “list00” match up with the values on daisylab
 - iii. If they match, no change is needed
 - iv. If they are off, the regressions in dairy lab for both the “continuous” and “gas bags” needed updated
 1. To update, do to the daisylab data folder (where the continuous program writes data too)
 2. Open Calabration Equations

3. Copy tab and relabel with current date
4. Update gas concentrate value to match new calibration
5. Update voltage value to in DaisyLab “list01” in the “Equation Calibration” program
6. Must do this for each calibration tank
7. Take the regression coefficients listed in each graph and put them into Daisylab
 - a. Double click the “Scaling00” module and update the “a” and “b” value with the new regression coefficients
 - b. The lightening bolts labeled 0, 1, and 2 represent channels 1, 2, and 3, respectively
8. Repeat step c to make sure scaling is correct.

MASS FLOW METERS SOP

1. New flow meter
 - a. Change standard temperature in flow meter to 0°C
 - i. Menu → Basic Config → stp/ntp → change Stan T value
 - b. Update pocket logger program
 - i. Add channel 2 for mass flow
 - ii. Use calibration sheet and add value for high flow number
2. Alicat suggests calibration every year
 - a. Use lamp runs to check,
 - b. If off, may need to send in for calibration

BOMB CALORIMETRY

1. Start up and running samples
 - a. Turn on bomb
 - i. Flip power switch
 - ii. Turn on heater (will take 30-45 minutes to warm up)
 - iii. Open O₂ and CO₂ tanks
 - b. Once bomb is warmed run a pre-test
 - c. Run a benzoic acid standard
 - i. Make sure value is 6318 ± 18 kcal/g
 - ii. If not, run another sample
 - iii. If the second standard is off, machine may need recalibrated
 - d. Run pre-weighed samples
 - i. Enter sample weight and spike (oil) weight
 1. When enter sample weight for urine an error message will pop-up, this can be ignored
 - ii. Allow ~ 10 minutes per capsule
 - iii. Record energy value
 - e. If a wrong sample weight or spike weight was entered, update values
 - i. Hit report -> select from list -> find sample -> update incorrect number
 - ii. Record recalculated energy value
2. Feeds, feces, refusal
 - a. Weigh 0.2 g of sample into clean dry bomb capsule, record
 - b. Add 0.4 g of oil to sample, record
 - c. Allow 2+ hours for oil to soak into sample
 - d. If sample is not completely saturated add more oil, record
3. Urine
 - a. Weigh 4.0 g of sample into clean dry bomb capsule
 - b. Place samples on metal pan with tape
 - c. Put sample in the oven in Ruminant set at 60°C (150° F)
 - d. After 4 hours start checking samples every 30 minutes
 - e. Once samples are just dry remove them
 - i. May have to remove some samples before other
 - ii. Over drying samples may cause energy loss
 - f. Add 0.4 g oil to dry samples

APPENDIX B: R CODE FOR OPTIMIZATION OF THE EXPERIMENTAL LAYOUT FOR CHAPTER 8:

```

library(data.table)

Blk = c(1,2,3)
Period = 1:4
Row = 1:8
layout.df <- expand.grid("Period" = Period, "Blk" = Blk, "Row" = Row)
layout.df <- layout.df[order(layout.df$Blk),]
cow <- data.frame("cow" = rep(1:24, times = 4))
cow <- cow[order(cow),]
n <- 1:96
cow.df <- cbind(layout.df, cow, n)
#Create treatment levels data frame
trt <- 1:16
x1 <- c(1,1,-1,-1,0,1,1,-1,-1,0,-1.2871,1.2871,0,0,0,0)
x2 <- c(1,-1,1,-1,0,1,-1,1,-1,0,0,0,-1.2871,1.2871,0,0)
x3 <- c(1,-1,-1,1,0,-1,1,1,-1,0,0,0,0,0,-1.2871,1.2871)
Rep <- 1:6

trt.df <- data.frame(n, trt)
factor.df <- data.frame(trt, x1, x2, x3)
cow.trt.df <- cbind(cow.df, trt.df)

#Penalty Function
Penalty.fun3 <- function(data.input, type = "a"){
  library(data.table)
  n <- data.frame("n" = 1:96)
  c <- data.frame("trt" = floor(data.input))
  e <- cbind(n, c)
  a <- merge(e, factor.df, by="trt")
  b <- merge(cow.df, a, by="n")
  dt <- data.table(b)

  #Each trt must appear 6 times, penalty of 25 points for each time
  this fails
  rep <- dt[, .N, by=trt]
  rep$rep.pen <- abs(6-rep$N)*25
  Trt.rep.pen <- sum(rep$rep.pen)

  #Rule 1: Each treatment must appear twice in each block.
  #gives count of the number of trt in a block.
  dt[, R1 := .N, by = list(Blk, trt)]
  R1.val <- dt[R1 != 2, .N] * 10

  #Rule 2: Each treatment is followed by another treatment only once.
  #function to create combination of sequential trt within a cow
  trt.combo.fun <- function(df) {
    df[order(cow, Period)]
    for (j in seq_along(1:4)){
      if (j == 1){
        df$trt.seq <- NA
      } else {
        df$trt.seq[j] <- paste(df$trt[j], df$trt[j-1], sep="-")
      }
    }
  }
}

```

```

    }
    return(df)
  }
  out<-by(dt, dt$cow, trt.combo.fun)
  dt <- do.call(rbind,out)

  #Penalty = 100 if a treatment within a cow is repeated
  dt[,R2 := uniqueN(trt.seq,na.rm = T)]
  R2.val <- ((72-(max(dt[, "R2"]))) * 2)

  #Rule 3: Each block is to contain 4 central point treatment (i.e.,
  treatment 5 and 10)
  #count # of center points in each blk
  dt[trt %in% c(5,10), R3 := .N, by =Blk]
  R3.val <- dt[R3!=4, .N]*10

  #Rule 4: A cow can receive no more than 1 central point treatment.
  #count # of center point for a cow
  dt[trt %in% c(5,10), R4 := .N, by =cow]

  R4.val <- dt[R4!=1, .N]*20

  #Rule 5: A cow cannot receive the same treatment twice.

  #count # of unique trt for a cow
  dt[, R5 := uniqueN(trt), by =cow]

  R5.val <- dt[R5!=4, .N]*10

  #Rule 6: Treatment sequences should minimize the frequency when 1 or
  more of the 3 factors
  #is either below or above the center point in all 4 periods within a
  block.
  #count # of levels above or below 0 of trt for a cow for each factor
  dt[x1 < 0, R6.x1.bel := .N, by = cow]
  dt[x1 > 0, R6.x1.gre := .N, by = cow]
  dt[x2 < 0, R6.x2.bel := .N, by = cow]
  dt[x2 > 0, R6.x2.gre := .N, by = cow]
  dt[x3 < 0, R6.x3.bel := .N, by = cow]
  dt[x3 > 0, R6.x3.gre := .N, by = cow]

  #penalty = 100 if N of level > or < 0 is 4 for any factor is 1
  if(max(dt[,c("R6.x1.bel", "R6.x1.gre", "R6.x2.bel", "R6.x2.gre",
  "R6.x3.bel", "R6.x3.gre")],
    na.rm = T) == 4) {
    R6.pen <- 100
  } else {
    R6.pen <- 0
  }

  #Rule 7: There must be no sequence in which a factor is at the same
  level across 3 periods within a block.
  #count # of unique levels of trt for a cow for each factor
  dt[, R7.x1 := uniqueN(x1), by =cow]
  dt[, R7.x2 := uniqueN(x2), by =cow]

