INTERRELATIONS BETWEEN DIETARY ENERGETICS AND NITROGEN EFFICIENCY USING ESSENTIAL AMINO ACID BALANCING IN LACTATING DAIRY CATTLE

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INTERRELATIONS BETWEEN DIETARY ENERGETICS AND NITROGEN EFFICIENCY USING ESSENTIAL AMINO ACID BALANCING IN LACTATING DAIRY CATTLE

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Improvements in nitrogen (N) utilization as it pertains to ruminant animals has been a long-held focus of many nutritionally based research programs. Traditional evaluation of this metric uses the relationship of a ruminant's productive N output, calculated as a sum of milk protein, lean muscle gain, and fetal growth in the event of pregnancy, relative to the intake of dietary CP or metabolizable supply (MP); however, the consideration of an animal's energetic status relative to N supply is often overlooked when optimizing N and amino acid (AA) use efficiency. Nonetheless, efforts have been made to incorporate the use of this relationship within the most recent version of the Cornell Net Carbohydrate and Protein System (CNCPS v.7). Findings recommend an optimal supply of each essential AA (EAA) relative to metabolizable energy (ME) which should be fed to maximize EAA efficiency without compromising lactation performance. The work described in this dissertation looks to explore these relationships under different conditions of nutrient supply to cattle. The first objective was to evaluate variation within the dataset used to calculate the optimized supply of EAA relative to ME. This was accomplished in the first study in which three diets varying in EAA supply relative to ME were fed in a longitudinal feeding trial and tested lactation performance and EAA efficiency of use. Cattle fed the diet which met the optimum supply of EAA relative to ME (NEU diet) were able to improve milk volume and energy corrected milk (ECM) yield while having the highest N use efficiency (NUE; 0.343) over the other diets fed. This work is in support of the previously optimized supply and was used to test the second objective of this dissertation, which included an evaluation of NUE when different levels of glucogenic nutrients and EAA are supplied.

The second feeding trial used a 2 x 2 factorial design, with two levels of dietary starch and two levels of EAA supply. Results suggested improvements in the milk protein output when cattle were fed more glucogenic nutrients; however, ECM yield was not different among diets due to improvements in milk fat yield for diets that were fed more lipogenic nutrients in substitution of glucogenic nutrients. Further changes in dry matter intake (DMI), due to shifts in rumen uNDF pool size, skewed feed efficiency results which might have altered the NUE of these diets unintentionally. Future work is required to avoid these unintended consequences and allow for the appropriate testing of varied glucogenic and lipogenic nutrient supplies. Lastly, this dissertation assessed previously recommended EAA supply relative to ME using optimized values for the efficiency of EAA use. This was performed by constructing a dataset from CNCPS v.7 predictions of the feeding trials described previously. Logistic models were fitted to describe the relationship between EAA supplied and CNCPS v.7 predictions for EAA requirements so that the optimized efficiency of use for these EAA could be ascertained. Once identified, loglogistic models were fitted to describe the relationship between EAA efficiency of use and the supply of EAA relative to ME and make predictions on the optimum supply of EAA relative to ME. Findings indicate similar results for the optimum efficiency of use and subsequent supply relative to ME for most EAA. Of importance was the ability to predict the optimum supply of Met and Lys to within 0.03 g/Mcal ME when these rederived numbers were compared to previous recommendations. Collectively, AA balancing can prove to be a useful tool in improving the productive efficiency of a herd and recommendations for the optimal supply of EAA relative to ME should be followed given their repeatability in different datasets used to calculate them.

BIOGRAPHICAL SKETCH

Andrew LaPierre was raised in the Champlain Valley on his family's dairy farm in Chazy, NY. As the child of a dairy farmer, he developed an appreciation for animal husbandry and learned the value of a hard work ethic from both his parents and grandparents. Andrew was a student of Chazy Central School which shares its founder with the William H. Miner Agricultural Institute, renowned for its research in production agriculture and natural resources. Frequent school visits to the institute provided exposure to the nature of applied agricultural research, prompting a desire to further his education in animal agriculture. Upon acceptance to Cornell University in the Fall of 2010, Andrew began his education in the dairy program with the intent of entering the dairy industry as a producer. His involvement in the Cornell Dairy Science Club, Dairy Fellows, and Alpha Gamma Rho gave him new insights on how the dairy industry operates given geographical and cultural differences. However, upon Dr. Mike Van Amburgh recommendation, Andrew spent a summer at Cornell where he began his time as a research assistant for several of Mike's graduate students, developing an appreciation for scientific rigor and proper research etiquette. He graduated from Cornell in 2014 with distinctions in research and went on to pursue his MS degree at the University of Illinois at Urbana-Champaign under the advisement of Dr. Jim Drackley. His time at Illinois was paramount towards his education in animal nutrition, statistical theory and experimental design, and gave him exposure to dairy industry as it exists in the Midwest. His thesis, titled: Effects of hydroxy vs. sulfate forms of trace minerals in milk replacer and starter on pre-ruminants, gave Andrew insights in micronutrition and stoked his enthusiasm for applied nutritional research. In cyclical fashion, Andrew returned to Dr. Van Amburgh's lab in 2016

to pursue his doctorate in ruminant nutrition, with emphasis in amino acid nutrition and systems.

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It has become apparent to me as to why a doctoral student's acknowledgements should always precede his or her dissertation, for without the support and encouragement from those mentioned here, I could not have ever hoped to have had a more complete and fulfilling program.

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To my parents, the words written here can never describe the love and appreciation I have for your support. My involvement and passion for agriculture would not have existed without your love for this industry and I thank you for teaching me the value of hard work. To my sister and brother, your support toward me and our family throughout my time as a graduate student has given the opportunity to make the most this program.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1 Overview

The nutrient profile of milk is one of the most balanced and nutritious profiles relative to any other naturally produced food source. Specifically, the digestible indispensable amino acid score (DIASS) of milk protein for human consumption has been rated higher than all plant-based protein sources (Mathai et al., 2017). This presents an opportunity for farmers within the ruminant production system to convert human inedible material into highly digestible protein (Thorning et al., 2016, Kim et al., 2019). With the steady increase in global demand for meat and milk, dairy production serves a vital role towards supplying these demands (FAO, 2017); however, these demands require an intensification of agricultural practices and care must be taken to ensure that adverse effects on the environment are not a consequence of this increased supply (FAO, 2018). These implications are a result of growing legislative pressure which mandate more sustainable practices be implemented on all farms (White and Hall, 2017).

Nitrogen (N) excretion is considered an environmental pollutant which has negative impacts on the quality of water ways and air supply (Dijkstra et al., 2013, Bodirsky et al., 2014, Lassaletta et al., 2016). As a contributor to N pollution, the dairy industry continues to make improvements toward minimizing its N excretion sourced from cattle through implementation of best management practices (BMP) ranging from the reduction in ammonia volatilization from manure that has been directly injected into soil used for crop production (Huijsmans et al., 2003) to the collection and recovery of ammonia emissions from long-term manure storage systems (Guarino et al., 2006, Ndegwa et al., 2008). Dietary modifications, often through the reduction of excess crude protein (CP) content, can also be employed in animal production systems as a first step in curbing N excretion through urine and feces (Klopfenstein et al., 2002, Satter et al.,

2002). Further, refinement in the dietary composition of protein N can increase gross N efficiency by improving the efficiency of conversion of dietary N into milk and milk protein. These improvements require the collective use of precision feeding techniques, nutrition models which use the most current knowledge on nutrient requirements, and producer compliance to allow for a consistent delivery of nutrients to cattle. Amino acid (AA) balancing has proven to be an viable technique which can boost both postabsorptive N efficiency and milk protein output in cattle (Lee et al., 2012b, Arriola Apelo et al., 2014b, Lee et al., 2015). Most of the previous research conducted using AA balancing techniques have balanced for either one or several of the essential AA (EAA) required by cattle, with most of the approaches centered around commonly limiting EAA such as Met (Noftsger and St-Pierre, 2003, Broderick et al., 2008), Lys (Donkin et al., 1989, Schwab, 1996), and His (Lee et al., 2012a, Lapierre et al., 2021), or balancing for branched chain AA (BCAA) (Korhonen et al., 2002, Yoder et al., 2019). However, opportunities exist to balance for all EAA, particularly under conditions when both requirements and supply of each EAA are appropriately described. Further, other bodies of research have suggested that efficiency of use for EAA can be improved when cattle are fed varying sources of dietary energy (Raggio et al., 2006, Cantalapiedra-Hijar et al., 2014b, Nichols et al., 2018). Improvements in our understanding on how the profile of energy yielding substrates can shift the efficiency of use for EAA, allows for more precise targets for the supply of EAA, according to EAA requirements, and can reduce the supply of any unproductive nutrients in the process.

This review will focus on the current knowledge regarding the metabolism of AA and explore efforts made to understand how their metabolism changes when supply and source of dietary energy is altered. Further, this review will discuss previous efforts made to optimize EAA

supply relative to energy and how the Cornell Net Carbohydrate and Protein System can aid in balancing for an optimized supply of EAA.

1.2 Protein, amino acid, and energy nutrition in ruminants

1.2.1 Protein and amino acid metabolism

In ruminant nutrition, dietary protein can be characterized into two main protein components: rumen degradable protein (RDP) and rumen undegradable protein (RUP), the former of which is proteolytically digested and deaminated by the rumen microbial population and used to produce a supply of microbial protein (NRC, 2001). It is well understood that protein degradation in the rumen increases as the supply of dietary RDP increases; however, this is predicated on the ability to feed a concomitant increase in fermentable carbohydrates so that optimal fermentation by the microbial population can occur (Russell et al., 1983, Ipharraguerre et al., 2005). Microbial protein serves as a large proportion of the total metabolizable protein (MP) supply and should be maximized to provide a highly digestible source of AA (Clark et al., 1992, Tamminga, 1993). Undegraded feed protein also contributes to total MP supply. Processing of raw feed ingredients, including heat and pressure treatment, can alter the digestibility of feeds within the rumen and provide a direct supply of AA to the animal through intestinal enzymatic activity. Further, the use of rumen protection, either through fat or polymer encapsulation, can protect nutrients like AA from microbial action and directly supply cattle with the protected nutrients These techniques have proven useful in balancing diets for EAA and allowing for the reduction in dietary CP while improving milk production and postabsorptive N efficiency (Broderick et al., 2008, Arriola Apelo et al., 2014a, Lee et al., 2015).

Once absorbed by the gastrointestinal tract, AA serve several important roles in protein metabolism, even beyond their need as substrates for protein synthesis. Of particular importance

is the role AA play in activating and inhibiting the mTORC1 and AMPK pathways, respectively (Dreyer et al., 2008, Suryawan et al., 2008, Pezze et al., 2016). Considered the master regulator of anabolic metabolism, activation of mTORC1 has been shown to be closely linked with protein synthesis in mammary epithelial cells (Appuhamy et al., 2014). Previous research has continually demonstrated mTORC1 activation in the presence of adequate BCAA supply (Suryawan et al., 2011, Pezze et al., 2016, Xu et al., 2019). Further, AA uptake and metabolism in the mammary gland follows a unique pattern originally described by Mepham (1982) and provides insights on how these AA should be fed to maximize milk protein and N efficiency. Briefly, group 1 AA are defined as having a mammary uptake to milk protein output ratio of 1:1 and include His, Met, Phe, Tyr, and Trp (Lapierre et al., 2012). The uptake to output ratio for these AA can vary slightly according to the supply status of other nutrients needed for milk protein synthesis. For instance, an uptake to output ratio above 1 suggests that these AA are being metabolized for other metabolic purposes within the mammary and are likely the result of other limiting nutrients. Alternatively, group 2 AA are defined as having an uptake to output ratio that is greater than 1 and include Arg, BCAA, Lys, and Thr. Excessive uptake of BCAA beyond milk protein output is likely contributed to cellular maintenance, regulation of anabolic pathways, and used as substrates for the synthesis of non-essential AA (NEAA) (Li et al., 2009, Lei et al., 2012). Mepham (1982) observed a decrease in the extraction of BCAA by the mammary gland as lactation progressed, suggesting that the demand for these BCAA decreases as the demand for synthesizing NEAA concomitantly decreases. Previous research has suggested that Lys is taken up in excess due an obligate level of catabolism, even when its supply is deficient (Lapierre et al., 2009). This catabolism is also thought to provide available N to support the synthesis of NEAA. Lastly, mammary uptake of Arg far exceeds the uptake of

any other EAA, where uptake to output can reach levels up to 2.5 times. Work using radio labeled Arg in porcine mammary tissue samples in an in vitro setting determined that major products of Arg catabolism are proline, ornithine, and urea as a results of the arginase pathway (O'Quinn et al., 2002). Proline content of bovine casein is 10.4% and is second only to glutamic acid which is 18.4% (Buňka et al., 2009). Improvements in Pro supply have been shown reduce the uptake of Arg by the mammary gland and suggest that opportunities exist to identify an optimum supply between Arg and Pro (Bruckental et al., 1991).

1.2.2 Glucogenic nutrients

Any nutrients which produce glucose, either through digestion or metabolism are considered glucogenic nutrients. These nutrients are considered vital for mammary metabolism as this tissue has an obligatory requirement for glucose (Mayes et al., 2003), metabolizing it primarily for the synthesis of lactose which accounts for the majority (between 62 to 83%) of total glucose uptake (Hanigan et al., 1992). Estimates suggest that cattle producing 60 kg/d of milk with a lactose content of 4.8% will drive mammary requirements for glucose to over 4 kg/d (Reynolds, 2005). Circulating glucose content is supported primarily by hepatic gluconeogenesis, accounting for over 80% of glucose appearance in lactating cattle (Bergman et al., 1970). In ruminants, the volatile fatty acid (VFA) propionate serves as the primary precursor for glucose synthesis by the liver. Once produced by ruminal microbial fermentation of non-structural carbohydrates (NSC), propionate is cleared via portal drainage and cleared by the liver at rate proportional to its supply (Reynolds, 2005). This clearance can be rather large as net hepatic removal of propionate from portal drainage can reach over 70% in transition cattle (Reynolds et al., 2003, Aschenbach et al., 2010).

Noncarbohydrate nutrients, including lactate, glycerol, and amino acids (excluding Lys and Leu but especially Ala), also serve as glucogenic nutrients which can be used for hepatic gluconeogenesis (Reynolds et al., 2003, Larsen and Kristensen, 2009). Amino acid contributions to hepatic gluconeogenesis can be variable based on the supply of glucogenic AA relative to the demand of those same AA for protein synthesis in the liver or other peripheral tissues, the supply of other glucogenic nutrients relative to the demand for glucose based on physiological state, and whether the AA is classified as essential or non- essential. Differences have been shown between the use of EAA and NEAA as glucogenic precursors, where 3% of the hepatic supply of EAA and between 10-25% of NEAA hepatic supply can be metabolized for glucose production (Lindsay, 1980). The greater conversion of NEAA supply to glucose is primarily due to the increased supply of Ala which is converted to pyruvate and used as a primary substrate source for glucose in the TCA cycle (Aschenbach et al., 2010). Demand for glucose by the mammary gland is highest during early and mid-lactation when cattle are typically observing peak milk yield (Lemosquet et al., 2009, Galindo et al., 2011). To meet the glucose demand by the mammary gland, emphasis should be taken to provide enough dietary glucogenic nutrients to allow for the production of propionate and stimulation of hepatic gluconeogenesis.

The effect of dietary starch and propionate production on milk protein output and N and EAA efficiency has also been described. Improvements in the net portal appearance of total AA (TAA) and higher net splanchnic release of AA have been observed in cattle fed diets with high a starch level (35% DM) relative to high fiber diets [NDF = 46% DM and starch = 4.5% DM (Cantalapiedra-Hijar et al., 2014a)]. This improved splanchnic release of AA resulted in improved milk N yield; however, the authors suggested that improvement in milk N output was due to improvements in microbial N flow through the duodenum. This suggests that

improvements of NSC might have affected milk yield by improving MP supply through increased microbial yield and through increased supply of propionate which in turn is used to supply glucose to the mammary through hepatic gluconeogenesis. Research on the additive effects of supplemental casein and propionate suggest that both sources of nutrients have the ability to improve milk protein yield but through different biochemical pathways (Raggio et al., 2006). Glucogenic nutrients have also been demonstrated as important signaling molecules for the downstream regulation of mTORC1 and AMPK pathways. Further, insulin and IGF-1 have been demonstrated as one of the primary activators of the mTORC1 pathway which is highly sensitive to cellular energy status (Jeyapalan et al., 2007, Suryawan and Davis, 2018, Sadria and Layton, 2021). Improvements in glucogenic nutrient supply can indirectly enhance energy signaling status and activation of MTORC1 through improved blood insulin concentrations meant to maintain glucose homeostasis.

1.2.3 Lipogenic nutrients

Nutrients required to synthesize endogenous fatty acids (FA) are considered lipogenic. Ruminal fermentation of structural carbohydrates, including cellulosic material, produce acetate and butyrate, both of which are considered lipogenic. The majority (~72%) of acetate produced in the rumen appears in the portal-drained viscera (Kristensen, 2001) and provides the primary substrate for lipogenesis in the mammary gland (Bauman et al., 1970) and peripheral tissues (Kristensen, 2005). Recent findings from a dose-response study in which acetate was infused into the rumen of cattle (Urrutia and Harvatine, 2017) indicates a quadratic response in milk fat output to increasing levels of circulating acetate. Further, linearly increased production of C16:0 FAs was observed and suggests that acetate is vital to FA chain elongation in *de novo* synthesis

of FA. Metabolism of acetate in the mammary gland produces acetyl-CoA which is the primary substrate needed in the isocitrate pathway (Bauman et al., 1970).

Changes in dietary FA levels can also contribute to lipogenic nutrient supply; however, careful considerations should be made when deciding which type of FA to supply. Polyunsaturated FA (PUFA) can be disruptive to proper rumen function through alterations in the biohydrogenation pathways by the bacterial population (Bauman et al., 2000). As a result of this alteration in rumen microbial biohydrogenation due to elevated levels of PUFA, an isomer shift occurs in the primary conjugated linoleic acid, from *cis*-9, *trans*-11 to *trans*-10, *cis*-12, and is produced as a byproduct of this altered biohydrogenation step. The presence of even slight increases in *trans*-10, *cis*-12 conjugated linoleic acid in circulation have been shown to elicit a significant milk fat depression response in cattle (Bauman and Griinari, 2001). Conversely, saturated fatty acids, including palmitic and stearic acid, are assumed to be rumen-inert and avoid metabolic interactions with rumen microbes, although recent research has suggested optimized levels of palmitic, stearic and oleic acids can improve aNDFom and FA digestibility over non-supplemented diets (Rico et al., 2017, de Souza et al., 2019). Postabsorptive interactions between protein supply and lipogenic nutrients have also been studied. In work which fed cattle with two levels of MP and abomassally infused either a supply of glucose or palm olein, cattle showed an improvement in ECM output when lipogenic nutrients were infused but did not observe any differences in milk N efficiency from low to high supply of MP (Nichols et al., 2019). Other research findings suggest that lipogenic nutrients lower or have no effect on milk protein output when compared to diets fed with a higher supply of glucogenic nutrients (Hammon et al., 2008, Boerman et al., 2015).

1.3 Amino acid predictions using the CNCPS

1.3.1 Amino acid requirements

The CNCPS makes predictions for cattle requirements regarding each EAA according to the demands needed to meet the physiological functions described, including growth, lactation, maintenance, and pregnancy (O'Connor et al., 1993, Van Amburgh et al., 2015). Most nutritional models, including the CNCPS, predict the supply of EAA needed to meet the net requirements for cattle and apply a 'coefficient of transfer' which describes the efficiency of which a given supply of EAA is used towards net requirements for cattle. Use of this coefficient of transfer assumes that the nutritional model does not describe 100% of cattle requirements and, by extension, calculates the necessary increase in supply of EAA over current model predicted net requirements to adequate meet all cattle requirements, regardless of whether they are defined by the model. Previous versions of the CNCPS applied separate transfer coefficients to individual EAA requirements (maintenance, lactation, etc.); however, the use of combined coefficients which describe the efficiency of transfer when total requirements are considered, has been recommended, given the complexity to optimize EAA supply when different efficiencies of transfer are applied to different physiological processes (Lapierre et al., 2007, Lapierre et al., 2021), and instituted in both the CNCPS v6.55 (Van Amburgh et al., 2015) and CNCPS v.7 (Higgs et al., 2014). For instance, current CNCPS parameters set the transfer efficiency for MP supply at 0.73 where predicted productive output is calculated based on the product of predicted MP supply and this coefficient. Recent recommendations suggest the use a of variable efficiency of transfer under different EAA supply conditions (Omphalius et al., 2019). The CNCPS v7 has the capability to predict the apparent efficiency of use for each EAA by taking the quotient of the predicted AA requirements (AAR) and the AA supplied (AAS) and has been employed toward an improved modeling of nutrient utilization in cattle

1.3.2 Predictions for optimized amino acid profile

The ability to create a robust EAA profile which satisfies the total requirements of lactating cattle has been the focus of several experiments (Lapierre et al., 2007, Haque et al., 2012). Work performed by Higgs and Van Amburgh (2016) has also focused on an optimized profile of EAA using the CNCPS v.7. Utilization of the model would improve both supply and requirement estimations for all EAA and would ideally improve the predictions for an optimum EAA profile. To do this, data from EAA infusion studies, which provided more accurate data on EAA supplied to cattle, were collected to build an evaluation dataset. Predictions for the EAA requirements of all treatment means from this dataset were done using the CNCPS v.7 and related to their described EAA supply. Non-linear model fit techniques, described by Doepel et al. (2004), were used to describe the relationship between EAA supply and model predicted requirements and deviate from the traditional 'broken stick' model that has been used previously to evaluate EAA supply to requirements (NRC, 2001). This relationship can be observed in Figure 1.1 which describes the relationship between digestible supply of Met and CNCPS v.7 predicted Met requirements using the dataset created by Higgs and Van Amburgh (2016). Doepel et al. (2004) suggested that the optimum transfer efficiency can be determined by utilizing third order differentials to solve for the 'upper critical limit' of model equation. Alternatively, this can be described as the point on the curve where the rate of change in the relationship between AAR and AAS had the most rapid decrease, indicating a marginalized increase in AAR in response to an increase in AAS. These relationships were performed for all EAA and results are described in Table 1.1. Comparisons of the optimum transfer efficiency for all EAA from this dataset were made to similar outputs by other research (Lapierre et al., 2007). All EAA transfer efficiencies were less in the Higgs results relative to Lapierre results, particularly when evaluating Met and Thr. It is possible that oversupply of the EAA within this

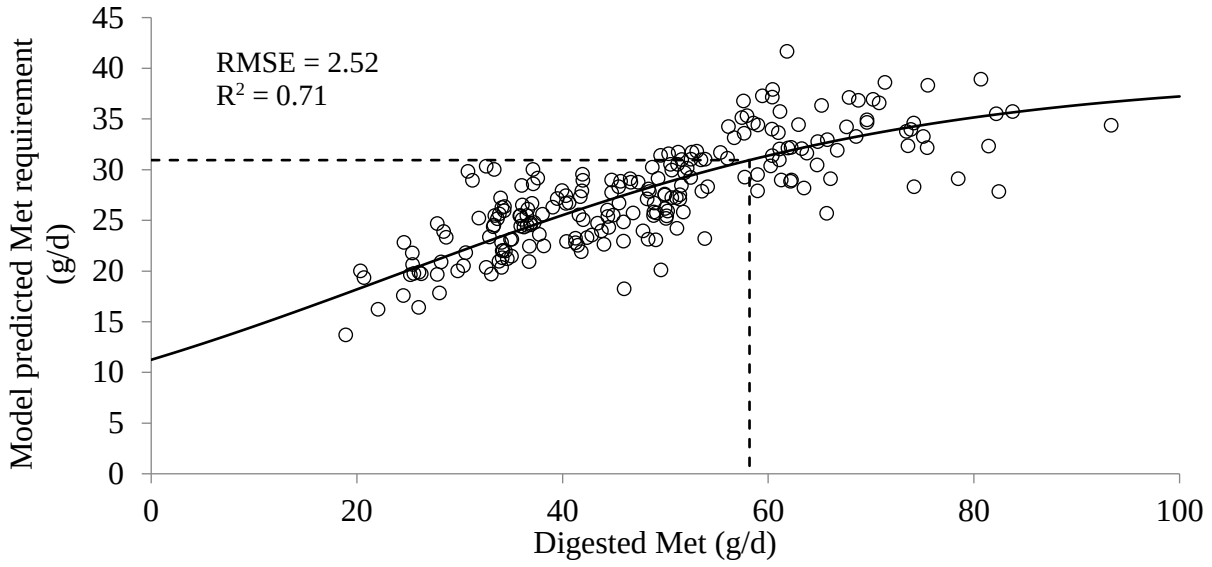

Figure 1.1. Logistic fit of model predicted Met requirement and Met supply. The dashed line represents the optimum ratio of Met requirement and Met supply. Adapted from (Higgs and Van Amburgh, 2016)

Table 1.1. Predicted optimum EAA transfer efficiency described by Higgs and Van Amburgh (2016) and Lapierre et al. (2007), optimum daily EAA supply and profile, optimum EAA supply to ME and relationship of Lys:EAA for both dairy and swine requirements.