```



```

dt[, R7.x3 := uniqueN(x3), by = cow]
R7.val<-sum(dt[R7.x1==1, .N], dt[R7.x2==1, .N], dt[R7.x3==1, .N])*10

#Rule 7: penalnty for trt combinations that occur more than 3 times
together.
trt.comb.per<-function(df) {
  d<-df
  e <- t(combn(d$trt, 2))
  d2<-data.table(c(paste(e[,1], e[,2], sep="-"), paste(e[,1],
e[,2], sep="-")))
}
R8.dt<-dt[,trt.comb.per(c(.BY, .SD)), by = .(Blk, Period)]
R8.dt[, R8 := .N, by = V1]
R8.dt$R8.pen <- ifelse(R8.dt[,R8] != c(4,6), ifelse(R8.dt[,R8] > 6,
R8.dt$R8*0.4, (6-R8.dt$R8)*0.8), 0)
R8.val <- sum(R8.dt$R8.pen)

#Create a penalty output table
var.pen<-(1-length(c$trt)/96)*100
unique.pen<-(16-length(unique(c$trt)))/16*100
pen.all <- sum(R1.val, R2.val, R3.val, R4.val, R5.val, R6.pen,
R7.val, R8.val, var.pen, unique.pen, Trt.rep.pen)
out <- c(pen.all, R1.val, R2.val, R3.val, R4.val, R5.val, R6.pen,
R7.val, R8.val, var.pen, unique.pen, Trt.rep.pen)
names(out) = c("all", "R1", "R2", "R3", "R4", "R5", "R6", "R7", "R8",
"var length", "Trt count", "Trt Rep")

if (type == "individual"){
  return(out)
} else if (type == "dt"){
  return(dt)
} else {
  return(pen.all)
}
}

#Genetic Algorithm to find optimal design
library(rgenoud)
val.limits<-matrix(c(1,16), nrow = 96, ncol = 2, byrow = T)
GA.OPT <- function(rep){
  out<-vector("list", rep)
  for(i in 1:rep){
    d <- genoud(Penalty.fun3, 96, data.type.int = T, Domains =
val.limits, pop.size = 10000,
wait.generations = 5, boundary.enforcement = 2,
solution.tolerance = 1, hard.generation.limit = T,
max.generations = 30)
    out[[i]] <- d$par
  }
  return(out)
}

#Run repeated optimization
system.time(solution1<-GA.OPT(3))

#Determine penalty of each solution

```

```

lapply(solution1, Penalty.fun3)

Penalty.fun3(solution1[[2]], type = "individual")
k<-Penalty.fun3(solution1[[2]], type = "dt")

write.csv(k,"C:/Users/dmorris11/Box Sync/Sync/Research/1901 - Energy x
Lys x E Source/opt.out 1 (836.8).csv")

solution.final<-
c(10,13,6,11,8,14,9,1,16,15,2,10,6,12,15,14,4,5,3,16,12,11,1,4,13,7,8,3
,2,9,5,7,

2,10,6,9,10,7,13,4,13,6,3,5,3,9,8,1,14,8,5,16,15,4,12,2,1,16,14,11,7,15
,11,12,

14,1,7,3,4,12,15,5,5,16,4,6,8,6,2,1,3,10,9,2,7,11,12,13,15,14,10,9,13,8
,11,16)
Penalty.fun3(solution.final, type = "individual")
solution.final.dt<-Penalty.fun3(solution.final, type = "dt")

#Quantify the number of occurrence of each treatment at the same time
trt.comb.per<-function(df) {
  d<-df
  e <- t(combn(d$trt, 2))
  d2<-data.table(c(paste(e[,1], e[,2], sep="-"),paste(e[,1],
e[,2],sep="-")))
}

tmp<-solution.final.dt[,trt.comb.per(c(.BY, .SD)), by = .(Blk, Period)]
tmp[, R8 := .N, by = V1]
tmp[, .N, by = R8]
solution.final.dt[, .N, by = trt]

library(crossdes)

solution.m1<-
matrix(c(10,13,6,11,8,14,9,1,16,15,2,10,6,12,15,14,4,5,3,16,12,11,1,4,1
3,7,8,3,2,9,5,7), ncol = 8)
solution.m2<-
matrix(c(2,10,6,9,10,7,13,4,13,6,3,5,3,9,8,1,14,8,5,16,15,4,12,2,1,16,1
4,11,7,15,11,12), ncol = 8)
solution.m3<-
matrix(c(14,1,7,3,4,12,15,5,5,16,4,6,7,6,2,1,3,10,9,2,8,11,12,13,15,14,
10,9,13,8,11,16), ncol = 8)
solution.m<-rbind(solution.m1,solution.m2,solution.m3)

out <- isGYD(solution.m, tables = T)
v<-out$`Concurrence w.r.t. rows`

o1<-isGYD(solution.m1, tables = T)$`Concurrence w.r.t. columns`
o2<-isGYD(solution.m2, tables = T)$`Concurrence w.r.t. columns`
o3<-isGYD(solution.m3, tables = T)$`Concurrence w.r.t. columns`
o.all<- o1+o2+o3

```

APPENDIX C: CALCULATION TO EXPERIMENTALLY ESTIMATE NEL

$DE = GE - \text{fecal energy}$

$ME = DE - \text{gas energy} - \text{urine energy}$

$TE = ME - \text{heat energy}$

$TE \text{ (Mcal of } NE_L/d) = \text{positive } TE \times k_L/k_G \text{ or negative } TE \times k_T$

$\text{Milk energy} = 9.29 \times \text{milk fat yield} + 5.71 \times \text{milk protein yield, and } 3.95 \times \text{milk lactose yield}$

$NE_L = \text{milk energy (} NE_L) + \text{maintenance energy (} NE_L) + TE (NE_L)$

Where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain, and k_L is the efficiency of utilizing ME intake for milk production. Values of 0.89, 0.75, and 0.66 were used for k_T , k_G , and k_L , respectively (Moraes et al., 2015). Maintenance energy is generally estimated as a coefficient times metabolic BW (BW raised to the 0.75 power). In NRC, 2001, the coefficient for maintenance is 0.080 Mcal per kg of metabolic BW.

APPENDIX D: POSTERS AND PRESENTATIONS

American Dairy Science Association Poster 2019 (CHAPTER 6:)

Effects of rumen-protected lysine and histidine on performance and energy and nitrogen partitioning in high hydrolyzed feather meal diets fed to Jersey cows

D. L. Morris and P. J. Kononoff

Department of Animal Science, University of Nebraska-Lincoln



INTRODUCTION

- Lys and His are commonly limiting AA in dairy cows
- Hydrolyzed feather meal (HFM) is:
 - High in CP (87–95%)¹
 - High in bypass CP (66–86% RUP)¹
 - Low in Lys (2.57% of CP)²
 - Low in His (1.15% of CP)²
- Increasing inclusion of HFM to 10% of diet DM decreased milk protein³

OBJECTIVES

- Determine effects of supplementing rumen-protected (RP) Lys and His on milk production and energy and N partitioning in lactating dairy cows fed HFM

MATERIALS and METHODS

- 12 multiparous Jersey cows (91 ± 18 DIM, 451 ± 44 kg BW)
- Triplicated 4 × 4 Latin square with 28 d periods
- 2 × 2 factorial treatment arrangement
 - 0 g/d of RP Lys (LYS0)
 - 70 g/d RP Lys (Ajipro-L; 23 g/d dLys; LYS+)
 - 0 g/d of RP His (HIS0)
 - 32 g/d of RP His (Balchem; 8 g/d dHis; HIS+)
- Measured:
 - DMI, milk production and components (4 d)
 - Urine and fecal output (4 d total collection)
 - O₂ consumption, CO₂ and CH₄ production (1 d headboxes-type indirect calorimeter)
 - Blood plasma AA
- Data analyzed with Imer function in R
- Fixed effects: Lys and His level and interaction
- Random effects: period, square, and cow nested in square

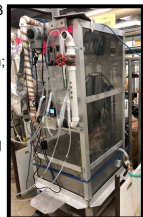


Table 1. Ingredient and chemical composition of the base TMR feed to all experimental cows (% DM)

Item	Base
Ingredient	
Corn Silage	39.0
Alfalfa hay	16.0
Hydrolyzed feather meal	5.0
Grain mix ¹	40.0
Composition (mean ± SD)	
DM	57.6 ± 2.5
CP	17.1 ± 0.42
NDF	32.4 ± 0.72
Starch	26.3 ± 1.20
Crude fat	5.3 ± 0.63

¹Supplied (% of the total diet) 17.5% ground corn, 9.3% soyhulls, 1.7% soybean meal, 3.0% molasses, 2.5% whey, 2.5% prilled fat, 0.55% urea, 0.09% RP Met, 2.86% mineral-vitamin mix.