EAA	Higgs and Van Amburgh 2016 AAR:AAS	Lapierre et al. (2007) AAR:AAS	AAS/d[1]	% TEAA	g/Mcal ME	Lys:EAA Dairy[2]	Lys:EAA Swine[3]
Arg	0.55	0.58	96.4	10.2%	2.04	1.49	1.85
His	0.70	0.76	43.9	4.5%	0.91	3.33	2.50
Ile	0.61	0.67	102.7	10.8%	2.16	1.40	1.78
Leu	0.67	0.61	158.3	17.1%	3.42	0.89	0.89
Lys	0.62	0.69	145.1	15.1%	3.03	1.00	1.00
Met	0.53	0.66	58.2	5.7%	1.14	2.66	3.71
Phe	0.53	0.57	103.4	10.7%	2.15	1.40	1.82
Thr	0.53	0.66	102.9	10.7%	2.14	1.41	1.49
Trp	0.58	--	28.1	2.9%	0.59	5.16	5.33
Val	0.62	0.66	118.8	12.4%	2.48	1.22	1.15

[1] Optimum EAA supply for the dataset described by (Higgs and Van Amburgh, 2016)
[2] Optimum Lys:EAA ratio the dataset described by (Higgs and Van Amburgh, 2016)
[2] Optimum Lys:EAA ratio for a lactating sow (NRC, 2012)

infusion dataset was common and could have skewed the results to reflect a depressed efficiency as the transfer efficiency for any EAA is often dictated by the supply of EAA (Hanigan et al., 1998). The optimum daily supply and EAA composition is provided for the infusion dataset used. These predicted supplies reflect the productive outputs of the dataset used to describe them. Average milk yield for this dataset is lower (26.3 kg) than that for most high-producing cattle in North America (USDA, 2020). The composition of Met and Lys relative to total EAA is in reasonable agreement with previous research (Rulquin et al., 1993, Schwab, 1996) and does suggest the profile of EAA can be applied toward a higher EAA which might be dictated due to increased lactation requirements.

1.3.3 Relationship between amino acids and energy supply within the CNCPS

Traditionally, protein and energy metabolism are thought of as separate entities when balancing nutrient supply; however, these nutrients interact on many physiological levels to ensure animal survival (Lobley, 2007). Metabolic flexibility of organs including the liver, mammary gland, and gastrointestinal tract allows dairy cattle to meet their energetic needs when nutrients are lacking by adaptively changing their metabolism to utilize high yielding energy substrates, including glucose and fatty acids, or AA when other substrates are lacking. Research has described the effect of metabolic flexibility with varying supplies of nutrients and across multiple species (Dunshea et al., 2005, Metcalf et al., 2008, Lemosquet et al., 2010). In spite of the collinearity of these two nutrients, their relationship seems more prevalent when exploring the relationship between digestible EAA and metabolizable energy (Higgs et al., 2014). Figure 1.2 describes the relationship between the efficiency of use for Met and the corresponding optimal supply relative to the ME supplied. This loglogistic relationship shows better model fit when compared to the supply of Met related to total MP and suggests ME supply should be

balanced in addition to balancing EAA. Data variation does exist around the optimum efficiency of use shown in Figure 1.2a and should be evaluated to confirm the optimum supply of EAA relative to ME.

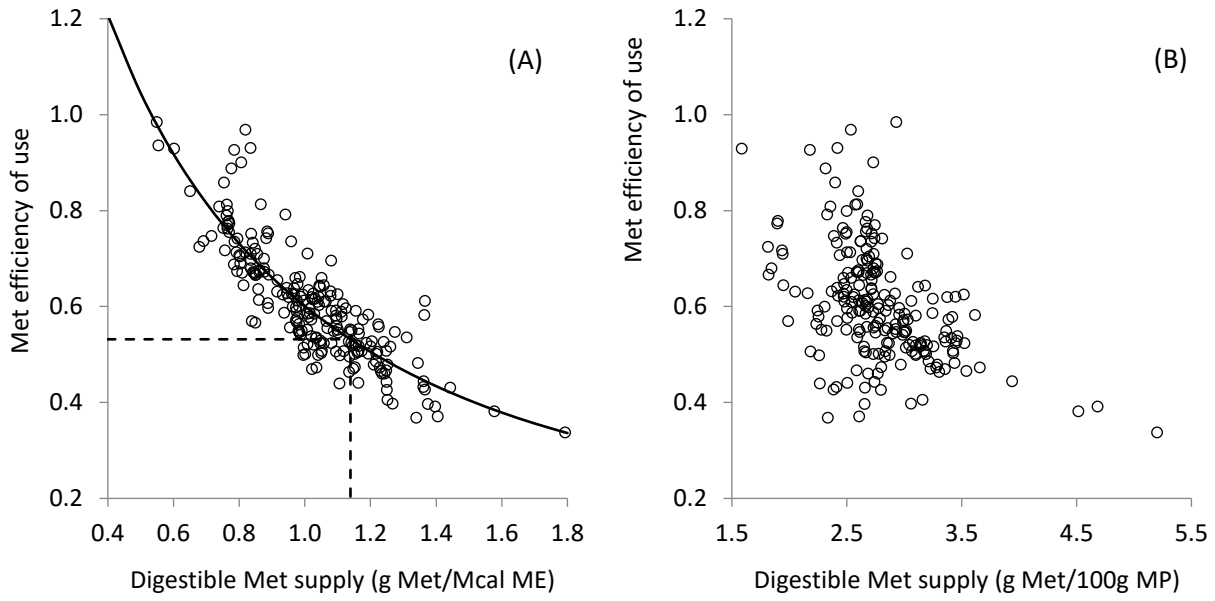

Figure 1.2. Relationship between CNCPS v.7 predicted efficiency of use for Met and Met supply relative to ME (A) or MP (B). The dashed line represents the recommended gram supply of Met per Mcal ME at the optimum efficiency of use for Met. Adapted from (Higgs and Van Amburgh, 2016)

1.4 Objectives

With consideration to what is currently known about AA metabolism and nutritional modeling in ruminant nutrition, the objectives of this body of work are:

1) Evaluate the variability in optimized supply of each EAA relative to ME as described by Higgs and Van Amburgh (2016) and provide recommendations on the confirmation or revision of these values.

2) Assess the efficiency of use for all EAA when diets are provided with different levels of glucogenic or lipogenic nutrients.

3) Rederive the optimum efficiency of use and supply relative ME for each EAA using data provided by objectives 1 and 2. Results from this work will be compared to previous recommendations.

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CHAPTER 2: Evaluating nitrogen efficiency and amino acid balancing relative to metabolizable energy in high producing dairy cattle.

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2.1 Abstract

To optimize nitrogen use efficiency (NUE), amino acid (AA) balancing can be one approach to improve milk and component yield and productive efficiency. Relationships between absorbed supply of each essential AA (EAA) and metabolizable energy (ME) intake have been identified and an optimized supply using these two parameters has been instituted to meet cattle requirements using ration formulation software. Variation exists around these optimized values, and questions involve both methodological and biological variance. The objective of this study was to evaluate differences in animal performance when supplied grams of each EAA relative to ME are varied around the optimized value for those AA. To achieve this, one hundred and forty-four high producing Holstein dairy cattle were assigned to 16 cow pens and randomly allocated to one of three diets: Neutral (NEU; 14.5% CP) which was formulated to contain the optimized supply of each EAA relative to ME, and two diets formulated one standard deviation below, Negative (NEG; 14% CP), and one standard deviation above, Positive (POS; 16% CP), the optimized supply according to the range in the dataset used to create the optimized supply fed in the NEU diet. No differences were observed in DMI among cows fed the three diets (25.9 – 26.4 kg/d); however, cattle fed the NEU and POS diet had improved milk yield, energy corrected milk (ECM), and fat corrected milk (FCM) yield over cattle fed the NEG diet ($P < 0.01$). Further,

cattle fed the NEU and POS diet had higher milk protein output (1.27 and 1.29 kg, respectively) compared to cattle fed NEG (1.14 kg). Body weight (BW), BW change, and body condition score (BCS) were not different among dietary groups. Milk N to feed N was highest in cattle fed the NEU diet (0.343; $P < 0.01$) compared to cattle fed the NEG (0.328) and POS (0.321) diets. Evaluation of nutrient supply in cattle using the CNCPS v.7 demonstrated that each diet was in energy excess when using actual milk output (1.1 – 4.8 Mcal/d excess ME supplied; $P = 0.31$). Negative MP supplies were predicted for both NEG and NEU diets, 140.8 and 155.5 g, respectively, whereas cattle fed the POS diet were in positive MP balance (163.8 g). Model predicted productive N to urinary N was highest in cattle fed the NEG and NEU diets (1.21 and 1.20, respectively), indicating improvements in NUE over cattle fed a greater supply of EAA to ME seen in the POS diet (1.05). Results from this study indicate that cattle fed the NEU exhibited comparable milk and component yield to cattle on the POS diet while being fed a lower rate of EAA supply and thereby improving their NUE over NEG and POS diets. Further indication from this study suggests that the use of results generated to make EAA supply to ME formulation for the NEU is acceptable for continued use in lactating cattle rations.

2.2 Introduction

Improvements in the collective understanding of protein and AA metabolism in ruminants has provided the means to develop more robust predictive equations for both animal requirements and nutritive supply of these nitrogenous molecules. Application of these predictions has become a useful technique not only for optimized efficiency of nitrogen (N) but for the avoidance of excessive excreted N that has detrimental environmental effects (Kebreab et al., 2002, Broderick et al., 2008, Cela et al., 2014). Further, these predictive equations have been built from a considerable volume of work that exists to understand the requirements of essential

amino acids (EAA) which are considered most limiting to lactating cattle, including work on the effect of Met and Lys supplementation (Noftsger and St-Pierre, 2003, Appuhamy et al., 2011, Chen et al., 2011, Whitehouse et al., 2013). Further research has suggested that His may be co-limiting with Met and Lys in high forage (Huhtanen et al., 2002) and low protein diets (Lapierre et al., 2008, Lee et al., 2012, Giallongo et al., 2017). These same approaches that were used to both refine the requirements and accurately describe the supply of Met, Lys, and His should also be applied to remaining EAA required for proper protein synthesis and metabolic function. Although literature data which concurrently formulates and evaluates a varying supply of each EAA relative to predicted requirements of the animal is lacking, work to improve this knowledge base would allow for the abandonment of current diet formulation criteria, including CP which can falsely predict the supply of each EAA under the assumption that the average N content of all protein in feeds is 16% (Schwab and Broderick, 2017). It has been a long-held belief that CP is not a requirement for cattle and that it exists only as an approximate nutrient value which comprises many protein and non-protein nitrogen (NPN) compounds. The advancement in feed chemistry techniques has allowed for the disaggregation of CP into more specific protein and NPN flows, each with distinct characteristics in size, function, digestibility, and AA composition. These improvements in our understanding of protein, AA, and N fractions has allowed for more accurate predictions yielded by currently existing nutritional models which aim to meet, but not exceed, nutrient requirements.

In an effort to transition to the dietary formulation and optimization of each EAA, changes have been made to the most recent research version of the CNCPS v.7 (Higgs and Van Amburgh, 2016) disaggregating CP into its constituent AA and describing their supply according to feed AA profiles and associated amino acid nitrogen (AAN) content. The results of these changes

allow for more accurate predictions of rumen N and AA supplies to the cow, particularly when combined with the estimation of endogenous protein flows (Ouellet et al., 2007, Marini et al., 2008), updated estimations of AA requirements and efficiency of use (Lapierre et al., 2007, Lapierre et al., 2012), and corrections in AA supply according to the multiple time point hydrolysis technique (Van Amburgh et al., 2017, Lapierre et al., 2019). Under the context of improved requirement and supply predictions by the CNCPS v7, an investigation with an AA infusion dataset (Higgs et al., 2014) alluded to a potential relationship between the supply of EAA and metabolizable energy (ME) in the diets fed. These relationships, using techniques published by Doepel et al. (2004), were found by regression analysis of EAA supply and CNCPS v7 predicted requirements for EAA. Solving for the upper critical limit of the second order derivative of that regression, determined the optimum efficiency of use for EAA, and interpolating that efficiency provides a solution for the supply of EAA per unit of ME at that optimum efficiency of use for productivity (Figure 2.1; Higgs et al., 2014). This technique can be applied to calculate the optimized efficiency of use for all EAA and determine the necessary supply for each EAA relative to ME, on a gram basis.

Recognition of the relationship between the supply of protein, or AA, and energy is not a novel idea, particularly when discussing mammalian metabolism. Metabolic flexibility, particularly in the mammary gland, allows dairy cattle to meet their energetic needs through either the use of high yielding energy substrates or N containing compounds (i.e. AA) when other substrates are lacking (Lobley, 2007). Studies have demonstrated that the supplementation of both propionate and casein have a greater, additive effect on milk yield in both cattle (Raggio et al., 2006) and lactating sows (Dunshea et al., 2005) than if either one was solely supplemented. In spite of the collinearity of these two nutrients, their relationship seems more

prevalent when exploring the relationship between digestible EAA and metabolizable energy (Higgs et al., 2014). The use of this relationship as a means to formulate diets has been established among other livestock production systems, particularly with swine (NRC, 2012). Depending upon the stage of life, the weight of animal, and its production (meat or milk), the Swine NRC provides specific tables containing ideal EAA profiles for a given animal which can then be related to a recommended energy content of the diet.

Work has been conducted to evaluate CNCPS v.7 performance when balancing diets for both rumen N requirements and EAA supply using the optimum supply of each EAA relative to ME (Higgs et al., 2014). Findings indicated that notwithstanding lower levels of CP (< 14% DM) in the diet, cattle maintained a high level of milk yield compared to herd mates fed higher CP diets (>16%) when supplied with adequate rumen N and balanced for EAA. However, the variation which exists around the predictions of each optimum supply of EAA relative to ME (Figure 2.1) has not been thoroughly tested which would confirm these predictions and label the variation as statistical error or provide revisions for these optimized EAA supplies.

With these advancements in AA balancing, the objective of this study was to continue the assessment of the relationship between the supply of each EAA and ME by testing both the optimized supply predicted by Higgs and Van Amburgh (2016) and the dataset variability that surrounds these optimums. We hypothesized that a diet which was optimized in the supply for each EAA relative to ME would allow cattle to simultaneously maximize milk component and ECM yield while optimizing NUE as compared to diets which might have a decreased or increased supply of EAA. Use of the CNCPS v7 to formulate diets in this study would not only allow for a more accurate testing of the range in EAA supply relative to ME around the predicted optimums (Figure 2.1) but would allow for the ability to maintain the relevant profile of EAA

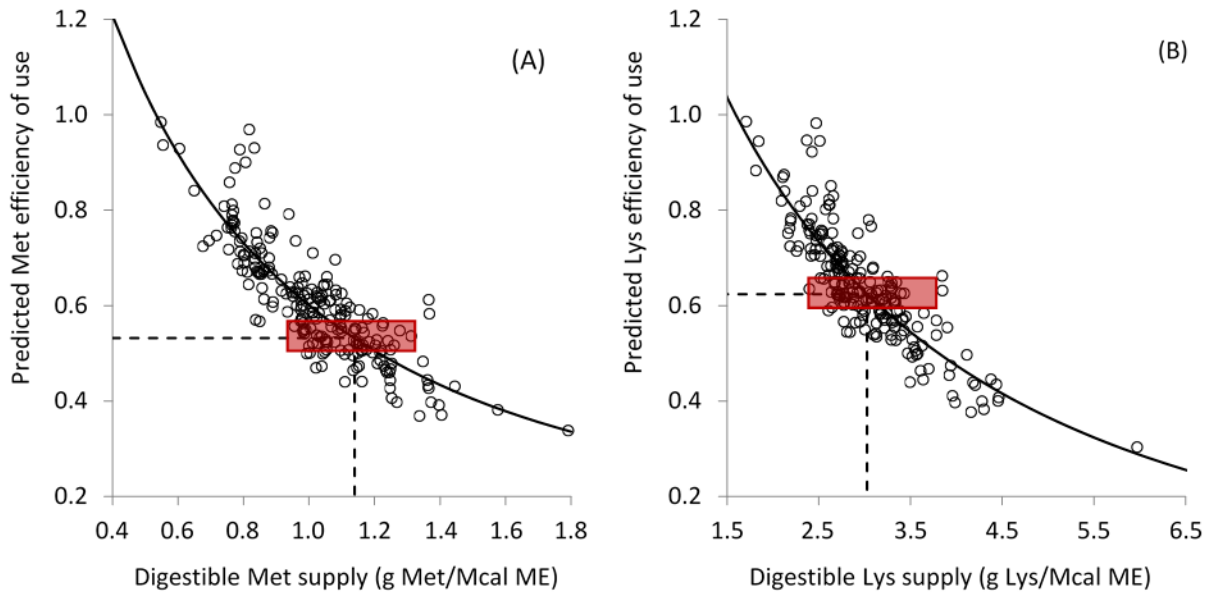


Figure 2.1. Relationship between model predicted EAA requirement: supply and EAA supply relative to ME for Met (A) and Lys (B). The dashed line in (A) & (B) represents the Met or Lys supply at the optimum ratio of model predicted Met or Lys requirement and supply. The red bar represents ± 1 standard deviation of AA supply relative to ME supply.

according to how it has been described in the total optimized supply for all EAA. Results from this study will also provide a means to evaluate the sensitivity of CNCPS v7 EAA supply predictions in the context of ME supply. Collectively, results will either provide confirmation for the optimized supply of each EAA relative to ME or suggest potential revisions to allow for further improvement in milk component yield and NUE.

2.3 Materials and methods

This feeding trial was performed from July – December 2018 at the Cornell University Ruminant Center, located in Harford, NY. All experimental protocols and data collection utilized throughout this trial was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee.

2.3.1 Animals, housing, and experimental design

One hundred and forty-four lactating Holstein dairy cattle (n=144) were enrolled in a 16-wk longitudinal study, including a one-week acclimation period, one week of covariate sampling, and 14-wk of experimental data collection. Cattle selected for the experiment averaged (mean ± SD) 2.88 ± 1.41 lactations, 101 ± 24 days in milk, 13,879 ± 2,949 kg of milk from previous lactation, and 696 ± 73 kg of body weight (BW) at the time of experimental enrollment. Due to a lack of mid-lactation animals at the beginning of the experiment, cattle were assigned to one of two enrollment periods (96 cattle in enrollment 1 and 48 cattle in enrollment 2), spaced three calendar weeks apart. Within each enrollment, cattle were randomly assigned to pens containing 3 primiparous and 13 multiparous cows each. Cattle were balanced for each pen by parity, days in milk, pre-trial and previous lactation milk performance, and BW. Summarized results of initial descriptive variables used to balance all pens and diets are found in Table 2.1 and Table 2.2, respectively. Cattle were fed the same diet throughout the acclimation and covariate period.

Initial measurements were taken during the covariate week and collected in a similar manner to the experimental data collection. All cows were housed in 16-stall pens for the entirety of the study where they had free access to water, feed, and stalls. All pens were fed once daily ranging from 0600 h to 0700 h where diets were fed ad libitum, targeted for a 5% refusal rate across pens, and refusal samples were collected and composited three times weekly at 0500 h. Cattle were milked three times daily at 0800, 1600, and 0000 h in a double-16 parallel parlor (De Laval Inc, Kansas City, MO).

2.3.2 Dietary treatments

Cattle were assigned to one of three diets throughout the 14 wk of experimental data collection. Dietary formulation was performed using the CNCPS v7 model. Formulation of the NEU diet was intended to provide the predicted optimum supply of each EAA relative to ME according to Higgs and Van Amburgh (2016) whereas the formulation of the NEG and POS diets targeted the lower and upper range in variation, respectively, which exists in the dataset used to calculate the optimum EAA supply fed in the NEU diet. As an additional objective to this experiment, it was decided that the composition of EAA fed to all cattle would be consistent to what was observed as the optimum EAA composition by Higgs and Van Amburgh (2016) to avoid any limited EAA supplies which may hinder lactation performance and NUE. . To do this, the standard deviation of data around the optimum Lys supply, which had the largest standard deviation among all EAA data, was used to establish targets for the Lys supply of the NEG and POS diets. Once Lys supply was determined for all diets, the ratio between Lys and all other EAA observed in the optimum EAA composition was used to formulate EAA supply for the lower (NEG) and upper (POS) ranges of EAA supply relative to ME. And because the ratio between EAA supply and ME can intuitively be influenced by either form of nutrients, it was

decided to have all diets be isocaloric, only changing the supply of EAA and MP supply in the diets. The NEU diet was considered balanced in both ME and MP supply, assuming 45 kg/d of ECM. Alternatively, the NEG and POS diets maintained a similar ME target relative to the NEU diet, but varied in their supply of EAA according to study objectives.

Prior to the experiment, commonly fed concentrate samples were sourced from a local commercial feed mill (Purina Animal Nutrition, LLC, Caledonia, NY) and submitted for wet chemistry analysis and fiber digestibility at a commercial feed lab (Cumberland Valley Analytical Services; Waynesboro, PA). Further, high protein, low fiber feedstuffs were analyzed for N intestinal digestibility according to the assay described by Ross (2013) where undigested N residue was analyzed using a combustion assay for estimation of N and AA availability (Leco FP928 N Analyzer, Leco Corp, St. Joseph, MI). Data generated from this analysis was used in substitution of the detergent system results (Neutral and acid detergent insoluble N) which were only used to evaluate protein digestibility in high fiber feed ingredients. The AA profiles of each feedstuffs fed in this experiment were sourced from Van Amburgh et al. (2017) which contained the maximum release of AA from hydrolysate samples after a series of multiple hydrolysis lengths ranging from 2 to 168 h of hydrolysis. Fiber digestibility results were used to predict fast and slow degrading fiber pool sizes and rates of digestion according to Raffrenato et al. (2019).

Forage DM for all diets was primarily corn silage, averaging 51% of total DM, with an average 9.5% DM inclusion of high moisture ear corn and average 7.5% DM inclusion of triticale silage. The remaining feed ingredients were delivered as three different protein premixes from the previously mentioned commercial feed mill and were fed to their respective diet. Each premix shared common ingredients fed at different inclusion levels, excluding the inclusion of a heated treated soy product fed exclusively in the NEU and POS mixes, blood meal

inclusion fed only in the POS mix, and rumen protected Met and blended Met and Lys products included in the POS mix. All diets were supplemented with urea (averaging 0.55% DM inclusion for all diets) to maintain adequate rumen NH_3-N levels and avoid the inhibition of microbial growth and aNDFom [NDF analysis conducted with sodium sulfite and amylase and ash corrected (Mertens, 2002)] digestibility. All cattle were fed the POS diet throughout the acclimation and covariate period, after which pens were randomly assigned to one of the three diets. Dietary ingredients were regularly monitored for any deviations in DM and ingredient inclusion rates were adjusted to match intended formulation of each diet (Table 2.3)

2.3.3 Sample collection and analysis

If cattle were of ill-health due to causes not related to the dietary treatments administered, they were moved to the farm's sick pen to receive treatment until deemed healthy. These animals were not sampled until they were moved back to their pen and on the dietary treatment for at least 2- wk. Animals were removed from the experiment if the illness influenced subsequent health and altered lactation performance over time.

Body weights and body condition score (BCS; 1-5 scale) were measured and assigned, respectively, once weekly on every animal immediately following the 1600 h milking. Body condition score was assigned by at least two trained scorers and averaged. Milk yield was recorded at every milking session by the parlor's software system (DelPro, De Laval Inc., Kansas City, MO) and summed to give total daily milk yields by animal. Milk samples were obtained every week at 3 consecutive milking sessions and analyzed for fat, true protein, total solids, milk urea nitrogen (MUN), and somatic cell count (Dairy One, Ithaca, NY). Milk chemical analysis was performed using Fourier transform infrared spectroscopy (Milkoscan 6000, Foss Electric, Hillerød, Denmark). Daily composition was calculated from both lab results

by using the weighted average of each session relative to the total daily milk yield and applied to each session's milk composition. Daily milk composition was used to determine ECM according to the equations given by Tyrrell and Reid (1965). Daily 3.5% FCM was also calculated using equations from NRC (2001).

Dry matter intake was measured for each pen as the daily amount of feed offered less the amount that was refused corrected for the DM of each forage ingredient, protein premix, and refusal sample and corroborated with the observed DM of each total mixed ration (TMR) sampled. Feed offered, feed refused, and any deviations in ingredient inclusion relative to intended formulation was recorded and stored by the farm's feeding management software (FeedWatch, Valley Agricultural Software, Tulare, CA). Differences in observed diets relative to formulated diets were averaged weekly and used to model nutrient availability to cattle. Samples of forages were collected three times weekly, composited by equal mass and a subsample was sent to a commercial feed lab for chemical analysis using near infrared spectroscopy (NIR) (Table 2.4). The remaining quantity was analyzed for DM using a forced air oven set at 50°C for 96 h, ground through a 1mm screen (Wiley Mill no. 4, Arthur H. Thomas, Philadelphia, PA), and stored for further chemical analysis immediately following the experiment. Measured DM was entered into the feeding software to maintain consistent inclusion of DM from each forage fed at their intended formulation. Total mixed rations of each diet were also sampled three times weekly, composited, subsampled for chemical analysis (Table 2.5) and dried and ground for future analysis. Protein premixes were sampled weekly and analyzed for DM and were sent for chemical analysis every 2 wk. Five separate deliveries of each protein premix were delivered to the farm, where samples of individual ingredients within the premixes were delivered and

Table 2.1. Initial descriptive statistics of cattle by pen within enrollment.

Parameter[1]	Enrollment 1						Enrollment 2					
Pen number	1	2	3	4	5	6	7	8	9		*P*	
Diet	POS[2]	NEU	POS	NEG	NEU	NEG	POS	NEG	NEU	SEM	Enroll	Pen
Lactation number	2.88	2.81	3.13	2.81	2.81	3.00	2.88	2.75	2.88	0.36	0.78	1.00
Days in milk	114.0	114.0	110.0	107.0	116.0	112.0	80.0	77.0	81.0	4.67	< 0.01	0.88
Bodyweight, kg	694.6	695.3	709.9	690.5	665.8	701.3	711.9	698.4	713.1	18.3	0.25	0.81
Milk yield, kg	44.1	46.2	45.7	47.1	45.4	43.8	45.4	45.9	47.7	1.85	0.47	0.84
Previous lactation stats[3], kg/lactation												
Milk yield	14359	14025	14840	13304	13198	13745	13602	13466	14403	834	0.88	0.82
Milk fat yield	567	489	554	498	509	536	520	495	565	35.1	0.97	0.53
Milk protein yield	438	413	449	409	415	423	416	412	444	26.0	0.99	0.90

[1] Data for each parameter were obtained one week prior to the adaptation period relative to each enrollment.
[2] NEG = Negative; NEU = Neutral; POS = Positive
[3] First lactation animals were not included in analysis given no previous lactation data.

Table 2.2. Initial descriptive statistics summarized for each diet.

	Diet				*P*	
Parameter[1]	Negative	Neutral	Positive	SEM	Enroll	Pen
Lactation number	2.84	2.82	2.95	0.21	0.77	0.90
Days in milk	93.0	98.0	96.0	2.71	< 0.01	0.38
Bodyweight, kg	699.2	693.7	708.0	10.8	0.25	0.63
Milk yield, kg	45.8	46.6	45.2	1.05	0.47	0.63
Previous lactation Stats[2], kg/lactation						
Milk Yield	13491	13861	14253	483	0.88	0.52
Milk Fat Yield	509	521	547	20.5	0.98	0.40
Milk Protein Yield	415	424	435	15.0	0.99	0.63

[1] Data for each parameter were obtained one week prior to the adaptation period relative to each enrollment.
[2] First lactation animals were not included in analysis given no previous lactation data.

Table 2.3. Feed ingredient inclusion (mean ± SD)[1] of experimental diets.

	Diet		
Ingredient, % DM	Negative	Neutral	Positive
Corn silage	51.7 ± 0.9	52.0 ± 0.9	51.0 ± 0.9
High moisture ear corn	9.7 ± 0.4	9.7 ± 0.4	10.1 ± 0.4
Triticale forage	6.7 ± 0.6	6.7 ± 0.6	7.5 ± 0.6
Protein premix			
Canola meal	1.8 ± 0.04	9.1 ± 0.2	6.3 ± 0.2
Corn meal	6.4 ± 0.1	6.4 ± 0.2	5.9 ± 0.1
Heat treated soybean meal	-	0.9 ± 0.02	3.6 ± 0.1
Blood meal	-	-	3.1 ± 0.1
Soybean hulls	9.2 ± 0.2	3.8 ± 0.1	2.8 ± 0.1
Soybean meal	8.1 ± 0.2	5.5 ± 0.1	2.7 ± 0.1
Dextrose	1.6 ± 0.04	1.6 ± 0.04	2.2 ± 0.1
Saturated fatty acid[3]	0.7 ± 0.02	0.7 ± 0.02	0.9 ± 0.02
Calcium carbonate	0.8 ± 0.02	1.0 ± 0.02	1.0 ± 0.03
Sodium bicarbonate	0.7 ± 0.02	0.7 ± 0.02	0.8 ± 0.02
Urea	0.6 ± 0.01	0.5 ± 0.01	0.5 ± 0.01
Min AD	1.1 ± 0.02	0.5 ± 0.01	0.6 ± 0.02
White salt	0.3 ± 0.01	0.3 ± 0.01	0.2 ± 0.01
Magnesium oxide	0.1 ± 0.002	0.1 ± 0.003	0.2 ± 0.005
Rumen protected methionine[4]	-	-	0.1 ± 0.002
Rumen protected methionine and lysine[5]	-	-	0.1 ± 0.001
Vitamin and mineral mix[6]	0.2 ± 0.005	0.2 ± 0.005	0.2 ± 0.005

[1] Composition obtained from commercial mill data and on-farm feeding management software.
[2] SOYPLUS (West Central Cooperative, Ralston, IA).
[3] ENERGY BOOSTER 100 (MSC Company, Dundee, IL).
[4] Smartamine M (Adisseo USA Inc, Alpharetta, GA)
[5] Smartamine ML (Adisseo USA Inc, Alpharetta, GA)
[6] Included: (% DM) 27.37 % Ca; 0.48% Mg; 0.08% K; 4.53 % S; 222.9 ppm Fe; 24,997.9 ppm Zn; 5,765.2 ppm Cu; 18,473.7 ppm Mn; 134.5 ppm Se; 568.1 ppm Co; 568.1 ppm I; 2,022 KIU/kg Vitamin A; 562 KIU/kg Vitamin D; 9,661 IU/kg Vitamin E.

analyzed for chemical analysis, aNDFom digestibility, and N intestinal digestibility when applicable (Table 2.5).

Fecal spot samples were collected on wk 7 and wk 12 of the experiment from a subset of randomly selected cows, 2 primiparous and 6 multiparous, from each pen for diet digestibility determination and calculations. Eight samples (~500 g of wet weight each) from each cow selected were collected over a 3-d period (Day 1: 1300 h and 1900 h; Day 2: 0100 h, 0700 h, 1600 h, and 2200 h; Day 3: 0400 h and 1000 h) composited by timepoint and pen, and was frozen at -20°C. Samples were thawed several days following the last day of sampling, thoroughly mixed, and a subsample (~1.5 kg of wet weight) was dried 50°C for 96 h, and ground through a 1mm screen. Processed fecal samples were analyzed for aNDFom and undigested, amylase and ash corrected NDF (uNDFom) following a 240 h *in vitro* incubation with rumen fluid based on the method described by Raffrenato et al. (2018). Data generated from these analyses were used to determine apparent total tract digestibility of DM, OM, aNDFom, and potentially digestible, amylase and ash corrected NDF (pdNDFom) using uNDFom as an internal marker (Huhtanen et al., 1994).

Cattle that were fecal sampled also had blood samples collected during the covariate period and every 2 wk following the initial sample. Samples were collected 10 h after feeding via the coccygeal vein using vacutainer tubes containing lithium heparin, placed on ice until the remaining samples were collected (averaging 20 min), centrifuged at $3{,}000 \times g$ for 20 min at 4°C, and plasma was harvested. Aliquots of plasma was stored in microcentrifuge tubes and immediately frozen at -20°C for later analysis. A separate aliquot was mixed with equal parts of 5-sulfosalicylic acid (SERAPREP, Pickering Laboratories, Mountain View, CA), containing DL-glucosamic acid added as an internal standard, to effectively precipitate protein from the sample

Table 2.4. Chemical composition (mean ± SD)[1] and aNDFom digestibility of forages fed in experiment.

Parameter[2]	Corn silage	High moisture ear corn	Triticale silage
Dry matter, %	38.0 ± 1.9	64.7 ± 3.3	23.8 ± 2.0
CP, % DM	6.9 ± 0.2	8.1 ± 0.6	12.3 ± 0.5
Soluble protein, % CP	52.4 ± 5.4	46.6 ± 5.7	78.9 ± 5.5
NH3-N, % Soluble protein	22.1 ± 2.0	24.9 ± 2.0	21.6 ± 4.5
NDIP, % CP	11.4 ± 0.9	7.6 ± 1.4	9.2 ± 2.4
ADIP, % CP	9.8 ± 1.0	5.6 ± 0.8	6.8 ± 1.0
aNDFom, % DM	37.7 ± 1.7	24.1 ± 2.8	58.3 ± 2.9
30h uNDFom, % aNDFom	44.1 ± 2.8	-	27.7 ± 3.0
120h uNDFom, % aNDFom	33.9 ± 2.5	-	21.9 ± 3.0
240h uNDFom, % aNDFom	30.6 ± 2.9	-	18.4 ± 2.9
ADF, % DM	22.5 ± 1.3	11.8 ± 2.3	37.3 ± 1.7
Lignin, % DM	2.8 ± 0.3	1.5 ± 0.3	3.8 ± 0.3
Acetic acid, % DM	1.5 ± 0.5	1.2 ± 0.3	2.6 ± 0.7
Propionic acid, % DM	0.1 ± 0.07	-	-
Butyric acid, % DM	-	-	0.6 ± 0.11
Lactic acid, % DM	3.2 ± 0.6	1.1 ± 0.3	7.1 ± 1.3
Ethanol soluble sugars, % DM	1.0 ± 0.4	1.4 ± 0.3	1.9 ± 0.6
Starch, % DM	38.6 ± 1.9	62.3 ± 3.2	0.7 ± 0.28
Soluble fiber, % DM	6.2 ± 0.5	-	7.1 ± 1.5
Ether extract, % DM	2.6 ± 0.1	3.1 ± 0.3	3.5 ± 0.2
Ash, % DM	2.7 ± 0.5	1.9 ± 0.2	6.9 ± 1.1
AA N, % Total N[3]			
Arginine	4.0	10.8	4.9
Histidine	2.4	6.2	3.2
Isoleucine	2.4	2.7	2.7
Leucine	5.9	8.3	4.5
Lysine	2.7	4.4	4.8
Methionine	1.6	2.2	1.8
Phenylalanine	3.4	3.3	3.5
Threonine	2.2	2.8	2.4
Tryptophan	0.9	1.5	1.4
Valine	3.6	4.0	3.9
EAA N	29.1	46.2	33.1
Alanine	7.9	7.5	5.1
Aspartic acid	2.8	4.8	5.9
Cysteine	1.4	2.0	1.5
Glutamic acid	5.5	11.2	3.7
Glycine	4.4	5.0	4.9
Proline	6.0	9.2	5.8
Serine	2.2	4.4	2.6
Tyrosine	1.5	2.2	1.8
NEAA N	31.7	46.3	31.3
AA N, % Total N	60.8	92.5	64.4

[1] Analyzed values obtained from 17 weekly samples for each forage ingredient.

[2] ADIP = Acid detergent insoluble protein; NDIP = Neutral detergent insoluble protein; aNDFom = amylase and ash corrected neutral detergent fiber; uNDFom = undigested neutral detergent fiber analyzed after the specified number of hours.

[3] Values procured from Van Amburgh et al. (2017)

Table 2.5. Chemical composition (mean ± SD)[1] of concentrate ingredients fed in experiment.

Parameter[2]	Blood meal	Canola	Corn meal	SoyPlus	Soybean meal	Soybean hulls	Wheat midds
Dry matter, %	92.3 ± 1.3	90.9 ± 0.9	90.6 ± 1.2	91.4 ± 0.9	92.5 ± 1.0	90.9 ± 0.6	90.2 ± 0.4
CP, % DM	95.5 ± 1.6	41.5 ± 0.4	8.2 ± 0.2	46.7 ± 2.1	52.6 ± 0.4	13.2 ± 0.7	17.4 ± 0.6
Soluble protein, % CP	5.2 ± 4.0	14.7 ± 3.7	13.2 ± 4.0	9.3 ± 2.7	18.1 ± 1.5	21.9 ± 3.4	34.7 ± 1.1
NDIP, % CP	-	13.2 ± 3.6	9.5 ± 0.9	14.1 ± 1.7	1.0 ± 0.1	27.6 ± 3.8	22.0 ± 0.1
ADIP, % CP	-	5.8 ± 0.5	5.2 ± 0.8	1.4 ± 0.2	0.7 ± 0.08	8.7 ± 1.0	3.9 ± 0.1
uNitrogen[3], % Total N	27.5 ± 4.3	21.2 ± 3.0	N/A	14.5 ± 2.2	9.5 ± 2.8	N/A	N/A
aNDFom, % DM	-	25.8 ± 3.5	8.7 ± 0.6	19.2 ± 2.4	7.8 ± 0.2	67.9 ± 3.2	44.2 ± 3.6
12h uNDFom, % aNDFom	-	-	-	-	-	61.6 ± 1.5	-
72h uNDFom, % aNDFom	-	-	-	-	-	6.3 ± 0.6	-
120h uNDFom, % aNDFom	-	-	-	-	-	5.7 ± 0.3	-
ADF, % DM	-	21.0 ± 0.5	3.2 ± 0.4	10.5 ± 2.2	5.1 ± 0.36	50.0 ± 1.5	12.8 ± 0.1
Lignin, % DM	-	8.5 ± 0.1	0.7 ± 0.2	1.5 ± 1.0	0.4 ± 0.30	0.9 ± 0.13	3.8 ± 0.5
Ethanol soluble sugars, % DM	0.40 ± 0.28	9.3 ± 0.7	2.1 ± 0.4	12.8 ± 1.2	12.4 ± 1.0	3.0 ± 0.7	5.2 ± 1.2
Starch, % DM	0.05 ± 0.03	0.6 ± 0.14	76.0 ± 1.8	0.6 ± 0.07	0.56 ± 0.09	1.0 ± 0.3	18.3 ± 1.3
Soluble fiber, % DM	-	-	-	-	-	-	-
Ether extract, % DM	0.1 ± 0.05	3.6 ± 0.5	3.6 ± 0.3	4.7 ± 1.1	1.3 ± 0.2	2.0 ± 0.2	3.6 ± 0.2
Ash, % DM	5.5 ± 3.6	7.6 ± 0.1	1.7 ± 0.3	6.8 ± 0.4	7.3 ± 0.4	5.0 ± 0.3	6.1 ± 0.1

N/A = Not analyzed.

Table is continued on next page.

Table 2.5.(continued) Chemical composition (mean ± SD)[1] of concentrate ingredients fed in experiment.

Parameter[2]	Blood Meal	Canola	Corn Meal	SoyPlus	Soyhulls	Soybean Meal	Wheat Midds
AA N, % Total N[4]							
Arginine	8.7	12.3	10.8	14.0	10.4	14.4	14.5
Histidine	9.8	4.4	6.2	4.2	5.2	4.5	4.7
Isoleucine	0.5	2.7	2.7	3.0	2.9	3.1	2.2
Leucine	8.2	4.6	8.3	5.0	5.0	5.1	4.1
Lysine	11.0	6.2	4.4	6.6	8.9	7.1	5.1
Methionine	1.2	1.8	2.2	1.1	1.4	1.3	1.5
Phenylalanine	4.2	2.7	3.3	3.0	2.8	3.2	2.6
Threonine	3.3	2.9	2.8	2.5	2.6	2.7	2.1
Tryptophan	3.4	2.2	1.5	1.9	2.4	2.0	2.5
Valine	6.1	3.8	4.0	3.7	3.9	3.6	3.5
EAA N	56.4	43.6	46.2	45.0	45.5	47.0	42.8
Alanine	7.4	4.0	7.5	3.8	4.3	3.8	4.3
Aspartic acid	6.0	4.3	4.8	6.5	5.9	6.7	4.3
Cysteine	0.9	2.0	2.0	1.0	1.8	1.2	1.7
Glutamic acid	4.8	9.6	11.2	9.6	5.9	9.9	10.0
Glycine	4.5	5.6	5.0	4.7	11.0	4.6	5.9
Proline	4.7	6.0	9.2	4.2	5.8	3.7	6.4
Serine	4.2	3.4	4.4	3.8	5.2	4.1	3.4
Tyrosine	2.0	1.9	2.2	2.2	2.7	2.2	1.9
NEAA N	34.5	36.8	46.3	35.8	42.6	36.2	37.9
Total AA N	90.9	80.4	92.5	80.8	88.1	83.2	80.7

[1] Analyzed values obtained from 5 batch samples per feed ingredient when new protein premix batches delivered.

[2] ADIP = Acid detergent insoluble protein; NDIP = Neutral detergent insoluble protein; aNDFom = amylase and ash corrected neutral detergent fiber; uNDFom = undigested neutral detergent fiber analyzed after the specified number of hours.

[3] Values obtained through use of intestinal digestibility assay (Ross, 2013)

[4] Values obtained from Van Amburgh et al. (2017)

and analyze for plasma AA analysis. Once dosed, the sample was vortexed for several seconds, placed on ice for 16 h while vortexing occasionally, centrifuged at 15,800 × g for 10 min at 4°C, and collecting the supernatant frozen at -20°C until analysis. Plasma urea nitrogen (PUN) analysis was measured as described by Chaney and Marbach (1962) using an enzymatic colorimetric assay based on a purchased commercial kit (No. 640; Sigma-Aldrich, St. Louis, MO).

2.3.4 Power calculation and statistical analysis

A major outcome of interest for this experiment was the ECM output for the diets fed. Under initial experimental assumptions, cattle produced a daily average 45 kg of ECM according to their dietary formulations, the standard deviation around this mean was estimated at 3.7 kg, and the desired observable difference was established at 3 kg. To achieve the necessary statistical power for this experiment, set by the researchers to be a minimum of 80% under an alpha of 0.05 using a F-distribution test, it was determined that each diet would need a minimum of three, 16-cow pens for a total of 144 cows under this experimental design.

All data were analyzed using SAS version 9.4 (SAS Institute Inc. Cary, NC). Forage, feed ingredients, and TMR samples were summarized over the experiment using PROC TABULATE. Under the assumptions of the experimental design, pen was designated as the experimental unit whereas cattle within the experiment were classified as subsample observations within each pen (St-Pierre, 2007, Bello et al., 2016). Evaluation of pens and diets balanced for cattle parameters was performed using PROC GLM, testing the effect of enrollment and pen within enrollment, shown in Table 2.1, and diet, shown in Table 2.2. Multiple comparisons using least squared mean differences were adjusted using Tukey's adjustment. With the exception of BCS, all variables of interest were analyzed using PROC MIXED. Body condition score was analyzed

using a non-parametric analysis (PROC NPAR1WAY) where the fixed effect of diet was used for analysis of differences. Any dependent observations made at the cow-level, including lactation performance results, animal characteristic changes, and blood metabolites, the following statistical model was used under the mixed-model analysis:

$$Y_{ijlkmn} = \mu + E_i + T_j + W_k + EW_{ik} + TW_{jk} + P_{l:j} + PW_{kl:i} + C_{m:lj} + L_n + BX_{jlm} + \epsilon_{ijklmn}$$

where $Y_{ijlkm} =$ the dependent variable, $\mu =$ the overall mean, $E_i =$ the fixed effect of the ith Enrollment, $T_j =$ the fixed effect of jth dietary treatment, $W_k =$ the fixed effect of kth experimental week, $P_{l:j} =$ the random effect of lth pen within the jth dietary treatment, $C_{m:lj} =$ the random effect of mth cattle within the lth pen and the jth dietary treatment, $L_n =$ the fixed effect of nth lactation group (grouped as, 1st lactation, 2nd lactation, and $\geq$ 3rd lactation), $BX_{jlm} =$ the covariate adjustment for each cow sampled, and $\epsilon_{ijklmn} =$ the residual error. When analyzing pen-level observations, including DMI, diet digestibility, and productive efficiency, cattle specific variables were dropped from the previous model and available covariate measurements were applied on a pen-level basis. Statistical analysis of initial dependent variable measurements, either on a cow or pen-level, and non-repeating measurements, including changes in BW and BCS, did not have a covariate or repeated measurement analysis. Normally distributed residuals and homogeneity of variances were satisfied for all statistical models used for data analysis. For all statistical analysis, degrees of freedom were adjusted using the Kenwood-Rogers option to account for any missing data in the analysis. Covariance matrix structures were determined for each repeated measurement analysis according to best model fit

as determined by the lowest Akaike information criteria (AIC) where the null model likelihood test was significant. Diet comparisons were performed using least squares means and the Tukey's adjustment for multiple comparisons. Statistical significance was designated at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

2.4 Results

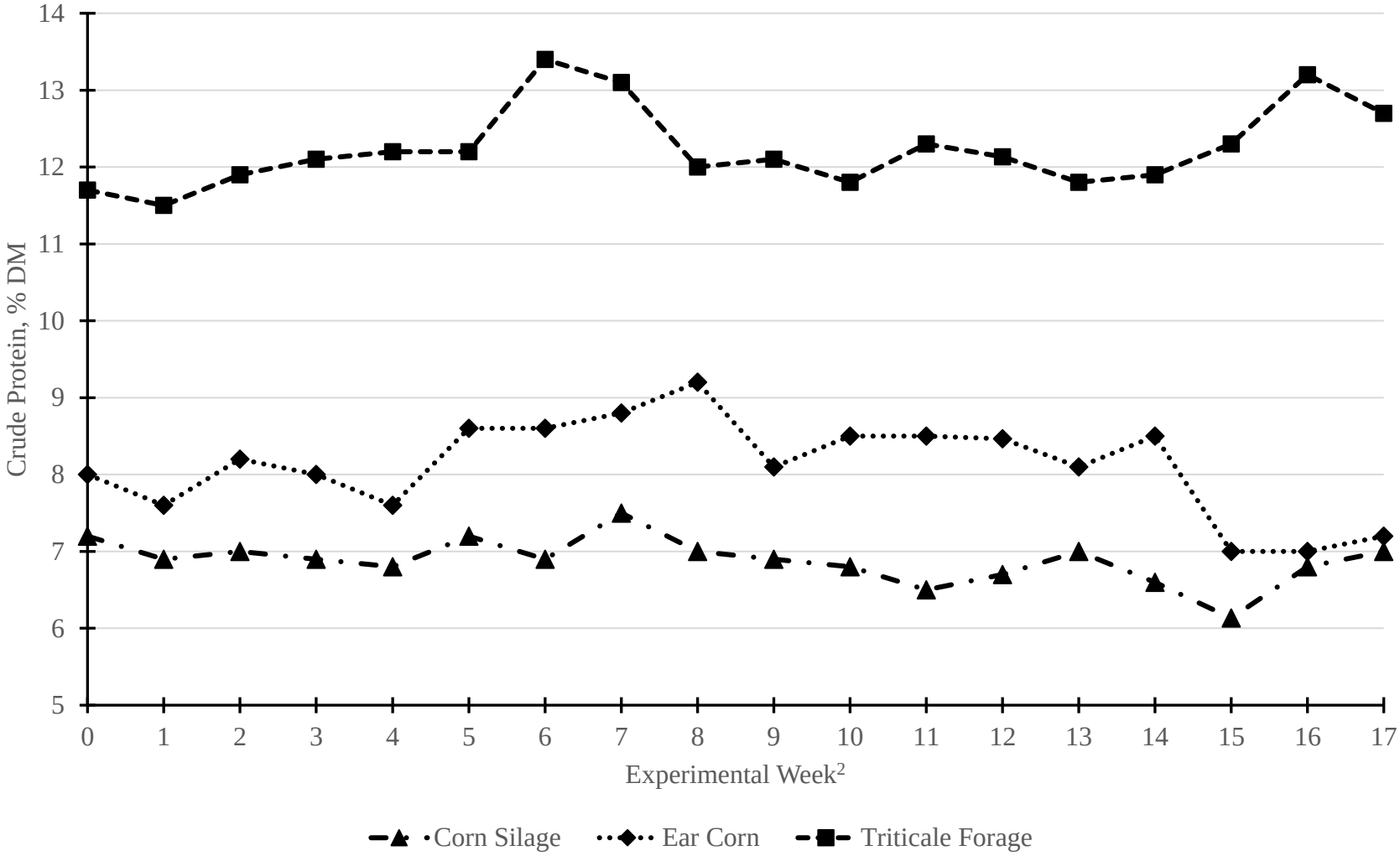
2.4.1 Diets, intake, and digestibility

Variability of ingredient composition relative to the formulated diets was minimal, with the highest variability exhibited in corn silage inclusion rates (Standard deviation averaging 0.9% of dry matter across all diets; Table 2.3). Because most of the ingredients fed were within the protein premix, their variability across the experiment followed a similar trend within each diet. The corn silage averaged 37.7% aNDFom, 30.6% of which was uNDFom, and a starch content of 38.6% of DM. On wk 8 of the experiment, a new hybrid of corn silage was fed to cattle; however, the shift in chemical composition was negligible and the inclusion of this forage remained similar to previous weeks. High moisture ear corn averaged 24.0 % aNDFom, 11.8 % ADF, and 61.5 % starch; however, the range of starch content, and subsequently the aNDFom content due to nutrient dilution, was noticeably high, ranging from 54% to 67% and 19% to 30%, respectively (Data not shown). The inclusion rate of this feed was modestly adjusted, increasing 0.25% of DM from week 8 to 10 to account for the dilution of starch content and maintain ME supply. Triticale forage averaged 58.3% aNDFom, 18.4% uNDFom, and 11.5% CP. It is important to note that diets were formulated with triticale forage which contained 16.0% CP, a nearly 30% increase in protein content over the observed measurements; however, with similar inclusions of this forage across all diets, the average level of NDF matching the formulated level, and a large proportion of N being supplied by non-forage sources, the inclusion level was kept at

its original rate to maintain proper rumen fill among all diets. Ranges in the protein content of each forage are reasonably small with noticeable increases in triticale on wk 4 and 5 and a sudden drop in corn silage on wk 15-17 (Figure 2.2).