Table 2. Chemical composition of hydrolyzed feather meal (% DM)¹

Item	Hydrolyzed feather meal
DM	91.5 ± 0.32
CP	89.7 ± 2.20
Crude fat	9.0 ± 1.64
Ca	0.50 ± 0.24
P	0.28 ± 0.13
S	2.38 ± 0.27

¹Three source (American Proteins Inc., Cumming, GA; Pilgrim's, Greeley, CO; Simmons Foods, Siloam Springs, AR)

Table 3. Estimated MP and AA balance in dairy cows supplemented with RP Lys and His¹

Item ²	LYS0	LYS+	HIS0	HIS+
MP supply	1958	1958	1946	2000
MP balance	136	102	137	115
dLys supply ³	118	118	140	140
dLys balance ⁴	-2	-4	21	19
dHis supply ³	38	46	38	47
dHis balance ⁴	-2	5	-2	6

¹LYS0 = 0 g/d RP Lys, LYS+ = 70 g/d RP Lys (Ajipro-L), HIS0 = 0 g/d RP His, HIS+ = 32 g/d RP His (Balchem)

²Estimated with NRC, 2001 using actual intake and using manufacturer specifications and mobile bag for dAA supply.

³Includes dietary, microbial and 23 g of dLys or 8 g of dHis

⁴Recommendations for Lys and His were calculated as 6.6 % and 2.2 % of MP, respectively

RESULTS

Table 4. DMI, milk production and composition of lactating Jersey cows fed a diet containing 5% feather meal and supplemented with RP Lys and His¹

Item	LYS0	LYS+	HIS0	HIS+	SEM	P-Value ²
DMI, kg	18.4	18.4	18.3	18.8	0.83	0.64
Milk yield, kg	21.4	22.2	21.7	22.7	2.04	0.36
ECM, kg	29.5	29.4	29.2	30.5	2.16	0.55
ECM/DMI	1.61	1.61	1.60	1.62	0.067	0.93
Fat, %	6.13	5.75	5.85	5.75	0.359	0.31
Fat, kg	1.28	1.25	1.26	1.30	0.094	0.64
Protein, %	3.65	3.62	3.57	3.59	0.135	0.14
Protein, kg	0.775	0.789	0.768	0.812	0.051	0.66
MUN, mg/dL	17.2	16.1	17.1	16.5	0.76	0.59

¹LYS0 = 0 g/d RP Lys, LYS+ = 70 g/d RP Lys (Ajipro-L), HIS0 = 0 g/d RP His, HIS+ = 32 g/d RP His (Balchem)

²L = Lys, H = His, no L × H interaction (P > 0.29)

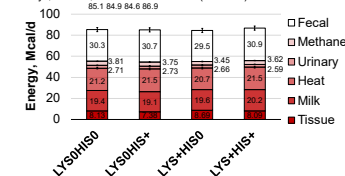


Figure 1. Effects of supplementing RP Lys and His on energy partitioning. For methane, Lys P = 0.05, all other effects P > 0.27.

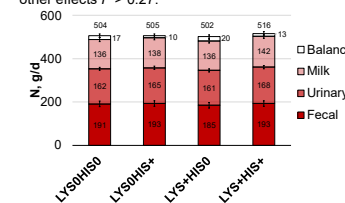


Figure 2. Effects of supplementing RP Lys and His on N partitioning. No effects observed (P > 0.16).

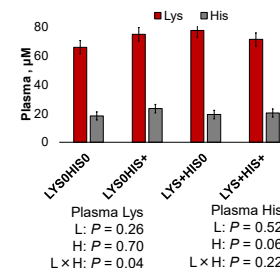


Figure 3. Effects of supplementing RP Lys and His on plasma Lys and His.

CONCLUSIONS

- RP Lys in a HFM diet had no effect on DMI, milk production, N and energy utilization
- Supplementing RP His to a HFM diet increased:
 - milk yield
 - milk protein yield
 - decreased MUN
 - plasma His
- Lys supply from HFM may be adequate or Lys supply might not have been adequate
- His may be a potentially limiting AA in HFM

REFERENCES

- Buse, Morris, and Kononoff 2019. JDS Abstr. M14
- NRC, 2001.
- Judy and Kononoff, 2018. JDS Abstr. 321.
- Lee et al., 2012. JDS 95:6042.

Acknowledgments



American Dairy Science Association 2019 Presentation (CHAPTER 7:)

The effects of isoenergetic high-starch or high-fat diets on energy and nitrogen partitioning and utilization in late-lactation Jersey cows

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²Department of Biological Systems Engineering, University of Nebraska–Lincoln

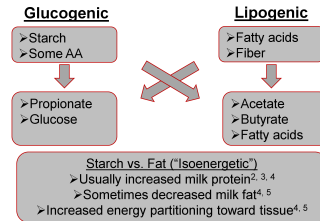
³Department of Animal and Food Sciences, Texas Tech University

⁴Department of Animal Science, The Pennsylvania State University

N

1

Not all energy is created equally¹



¹Van Knegsel et al., 2007a; ²Gum et al., 1996; ³Drackley et al., 2003; ⁴Boerman et al., 2015; ⁵Van Knegsel et al., 2007b

N

2

Can Energy Source Affect Energetic Efficiency?

- Reduced heat increment with fat?
- NEL/ME = 0.77 for Ca-salts¹
- NEL/ME = 0.66 for diets²
- Will supplemental fat increase energetic efficiency?



DE = Digestible energy
ME = Metabolizable energy
NEL = Net energy of lactation

¹Andrew et al., 1991; ²NRC, 2001

N

3

Objectives

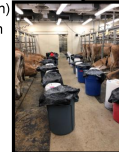
Determine the effect of a high-starch or an isoenergetic high-fat diet on energy and N partitioning and efficiency of energy utilization in late-lactation Jersey cows

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Materials and Methods

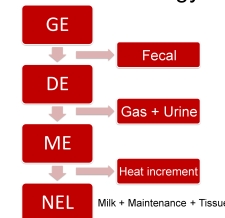
- Twelve multiparous Jersey (192 ± 11 DIM)
- Cross-over design with two 28-d periods
- Measured:
 - DMI, milk production and composition (4 d)
 - Urine and fecal output (4 d, total collection)
 - O₂ consumption, CO₂ and CH₄ production (1 d, headbox-style indirect calorimeters)



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Energy Calculations



- Heat production = gas exchange (Brouwer, 1965)
- Maintenance (Mcal) = 0.080 × BW^{0.75} (NRC, 2001)
- Tissue = ME – heat production – Milk
- Heat increment = Heat production – Maintenance