Diets were formulated to be iso-caloric, increasing in supply of each EAA from the NEG to POS diet. Average dietary CP for each diet was not different from respective formulations, except for the NEG diet, which tested higher than expected (Formulated CP: NEG = 13.5%; NEU = 14.5%; POS = 16.0%). Evaluation of CP over time for all diets shows variation that was higher than anticipated, with average standard deviation across all diets at 0.65% of DM (Figure 2.3). Despite this variation, the analyzed CP of all diets remained in the same order as the intended formulations, with NEG, NEU, and POS diets in order of increasing protein content. Neutral detergent insoluble protein did increase from NEG to POS but the ADIP values remained constant among treatments, suggesting that N digestibility across treatments was consistent but that the POS and NEU diets had a greater intake of protein B2 than the NEG diet. To improve EAA supply for the NEU and POS diets, the inclusion of heat-treated soy, animal by-products, and rumen protected AAs were added to the diets (Table 2.3), inherently increasing the MP supply from feed. To maintain DMI while decreasing the supply of EAA to the NEG diet, a larger inclusion rate of soy hulls was added relative the other diets fed. Subsequently, a dilution between protein, aNDFom, and starch occurred where NEG tested for higher levels of aNDFom and starch compared to the other diets. Further, the uNDF was significantly lower in the NEG diet than NEU and POS ($P < 0.01$), decreasing the intake of dietary uNDF and improving the diet's aNDFom and pdNDFom digestibility over the other two diets (Table 2.7; $P < 0.01$) which were not significantly different than each other. Dry matter and aNDFom intake were not

Figure 2.2. Analyzed weekly[1] crude protein of forages fed in diets over the duration of the experiment.



[1] Forages were submitted for analysis once weekly and have no measure of variability within a given week.
[2] Cattle enrolled in enrollment 1 were fed forages from experimental week 0 to 14 and cattle enrolled in enrollment 2 were fed forages from experimental week 3 to 17.

Table 2.6. Comparison of chemical composition for experimental diets varying in essential amino acid supply relative to ME.

	Diet[2]				*P*
Parameter	Negative	Neutral	Positive	SEM	Diet
Dry matter, %	44.6	44.4	44.6	0.21	0.68
Crude protein, % DM	14.0^{c}	14.7^{b}	15.9^{a}	0.10	< 0.01
Soluble protein, % CP	42.8^{a}	40.2^{b}	37.2^{c}	0.34	< 0.01
Ammonia, % SP	13.3^{ab}	14.3^{a}	12.1^{b}	0.50	0.01
NDIP, % CP	15.1^{c}	15.6^{b}	18.9^{a}	0.11	< 0.01
ADIP, % CP	5.68^{abx}	5.86^{a}	5.43^{by}	0.08	< 0.01
RUP, % CP	28.6^{c}	29.9^{b}	31.4^{a}	0.17	< 0.01
Ethanol soluble sugar, % DM	3.88	4.00	3.89	0.08	0.50
Starch, % DM	29.9	29.4	29.3	0.20	0.11
Starch digestion 7hr, % Starch	78.6^{a}	76.3^{b}	78.6^{a}	0.32	< 0.01
aNDFom, % DM	32.5^{a}	31.1^{b}	31.5^{b}	0.22	< 0.01
uNDF 24hr, % NDF	44.5^{c}	48.1^{b}	49.3^{a}	0.28	< 0.01
uNDF 240hr, % NDF	25.3^{b}	28.9^{a}	28.5^{a}	0.24	< 0.01
ADF, % DM	20.9^{a}	20.0^{b}	19.9^{b}	0.16	< 0.01
Lignin, % DM	2.62^{bx}	3.01^{a}	2.76^{aby}	0.05	< 0.01
Ash, % DM	6.57^{b}	6.89^{a}	6.51^{b}	0.05	< 0.01
Ether extract, % DM	3.49^{c}	3.61^{b}	3.75^{a}	0.04	< 0.01
NE_L, Mcal/kg	1.68^{b}	1.70^{a}	1.71^{a}	0.01	< 0.01

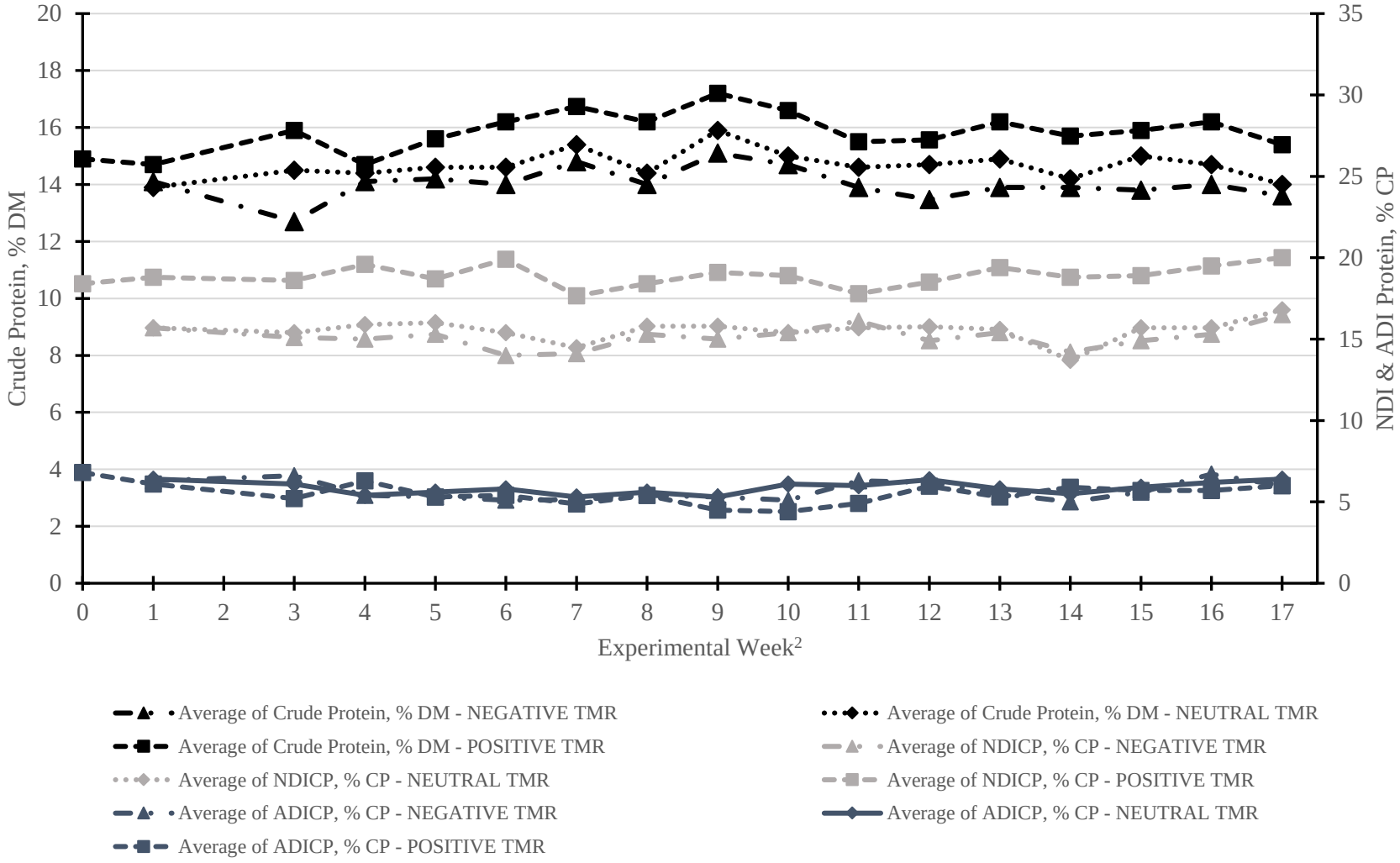
[1] Analyzed values obtained from 17 weekly samples for each total mixed ration.

[2] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Figure 2.3. Analyzed weekly[1] crude protein, neutral detergent insoluble protein, and acid detergent insoluble protein of total mixed rations for each diet over the duration of the experiment.



[1] Forages were submitted for analysis once weekly and have no measure of variability within a given week.
[2] Cattle enrolled in enrollment 1 were fed forages from experimental week 0 to 14 and cattle enrolled in enrollment 2 were fed forages from experimental week 3 to 17.

different among diets throughout the experiment (Table 2.8) and the digestibility trial (Table 2.7).

2.4.2 Animal performance and feed efficiency

Milk yield, ECM, and FCM yield were not different among pens during the covariate period of the experiment ($P > 0.10$; Figure 2.4). Two weeks after diet assignments to pens, cattle fed the NEG diet showed a significant decrease in ECM relative to cattle fed the NEU and POS diets ($P < 0.05$; Table 2.8 and Figure 2.4). Cattle fed the POS diet showed a numerically higher output of ECM over cattle fed the NEU diet; however, statistical analysis shows no significant difference between these two diets throughout the experiment ($P = 0.40$), apart from wk 14. Milk component yields follow a similar trend to ECM, where cattle fed the NEG diet producing nearly 100 g/d less of both milk fat and true protein compared to the NEU and POS diets, whereas NEU and POS cattle did not exhibit different outputs in component yields (Table 2.8). Concentration of milk urea nitrogen (MUN) increased across treatments from NEG to POS with a range of 10.5 to 13.6 mg/dL (Table 2.8). These results correlate with PUN concentrations of cattle which increased from NEG to POS diet.

Initial BW were not different among pens and treatments (Table 2.1 and Table 2.2). Further, average BW was not different among treatments (Table 2.8); however, the rate of weekly weight gain was numerically lower in the NEG diet than cattle which were fed either the NEU or POS diets. Initial and final BCS were not different among treatments, indicating no effect of diet on body composition or mobilization of body reserves to support lactation requirements.

Milk yield to DMI was affected by the improvement of EAA to ME as cattle that were fed the NEG diet were less efficient in their milk production than those fed the NEU and POS, both of which were not different from one another (Table 2.8). This relationship was also observed

Table 2.7. Effect of varying essential amino acid supply relative to metabolizable energy on apparent total tract nutrient intake and digestibility.

	Diet[1]				*P*	
Parameter	Negative	Neutral	Positive	SEM	Enrollment	Diet
Nutrient intake, kg/d						
Dry matter	26.2	26.6	26.6	0.33	0.02	0.65
Organic matter	24.3	24.8	24.7	0.31	0.02	0.51
aNDFom[2]	9.29	9.42	9.56	0.11	0.02	0.30
pdNDFom[3]	7.08	6.98	7.18	0.08	0.01	0.30
uNDFom[4]	2.21[a]	2.44[b]	2.38[b]	0.03	0.02	0.01
Apparent digestion, %						
Dry matter	73.7[a]	68.6[b]	68.3[b]	0.59	0.35	< 0.01
Organic matter	75.6[a]	71.1[b]	70.8[b]	0.58	0.24	< 0.01
aNDFom	55.9[a]	47.5[b]	49.4[b]	0.77	0.18	< 0.01
pdNDFom	73.4[a]	64.3[b]	66.1[b]	1.04	0.19	< 0.01

[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.
[2] aNDFom = amylase and ash corrected neutral detergent fiber
[3] pdNDFom = potentially digestible NDF, amylase and ash corrected.
[4] uNDF = undigested NDF, amylase and ash corrected.
[ab] Within a row, means without a common superscript differ ($P < 0.05$)

Table 2.8. Intake, lactation performance, and feed efficiency for animals fed experimental diets varying in essential amino acid supply relative to ME.

	Diet[1]				*P*[2]		
Parameters	Negative	Neutral	Positive	SEM	Enroll	Diet	Diet x Week
Intake and lactation performance, kg/d							
Dry matter intake	25.9	26.4	26.4	0.27	0.41	0.37	0.10
Energy corrected milk yield[3]	40.3[b]	43.3[a]	44.2[a]	0.51	0.01	< 0.01	0.30
3.5% fat corrected milk[4]	41.0[b]	43.7[a]	44.6[a]	0.55	0.01	< 0.01	0.48
Milk yield	37.6[b]	40.5[a]	41.6[a]	0.40	0.37	< 0.01	0.02
True protein yield	1.14[b]	1.27[a]	1.29[a]	0.01	0.23	< 0.01	0.01
Fat yield	1.54[y]	1.61[x]	1.65[x]	0.03	0.05	0.07	0.67
Lactose yield	1.79[b]	1.93[a]	1.97[a]	0.02	< 0.01	< 0.01	0.01
Milk composition, %							
True protein	3.08[b]	3.17[a]	3.15[a]	0.02	0.01	< 0.01	0.42
Fat	4.17	4.09	4.08	0.08	0.07	0.69	0.81
Lactose	4.80	4.80	4.80	0.02	< 0.01	0.99	0.03
Milk urea nitrogen, mg/dL	10.5[c]	11.2[b]	13.6[a]	0.14	< 0.01	< 0.01	< 0.01
Plasma urea nitrogen, mg/dL	8.5[b]	10.3[ab]	12.0[a]	0.56	0.09	0.16	0.02
Body weight and condition							
Overall bodyweight, kg	710	711	714	2.4	< 0.01	0.40	0.01
Final bodyweight, kg	718	727	724	4.9	< 0.01	0.43	-
Body weight change, kg·wk^{-1}	1.73	2.39	2.14	0.35	< 0.01	0.43	-
Final BCS, 1-5 scale	2.89	2.90	2.91	-	-	0.71	-
Feed efficiency calculations							
Milk Yield:DMI	1.47[b]	1.57[a]	1.59[a]	0.02	0.71	< 0.01	0.13
ECM:DMI	1.58[b]	1.68[a]	1.69[a]	0.02	0.26	< 0.01	0.62
FCM:DMI	1.61[by]	1.69[ax]	1.71[a]	0.02	0.25	0.04	0.71
Milk N:Feed N	0.328[b]	0.343[a]	0.321[b]	0.004	< 0.01	< 0.01	< 0.01

[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

[2] The main effect of week is highly significant ($P < 0.01$) for all parameters which contain this effect in its statistical model and is omitted from this table.

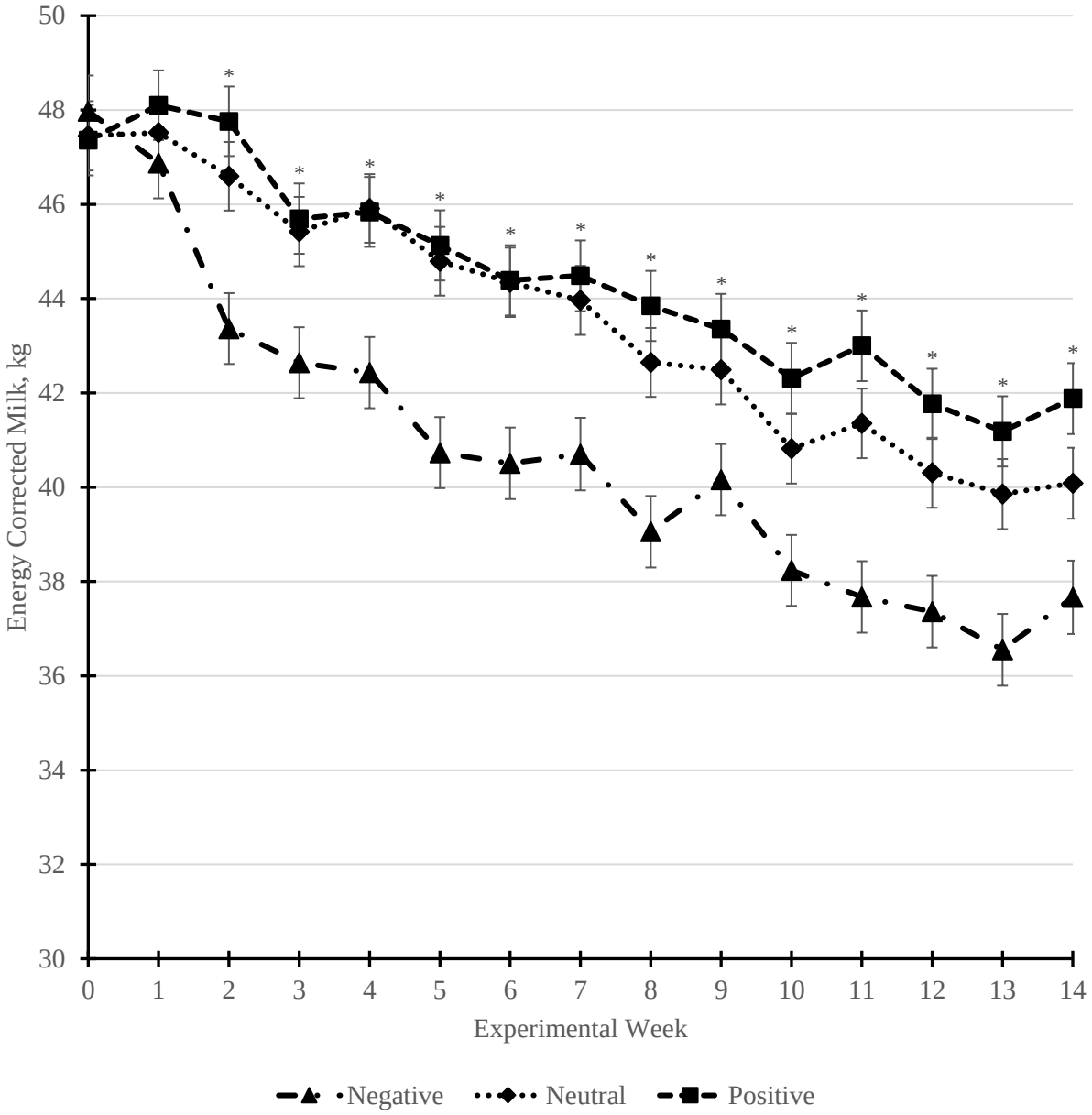
[3] Calculated using equations generated by Tyrrell and Reid (1965).

[4] Calculated using equation adapted from NRC (2001).

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

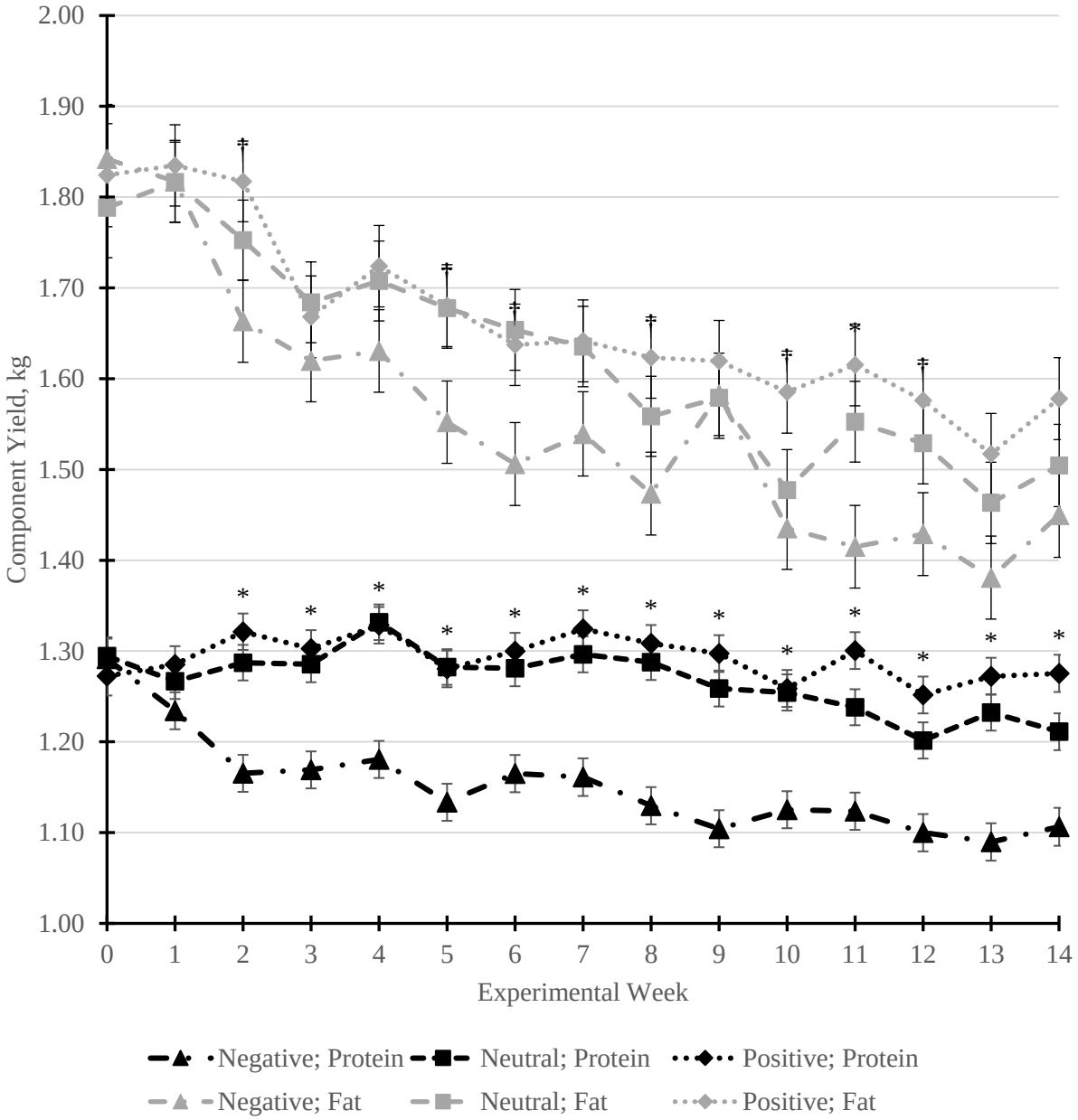
Figure 2.4. Effect of varying essential amino acid supply relative to metabolizable energy[1] on energy corrected milk



[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

* Indicates differences among treatments within a week ($P < 0.05$)

Figure 2.5. Effect of varying essential amino acid supply relative to metabolizable energy[1] on milk fat and true protein yield



[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

* Within a response variable, indicates differences among treatments on a given week ($P < 0.05$)

† Within a response variable, indicates differences among treatments on a given week ($P < 0.10$)

when evaluating efficiencies using ECM to DMI and to a lesser degree when evaluating FCM to DMI. Although each treatment had an efficiency of milk N to feed N over 30 %, cattle fed the NEU diet observed the highest NUE (0.343) which was greater than either the NEG or POS diets ($P < 0.05$). Milk N to feed N was not different in cattle fed the NEG or POS diet ($P = 0.47$).