N

6

Experimental Diets

➤ STA = High starch; HFA = High fat

Items, % of DM	STA	HFA	Items, % of DM	STA	HFA
Corn silage	38.1	38.1	CP	15.5 (0.52)	16.0 (0.35)
Alfalfa hay	21.0	21.0	NDF	31.8 (3.19)	41.7 (1.90)
Ground corn	22.5	2.5	Starch	30.8 (0.42)	16.8 (0.85)
Soyhulls	4.1	6.5	Fatty acids	1.88 (0.02)	4.06 (0.14)
Soybean meal	11.5	10.9	NEL, Mcal/kg ¹	1.56	1.54
Bypass Soy ¹	—	0.6	*NRC, 2001 using mean production and measured composition		
Cottonseed hulls	—	12.5			
Distillers	—	2.5			
Fat ¹	—	2.6			
Other ²	2.8	2.8			

¹Soypass, Energy Booster 100
²Rumen-protect Lys and Met, vitamins and minerals



N

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Statistical Analysis

- Imer function of R
- Fixed: treatment
- Random: period, cow, and error
- Significance: $P \leq 0.05$
- Trends: $P \leq 0.15$



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Energy Utilization

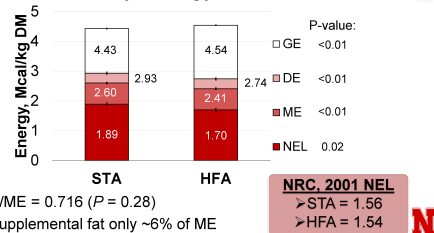
Items, Mcal/d	STA	HFA	SEM	P-Value
Feces	26.0	31.7	1.16	<0.01
Methane	3.70	3.66	0.26	0.83
Urine	2.07	2.44	0.11	0.04
HP	20.6	20.7	0.74	0.81
Milk	19.1	18.9	1.48	0.82
TE	5.97	3.10	1.30	0.16
TE _p	0.56	0.58	0.26	0.95
TE _f	5.41	2.51	1.16	0.12
RQ, L CO ₂ /L O ₂	1.09	1.05	0.012	<0.01

¹STA = high-starch diet; HFA = high-fat diet.

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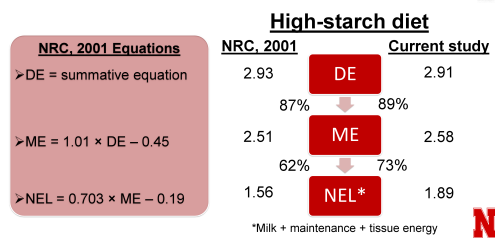
Dietary Energy Content



➤ NEL/ME = 0.716 ($P = 0.28$)
➤ Supplemental fat only ~6% of ME

10

NRC, 2001 vs. Measured NEL (Mcal/kg DM)



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Nutrient Digestibility

Items, Mcal/d	STA	HFA	SEM	P-Value
DM	66.7	61.7	1.06	<0.01
NDF	43.7	43.8	1.09	0.93
CP	64.4	65.2	1.02	0.23
Starch	94.5	97.0	0.48	<0.01
Fatty acids	63.7	60.5	1.57	0.18
18C fatty acids	67.9	61.2	1.60	<0.01
Energy	66.0	60.4	0.92	<0.01

¹STA = high-starch diet; HFA = high-fat diet.

N

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Nitrogen Utilization

Nebraska
Lincoln

Items, Mcal/d	Treatment ¹		SEM	P-Value
	STA	HFA		
N Intake	434	455	21.4	0.16
Fecal N	154	158	6.7	0.52
Urinary N	123	150	6.4	<0.01
Milk N	141	131	10.5	0.03
Retained N	16	15	7.3	0.92

¹STA = high-starch diet; HFA = high-fat diet.



N

13

DMI, Milk Production and Composition

Nebraska
Lincoln

Items, Mcal/d	Treatment ¹		SEM	P-Value
	STA	HFA		
DMI, kg/d	17.3	17.6	0.75	0.55
Milk yield, kg/d	19.7	18.9	1.38	0.12
ECM, kg/d ³	27.2	27.1	1.99	0.84
Fat, %	5.93	6.37	0.15	<0.01
Fat, kg/d	1.17	1.20	0.094	0.29
Protein, %	4.03	3.93	0.10	0.13
Protein, kg/d	0.791	0.740	0.050	0.07

¹STA = high-starch diet; HFA = high-fat diet.

N

14

Conclusions

Nebraska
Lincoln

- Increased starch compared to fat:
 - Increased NEL
 - Increased milk protein
 - Increased N partitioning toward milk
 - Decreased urinary N excretion
- Increased fat did not affect energetic efficiency
- NRC, 2001 underestimated NEL

N

15

Questions?



16

Prediction of heat production of lactating Jersey cows

D. Logan Morris¹, Robin White², and Paul Kononoff¹

¹Department of Animal Science, University of Nebraska–Lincoln

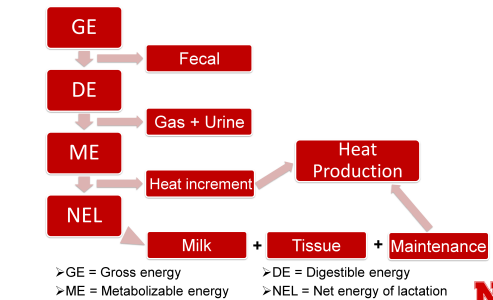
²Department of Animal and Poultry Science, Virginia Tech



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1

Energy System¹

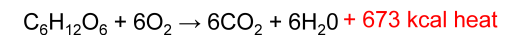


N

2

What is Heat?

➤ Energy associated with the inefficiency of conversion of a product to a substrate¹



¹Baldwin, 1995

3



N

What is Heat?

➤ Energy associated with the inefficiency of conversion of a product to a substrate¹



➤ Heat production (HP): total heat energy from whole-body metabolism
 ➤ Gas exchange²

➤ Heat increment (HI): increase in heat that occurs with production
 ➤ HP – Maintenance energy

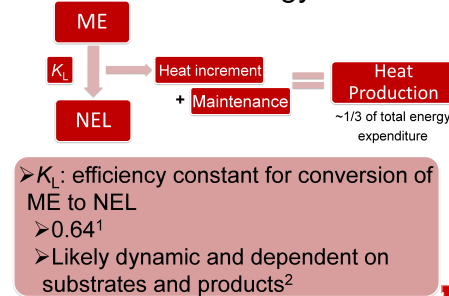


N

¹Baldwin, 1995; ²Brouwer, 1995

4

Heat Energy



N

¹NRC, 2001; ²Baldwin, 1995

5

Objectives

To derive models that estimate and explain variation in HP and HI of lactating Jersey cows.

Hypothesis

HP and HI could be estimated from DMI and that the addition of milk component yield, nutrient digestibility and urinary N excretion would improve models

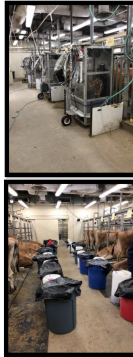


N

6

Dataset

- Lactating Jersey Cows
- Nine energy balance experiments
- 297 individual animal observations
- Measured:
 - DMI, milk production and composition (4 d)
 - Urine and fecal output (4 d, total collection)
 - Nutrient digestibility
 - O₂ consumption, CO₂ and CH₄ production (1 d or 2 d, headbox-style indirect calorimeters)



7

Headbox-Style Indirect Calorimeters



- Negative pressure system
- Continuous representative gas sample

N

8

Key Calculations

- Heat production (kcal/d):¹
 - $3.866 \times \text{O}_2 (\text{L}) + 1.200 \times \text{CO}_2 (\text{L}) - 0.518 \times \text{CH}_4 (\text{L}) - 1.431 \times \text{Urinary N (g)}$
- Heat increment:
 - Heat production – Net energy for maintenance (**NEm**)
- NEm:
 - Literature range from 0.073 to 0.140 Mcal/Metabolic BW²
 - Assumed 0.100 Mcal/Metabolic BW