2.4.3 Amino acid and nitrogen balances and model outputs

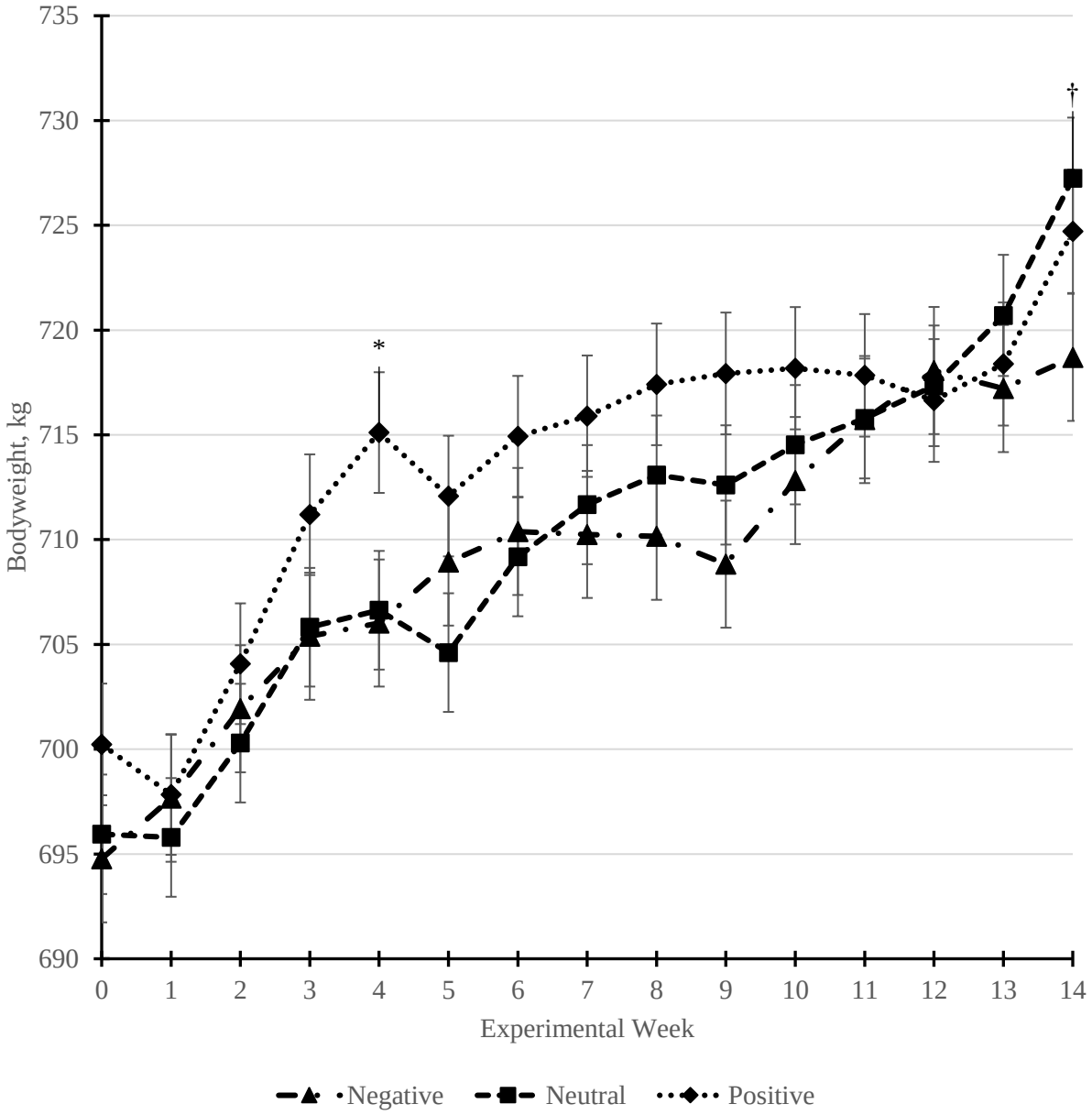
Variability in ingredient inclusion rate, analyzed feed chemistry, and mixing error for all diets yielded variation in EAA supply relative to formulation targets (Table 2.9). Within the NEG diet, each predicted EAA supply, apart from Arg, Met, and Val, was higher than intended amounts. Notably, metabolizable supply of Lys was predicted to be 15 g higher than formulation (201.5 supplied vs 185.7 g formulated), whereas Met was supplied at nearly the same quantity as what was formulated. Supplied EAA for the NEU diet were much closer to intended formulations; however, metabolizable supply of Met was supplied at a rate of nearly 7 g less than its formulation. A consistent increase in the supply of each EAA was not always observed when moving from the NEU to POS diet. Isoleucine and Trp were two EAA where their supplies were less in the POS diet than the NEU diet. Further, only a slight increase in Arg was observed when comparing NEU and POS diets. Methionine supply was also lower than anticipated for the POS diet; however, an increase in supply was detected between NEU and POS diets.

Model predicted supply of EAA relative to ME illustrates that all EAA supplies are greater than the formulated targets needed for the NEG diet. Differences between the formulated and model predicted values are due to numerically lower ME supply that was predicted for the NEG diet. Dietary formulation assumed about 70 Mcal/d of ME for each of the three diets; however, all three diets were less than their targets given the actual DMI of cattle, with the NEG diet supplying 67.2 Mcals of ME (Table 2.10). Similar outcomes were observed for both the NEU

and POS diets where most of the EAA supplies relative to ME exceed their targets. Predicted Met supply was lower in the NEU diet than what was expected (Table 2.9); however, this was also true for the POS diet. The Lys:Met ratio was different among all treatments ($P < 0.01$; Table 2.9), with the ratio being higher for each treatment relative to their respective targets. Irrespective of the differences of model predicted EAA supply relative to formulations, the supply of EAA increased from the NEG to POS diets, apart from Ile and Trp within the POS diet.

In addition to expressing EAA supply on a gram and gram to ME basis, the composition of EAA supplied to each diet was also reviewed (Table 2.10). As a percent of total EAA, nearly all EAA were reasonably close to optimum composition described previously. Variation from this intended optimum was similar for NEG and NEU diets, where the average deviation was 0.29 and 0.28 percentage units, respectively. Conversely, higher variation was observed in the POS diet, where the average deviation was 0.56 percentage units from the optimum. Larger variation in this diet is due to a substitution of greater levels of Leu for lower levels of Ile. Similar to swine nutrition techniques (NRC, 2012) all EAA were compared to supply of Lys, which is typically most limiting in ovine species, and comparisons were made to the targeted profile. All EAA supplies were meant to increase or decrease proportionally to the NEU diet to maintain the same EAA profile. The elevated supply of Lys in the NEG diet caused both Lys:Arg and Lys:MET to increase 9% over the targeted profile. Alternatively, a higher supply of Trp in this diet caused Lys:Trp to decrease 13%. A decreased supply of Met and increased supply of Arg and Trp in the NEU diet caused Lys:Met to increase 8.7% and Lys:Arg and Lys:Trp to decrease -6% and -11.5%, respectively. A substantial decrease in Ile and Trp and modest decrease in Arg and Met supply caused Lys:Ile, Lys:Trp, and Lys:Met to increase 20.3%, 14.6%, 8.6%, and

Figure 2.6. Effect of varying essential amino acid supply relative to metabolizable energy[1] on average body weight.



[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

* Indicates differences among treatments within a week ($P < 0.05$)

† Indicates differences among treatments within a week ($P < 0.10$)

6.2%, respectively for the POS diet. Further the increase in His supply for the POS diet caused Lys:His to decrease 14.7% from the intended profile.

Metabolizable energy supply was in excess relative to the model predicted requirements of milk production (Table 2.11) indicating that animals were not limited by energy supply. Conversely it was shown that the MP balance for the NEG and NEU diets were negative and different than the MP supply predicted for the POS diet ($P < 0.01$). Microbial protein supply was not different among diets ($P = 0.32$) although MP from feed sources increased as diet improved in EAA supply ($P < 0.01$), diluting the proportion of MP from microbial sources. Model predictions further indicated the cattle fed the NEG and NEU diets had higher efficiency of MP (76.6% and 76.8%, respectively) and EAA (60.9% and 61.9%, respectively) compared to cattle fed the POS ($P < 0.01$). Conversely, model predicted mean rumen ammonia content was significantly lower for the NEG (7.9 mg/dL) cattle when compared to NEU (9.1 mg/dL) and POS cattle (8.9 mg/dL).

Nitrogen intake increased with increasing EAA supply, ranging from 563 g in the NEG diet to 683 g in the POS diet (Table 2.11). Predicted fecal and urinary N excretion was different among all treatments with excretion increasing with additional N intake ($P < 0.01$). Productive N use, defined as N used for milk protein synthesis, lean tissue growth, and fetal growth demands, was higher for both NEU and POS diets compared to the NEG diets ($P < 0.01$; Table 2.10) but was not different between each other ($P = 0.96$). Alternatively, productive N to urinary N was significantly higher for cattle fed NEG (1.21) and NEU (1.20) over POS (1.05) cattle but was not different from each other.

Table 2.9. Predicted essential amino acid supply, expressed as daily metabolizable grams and grams per megacalorie of metabolizable energy for each diet fed.

	Diet[1]							*P*
Parameter[2]	Negative		Neutral		Positive		SEM	Diet
Metabolizable supply, g·d^{-1}	Target	Predict	Target	Predict	Target	Predict		
Arginine	143.9	141.1^{b}	144.9	153.2^{a}	164.5	154.1^{a}	1.6	< 0.01
Histidine	56.0	60.6^{c}	64.7	66.1^{b}	73.0	87.1^{a}	0.7	< 0.01
Isoleucine	131.8	146.0^{b}	153.5	155.2^{a}	173.7	146.9^{b}	1.7	0.02
Leucine	209.1	223.9^{c}	243.0	239.2^{b}	275.7	285.5^{a}	2.6	< 0.01
Lysine	185.7	201.5^{c}	215.3	214.0^{b}	243.8	248.1^{a}	2.3	< 0.01
Methionine	69.5	69.5^{c}	81.0	74.1^{b}	92.2	88.3^{a}	0.8	< 0.01
Phenylalanine	131.8	148.4^{c}	152.8	155.3^{b}	173.0	178.3^{a}	1.7	< 0.01
Threonine	131.1	142.6^{c}	152.1	154.6^{b}	172.3	166.8^{a}	1.6	< 0.01
Tryptophan	36.1	45.1^{ay}	41.9	47.0^{ax}	47.5	42.2^{b}	0.5	< 0.01
Valine	160.9	157.9^{c}	176.2	170.6^{b}	199.9	196.3^{a}	1.8	< 0.01
Lys:Met	2.67	2.90^{ax}	2.66	2.89^{ay}	2.65	2.81^{b}	0.003	< 0.01
Grams EAA·Mcal ME^{-1}								
Arginine	2.03	2.10^{b}	2.04	2.23^{a}	2.32	2.25^{a}	0.01	< 0.01
Histidine	0.79	0.90^{c}	0.91	0.96^{b}	1.03	1.27^{a}	0.003	< 0.01
Isoleucine	1.86	2.17^{c}	2.16	2.26^{b}	2.45	2.15^{a}	0.01	< 0.01
Leucine	2.95	3.33^{c}	3.42	3.49^{b}	3.89	4.17^{a}	0.01	< 0.01
Lysine	2.62	3.00^{c}	3.03	3.12^{b}	3.44	3.62^{a}	0.01	< 0.01
Methionine	0.98	1.04^{c}	1.14	1.08^{b}	1.30	1.29^{a}	0.003	< 0.01
Phenylalanine	1.86	2.21^{c}	2.15	2.26^{b}	2.44	2.60^{a}	0.01	< 0.01
Threonine	1.85	2.12^{c}	2.14	2.25^{b}	2.43	2.44^{a}	0.01	< 0.01
Tryptophan	0.51	0.67^{c}	0.59	0.69^{b}	0.67	0.62^{a}	0.002	< 0.01
Valine	2.27	2.35^{c}	2.48	2.49^{b}	2.82	2.87^{a}	0.01	< 0.01
Metabolizable energy, Mcal·kg^{-1}	2.57	2.60^{b}	2.58	2.62^{a}	2.58	2.61^{a}	0.004	< 0.01

[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

[2] Predicted parameters obtained from CNCPS v.7 model outputs

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Table 2.10. Predicted essential amino acid composition as a percent of total essential amino acids and as Lys:EAA for diets varying in essential amino acid supply relative to ME.

		Diet[1]				*P*
EAA composition, % EAA[2]	Optimum[3]	Negative	Neutral	Positive	SEM	Diet
Arginine	10.2	10.6[b]	10.7[a]	9.7[c]	0.005	< 0.01
Histidine	4.5	4.5[c]	4.6[b]	5.5[a]	0.006	< 0.01
Isoleucine	10.8	10.9[a]	10.9[a]	9.2[b]	0.008	< 0.01
Leucine	17.1	16.8[b]	16.7[b]	17.9[a]	0.008	< 0.01
Lysine	15.1	15.1[b]	15.0[c]	15.6[a]	0.006	< 0.01
Methionine	5.7	5.2[b]	5.2[b]	5.5[a]	0.003	< 0.01
Phenylalanine	10.7	11.1[b]	10.9[c]	11.2[a]	0.004	< 0.01
Threonine	10.7	10.7[b]	10.8[a]	10.5[c]	0.003	< 0.01
Tryptophan	2.9	3.4[a]	3.3[b]	2.7[c]	0.002	< 0.01
Valine	12.4	11.8[b]	11.9[a]	12.3[c]	0.002	< 0.01
Lys:EAA						
Arginine	1.49	1.43[b]	1.40[c]	1.61[a]	0.001	< 0.01
Histidine	3.33	3.32[a]	3.24[b]	2.85[c]	0.004	< 0.01
Isoleucine	1.40	1.38[b]	1.38[b]	1.69[a]	0.001	< 0.01
Leucine	0.89	0.90[a]	0.89[b]	0.87[c]	0.001	< 0.01
Lysine	1.00	1.00	1.00	1.00	-	-
Methionine	2.66	2.90[a]	2.89[a]	2.81[b]	0.002	< 0.01
Phenylalanine	1.40	1.36[c]	1.38[b]	1.39[a]	0.001	< 0.01
Threonine	1.41	1.41[b]	1.38[c]	1.49[a]	0.000	< 0.01
Tryptophan	5.16	4.47[c]	4.56[b]	5.87[a]	0.005	< 0.01
Valine	1.22	1.28[a]	1.25[c]	1.26[b]	0.001	< 0.01

[1] Neutral = All EAA are supplied relative to data generated by Higgs and Van Amburgh (2016); Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.
[2] Predicted parameters obtained from CNCPS v.7 model outputs
[3] Optimum EAA composition calculated by Higgs and Van Amburgh (2016)
[ab] Within a row, means without a common superscript differ ($P < 0.05$)
[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Table 2.11. Selected CNCPS v.7 model outputs for diets varying in essential amino acid supply relative to ME.

	Diet[1]				*P*
Parameter	Negative	Neutral	Positive	SEM	Diet
DMI[2], kg·d^{-1}	25.9	26.2	26.2	0.3	0.57
Actual milk[2], kg·d^{-1}	37.2^{b}	40.4^{a}	40.8^{a}	0.4	< 0.01
ME allowable milk, kg	41.1^{y}	42.5^{x}	41.5^{xy}	0.4	0.10
MP allowable milk, kg	34.5^{c}	37.5^{b}	45.4^{a}	0.5	< 0.01
ME/MP first limiting, kg	34.5^{c}	37.5^{b}	41.5^{a}	0.5	< 0.01
ME/MP allowable average, kg	37.8^{c}	40.0^{b}	43.5^{a}	0.4	< 0.01
ME supply, Mcals·d^{-1}	67.2	68.7	68.5	0.7	0.31
ME required, Mcals ME·d^{-1}	62.4^{c}	65.9^{b}	67.4^{a}	0.8	0.02
ME balance, Mcals ME·d^{-1}	4.8^{a}	2.7^{bx}	1.1^{by}	0.4	0.01
MP supply, g·d^{-1}	2549^{c}	2751^{b}	3090^{a}	29.9	< 0.01
MP required[3], g·d^{-1}	2690^{b}	2907^{a}	2926^{a}	39.8	0.01
MP balance, g·d^{-1}	-140.8^{a}	-155.5^{a}	163.8^{b}	28.5	< 0.01
MP RUP, g·d^{-1}	936^{c}	1105^{b}	1484^{a}	13.4	< 0.01
MP microbial, g·d^{-1}	1614	1646	1606	17.9	0.32
MP microbial, %	60.4^{a}	56.7^{b}	48.8^{c}	0.1	< 0.01
EAA apparent efficiency[4], %	60.9^{b}	61.9^{a}	55.7^{c}	0.7	< 0.01
MP apparent efficiency[5], %	76.6^{a}	76.8^{a}	68.9^{b}	0.8	< 0.01
Mean rumen NH_3, mg·dl^{-1}	7.9^{b}	9.1^{a}	8.9^{a}	0.1	< 0.01
Mean bacterial growth depression, %	10.8^{a}	5.9^{b}	5.5^{b}	0.3	< 0.01
RDP, % DM	9.9^{b}	10.3^{a}	9.5^{c}	0.01	< 0.01
RUP, % DM	3.7^{c}	4.4^{b}	6.7^{a}	0.02	< 0.01
NDF intake, kg	8.72^{ax}	8.36^{by}	8.09^{b}	0.09	0.01
NDF digested at rumen, %	58.4^{a}	56.9^{b}	57.0^{b}	0.08	< 0.01
Starch intake, kg	7.95	8.09	7.98	0.08	0.48
Starch digested at rumen, %	82.5	82.3	82.6	0.09	0.17
Rumen NDF pool size and fluxes					
uNDF pool size, kg·kg^{-1} BW	0.684^{b}	0.749^{a}	0.728^{a}	0.04	< 0.01
uNDF flux, (kg·d^{-1}) ·kg^{-1} BW	0.290^{b}	0.313^{a}	0.298^{b}	0.003	0.01
Total NDF pool size, kg·kg^{-1} BW	1.21^{xy}	1.22^{x}	1.18^{y}	0.01	0.08
Nitrogen accounting, grams					
Nitrogen intake	563.1^{c}	617.6^{b}	682.6^{a}	5.8	< 0.01
Total fecal nitrogen	231.3^{c}	243.7^{b}	255.8^{a}	2.4	< 0.01
Total urinary nitrogen	148.8^{c}	167.6^{b}	193.4^{a}	2.6	< 0.01
Productive nitrogen	178.9^{b}	200.0^{a}	201.4^{a}	3.6	0.01
Productive N to Urinary N, g·g^{-1}	1.21^{a}	1.20^{a}	1.05^{b}	0.04	0.04

[1] Neutral = All EAA are supplied relative to data generated in Higgs; 2014; Negative = Each EAA supply are shifted 1 standard deviation below the Neutral supply; Positive = Each EAA supply are shifted 1 standard deviation above the Neutral supply.

[2] Observed means across experiment.

[3] Model predicted MP requirements under the assumption of a 73% efficiency of use for MP supplied.

[4] Calculated as a weighted average using the apparent efficiency of use for each EAA.

[5] Apparent efficiency of use = MP Required/MP supply

2.5 Discussion

Objectives of this study were to evaluate milk and component yield and the NUE of cattle fed varying supplies of EAA relative to the ME supplied. The ability to predict and balance for individual EAA supplies in diets has pronounced implications towards the elimination of excess protein fed in diets. Further, the use of CP to assess adequate N and AA supply is no longer warranted as its use leads to inaccurate predictions of EAA supply using the assumption that all feed ingredient protein has the same AA profile, expressed as a protein N content of 16% (Schwab et al., 2014).

Dietary N content and intake are the primary driver of NUE (Huhtanen and Hristov, 2009); however, it has also been shown that increases in digestibility and subsequent energy availability can further improve NUE (Reynal et al., 2003, Rotz, 2004). Based on results from previous work evaluating the relationship between EAA supply and ME within the structure of the CNCPS v7, it was hypothesized that the NEU diet would provide the optimized balance between EAA and energy to allow cattle to simultaneously be more efficient with the EAA they consumed and avoid any loss in ECM yield. Evaluation of the CP contents of these diets shows that the POS diet (15.9% DM) would typically be observed in most North American, corn silage-based diets with the NEG (14% DM) and NEU (14.7% DM) diets considered too low for most high producing dairy cattle. Yet, when describing NUE using the metric of milk N output compared to dietary N intake, all cattle had a NUE efficiency above 30%. Among commercial herds a large variation in NUE efficiency can be observed, averaging 25% with a range of 40% around that average (Jonker et al., 2002, Gourley et al., 2012). Improvements in NUE through the reduction dietary CP and use EAA balancing serves as one of many on-farm BMP which benefit farmers through increased profitability and a reduction in N excretion into the

surrounding environment (Ndegwa et al., 2008, van der Stelt et al., 2008, Fadul-Pacheco et al., 2017). Under the context of this study, each diet benefited from the use of balancing EAA relative to ME as milk N to feed N was greater than 30% for these diets, with the NEU diet providing the highest NUE efficiency (34.3%; Table 2.8).

Supplying the appropriate quantity of each EAA proved challenging for all diets fed in this study (Table 2.9). It is crucial to preface that all diets were formulated to supply around 70 Mcal/d of ME to cattle; however, CNCPS v7 predicted ME supply for these diets showed a 1.5 to 2.8 Mcal/d difference, altering the grams of each EAA to ME ratio (Table 2.10). Post-study model evaluation of supplies of EAA to ME within these diets are higher than what was originally formulated for. The supply of each EAA was higher in the NEG than was formulated for, apart from Met, which was supplied at formulated targets. Methionine is regarded as the most limiting EAA in dairy cattle and it's plausible a supplemental increase to match the relative increase in the other EAA fed in the NEG diet would have boosted milk yield (Noftsger and St-Pierre, 2003, Lee et al., 2012) or milk components (Chen et al., 2011). Typically being regarded as a group 1 AA, improvements in mammary uptake of achieved through improvement in metabolizable Met supply would have most likely resulted in improved milk performance (Lapierre et al., 2012). Several of the predicted EAA supplies relative to ME for the NEG diet are more similar to what was formulated for the NEU diet; however, the improvement in Met supply in the NEU diet from the NEG is likely why there is a significant increase in lactation performance from NEG to NEU. Haque et al. (2015) supplied a similar profile of EAA fed in the NEU diet, except for supplying His at a greater level than Met and obtained similar results for component yield and N efficiency. Several other studies and reviews have demonstrated the need to improve His supply to overcome any limitations in supply (Lee et al., 2012, Giallongo et

al., 2017, Lapierre et al., 2021). An increase in supply of nearly all EAA can be observed when moving from the NEU to POS diets. Due to availably of feedstuffs and the need to maintain a proper balance of other diet components, Ile and Trp did not increase in supply from NEU to POS and might have been limiting relative to the supply of the other EAA. Several studies have indicated that the supplementation of branch chain AA (BCAA) does not improve milk production and likely stimulates other body protein synthesis (Mackle et al., 1999, Appuhamy et al., 2011, Yoder et al., 2019). Tryptophan supplementation is rarely evaluated in ruminant models; however, the relative supply of Trp in all diets fed was higher than what is typically observe when formulating diets for lactating dairy cows.

Formulating diets for the proper EAA profile is also crucial in reducing excessive protein and N and improving NUE. Relating individual EAA requirements to Lys is a frequent formulation technique adopted by swine nutritionists (NRC, 2012) who have determined Lys as the most limiting in lactating diets (Kim et al., 2009). Adherence to the targeted EAA profile, which was the same for all diets in this study, proved to be difficult even for formulated diets. The ability to reach the targeted supplies for each EAA relative to ME is based on the ability to source and balance the appropriate feed ingredients which will meet other nutrient requirements while having complimentary EAA profiles to meet, but not exceed EAA requirements. Under the controlled setting of the research farm, variations in predicted Lys to each EAA supply ranged from -15 to 25% of intended supplies (Table 2.10). Methionine, Trp, and His were frequently misaligned in the profile of almost all diets fed in this experiment; however, because requirements for these EAA are relatively smaller than other EAAs, quantitative differences in their gram supply would cause more impactful variations in the total EAA profile. Other deviations from the targeted profile are a result of altered Lys supply which causes the ratio of

Lys to each EAA to vary more, as was observed in the NEG diet. Future feed technologies might look to provide rumen protected AA beyond the most-limiting EAAs to assist in balancing the EAA profile fed and reducing the inclusion rate of other feed ingredients which may help balance some EAA but exceed the balance of others. Further, the feasibility of balancing for all EAA relative to ME under a commercial farm setting should be evaluated given the level of variability in EAA supply under a controlled research setting. Currently, many producers are balancing for Met and Lys supply; however, given the variability of feed EAA composition coupled with variable feed costs, balancing for only a subset of EAA might be warranted.

The evaluation of milk and component yield through use of amalgamating metrics such as ECM or FCM allows for a more biologically inclusive representation of performance. Ruminants have the ability to be metabolically flexible and utilize nutrients in a variety of pathways depending upon nutrient limitations and prioritizing nutrients to particularly pathways to optimize for energetic efficiency (Lobley, 2007, Lemosquet S. et al., 2011). For this reason, ECM is used as the primary lactation performance metric given the consideration of milk yield, fat, and protein component yield. Cattle performance throughout this experiment showed comparable ECM production for both NEU and POS cattle, with comparable persistency. Energy corrected milk of cattle fed the NEG decreased after two weeks on study, indicating the mammary gland likely adjusted its affinity and uptake of EAA during that period but could not meet the demand of milk production due to a limited nutrient supply (Arriola Apelo et al., 2014).

Predicted mean rumen N concentration increased with increasing levels of N intake among treatments. At the lowest concentration, 7.9 mg/dL in the NEG diet, model predictions estimated a mean bacterial growth depression of 10.8% relative to the NEU and POS diets which averaged a 5.7% reduction in microbial mass (Table 2.10). Work from Satter and Slyter (1974) suggests

that a minimum rumen ammonia level of 5 mg/dL is needed to maintain sufficient microbial growth and subsequent MP from microbial sources. All diets were supplied with urea at a rate of 0.5% DM to avoid approaching this threshold in the rumen which likely inhibit NDF digestibility and decrease DMI as observed in other literature meant to evaluate low levels of CP and the effect on NUE (Olmos Colmenero and Broderick, 2006). Dry matter intake and subsequent aNDFom intake was slightly lower in NEG cattle during the digestibility trial; however, no difference was observed when compared to intakes from NEU and POS cattle ($P = 0.65$ and $P = 0.30$, respectively; Table 2.7). Further, apparent digestibility of these diets indicates that pdNDFom digestibility was highest in the NEG ($P < 0.01$) and is mostly the results of a higher inclusion rate of soy hulls in the diet which is known to have a higher extent of digestibility over other high fiber ingredients (Cunningham et al., 1993, Ipharraguerre et al., 2002). The predicted MP supply from microbial yield was not different among treatments ($P = 0.32$; Table 2.10). To improve EAA supply relative to ME from the NEG to POS diets, feeds that would typically resist ruminal degradation and supply RUP were used (Table 2.3). The prediction of MP supply from feed were observed in dietary evaluations using the CNCPS v7, where supply ranged from 936 g in the NEG diet to 1484 g in the POS diet (Table 2,10). Improvements in MP from RUP sources are often associated with higher levels of ECM and milk protein output (Santos et al., 1998, Volden, 1999, Noftsger and St-Pierre, 2003).

Total MP balance was negative for both the NEG and NEU diets; however, the cattle fed the NEU diet made a comparable amount of milk and components relative to the POS diet which was in excess over 150 g. The efficiency of MP to net protein (NP) is set to 73% within the CNCPS v.7 (Higgs et al., 2014) and is used to determine requirements of MP based on lactation, maintenance, and other biological process which required N. The model is also capable of

describing the apparent efficiency of use for both total MP supply as well as the supply of each EAA supplied. Metabolizable protein use efficiency within this study ranged from 68.9 % in the POS diet to 76.8% in the NEU diet, suggesting that although the use of an efficiency of MP use at 73% is biologically relevant, cattle might have varied their efficiency based on a limiting supply of nutrients (Metcalf et al., 2008, Sinclair et al., 2014). The results of the NEG and NEU diets agree with Higgs et al. (2014), who observed a nearly 10% increase in efficiency of use for MP when diets ranged from -140 to -310 g of MP balance. The efficiency of use for EAA followed a similar trend to MP and ranged from 55.7% in POS diet to 61.9 % in the NEU diet. Determination of this number is based on the weighted average of the efficiency of use for each EAA in the diets fed weighted by the proportional supply of each EAA to total EAA supplied. When using the optimized supply of EAA to ME according to Higgs et al. (2014), the apparent efficiency of use for EAA is 59%; however, the NEU diet, which was formulated to evaluate these optimum supplies, observed a 5% increase over the optimized efficiency of EAA use… Again, it is worth recognizing that cattle fed the NEU diet had nearly all their EAA supplies met relative to formulated targets, except for Met.

2.6 Conclusion

The ability to improve NUE while maintaining an improved lactation performance is obtainable when balancing for the optimum EAA supply relative to ME. Cattle fed the NEU diet had similar ECM yield and component production while having a reduced CP and EAA supply when compared to the POS diet. These results indicate that milk and component yield decrease as the supply of EAA to ME decreases to levels fed in the NEG diet. Further, cattle which were fed the increased supply of EAA to ME fed in the POS diet observed a marginal improvement in milk and component yield over cattle fed the NEU diet, reducing their NUE. Model estimates

were sensitive to these changes in EAA supply and predicted the appropriate milk response observed from each diet fed. Collectively, results support the experimental hypothesis and reinforce the recommended gram supply of each EAA relative ME proposed by Higgs and Van Amburgh (2016) as the optimized supply for lactating dairy cattle. Improvements in EAA requirements coupled with better description of EAA supply from both microbial and feed supplies allow for more accurate model predictions, leading to reduced N excretion, improved productive efficiency, and an optimized farm profitability.

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CHAPTER 3: THE EFFECT OF DIETARY STARCH LEVELS AND ENERGY PROVIDING NUTRIENT SOURCES ON AMINO ACID REQUIREMENTS OF LACTATING DAIRY CATTLE.

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3.1 Abstract

Efforts to optimize nitrogen use efficiency (NUE) and improve amino acid (AA) balancing in lactating dairy cattle, particularly when describing the supply and requirements of essential AA (EAA), have demonstrated a relationship between metabolizable AA supply and metabolizable energy (ME). Additional data has demonstrated that gluconeogenic versus lipogenic substrates providing ME might alter the efficiency of EAA use. The experimental objective was to evaluate energy-corrected milk (ECM) yield in cattle fed diets with two levels of dietary starch and two levels of EAA supply. Lactating cattle (n = 192; 2.7 ± 1.4 lactations, 94 ± 26 DIM, 681 ± 85 kg BW) were blocked in 16 cow pens (n = 6) by parity, DIM, and BW and enrolled for 60 d in either one of two blocks of a randomized complete, unbalanced block experiment. Pens were fed a common diet during a 10-d covariate period and randomly assigned to one of four diets. Diets were fed containing either two levels of dietary starch (23% [LS] and 29% [HS] DM) and two levels of EAA (100% [100] and 105% [105] of requirements as described by CNCPS v7). Further, diets were formulated to be isocaloric and balanced for EAA supply, with the 100% MP diets formulated for the optimum gEAA/Mcal ME and the 105% diets formulated at 5% excess of the optimum. Cattle fed a HS diet consumed more DM (28.3 kg) than cattle fed a LS diet (26.2 kg; $P < 0.01$). Energy-corrected milk was highest in cattle consuming the 105% of EAA supply with HS 105 cattle tending to have a higher yield of ECM over HS 100 and LS 100 cattle.

Milk protein yield was significantly improved by higher levels of both dietary starch ($P < 0.01$) and EAA supply ($P = 0.01$), with starch level having the greater influencer on yield. Low starch diets, which were supplemented with a fat source to maintain caloric density relative to HS diets, had improved milk fat yield over HS diets ($P < 0.01$). Feed efficiency was greatest in LS diets due to lower intakes while maintaining comparable ECM to HS diets. Further, NUE was not different among diets fed in this study ($P = 0.59$); however, numerical difference shows the greatest improvement in efficiency when HS diets were supplied with a higher supply of EAA. Collectively, diets fed different sources of glucogenic or lipogenic nutrients can regulate different quantities of components but have a similar lactation performance when evaluating cattle in an ECM basis.

3.2 Introduction

The recognition of numerous metabolic interactions between energy and amino acid (AA) supply has become relevant regarding the efficiency of milk production (Lobley, 2007). Although these interactions at the post-absorptive levels can be complex, understanding their net supply relative to cattle requirement can improve precision feeding techniques, which in turn improves animal performance while reducing excessive nitrogen and EAA supply (Lapierre et al., 2006). Nitrogen and EAA use efficiency are metrics used to assess the amount the productive responses, typically observed as milk yield and milk component output, relative to the gross supply of N and EAA. Previous calculations indicate that the efficiency of use for productive N averages around 25% of feed N intake and is highly variable, with ranges between 10% and 40% (Huhtanen and Hristov, 2009, Calsamiglia et al., 2010). Dijkstra et al. (2013) suggested that the upper limit for N efficiency of use for lactating cattle is above 40% and can be

achieved through accurate predictions of AA requirements as well as a proper understanding of their biochemical interactions with energy and energy signaling mechanisms.

The disaggregation of metabolizable protein (MP) into the supply of each AA has become a precision feeding method to improve AA efficiency of use. Currently, the CNCPS v.7 predicts the requirements of each essential amino acid (EAA) and calculates the net supply of these EAA to help assess first limiting nutrients. Further, the model expresses the requirements of each EAA relative to metabolizable energy (ME) to account for the energy demands for milk yield (Higgs and Van Amburgh, 2016). Application of this approach allows for a reduction in the MP supply, inherently decreasing dietary crude protein (CP) and improving NUE and allowing for the calculation of N requirements in two compartments, the rumen N requirement and the post-absorptive EAA requirement. Previous studies have formulated diets that are targeted for an optimum supply of EAA relative to ME and are lower in CP (13.5-14.5% DM) without compromising milk or protein yield relative to diets that have higher supply of EAA relative to ME and are consequently elevated in CP (Higgs et al., 2014, LaPierre et al., 2019). As a result, the NUE has improved for these diets which are targeted for the optimum supply of EAA; however, this improvement in efficiency is still not at the upper level described by Dijkstra et al. (2013).

To improve N and EAA efficiency of use in dairy cattle, it is important to evaluate the level of glucogenic nutrients available to the animal. Literature has shown that increasing levels of glucogenic nutrients, including ruminally produced propionate and intestinally available glucose, has improved the transfer efficiency of AA from the gastrointestinal tract to the mammary gland and allowed for greater yields of milk and milk protein (Lemosquet et al., 2009, Rius et al., 2010b, Cantalapiedra-Hijar et al., 2016). Further, glucogenic nutrients might support N retention

in peripheral tissue in the presence of elevated AA supply, improving the efficiency of productive use for N beyond the amount needed for milk protein production (Curtis, 2018, Nichols et al., 2018a). Given the current understanding of glucogenic nutrients on AA utilization in dairy cattle, our objective was to evaluate the efficiency of use for EAA and N when supplying two levels of glucogenic nutrients in the form of ruminally produced propionate via differences in dietary starch, in addition to two levels of EAA supply. The EAA were formulated relative to ME (g digestible AA/Mcal ME) to assess if the optimum ratio of each EAA supply relative to ME changed with the addition of glucogenic nutrients. Our hypothesis was that cattle fed the higher level of glucogenic nutrients without the larger supply of EAA would have increased N efficiency through improved ECM over cows with lower levels of glucogenic nutrients.

3.3 Materials and methods

This feeding trial was performed from December 2019 – April 2020 at the Cornell University Ruminant Center, located in Harford, NY. All experimental protocols and data collection utilized throughout this trial was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee.

3.3.1 Animals, housing, and experimental design

One hundred and ninety-two lactating Holstein dairy cattle (n=192) were enrolled in a 9-wk randomized complete, unbalanced design, including a 1 wk acclimation period, 1 wk of covariate sampling, and 7 wk of experimental data collection. Given restrictions in barn space, two enrollment periods were performed sequentially to obtain the necessary cattle numbers for statistical power. Cattle selected for the experiment averaged (mean ± SD) 2.7 ± 1.4 lactations, 94 ± 26 days in milk, 12,155 ± 2,259 kg of milk from previous lactation, and 681 ± 85 kg of

body weight (BW) at the time of experimental enrollment. Within each enrollment, cattle were randomly assigned to pens containing 4 primiparous and 12 multiparous cows each. Cattle were balanced for all pens by parity, days in milk, pre-trial and previous lactation milk performance, and BW. Data in tables 3.1 and 3.2 give the initial descriptive variables for each pen and diet, respectively, which were used to balance them. Cattle were fed the same diet throughout the acclimation and covariate period. Initial measurements were taken during the covariate week and collected in a similar manner to the experimental data collected. Any one cow was housed in a 16 stall, 16 headlock pen for the entirety of the study where they had free access to water, feed, and bedding at all times. All pens were fed once daily ranging from 0630 h to 0730 h where diets were fed ad libitum, targeted for a 5% refusal rate among pens, and refusal samples were collected and composited three times weekly at 0530 h. Cattle were milked three times daily starting at 0800, 1600, and 0000 h in a double-16 parlor (De Laval Inc, Kansas City, MO).

3.3.2 Dietary treatments

Dietary formulation was performed using version 7 of the Cornell Net Carbohydrate and Protein System (CNCPS) model. Dietary treatments included a 2 x 2 factorial design which utilized two different levels of dietary starch, formulated at 23% DM (LS) and 29% DM (HS), and two different levels of EAA supplied, formulated at 100% and 105% of optimized EAA supply to ME according to Higgs and Van Amburgh (2016) (100 and 105, respectively). All diets were formulated to be isocaloric, assuming 45 kg of ECM. To maintain equal quantities of ME, diets formulated for LS were supplemented with a high saturated fatty acid blend supplement (Table 3.3). Diets formulated for 100 % of EAA supplied were also formulated under the assumption of 45 kg of ECM; however, the 105% assumed 47.5 kg of ECM. All cattle were fed the HS 100 diet throughout the acclimation and covariate period and pens were then

Table 3.1. Initial descriptive statistics of cattle by pen within enrollment 1 (panel A) and enrollment 2 (panel B).

	Panel A					
Parameter[1]	Enrollment 1					
Pen number	1	2	3	4	11	12
Diet	LS 105	LS 105	HS 105	HS 105	HS 100	LS 100
Lactation number	2.50	2.63	2.71	2.63	2.67	2.75
Days in milk	102	100	99	100	99	99
Bodyweight, kg	685	676	697	683	682	688
Milk yield, kg	43.6	45.9	44.3	44.1	44.6	44.1
Previous lactation stats[2], kg/lactation						
Total milk yield	12127	12367	12059	12303	12130	12179
Total milk fat yield	479	492	488	478	508	478
Total milk protein yield	364	386	373	385	375	366

	Panel B							*P*	
Parameter[1]	Enrollment 2						SEM	Enroll	Pen
Pen number	2	3	4	10	11	12	-	-	-
Diet	HS 100	HS 100	HS 105	LS 100	LS 100	LS 105	-	-	-
Lactation number	2.75	2.75	2.69	2.69	2.75	2.71	0.35	0.71	0.94
Days in milk	86	86	94	86	91	89	6.4	< 0.01	0.92
Bodyweight, kg	674	687	678	676	665	679	21.6	0.48	0.98
Milk yield, kg	47.2	49.1	46.4	47.4	47.3	47.9	1.8	< 0.01	0.99
Previous lactation stats[2], kg/lactation									
Total milk yield	11969	12068	12397	11933	12022	12285	668	0.83	0.95
Total milk fat yield	477	473	471	504	483	491	27.7	0.79	0.93
Total milk protein yield	370	373	375	366	371	374	19.3	0.75	0.94

[1] Data for each parameter were obtained one week prior to the adaptation period relative to each enrollment.
[2] First lactation animals were not included in analysis given no previous lactation data.

Table 3.2. Initial descriptive statistics summarized for each diet.

	Diet[1]					*P*	
Parameter[2]	HS 100	HS 105	LS 100	LS 105	SEM	Enroll	Diet
Lactation Number	2.71	2.68	2.72	2.62	0.20	0.79	0.99
Days in Milk	92	96	94	95	3.7	0.01	0.87
Bodyweight	682	685	677	678	12.5	0.53	0.97
Milk Yield	46.5	45.4	45.7	46.3	1.0	0.01	0.89
Previous Lactation Stats[3], kg/lactation							
Total Milk Yield	12062	12245	12048	12257	381	0.97	0.97
Total Milk Fat Yield	488	478	489	487	15.9	0.69	0.96
Total Milk Protein Yield	373	377	368	375	11.0	0.89	0.95

[1] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
[2] Data for each parameter were obtained one week prior to the adaptation period relative to each enrollment.
[3] First lactation animals were not included in analysis given no previous lactation data.

assigned to one of four diets throughout the 7 wk of experimental data collection.

Prior to the experiment, typical concentrate samples fed in Northeastern US diets were sourced from a local commercial feed mill (Purina Animal Nutrition, LLC, Caledonia, NY) and submitted for wet chemistry analysis and fiber digestibility at a commercial feed lab (Cumberland Valley Analytical Services; Waynesboro, PA). The Ross N intestinal digestibility assay (Ross et al., 2013) was employed to evaluate undigested N residue (uN) and was analyzed using a combustion assay for estimation of N and AA availability (Leco FP928 N Analyzer, Leco Corp, St. Joseph, MI). Data generated from this analysis was used in place of the detergent system results (Neutral and acid detergent insoluble N) which were only used to evaluate protein digestibility in high fiber containing feed ingredients. The EAA profiles of each feedstuffs fed in this experiment were sourced from Van Amburgh et al. (2017) which contained the maximum release of EAA from hydrolysate samples after a series of multiple hydrolysis lengths ranging from 2 to 168 h of hydrolysis. Fiber digestibility results were used to predict fast and slow degrading fiber pool sizes and rates of digestion according to Raffrenato et al. (2019).

The forage inclusion rate varied among HS and LS diets, with formulated inclusions levels being 10 percentage units less for HS diets than LS diets (50% vs 60% of diet DM, respectively). Corn silage was the primary forage used in all diets with a mixed mostly legume (MML) silage used for the remaining forage DM (Table 3.3). The remaining concentrate ingredients were sourced from the same feed mill and used at varied levels to create four separate premixes, each fed to one of the four diets in the experiment. Premixes formulated for the HS diets utilized a high-quality steam flaked corn (O'Brien and Sons; Hamburg, NY) and finely ground corn meal as the primary sources of dietary starch. As mentioned previously, only premixes used to feed LS diets had an inclusion of a high palmitic fat blend (Energy Booster HP; MSC Company,

Table 3.3. Ingredient composition (mean ± SD)[1] of experimental diets

	Diet[2]			
Ingredient, % DM	HS 100	HS 105	LS 100	LS 105
Corn silage	42.3 ± 0.2	39.8 ± 0.2	52.5 ± 0.4	49.7 ± 0.2
Mixed mostly legume silage	9.5 ± 0.2	7.4 ± 0.6	8.0 ± 0.3	9.8 ± 0.4
Premix				
Steam flaked corn	12.4 ± 0.1	12.2 ± 0.1	4.2 ± 0.04	4.5 ± 0.1
Soybean meal	10.9 ± 0.1	6.9 ± 0.1	7.3 ± 0.1	9.7 ± 0.1
Wheat midds	7.3 ± 0.04	3.8 ± 0.03	4.3 ± 0.04	4.4 ± 0.05
Corn meal	5.4 ± 0.03	7.9 ± 0.07	2.1 ± 0.02	3.2 ± 0.04
Soybean hulls	3.6 ± 0.02	7.4 ± 0.07	0.7 ± 0.01	1.4 ± 0.02
Beet pulp pellets	1.9 ± 0.01	-	6.9 ± 0.06	4.6 ± 0.06
Canola meal	1.9 ± 0.01	7.6 ± 0.07	3.6 ± 0.03	1.2 ± 0.01
Heat treated soybean meal[3]	1.0 ± 0.005	3.0 ± 0.03	5.5 ± 0.05	7.4 ± 0.09
Dextrose	0.38 ± 0.002	0.38 ± 0.003	0.19 ± 0.002	-
Saturated fatty acid supplement[4]	-	-	1.34 ± 0.01	0.97 ± 0.01
Urea	0.19 ± 0.001	0.17 ± 0.002	0.23 ± 0.002	0.19 ± 0.002
Rumen protected methionine[5]	0.08 ± 0.0004	0.08 ± 0.001	0.09 ± 0.001	0.10 ± 0.001
Rumen protected methionine & lysine[6]	0.04 ± 0.0002	0.08 ± 0.001	-	0.04 ± 0.001
Calcium carbonate	1.1 ± 0.01	1.1 ± 0.01	1.1 ± 0.01	1.0 ± 0.01
Sodium bicarbonate	0.78 ± 0.004	0.8 ± 0.01	0.76 ± 0.01	0.76 ± 0.01
Min AD	0.55 ± 0.003	0.58 ± 0.01	0.57 ± 0.01	0.55 ± 0.01
Salt white	0.37 ± 0.002	0.34 ± 0.003	0.33 ± 0.003	0.33 ± 0.004
Vitamin and mineral mix[7]	0.21 ± 0.001	0.21 ± 0.002	0.21 ± 0.002	0.22 ± 0.003
Magnesium oxide	0.13 ± 0.001	0.13 ± 0.001	0.12 ± 0.001	0.12 ± 0.002

[1] Composition obtained from commercial mill data and on-farm feeding management software.
[2] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
[3] SOYPLUS (West Central Cooperative, Ralston, IA).
[4] ENERGY BOOSTER HP (40% C16:0, 40% C18:0, 10% C18:1 *cis*-9; MSC Company, Dundee, IL).
[5] Smartamine M (Adisseo USA Inc, Alpharetta, GA)
[6] Smartamine ML (Adisseo USA Inc, Alpharetta, GA)
[7] Included: (% DM) 27.37 % Ca; 0.48% Mg; 0.08% K; 4.53 % S; 222.9 ppm Fe; 24,997.9 ppm Zn; 5,765.2 ppm Cu; 18,473.7 ppm Mn; 134.5 ppm Se; 568.1 ppm Co; 568.1 ppm I; 2,022 KIU/kg Vitamin A; 562 KIU/kg Vitamin D; 9,661 IU/kg Vitamin E and Rumensin (Elanco Animal Health, Greenfield, IN) at 300 mg per head per day.

Dundee, IL) to improve the ME supply in those diets. All diets were fed urea at an average inclusion rate of 0.19% DM to avoid the possibility of low rumen N availability. Further, all diets were fed rumen protected products either to supply Met and/or Lys post-ruminally (Smartamine M and Smartamine ML; Adisseo, Alpharetta, GA).

3.3.3 Sample collection and analysis

If cattle were diagnosed as sick due to causes unrelated to the dietary treatments, they were transferred to the farm's hospital pen to receive treatment until considered healthy. Sick animals were not sampled until they moved back to their pen and consumed their dietary treatment for at least 2-wk. Animals were removed from the experiment if an acute illness influenced subsequent health and altered lactation performance over time. Data collected on these animals before their illness was used for statistical analysis.

Body weights and body condition score (BCS; 1-5 scale) were measured and assessed, respectively, once weekly on every animal immediately following the 1600 h milking. Body condition score was assigned by at least two trained scorers and the results averaged prior to analysis. Milk yield was recorded at every milking session by the parlor's software system (DelPro, De Laval Inc., Kansas City, MO) and summed to give total daily milk yields by animal. Milk samples were obtained every week using 3 consecutive milkings and analyzed for fat, true protein, total solids, milk urea nitrogen (MUN), and somatic cell count (Dairy One, Ithaca, NY). Chemical analysis was performed using Fourier transform infrared spectroscopy (Milkoscan 6000, Foss Electric, Hillerød, Denmark). Throughout the second enrollment of the experiment, sixteen cattle from each diet (n=64) had their milk subsampled and sent to an alternative milk testing facility for milk components and fatty acid profiles and characteristics, including the proportion of *de novo* (defined as fatty acids containing between 4 and 15 carbons), mixed

(defined as fatty acids containing 16 carbons), and preformed (defined as fatty acids containing more than 18 carbons) fatty acids produced by animals (David Barbano, Department of Food Sciences, Cornell University, Ithaca, NY). Milk analysis was performed using Fourier transform infrared (FTIR) technology (CombiScope FTIR, Delta Instruments, Leiden, Netherlands). Daily milk composition was used to determine ECM according to the equations from Tyrrell and Reid (1965). Daily 3.5% FCM was also calculated using equations from NRC (2001).

Dry matter intake was measured for each pen as the daily amount of feed offered less the amount refused corrected for the DM of each forage ingredient, protein premix, and refusal sample and corroborated with the observed DM of each total mixed ration (TMR) sampled. Feed offered, feed refused, and any deviations in ingredient inclusion relative to intended formulation was recorded and stored by the farm's feeding management software (FeedWatch, Valley Agricultural Software, Tulare, CA). Differences in observed diets relative to formulated diets were averaged weekly and used to model nutrient availability for cattle. Samples of forages were collected three times weekly, composited by equal mass and a subsample was sent to a commercial feed lab for chemical analysis using near infrared spectroscopy (NIR) (Table 3.4). The remaining quantity was analyzed for DM using a forced air oven set at 50°C for 96 h, ground through a 1 mm screen (Wiley Mill no. 4, Arthur H. Thomas, Philadelphia, PA), and stored for further chemical analysis immediately following the experiment. Calculated DM was entered into the feed management software to maintain consistent inclusion of DM from each forage fed. When new batches of premix were delivered, samples of individual ingredients within premixes were delivered and analyzed for chemical analysis, fiber digestibility, and intestinal N digestibility when applicable (Table 3.5). To evaluate compliance of nutrient delivery provided by the premixes, samples were obtained weekly and analyzed for DM and

were composited every two weeks and sent for chemical analysis (Table 3.6). The TMR of each diet was also sampled three times weekly, composited, subsampled for chemical analysis, and dried and ground for future analysis (Table 3.7).