¹Brouwer, 1965; Moe et al., 1971; Birnie et al., 2000; Kebreab et al., 2003; Foth et al., 2015; Moraes et al., 2015

9

N

Models

Nebraska
Linux

- Y = heat production or heat increment (Mcal/d)
- 1) **INTAKE**: $y = \text{DMI (kg/d)}$
 - 2) **INTAKE+MILK**: $y = \text{DMI} + \text{fat yield} + \text{protein yield} + \text{lactose yield (kg/d)}$
 - 3) **DIGESTED**: $y = \text{dNDF} + \text{dCP} + \text{dSTA (kg/d)}$
 - 4) **INTAKE+DIGESTED**: $y = \text{DMI (kg/d)} + \text{dNDF} + \text{dCP} + \text{dSTA (as \% of DMI)}$
 - 5) **INTAKE+MILK+DIGESTED**: $y = \text{model 2} + \text{model 4}$
 - 6) **INTAKE+MILK+UN**: $y = \text{model 2} + \text{urinary N excretion (kg/d)}$
- Metabolic body weight (BW^{0.75}) included in all heat production models
 - Random effects: study (n = 9), cow (n = 55), and period within block and study (n = 36).

N

10

Models

Nebraska
Linux

- BCS nor DIM were significant
- dFA (n = 87)
- DE and ME intake vs. DMI

N

11

Model Derivation and Evaluation

Nebraska
Linux

- Fit with the lmer function of R
- Variables removed when parameter estimates were not different from 0 ($P > 0.20$)
- Variance inflation factor < 5
- Evaluate with concordance without random effects (**uCCC**) and error variance
- Monte Carlo cross validation
 - 1000 iterations of 60:40 derivation:evaluation split by experiment
 - Root mean squared prediction error (**RMSPE**)
 - Decomposed RMSPE into slope and mean bias

N

12

Descriptive Stats

Item	Mean	SD	Minimum	Maximum
Heat production, Mcal/d	22.7	3.0	15.9	29.9
Heat increment, Mcal/d	12.9	2.9	6.3	19.4
Maintenance, Mcal/d	9.8	0.72	8.0	11.6
DIM	183	72.5	44	410
Parity	3.0	0.95	2.0	5.0
BW, kg	453	44	342	568
Metabolic BW, kg ^{0.75}	98.1	7.2	79.5	116.4
DMI, kg/d	18.2	2.56	9.6	25.0
Milk, kg/d				
Yield	24.9	5.58	7.8	43.0
Fat	1.39	0.297	0.48	2.19
Protein	0.90	0.168	0.33	1.29
Lactose	1.19	0.289	0.33	2.10
Apparent digested, kg/d				
CP	2.24	0.41	1.09	3.39
NDF	2.87	0.72	0.81	5.06
Starch	4.01	1.10	1.52	6.62

Nebbraska
Lincoln

N

13

Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	-1.02 ^a	-2.36 ^a	-0.14 ^a	3.58	2.14 ^a	-1.71 ^a
Metabolic BW	0.135	0.144	0.162	0.128	0.137	0.121
DMI, kg/d	0.567	0.403	0.591	0.448	0.400	0.400
Milk fat, kg/d		0.99		1.04	1.15	
Milk protein, kg/d		2.37		1.82	2.95	
aCP, kg/d			1.64			
aSTA, kg/d			0.763			
aNDP/DMI, %				-0.143	-0.139	
aCP/DMI, %				-0.155	-0.152	
Urinary N excretion, kg/d						5.68
Fit statistics						
aCCC	0.83	0.82	0.79	0.82	0.80	0.83
Error variance	1.47	1.44	1.60	1.44	1.44	1.42
Cross validation						
RMSPE, % mean	11.2	11.1	13.6	12.0	12.4	11.5
Mean bias, % RMSPE	17.9	18.8	17.8	18.8	19.3	17.9
Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 ($P > 0.20$)

- Similar uCCC
- Increased error variance for DIGESTED
- No additional info from MILK, DIGESTED or UN

N

14

Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	-1.02 ^a	-2.36 ^a	-0.14 ^a	3.58	2.14 ^a	-1.71 ^a
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Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 ($P > 0.20$)

- Digestibility variables increased RMSPE (12.7 vs. 11.2% of mean)
- Increased slope bias (11.9 vs 1.4% of RMSPE)

N

15

Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	-1.02 ^a	-2.36 ^a	-0.14 ^a	3.58	2.14 ^a	-1.71 ^a
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Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 ($P > 0.20$)

- Non-zero intercept for INTAKE+DIGESTED
- May have contributed to slope bias

N

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Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	-1.02 ^a	-2.36 ^a	-0.14 ^a	3.58	2.14 ^a	-1.71 ^a
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Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 ($P > 0.20$)

- Metabolic BW and DMI explain most variation
- NEM between 0.073–0.140 Mcal/kg of MBW¹
- Most of HP from MBW is NEM

N

¹Moe et al., 1971; Birnie et al., 2000; Kebreab et al., 2003; Fohr et al., 2015; Moraes et al., 2015

17

Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
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Milk fat, kg/d		0.99		1.04	1.15	
Milk protein, kg/d		2.37		1.82	2.95	
aCP, kg/d			1.64			
aSTA, kg/d			0.763			
aNDP/DMI, %				-0.143	-0.139	
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Mean bias, % RMSPE	17.9	18.8	17.8	18.8	19.3	17.9
Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 ($P > 0.20$)

- HP for milk protein > milk fat
- Energetic efficiency for preformed FA is 94–97%¹

N

¹Baldwin, 1985

18

Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	-1.02 ^a	-2.36 ^a	-0.14 ^a	3.58	2.14 ^a	-1.71 ^a
Metabolic BW	0.135	0.144	0.162	0.128	0.137	0.121
DMI, kg/d	0.567	0.403		0.591	0.448	0.400
Milk fat, kg/d		0.99			1.04	1.15
Milk protein, kg/d		2.37			1.82	2.95
dCP, kg/d			1.64			
dN/A, kg/d			0.763			
dND/DMI, %				-0.143	-0.139	
dCP/DMI, %				-0.155	-0.152	
Urinary N excretion, kg/d						5.68
Fit statistics						
adj R ²	0.83	0.82	0.79	0.82	0.80	0.83
Error variance	1.47	1.44	1.60	1.44	1.44	1.42
Cross validation						
RMSPE, % mean	11.2	11.1	13.6	12.0	12.4	11.5
Mean bias, % RMSPE	17.9	18.8	17.8	18.8	19.3	17.9
Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 (P > 0.20)

- With DMI, negative coefficients???
- DMI may explain most of the variation

N

19

Heat Production

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	-1.02 ^a	-2.36 ^a	-0.14 ^a	3.58	2.14 ^a	-1.71 ^a
Metabolic BW	0.135	0.144	0.162	0.128	0.137	0.121
DMI, kg/d	0.567	0.403		0.591	0.448	0.400
Milk fat, kg/d		0.99			1.04	1.15
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dCP, kg/d			1.64			
dN/A, kg/d			0.763			
dND/DMI, %				-0.143	-0.139	
dCP/DMI, %				-0.155	-0.152	
Urinary N excretion, kg/d						5.68
Fit statistics						
adj R ²	0.83	0.82	0.79	0.82	0.80	0.83
Error variance	1.47	1.44	1.60	1.44	1.44	1.42
Cross validation						
RMSPE, % mean	11.2	11.1	13.6	12.0	12.4	11.5
Mean bias, % RMSPE	17.9	18.8	17.8	18.8	19.3	17.9
Slope bias, % RMSPE	1.4	1.4	13.4	10.5	11.7	15.4

^aNot different from 0 (P > 0.20)