Fecal spot samples were collected during the covariate, wk 3, and wk 6 for each enrollment of the experiment from a subset of randomly selected cows, 2 primiparous and 6 multiparous, from each pen for diet digestibility calculations. Eight samples (~500 g of wet weight each) from each cow selected were collected over a three-day period (Day 1: 0100 h, 0700 h, 1300 h, and 1900 h; Day 2: 0400 h, 1000 h, 1500 h, and 2200 h; composited by timepoint and pen, and was frozen at -20°C. Samples were thawed several days following the last day of sampling, thoroughly mixed, and a subsample (~1.5 kg of wet weight) was dried 50°C for 96 h and ground through a 1 mm screen. Processed fecal samples were analyzed for aNDFom (NDF analysis conducted with sodium sulfite and amylase and ash corrected (Mertens, 2002) and undigested NDF (uNDFom) following a 240 h *in vitro* incubation with rumen fluid based on the method described by Raffrenato et al. (2018). Data generated from these analyses were used to determine apparent total tract digestibility of DM, OM, and aNDFom using uNDFom as an internal marker (Huhtanen et al., 1994). Daily rumination times (Smartbow; Zoetis, LLC, Parsippany, NJ) were also collected on all cattle throughout the entirety of the experiment to evaluate chewing activity.

Cattle that were fecal sampled also had blood samples collected during the covariate period and every 2 wk following the initial sample. Samples were collected 10 h after feeding via the coccygeal vein using vacutainer tubes containing lithium heparin, placed on ice until the remaining samples were collected (averaging 20 min), centrifuged at $3{,}000 \times g$ for 20 min at 4°C, and plasma was harvested. Aliquots of plasma was stored in microcentrifuge tubes and immediately frozen at -20°C for later analysis. A separate aliquot was mixed with equal parts of

Table 3.4. Chemical composition (mean ± SD)[1] and aNDFom digestibility of forages fed in experiment.

Parameter[2]	Corn silage	Mixed mostly legume silage
Dry matter, %	28.2 ± 1.0	24.5 ± 2.8
CP, % DM	7.3 ± 0.3	18.7 ± 1.5
Soluble protein, % CP	63.5 ± 3.0	60.2 ± 2.6
NH3-N, % Soluble protein	18.9 ± 0.9	30.0 ± 4.8
ADIP, % CP	9.3 ± 0.7	14.6 ± 1.2
NDIP, % CP	7.6 ± 0.7	8.4 ± 0.5
aNDFom, % DM	38.5 ± 2.3	48.0 ± 3.4
30h uNDFom, % aNDFom	41.4 ± 1.7	34.4 ± 1.3
120h uNDFom, % aNDFom	32.8 ± 2.7	27.2 ± 2.1
240h uNDFom, % aNDFom	29.1 ± 3.4	23.5 ± 1.6
ADF, % DM	23.4 ± 1.5	34.8 ± 1.9
Lignin, % DM	2.6 ± 0.2	5.2 ± 0.3
Acetic acid, % DM	3.8 ± 0.5	5.7 ± 1.1
Propionic acid, % DM	0.1 ± 0.03	0.1 ± 0.02
Butyric acid, % DM	-	1.1 ± 0.6
Lactic acid, % DM	7.1 ± 0.5	4.7 ± 0.8
Ethanol soluble sugars, % DM	0.5 ± 0.27	2.0 ± 0.9
Starch, % DM	32.3 ± 2.4	1.2 ± 0.3
Soluble fiber, % DM	5.1 ± 0.4	7.1 ± 1.1
Ether extract, % DM	3.2 ± 0.2	4.3 ± 0.4
Ash, % DM	3.4 ± 0.5	11.6 ± 1.1

[1] Analyzed values obtained from 16 weekly samples for each forage ingredient.
[2] ADIP = Acid detergent insoluble protein; NDIP = Neutral detergent insoluble protein; aNDFom = amylase and ash corrected neutral detergent fiber; uNDFom = undigested neutral detergent fiber analyzed after the specified number of hours. Amino acid values for feeds are the same as those expressed in Chapter 2.

Table 3.5. Chemical composition (mean ± SD)[1] of concentrate ingredients fed over the experimental period.

Parameter[2]	BP	C	CM	SBM	SBH	SP	SFC	WM
Dry matter, %	89.9 ± 0.8	88.8 ± 1.0	88.4 ± 1.2	88.0 ± 0.4	88.9 ± 1.1	88.6 ± 0.3	88.6 ± 2.6	88.5 ± 1.1
CP, % DM	9.3 ± 0.3	41.8 ± 0.7	8.5 ± 0.4	53.0 ± 0.5	11.7 ± 0.7	46.4 ± 0.7	8.0 ± 0.3	19.4 ± 1.6
Soluble protein, % CP	13.6 ± 4.2	20.7 ± 2.0	14.8 ± 2.2	20.1 ± 3.9	25.6 ± 4.9	11.3 ± 1.4	9.3 ± 3.5	37.3 ± 3.2
NDIP, % CP	61.4 ± 4.2	12.7 ± 1.2	6.3 ± 1.7	1.5 ± 0.4	32.6 ± 3.0	10.6 ± 1.8	9.8 ± 1.7	15.4 ± 2.5
ADIP, % CP	39.3 ± 6.3	5.9 ± 0.7	4.7 ± 1.2	0.8 ± 0.2	9.8 ± 1.4	1.3 ± 0.4	6.2 ± 1.3	3.5 ± 0.5
uNitrogen, % Total N	N/A	18.3 ± 1.9	N/A	8.6 ± 1.1	N/A	13.1 ± 1.5	N/A	8.7 ± 2.1
aNDFom, % DM	42.7 ± 1.6	28.9 ± 1.3	10.6 ± 1.1	8.0 ± 0.6	71.5 ± 1.7	16.8 ± 0.9	10.0 ± 0.8	40.4 ± 1.6
ADF, % DM	31.6 ± 2.8	20.5 ± 1.0	4.1 ± 0.7	5.5 ± 0.5	49.7 ± 1.6	8.2 ± 1.4	4.3 ± 0.4	12.5 ± 0.9
Lignin, % DM	4.4 ± 1.4	8.5 ± 1.1	1.8 ± 0.6	0.7 ± 0.3	1.9 ± 0.4	1.0 ± 0.5	1.7 ± 0.4	4.0 ± 0.8
Ethanol soluble sugars, % DM	6.5 ± 1.7	7.8 ± 1.1	1.7 ± 0.5	9.8 ± 1.3	1.0 ± 0.4	9.7 ± 1.1	1.1 ± 0.2	4.1 ± 0.7
Starch, % DM	0.3 ± 0.2	0.6 ± 0.2	72.9 ± 1.5	0.5 ± 0.2	0.3 ± 0.2	0.5 ± 0.3	75.0 ± 1.7	20.8 ± 2.4
Soluble fiber, % DM	26.8 ± 3.0	8.6 ± 1.5	0.7 ± 0.08	19.7 ± 2.1	7.9 ± 2.1	12.2 ± 1.6	2.8 ± 1.9	4.2 ± 2.2
Ether extract, % DM	1.0 ± 0.2	3.7 ± 0.4	4.1 ± 0.5	1.6 ± 0.3	2.1 ± 0.5	6.6 ± 0.4	3.2 ± 0.4	4.1 ± 0.5
Ash, % DM	10.4 ± 1.8	8.0 ± 0.4	1.6 ± 0.5	7.3 ± 1.0	4.8 ± 0.5	7.0 ± 0.5	1.5 ± 0.4	5.5 ± 1.1

BP = Beet pulp, C = Canola, CM = Corn meal, SBM = Soybean meal, SBH = Soybean hulls, SP = SoyPlus, SFC= Steam flaked corn, WM = Wheat Midds.
N/A = Not analyzed
Table is continued on next page.

Table 3.5. (continued) Chemical composition (mean ± SD)[1] of concentrate ingredients fed over the experimental period.

Parameter[3]	BP[4]	C	CM	SBM	SBH	SP	SFC	WM
AA N, % Total N								
Arginine	3.7	12.3	10.8	14.4	10.4	14.0	12.0	14.5
Histidine	3.1	4.4	6.2	4.5	5.2	4.2	6.8	4.7
Isoleucine	3.5	2.7	2.7	3.1	2.9	3.0	3.3	2.2
Leucine	5.9	4.6	8.3	5.1	5.0	5.0	10.4	4.1
Lysine	5.8	6.2	4.4	7.1	8.9	6.6	5.5	5.1
Methionine	1.6	1.8	2.2	1.3	1.4	1.1	2.5	1.5
Phenylalanine	3.6	2.7	3.3	3.2	2.8	3.0	3.9	2.6
Threonine	4.5	2.9	2.8	2.7	2.6	2.5	3.1	2.1
Tryptophan	1.1	2.2	1.5	2.0	2.4	1.9	1.6	2.5
Valine	5.5	3.8	4.0	3.6	3.9	3.7	4.7	3.5
EAA N	38.3	43.6	46.2	47.0	45.5	45.0	53.8	42.8
Alanine	-	4.0	7.5	3.8	4.3	3.8	8.5	4.3
Aspartic Acid	-	4.3	4.8	6.7	5.9	6.5	5.1	4.3
Cysteine	-	2.0	2.0	1.2	1.8	1.0	2.1	1.7
Glutamic Acid	-	9.6	11.2	9.9	5.9	9.6	12.9	10.0
Glycine	-	5.6	5.0	4.6	11.0	4.7	5.9	5.9
Proline	-	6.0	9.2	3.7	5.8	4.2	9.4	6.4
Serine	-	3.4	4.4	4.1	5.2	3.8	4.8	3.4
Tyrosine	-	1.9	2.2	2.2	2.7	2.2	2.3	1.9
NEAA N	N/A	36.8	46.3	36.2	42.6	35.8	51.0	37.9
Total AA N	N/A	80.4	92.5	83.2	88.1	80.8	104.8	80.7

[1] Analyzed values obtained from 7 batch samples per feed ingredient when new protein premix batches delivered.
[2] ADIP = Acid detergent insoluble protein; NDIP = Neutral detergent insoluble protein; aNDFom = amylase and ash corrected neutral detergent fiber; uNDFom = undigested neutral detergent fiber analyzed after the specified number of hours.
[3] Values procured from Van Amburgh et al. (2017)
[4] EAA values for beet pulp are feed CNCPS feed library values.

Table 3.6. Chemical composition (mean ± SD)[1] of grain mixes fed over the experimental period.

	Diet[2]			
Parameter	HS 100	HS 105	LS 100	LS 105
Dry matter, %	87.3 ± 0.3	87.7 ± 0.5	88.1 ± 0.7	88.1 ± 0.6
Crude protein, % DM	23.3 ± 1.4	22.3 ± 0.9	24.7 ± 3.1	28.9 ± 2.9
Soluble protein, % CP	27.2 ± 2.9	24.2 ± 4.2	22.3 ± 2.9	22.6 ± 2.4
NDIP, % CP	6.2 ± 1.1	9.6 ± 1.0	13.9 ± 4.5	8.5 ± 1.6
ADIP, % CP	2.1 ± 1.5	2.8 ± 1.5	7.1 ± 4.2	1.8 ± 0.5
Ethanol soluble sugars, % DM	4.4 ± 0.3	4.2 ± 0.5	7.0 ± 0.7	7.0 ± 0.9
Starch, % DM	35.6 ± 6.6	32.5 ± 6.2	14.0 ± 1.1	18.7 ± 1.1
NFC, % DM	48.2 ± 5.7	44.3 ± 5.0	36.7 ± 2.7	39.4 ± 3.2
NDF, % DM	18.4 ± 3.3	23.7 ± 4.0	24.9 ± 2.9	19.1 ± 2.4
ADF, % DM	10.0 ± 1.4	14.4 ± 2.4	16.8 ± 3.3	11.3 ± 2.0
Lignin, % DM	1.8 ± 0.3	2.3 ± 0.2	2.7 ± 0.3	2.0 ± 0.4
Ash, % DM	8.8 ± 0.9	8.6 ± 0.6	12.3 ± 0.8	10.3 ± 1.2
Ether extract, % DM	2.8 ± 0.6	3.2 ± 0.3	4.7 ± 1.3	4.7 ± 0.1
NE_L, Mcal/kg	1.74 ± 0.03	1.70 ± 0.03	1.66 ± 0.07	1.77 ± 0.05

[1] Analyzed values obtained from 14 weekly samples for each total mixed ration.

[2] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).

5-sulfosalicylic acid (SERAPREP, Pickering Laboratories, Mountain View, CA), containing DL-glucosamic acid added as an internal standard, to effectively precipitate protein from the sample and analyze for plasma AA analysis. Once dosed, the sample was vortexed for several seconds, placed on ice for 16 h while vortexing occasionally, centrifuged at 15,800 × g for 10 min at 4°C, and the supernatant frozen at -20°C until analysis. Plasma urea nitrogen (PUN) analysis was measured as described by Chaney and Marbach (1962) using an enzymatic colorimetric assay based on a purchased commercial kit (No. 640; Sigma-Aldrich, St. Louis, MO). Blood insulin levels were also evaluated on all samples collected using a commercial radioimmunoassay (RI-13K; Millipore Sigma, Burlington, MA).

3.3.4 Power calculation and statistical analysis

The major outcome of interest for this experiment was the ECM output and NUE from the dietary treatments. Similar to the experimental assumptions described in Chapter 2, cattle would yield a daily average of 45 kg of ECM according to their dietary ME formulations with a standard deviation estimated at 3.7 kg and a desired experimental difference was 3 kg. To achieve the necessary statistical power of 80% under an alpha of 0.05 using a F-distribution test, it was determined that each dietary treatment would need a minimum of three, 16-cow pens for a total of 12 pens and 192 cows for the experiment. Given pen limitations at the time the experiment was conducted, two diets (HS 105 and LS 105) were fed to two pens each whereas the other diets (HS 100 and LS 100) were fed to only one pen each during the first enrollment period. This allocation was reversed in enrollment two and allowed for the necessary number of three pens per diet to be observed.

All data were analyzed using SAS version 9.4 (SAS Institute Inc. Cary, NC). Forage, feed ingredients, protein premixes, and TMR samples were summarized over the experiment using

PROC TABULATE. Pen was designated as the experimental unit whereas cattle within the experiment were classified as subsample observations within each pen (St-Pierre, 2007, Bello and Renter, 2017). Evaluation of pens and diets balanced for cattle parameters was done using PROC GLM, testing the effect of enrollment and pen within enrollment, as shown in Table 3.1, and diet, as shown in Table 3.2. Multiple comparisons in least squared mean differences were adjusted using Tukey's adjustment. With the exclusion of BCS, all variables of interest were analyzed using PROC MIXED. Body condition score was analyzed using a non-parametric analysis (PROC NPAR1WAY) where the fixed effect of diet was used for analysis of differences. Under any dependent observations made at the cow-level, including lactation performance, animal characteristic changes, and blood metabolites, the following statistical model was used under the mixed-model analysis:

$$Y_{ijlkmno} = \mu + E_i + S_j + A_k + SA_{jk} + W_l + EW_{il} + SW_{jl} + AW_{kl} + SAW_{jkl} + P_{m:jk:i} + PW_{lm:jk:i} + C_{n:jk:i} + L_o + BX_{jkmn} + \epsilon_{ijklmno}$$

where $Y_{ijlkmno}$ = the dependent variable, μ = the overall mean, E_i = the fixed effect of the ith Enrollment, S_j = the fixed effect of jth dietary starch level, A_k = the fixed effect of kth dietary EAA supply level, W_l = the fixed effect of lth experimental week, $P_{m:jk:i}$ = the random effect of mth pen within the jth starch level by kth EAA level and the ith enrollment, $C_{n:jk:i}$ = the random effect of nth cattle within the mth pen, the jth starch level by kth EAA level, and the ith enrollment, L_n = the fixed effect of oth lactation group (grouped as, 1st lactation, 2nd lactation, and ≥ 3rd lactation), BX_{jkmn} = the covariate adjustment for each cow sampled, and $\epsilon_{ijklmno}$ = the residual

error. When analyzing pen-level observations, including DMI, diet digestibility, and productive efficiency, cattle specific variables were dropped from the previous model and any available covariate measurements were applied on a pen-level basis. Statistical analysis of initial dependent variable measurements, either on a cow or pen-level, and non-repeating measurements, including changes in BW and BCS, did not have a covariate or repeated measurement analysis. The milk samples which were subsampled during the second enrollment period had the effect of enrollment dropped from the model described above. All data satisfied model assumptions of having normally distributed residuals that were homogenously distributed across diets. For all statistical analysis, degrees of freedom were adjusted using the Kenwood-Rogers option to account for any missing data in the analysis. Covariance matrix structures were determined for each repeated measurement analysis according to best model fit as determined by the lowest Akaike information criteria (AIC) where the null model likelihood test was significant. Diet comparisons were performed using least square means and the Tukey's adjustment for multiple comparisons. Statistical significance was designated at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

3.4 Results

Analysis of initial variables used for determining balanced pens and diets are shown in Table 3.1 and 3.2. Results indicate that both pens and dietary treatments were balanced for all variables of interest ($P > 0.10$). Both tables do, however, show significant differences between enrollment 1 and 2 when analyzing for DIM and previous week's milk yield ($P < 0.01$). Simultaneous significant results of these two variables are not coincidental as the DIM for cows in enrollment 2 were closer to peak milk lactation. These differences among enrollment did not

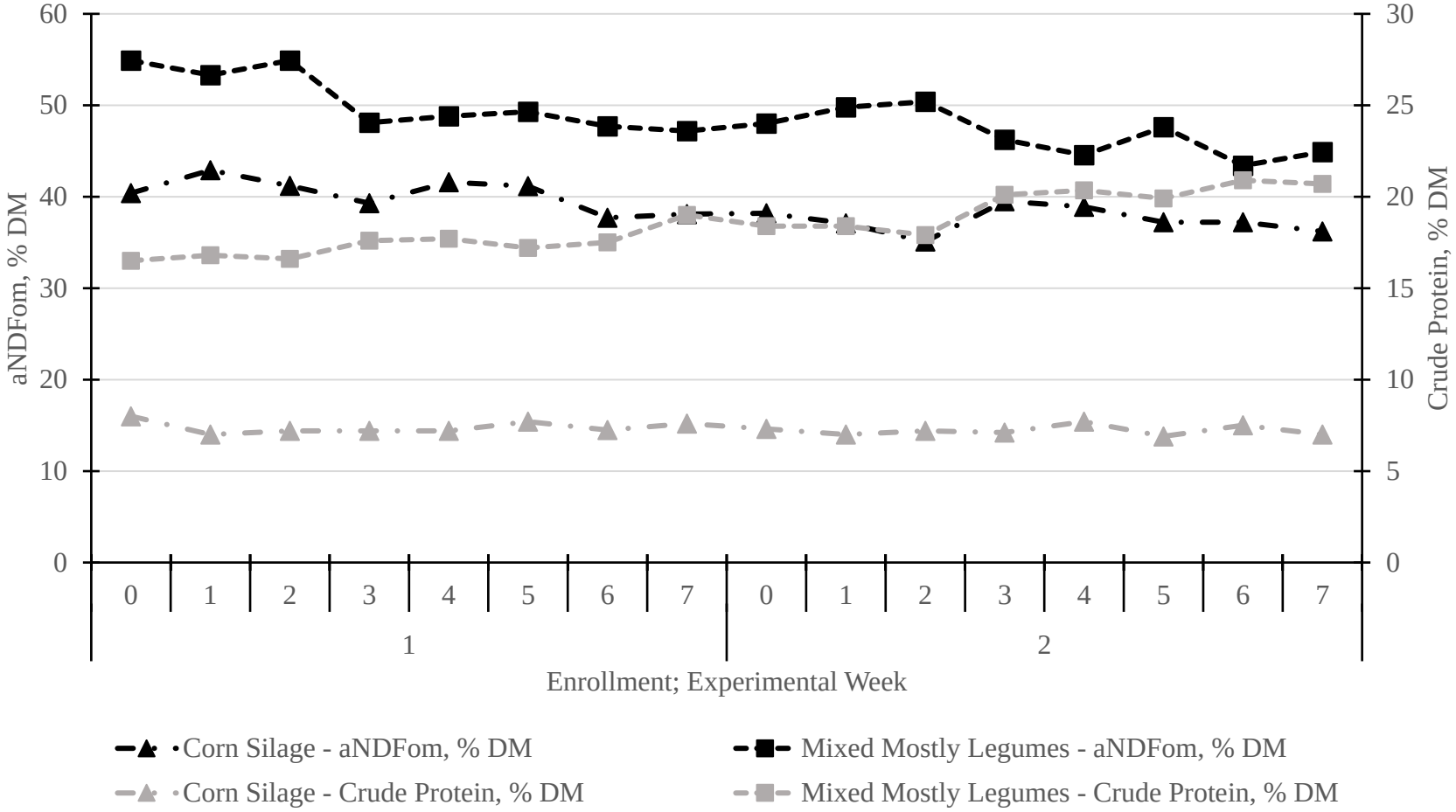
indicate any differences in the main effect of diet (Table 3.2) although numerical differences can be observed in milk yield and previous lactation total milk yield.

3.4.1 Diets, intake, and digestibility

Shifts in forage DM and ingredient inclusion rate throughout the experiment showed little impact on dietary ingredient inclusion for all diets fed (Table 3.3). Premix ingredients for each diet had variability according to their inclusion rate within that premix. Corn silage averaged 38.5 % aNDFom, 32.3 % starch, and 7.3 % CP all of which were similar to formulated values although a new bunker silo was opened on wk 5 of enrollment 1. Conversely, the MML silage averaged 48.0 % aNDFom and 18.7 % CP where formulated values were 54.6 % and 16.0 %, respectively. Throughout the experiment, diets transitioned between 3 different bunk silos of MML silage, changing on wk 3 in enrollment 1 and wk 4 of enrollment 2. Significant shifts in aNDFom and CP content for this forage were observed on these weeks, contributing to the observed variability (Figure 3.1).

Feed chemistry for ingredients found in dietary premixes are in Table 3.5. The mean protein digestibility, analyzed as NDIP and ADIP, was similar for all ingredients when compared to their formulated values, apart from the beet pulp, which had observably improved protein digestibility yet tended to have more variable results. Further, mean aNDFom values for beet pulp was nearly 7% higher than as formulated. These shifts in nutritional values have larger implications on the LS premixes where this ingredient was fed at a higher inclusion rate relative to HS premixes. The remaining feed ingredients showed minor shifts in nutrient values and do not appear to impact diet nutrient targets. Alternatively, feed chemistry for diet premixes exhibited higher variability than anticipated (Table 3.6). Of particular concern is the variation in CP for LS

Figure 3.1. Analyzed weekly[1] aNDFom and crude protein of forages fed in dietary treatments over the duration of the experiment.



[1] Forages were submitted for analysis once weekly and have no measure of variability within a given week.

Table 3.7. Chemical composition (mean ± SD)[1] of diets analyzed over the entire experiment.