- 5.68 Mcal of HP/kg of N excretion
- Excess N intake increases HP^{1,2}
- Due to AA catabolism not ureagenesis³

¹Tyrrell et al., 1970; ²Reed et al., 2017; ³Firkins and Reynolds, 2005

20

Heat Increment

Item	INTAKE	INTAKE+ MILK	DIGESTED	INTAKE+ DIGESTED	INTAKE+ MILK+ DIGESTED	INTAKE+ MILK+ UN
Model	1	2	3	4	5	6
Variable						
Intercept	1.80	1.49	5.30	5.97	5.58	-0.034 ^a
DMI, kg/d	0.598	0.473		0.615	0.510	0.422
Milk fat, kg/d			3.04		2.57	1.85
Milk protein, kg/d				1.81		2.99
dCP, kg/d				0.827		
dN/A, kg/d						
dND/DMI, %				-0.147	-0.140	
dCP/DMI, %				-0.156	-0.162	
Urinary N excretion, kg/d						5.93
Fit statistics						
adj R ²	0.84	0.81	0.80	0.82	0.81	0.83
Error variance	1.48	1.47	1.62	1.44	1.44	1.43
Cross validation						
RMSPE, % mean	12.7	12.1	21.6	20.8	20.6	20.1
Mean bias, % RMSPE	18.1	17.8	18.9	17.9	17.5	17.2
Slope bias, % RMSPE	3.0	2.7	12.1	9.3	9.6	14.4

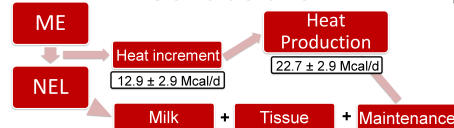
^aNot different from 0 (P > 0.20)

- Similar to HP models
- Increased RMSPE (20.6 vs. 12.0% of mean)
- Non-zero intercepts
- Is 0.100 Mcal/MBW for NEM too small?

N

21

Conclusions



- $HP = -1.02 + 0.135 \times MBW + 0.567 \times DMI$
 $HP = -2.36 + 0.144 \times MBW + 0.403 \times DMI$
 $0.99 \times \text{milk fat yield} + 2.37 \times \text{milk protein yield}$
- Prediction error for HP was ~12%
 - Prediction error for HI was ~20%
 - Inclusion of milk component yields, digestibility or urinary N excretion did not improve models

N

22


Questions?



DLMorris@unl.edu

23

American Dairy Science Association 2020 Presentation (CHAPTER 5:)




Relationship between urinary energy and N excretion in lactating Jersey cows

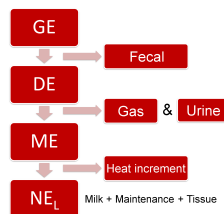
D. L. Morris,¹ J. L. Firkins,² W. P. Weiss,² and P. J. Kononoff¹
¹University of Nebraska–Lincoln
²Ohio State

N

1



Net Energy System



Estimation methods

Summative equation¹


$$ME = 1.01 \times DE - 0.45$$

$\approx 85\%$ of DE²


¹Weiss and Tebbe, 2019; ²Moe et al., 1971 & NRC, 2001

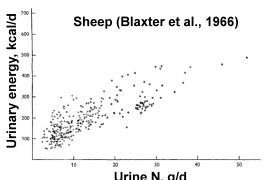
N

2




Urinary energy





N

3




Objectives

Develop equations to describe the relationship between urinary N and energy

N

4




Materials and Methods

- Animal-period data from Jersey (n = 134)
- Total collection with catheters
- Samples acidified to pH < 5.0
- Analyzed for N on an as-is basis
- Analyzed for gross energy with bomb calorimeter


Stats

- Random effect of cow and period within study
- Regressions between urinary energy and N
- Additional regression with DMI (% of BW) and BW



N

5

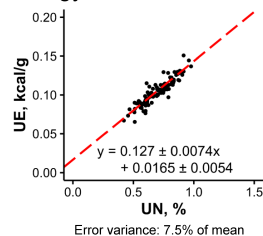


Dataset (n = 134)

Item	Mean	SD	Min	Max
d in milk	209	63	88	346
BW, kg	461	54	363	606
DMI, kg/d	18.3	2.4	11.6	24.6
ECM, kg/d	29.4	5.6	14.8	48.2
Urine				
Energy, kcal/g	0.104	0.021	0.035	0.155
Energy, kcal/d	2381	314	1390	3160
N, %	0.69	0.14	0.20	0.98
N, g/d	158	24	85	220
N, % of N intake	32.7	4.61	20.6	59.5

6

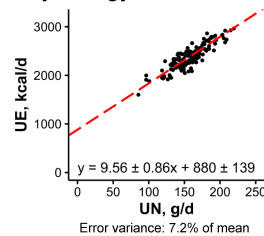
Urinary energy and N concentration



N

7

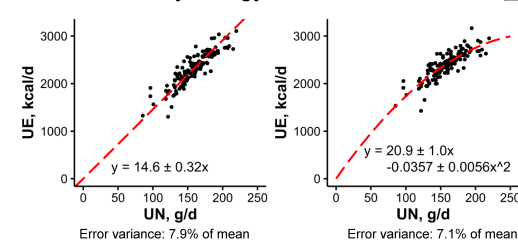
Urinary energy and N excretion



N

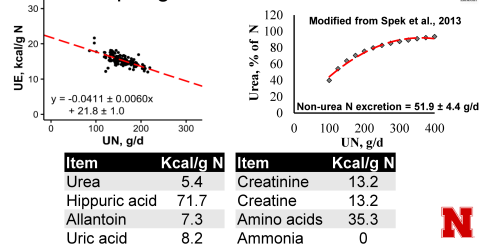
8

Urinary energy and N excretion



9

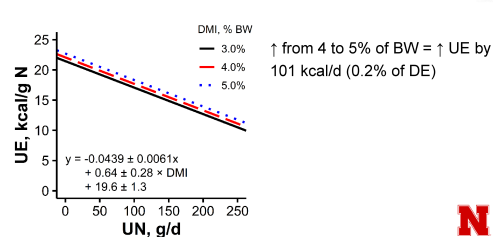
UE per g N and UN excretion



N

10

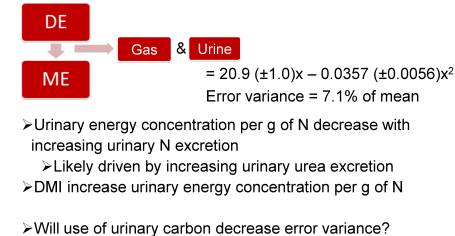
Effects of DMI



N

11

Conclusions



N

12

Exit Seminar

ENERGY METABOLISM IN JERSEY COWS: IMPROVING OUR UNDERSTANDING OF ENERGY REQUIREMENTS AND UTILIZATION

Logan Morris
September 15th, 2020

N

1

What is energy?

- Ability of a feed to support work done or products formed
- Amount of heat released when something is completely burned

1 lbs



=

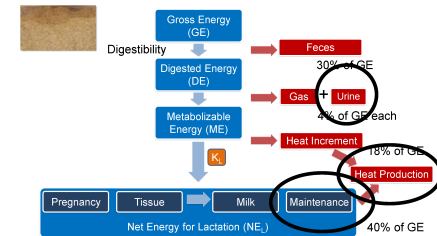
1 lbs



➤ Will they lead to the same amount of milk production?
➤ NO

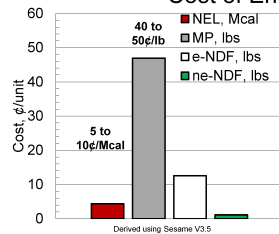
2

Net energy for lactation system



3

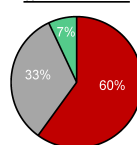
Cost of Energy



St-Pierre and Weiss, 2011; Buckeye dairy news; Tebbe, 2020

- 1000 lbs Jersey
- 40 lbs DMI

% of ration cost



4

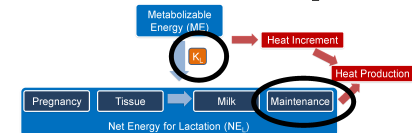
General Materials and Methods

- Multiparous Jersey
- Cross-over design with 28-d periods
- Measured:
 - DMI, milk production and composition
 - (4 d)
 - Urine and fecal output
 - (4 d, total collection)
 - O₂ consumption, CO₂ and CH₄ production
 - (1 d, headbox-style indirect calorimeters)



5

Maintenance and k_L



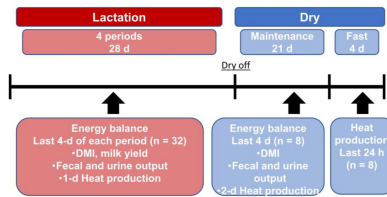
- Maintenance: energy required to conserve the state of an animal when no work is done
- Increasing over time¹
- K_L : efficiency constant for conversion of ME to NEL
- ~0.63 in NRC, 2001

¹Morales et al., 2015

6

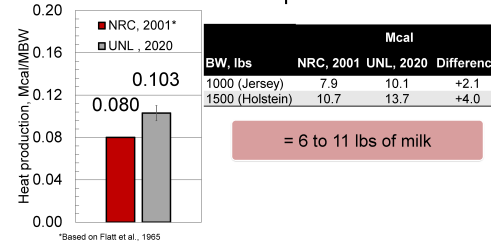
Methods

>8 non-pregnant Jersey cows



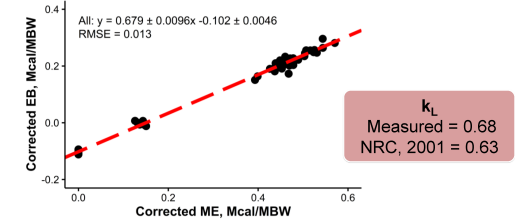
7

Maintenance requirements



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ME to NEL efficiency (k_L)



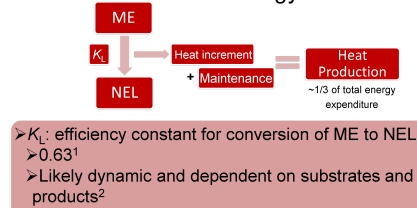
9

NRC, 2001 vs. "UNL" System

NRC, 2001	ME	UNL
46 Mcal	46 Mcal	46 Mcal
0.63	k_L	0.68
28.9 Mcal	NEL	31.2 Mcal
0.080 Mcal/MBW		0.103 Mcal/MBW
Maintenance: -7.8 Mcal		-10.1 Mcal
Milk energy: 21.1 Mcal		21.1 Mcal
	69.3 lbs of milk	

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Heat Energy



¹NRC, 2001; ²Baldwin, 1995

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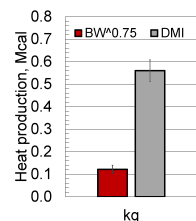
Dataset

- > Lactating Jersey Cows
- > Nine energy balance experiments
- > 297 individual animal observations
- > Measured:
 - > DMI, milk production and composition (4 d)
 - > Urine and fecal output (4 d, total collection)
 - > Nutrient digestibility
 - > O₂ consumption, CO₂ and CH₄ production (1 d or 2 d, headbox-style indirect calorimeters)
- > Fit several models



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Body weight and intake

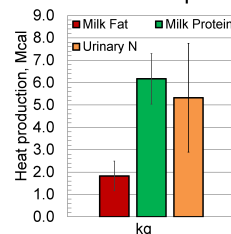


BW: 450 kg = 11.9 Mcal
DMI: 20 kg → 10.1 Mcal

➤ Increasing production will increase heat production

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Milk components and N excretion

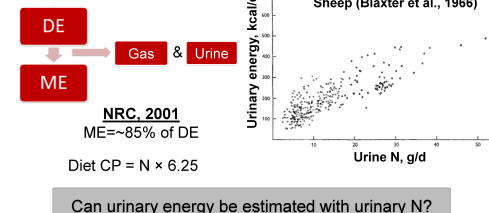


Fat: 1.4 kg → 2.6 Mcal
Protein: 0.9 kg → 5.6 Mcal
Urinary N: 190 g → 1.0 Mcal

➤ Milk protein take more energy to produce

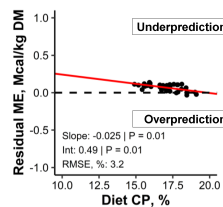
14

Urinary energy



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ME and diet CP



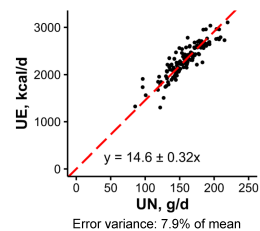
➤ N = 45 treatment means
➤ Lactating Jersey cows
➤ CP intake → Urine N
➤ Residual = measured ME – NRC, 2001 estimated ME
➤ $(1.01 \times \text{DE} - 0.45) + 0.0046 \times (\text{crude fat} - 3) = \sim 85\%$

➤ Current model does not account for loss in efficiency with increasing CP

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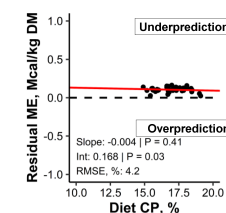
Urinary energy and N excretion

➤ N = 134
➤ Lactating Jersey cows
➤ CP intake → Urine N



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Practical implications of overfeeding CP



➤ N = 45 treatment means
➤ Residual = measured ME – estimated ME
➤ Urine energy = 14.6 × urine N
➤ $\text{CH}_4 = 0.294 \times \text{DMI} - 0.347 \times \text{crude fat} + 0.0409 \times \text{NDF digestibility}^1$

➤ CH₄ energy overestimated by 0.11 Mcal/kg DM
➤ Bias account for by estimate UE from UN

Nielsen et al., 2013
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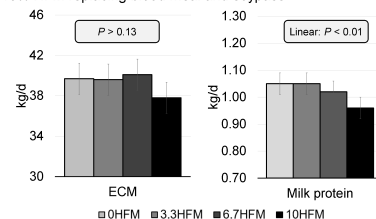
Halftime



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Hydrolyzed feather meal

> 0 to 10% HFM replacing blood meal and soyabass



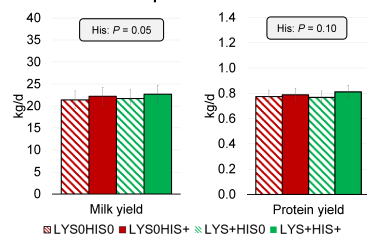
Morris et al., 2020 JDS:4206
20

Lys × His treatments

- > Lys and His are low in feather meal
- > Common limiting AA
- > 2×2 factorial design with 5% hydrolyzed feather meal diets
- > 0 g/d of RP Lys (**LYS0HIS0**)
- > 23 g of supplemental dLys (**LYS+HIS0**)
- > 8 g of supplemental dHis (**LYS0HIS+**)
- > Both (**LYS+HIS+**)

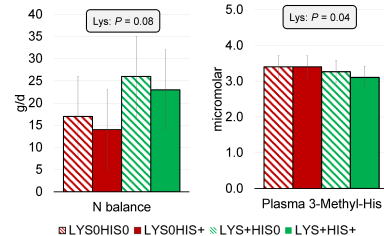
Morris and Kononoff, 2020 JDS:7110
21

Milk production



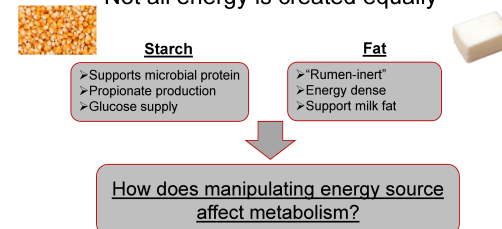
Morris and Kononoff, 2020 JDS:7110
22

N utilization



Morris and Kononoff, 2020 JDS:7110
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Not all energy is created equally



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Experimental diets

➤ STA = High starch; HFA = High fat

Items, % of DM	STA	HFA	Items, % of DM	STA	HFA
Corn silage	38.1	38.1	CP	15.5 (0.52)	16.0 (0.35)
Alfalfa hay	21.0	21.0	NDF	31.8 (3.19)	41.7 (1.90)
Ground corn	22.5	2.5	Starch	30.8 (0.42)	16.8 (0.85)
Soyhulls	4.1	6.5	Fatty acids	1.88 (0.02)	4.06 (0.14)
Soybean meal	11.5	10.9	NEL, Mcal/kg	1.55	1.56
Bypass Soy ¹	—	0.6			
Cottonseed hulls	—	12.5			
Distillers	—	2.5			
Fat ¹	—	2.6			
Other ²	2.8	2.8			

¹NRC, 2001 using mean production and measured composition

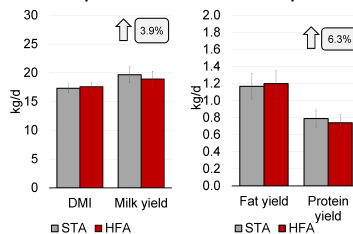
²Soypass: Energy Booster 100

³Rumen-protect Lys and Met; vitamins and minerals



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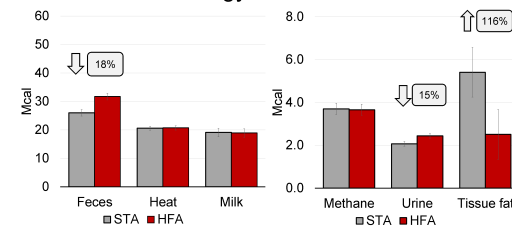
DMI, milk production and composition



Morris et al., 2020 JDS 4378

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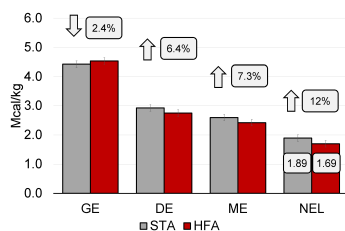
Energy utilization



Morris et al., 2020 JDS 4378

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Dietary energy content



NRC, 2001 NEL

➤ STA = 1.56

➤ HFA = 1.55

Morris et al., 2020 JDS 4378

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Response to rumen-protected Lys

Item	Milk protein %	Milk protein yield
Schwab et al., 1992	↑ (peak), — (other)	↑ (peak), — (other)
Paz and Kononoff, 2014	↑	—
Bernard et al., 2014	↑	—
Ariola Apello et al., 2014	—	↑
Giallongo et al., 2016	↑	—
Fleming et al., 2019	↑	—
Morris and Kononoff, 2020	—	—

➤ Milk protein synthesis is an energy dependent process¹

➤ Metabolizable protein and energy interact to affect milk protein yield^{2,3}

¹Cart et al., 2019; ²Oldham, 1984; ³Brun-Lefleur et al., 2010

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Treatments and stats

➤ Response surface experiment conducted to test the interaction between 3 factors:

➤ Dietary fatty acids (FA; 3.0 to 6.2%)

➤ 0 to 4% supplemental fat (57% C16:0, 21% C18:0, 11% C18:1)

➤ Replaced soyhulls

➤ Dietary starch (20.2 to 31.3%)

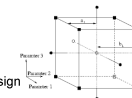
➤ Replaced soyhulls with corn grain

➤ Supplemental digestible Lys (dLys; 0 to 15.8 g/d)

➤ 5 levels of each factor arranged in a central composite design

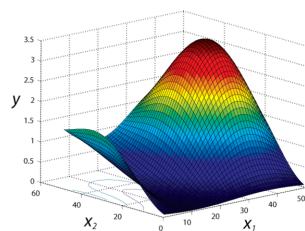
➤ 15 treatments

➤ Regressions with linear and quadratic effects and all two-way interactions



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Responses not treatment means



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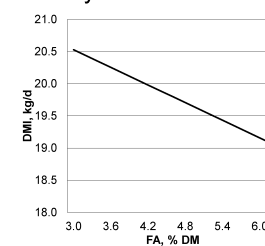
Descriptive stats

Item	Mean	SD	Min	Max
BW, kg	424	38	365	498
DMI, kg/d	19.7	2.1	14.9	24.7
ECM, kg/d	35.1	4.0	27.7	48.3
Fat, g/d	1415	186	1078	2167
Protein, g/d	981	126	758	1324

➤ On average, equal to a Holstein at 115 lbs of milk and 65 lbs DMI

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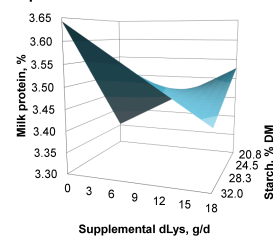
Dry matter intake



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Milk protein

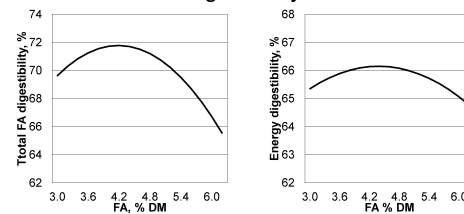
➤ Milk protein concentration in more sensitive than milk protein yield¹



¹Paz and Kononoff, 2014; Giallongo et al., 2016; Felming et al., 2019

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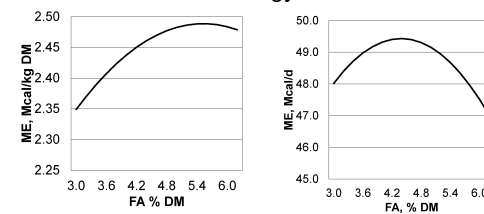
Digestibility



➤ 4.2% FA = ~ 0.65 lbs of supplemental fat

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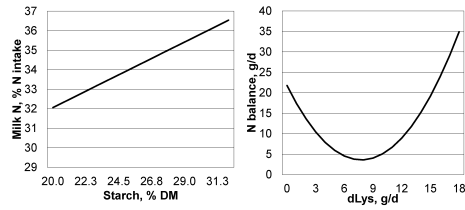
Energy



➤ 4.2% FA = ~ 0.65 lbs of supplemental fat

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N utilization



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Conclusions

- Maintenance requirements have likely increased but countered by increased efficiency
- Milk protein synthesis generates 2-fold the heat of fat synthesis
- Urinary energy can be estimated with high precision with N
- Increasing His increases milk protein in feather meal diets
- Increasing dietary starch increases milk protein yield and body fat gain
- Increasing Lys increase milk protein % in low but not high starch diets
- Lys may be preferentially used by muscle tissue

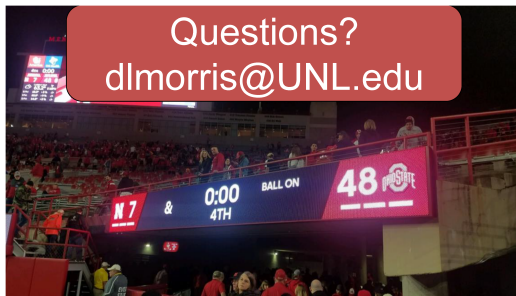
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Thanks!



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Questions?
dlmorris@UNL.edu



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