	Diet[2]					*P*		
Parameter	HS 100	HS 105	LS 100	LS 105	SEM	Starch	AA	S x AA
Dry matter, %	38.9^{b}	41.7^{a}	36.4^{c}	36.5^{c}	0.31	< 0.01	< 0.01	< 0.01
Crude protein, % DM	16.0^{bxy}	16.4^{abx}	15.9^{by}	16.6^{a}	0.16	0.70	< 0.01	0.52
Soluble protein, % CP	39.3^{abx}	37.3^{by}	40.1^{a}	39.5^{a}	0.35	< 0.01	< 0.01	0.06
Ammonia, % SP	13.6^{by}	12.7^{b}	14.7^{ax}	14.9^{a}	0.29	< 0.01	0.28	0.05
NDIP, % CP	14.0^{c}	14.3^{bc}	16.0^{a}	15.1^{ab}	0.29	< 0.01	0.31	0.07
ADIP, % CP	5.30^{b}	5.46^{b}	6.07^{a}	5.51^{b}	0.10	< 0.01	0.06	< 0.01
RUP, % CP	30.3^{b}	31.4^{a}	29.9^{b}	30.3^{b}	0.17	< 0.01	< 0.01	0.06
Ethanol soluble sugar, % DM	4.59^{abx}	4.63^{a}	4.28^{by}	4.52^{ab}	0.08	0.02	0.11	0.23
Starch, % DM	26.9	27.4	23.9	23.4	0.34	< 0.01	0.95	0.11
Starch digestion 7hr, % Starch	77.8^{a}	77.4^{abx}	76.8^{ab}	75.2^{by}	0.61	0.01	0.11	0.34
aNDFom, % DM	31.0^{aby}	30.6^{b}	32.2^{ax}	31.9^{a}	0.34	< 0.01	0.34	0.85
ADF, % DM	19.0^{b}	19.3^{ab}	20.0^{a}	19.8^{ab}	0.24	< 0.01	0.91	0.32
uNDF 24hr, % NDF	42.1^{a}	42.9^{abx}	43.3^{b}	42.0^{ay}	0.27	0.68	0.38	< 0.01
uNDF 240hr, % NDF	24.4^{b}	25.2^{b}	27.0^{a}	24.9^{b}	0.32	< 0.01	0.06	< 0.01
Lignin, % DM	2.72^{b}	2.94^{a}	3.03^{a}	2.95^{a}	0.04	< 0.01	0.05	< 0.01
Ash, % DM	7.53^{ab}	7.51^{by}	7.74^{abx}	7.82^{a}	0.07	< 0.01	0.70	0.49
Ether extract, % DM	3.65	3.63	4.51	4.45	0.04	< 0.01	0.28	0.62
TFA, % DM	2.42	2.50	3.08	3.04	0.05	0.59	< 0.01	0.64
C16:0, % TFA	16.0^{c}	17.3^{c}	24.2^{a}	22.0^{b}	0.5	< 0.01	< 0.01	0.33
C18:0, % TFA	3.55^{c}	3.99^{c}	5.54^{a}	4.89^{b}	0.15	0.01	< 0.01	0.48
NEL, Mcal/kg	1.72^{ab}	1.71^{b}	1.74^{a}	1.74^{a}	0.01	< 0.01	0.75	0.46

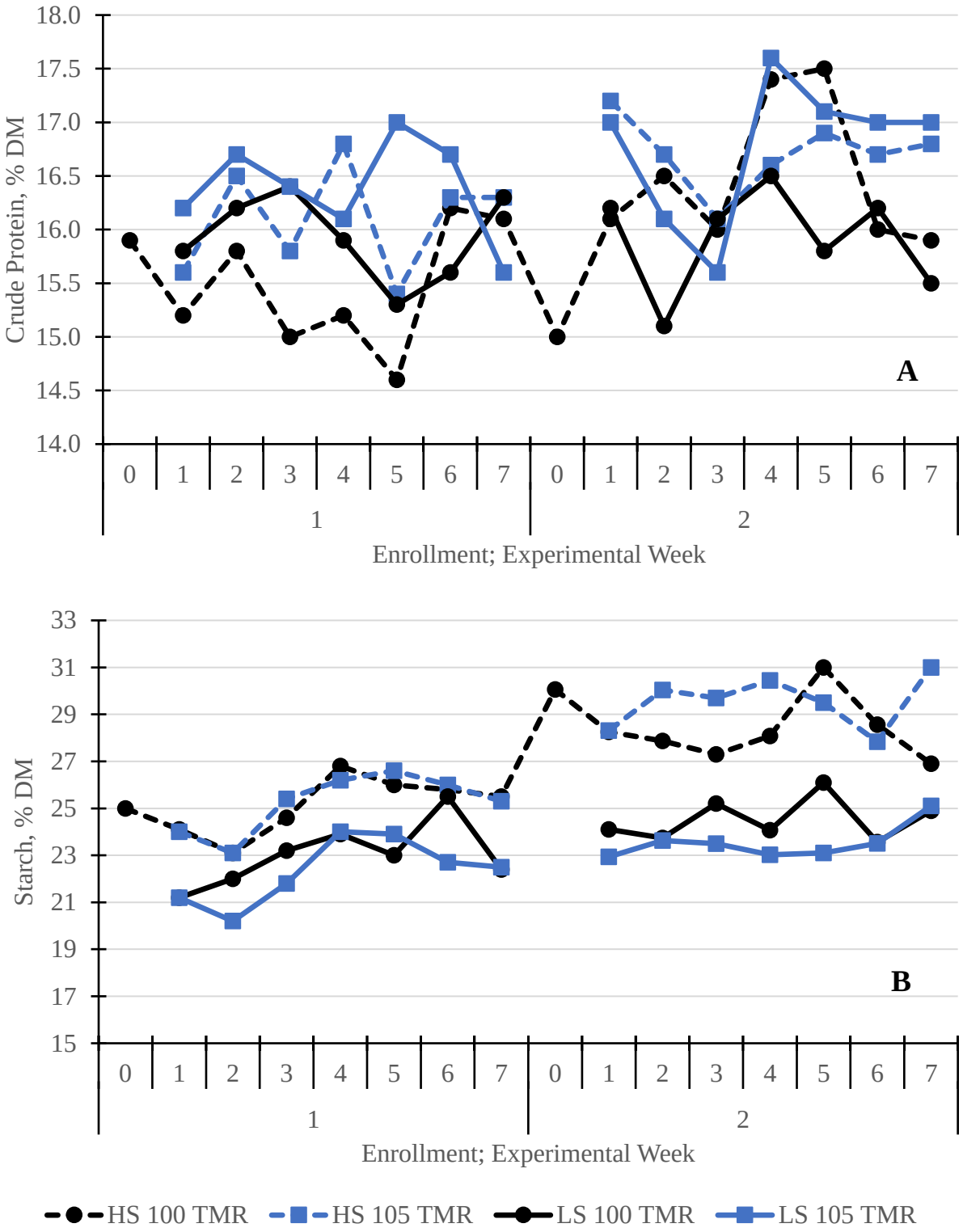
[1] Analyzed values obtained from 14 weekly samples for each total mixed ration.

[2] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Figure 3.2. Analyzed weekly[1] dietary crude protein (A) and starch (B) for diets fed over the experiment.



[1] Diets were submitted for analysis once weekly and have no measure of variability within a given week.

premixes, resulting in 3.1 and 2.9 % DM for LS 100 and LS 105, respectively. Further, NDIP and ADIP variability was reasonably minimal in all mixes except for the LS 100 mix, which had a larger inclusion of beet pulp (7.0 % DM) and is likely the contributor to this variation. Lastly, starch variability for HS mixes, but not LS mixes, displayed the highest amount of variability (6.6 % and 6.2 % DM for HS 100 and HS 105, respectively). Shifts in premix composition, and subsequent feed chemistry, might have occurred while in storage as particle size of some feed ingredients caused settling. For instance, the steam flaked corn, a major contributor to starch in all diets, had a larger particle size compared to the ground corn meal, soybean meal, and other lower particle size feeds and might have shifted to the top of the bin.

Mean nutrient measurements for diets fed are displayed in Table 3.7. Crude protein level, serving as a proxy for EAA supply, was significantly different for 100 and 105 diets ($P < 0.01$), although observed means were higher for all diets compared to intended formulation excluding LS 105. Throughout the experiment, observed weekly measurements of both CP and starch for diets tended to be more variable than anticipated (Figure 3.2). Unfortunately, the range of CP content for any one diet was 2% of DM with HS 100 having the highest range of 3%. Dietary starch was also significantly different between HS and LS levels ($P < 0.01$); however, average starch content for HS diets were less than the intended 29% DM formulation (26.9 and 27.4% DM for HS 100 and HS 105, respectively) whereas LS 100 had a slightly higher average (23.9 % DM) than the 23.3% DM target. High starch diets, but not LS diets, showed a higher than anticipated level of EE, averaging 3.64 % DM to the formulated 3.2 % DM.

Dry matter and organic matter intake were significantly higher in the digestibility trial for HS cattle over LS cattle ($P = 0.02$; Table 3.8) but their apparent digestibility was not different among diets ($P = 0.37$). Further, intake of aNDFom ($P = 0.46$), pdNDFom ($P = 0.76$), and uNDF ($P =$

0.14) were not different for diets. The HS 105 cattle had an increased intake in aNDFom over all other diets and the main effect of starch approached a statistical tendency ($P = 0.12$) indicating that cattle on the LS diets consumed slightly lower pdNDFom. Apparent digestibility of aNDFom and pdNDFom tended to be higher for the LS 105 diet over the LS 100 diet, creating a significant tendency for the main effect of EAA supply ($P = 0.06$ and $P = 0.07$, respectively). There were no significant differences in digestibility by enrollment or over weeks of sampling ($P > 0.10$).

Cattle fed the HS diet consumed 2 kg/d more DM than cows fed the LS diet ($P < 0.01$; Table 3.9). Significant differences in enrollment period and week were also observed; however, two- and three-way interactions between starch and/or AA level and week were not significant (Data not shown). Initial DMI shows no significant difference in enrollment period or subsequent dietary treatment allocation ($P > 0.10$). Throughout the first enrollment period, cattle fed the HS 100 diet had the highest DMI, averaging 29.0 kg/d, whereas cattle fed the HS 105 diet had the highest DMI in the second enrollment period, averaging 29.5 kg. Dry matter intake also increased between 2 and 2.3 kg/d from enrollment 1 to enrollment 2 for cattle fed the LS 100 and LS 105 diets, respectively.

3.4.2 Animal performance and feed efficiency

Yields of milk, ECM, and FCM were significantly greater for cattle fed the increased level of EAA supply ($P < 0.01$, $P = 0.01$, $P = 0.02$, respectively, Table 3.9). Cattle in enrollment 2 yielded more milk over enrollment 1 ($P < 0.01$) which agrees with initial parameters used to balance pens within enrollment (Table 3.1 and 3.2). Energy corrected milk throughout the experiment (Figure 3.2) shows that cattle fed the HS 105 diet had the highest yield starting on wk 2 of the experiment, followed by LS 105. Yields for HS and LS 100 cattle were similar

Table 3.8. Effect of varying dietary starch and amino acid supply on apparent total tract nutrient intake and digestibility.

	Diet					*P*				
Parameter	HS 100	HS 105	LS 100	LS 105	SEM	Enroll	Starch	AA	S x AA	Week
Nutrient intake, kg/d										
DM	27.8	28.2	26.1	25.8	0.68	0.06	0.02	0.88	0.61	0.08
OM	25.7^{xy}	26.2^{x}	24.0^{xy}	23.9^{y}	0.61	0.06	0.02	0.81	0.59	0.06
aNDFom[2]	9.37	9.72	9.29	9.24	0.26	0.64	0.31	0.61	0.46	0.20
pdNDFom[3]	7.20	7.37	6.88	7.02	0.18	0.46	0.12	0.37	0.76	0.34
uNDFom[4]	2.16	2.35	2.41	2.23	0.08	0.10	0.21	0.39	0.14	0.33
Apparent digestion, %										
DM	67.2	66.2	63.7	66.2	1.21	0.66	0.24	0.22	0.37	0.55
OM	70.1	68.9	66.7	68.8	0.98	0.74	0.23	0.27	0.36	0.55
aNDFom	46.0	45.6	43.7^{x}	49.0^{y}	1.15	0.31	0.86	0.06	0.35	0.85
pdNDFom	59.8	59.9	59.1^{x}	64.6^{y}	1.36	0.14	0.57	0.07	0.39	0.70

[1] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105)
[2] aNDFom = amylase and ash corrected neutral detergent fiber
[3] pdNDFom = potentially digestible NDF, amylase and ash corrected.
[4] uNDFom = undigested NDF, amylase and ash corrected.
^{xy} Within a row, means without a common superscript differ ($P < 0.10$)

throughout the experiment. Cattle fed a HS diet yielded more milk protein ($P < 0.01$); however, the effect of an increase EAA supply in both 105 diets had a greater impact on improving milk protein yield ($P < 0.01$). Cattle fed the HS 105 consistently yielded the highest amount of protein, followed by HS 100, LS 105, and LS 100. Fat yield was significantly affected by dietary starch levels ($P = 0.04$) and tended to be different for EAA supply ($P = 0.09$; Table 3.9). The cattle fed the LS 105 consistently produced the greatest quantity of fat, followed by similar yields in the HS 105 and LS 100 producing similar yields, and HS 100 producing consistently the lowest after wk 3 of the experiment (Figure 3.4). Milk composition of these components followed a similar trend as their respective yields, with the exception of a greater fat content for cows fed HS 100 versus HS 105 ($P = 0.06$; Table 3.9). Lactose yield followed a similar trend to milk yield and the proportion of lactose in milk was consistent among diets ($P = 0.41$). Milk urea nitrogen (MUN) levels shows significance for the main effect of starch, EAA, and their interaction. The lowest MUN levels were observed in LS 100 diets and the greatest in LS 105 diets.

Milk composition results from the Barbano lab are presented in Table 3.9. Energy corrected milk and 3.5 % FCM tended to be higher for cattle fed the HS diets ($P = 0.09$ and $P = 0.10$; Table 3.10). Further, cattle fed the elevated level of EAA tended to have a higher yield of 3.5% FCM ($P = 0.08$). Similar to results in Table 3.9, protein component yield was significantly different for the main effects of starch ($P < 0.01$) and EAA ($P = 0.01$) levels; however, the level of starch had a greater effect on protein yield than the level of EAA supply. Fat yield and composition were not different among diets ($P = 0.57$ and $P = 0.29$, respectively; Table 3.10). Evaluation of the specific fatty acid profile indicates that the proportion of palmitic acid is significantly lower in milk from cows fed HS 105 compared to all other diets ($P < 0.01$), while

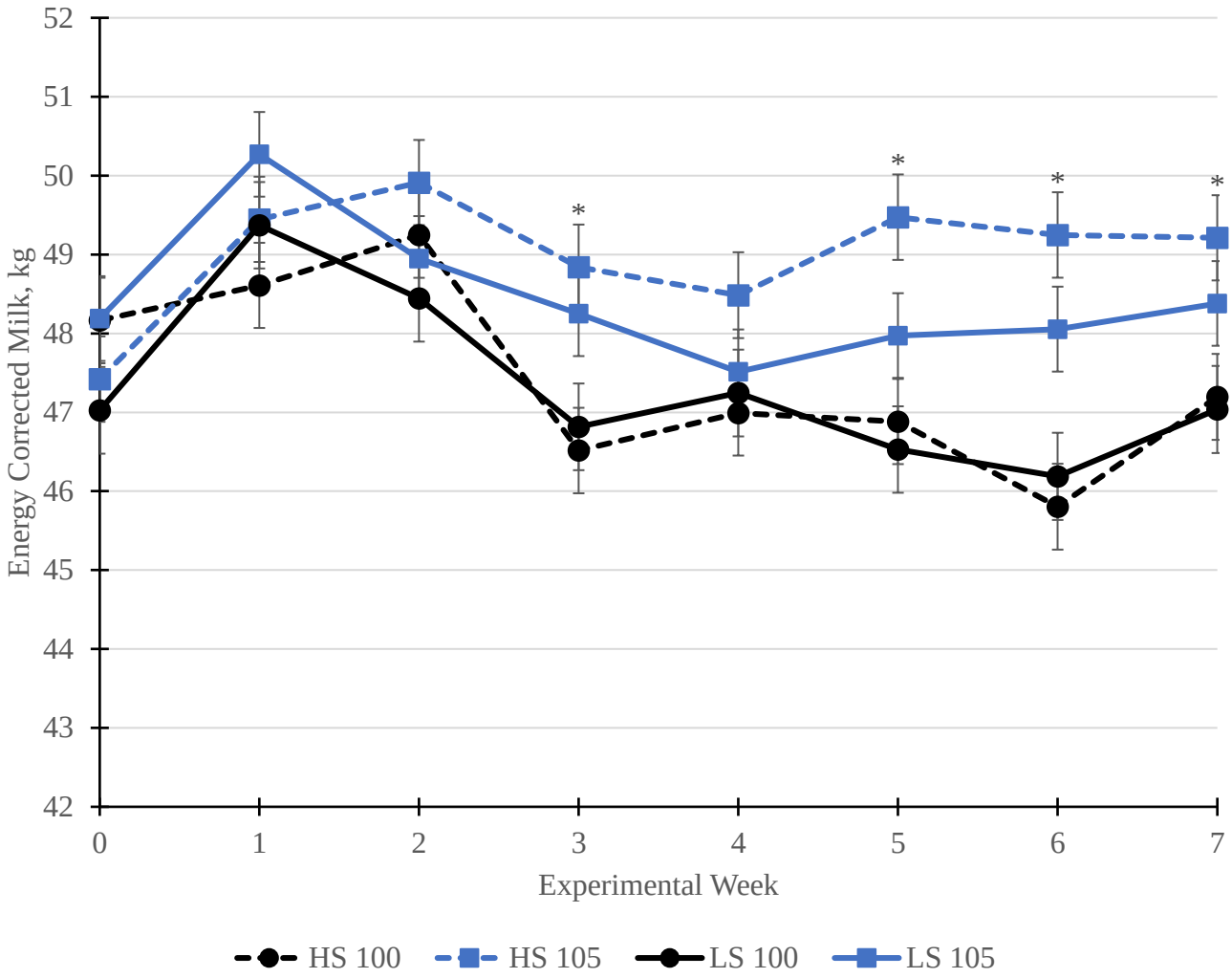
Table 3.9. Effect of dietary starch and EAA supply on intake, milk and component yield, feed efficiency, and blood metabolites in cattle on experiment.

	Diet[1]					*P*[2]				
Parameters	HS 100	HS 105	LS 100	LS 105	SEM	Enroll	Starch	AA	S X AA	Week
Intake and lactation performance, kg/d										
Dry matter intake	28.5	28.1	26.2	26.1	0.4	< 0.01	< 0.01	0.60	0.73	< 0.01
Milk yield	43.5^{b}	46.1^{a}	43.8^{b}	44.2^{b}	0.3	< 0.01	0.03	< 0.01	< 0.01	< 0.01
Energy corrected milk yield[3]	47.3^{y}	49.2^{x}	47.4^{y}	48.5^{xy}	0.5	0.02	0.45	0.01	0.39	< 0.01
3.5% FCM[4]	47.5^{y}	49.2^{x}	48.3^{xy}	49.3^{x}	0.5	0.06	0.37	0.02	0.50	< 0.01
True protein yield	1.40^{b}	1.49^{a}	1.33^{c}	1.38^{bc}	0.01	< 0.01	< 0.01	< 0.01	0.14	< 0.01
Fat yield	1.77^{b}	1.80^{ab}	1.81^{ab}	1.86^{a}	0.02	0.28	0.04	0.09	0.77	< 0.01
Lactose yield	2.14^{b}	2.28^{a}	2.16^{b}	2.18^{b}	0.01	< 0.01	0.03	< 0.01	< 0.01	< 0.01
Milk composition, %										
True protein	3.26^{a}	3.26^{a}	3.07^{c}	3.13^{b}	0.01	< 0.01	< 0.01	0.02	0.02	< 0.01
Fat	4.15^{b}	4.01^{c}	4.23^{a}	4.28^{a}	0.05	< 0.01	< 0.01	0.36	0.06	0.56
Lactose	4.93	4.94	4.94	4.94	0.01	0.70	0.73	0.44	0.41	< 0.01
Milk urea nitrogen, mg/dL	12.1^{bx}	11.1^{bcy}	10.7^{c}	13.9^{a}	0.2	< 0.01	0.02	< 0.01	< 0.01	< 0.01
Body weight and condition										
Overall body weight, kg	689	690	686	690	1.8	< 0.01	0.28	0.28	0.31	< 0.01
Final bodyweight, kg	697	699	695	696	12.4	0.85	0.81	0.89	0.96	-
Body weight change, kg/wk	2.8	2.4	2.5	2.7	0.40	< 0.01	1.00	0.87	0.40	-
Final BCS, 1-5 scale	3.01	2.98	3.00	2.96	-	-	-	-	0.81	-
Efficiencies										
Milk yield:DMI	1.55	1.61	1.65	1.73	0.07	0.08	0.03	0.16	0.80	< 0.01
ECM:DMI	1.70	1.73	1.82	1.88	0.07	0.22	0.01	0.29	0.71	< 0.01
FCM:DMI	1.71	1.72	1.86	1.91	0.07	0.37	< 0.01	0.44	0.67	< 0.01
Milk N:Feed N	0.319	0.328	0.326	0.328	0.011	0.10	0.64	0.48	0.59	< 0.01
Blood metabolites and rumination										
Plasma urea nitrogen, mg/dL	10.8^{b}	10.4^{b}	10.1^{b}	12.5^{a}	0.26	0.47	0.01	< 0.01	< 0.01	< 0.01
Insulin, uIU/mL	23.1	25.2	24.9	23.3	2.5	0.52	0.99	0.93	0.48	0.01
Daily rumination time, min	649	636^{x}	667^{y}	657	7.6	0.11	0.04	0.19	0.89	0.04

[1] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
[2] The interactions of enrollment by week, starch by week, amino acid supply by week, and starch by amino acid by week are omitted from this table.
[3] Calculated using equation published by Tyrrell and Reid (1965).
[4] Calculated using equation adapted from NRC (2001).
[ab] Within a row, means without a common superscript differ ($P < 0.05$)

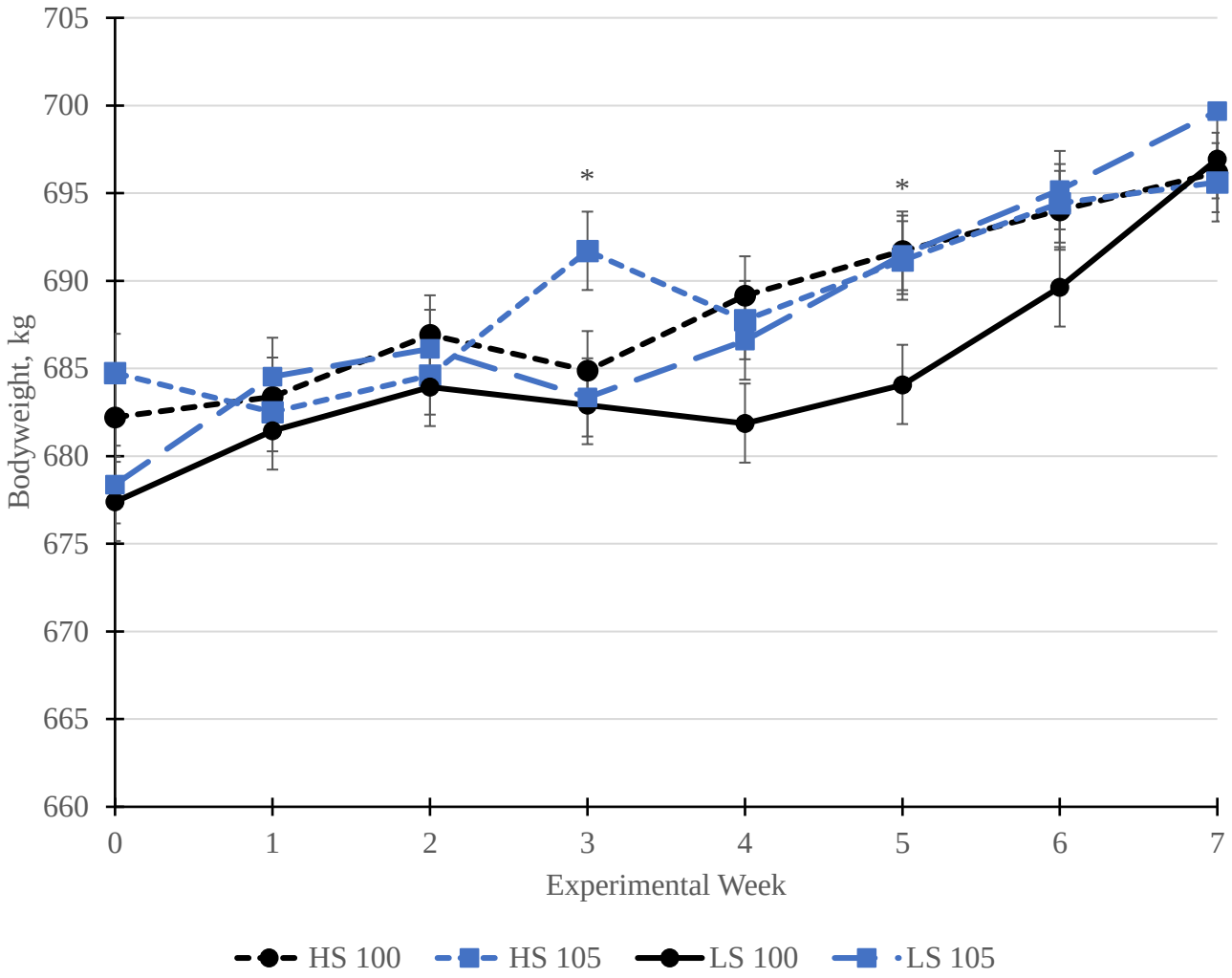
[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Figure 3.3. Effect of varying dietary starch level and essential amino acid supply relative to metabolizable energy[1] on energy corrected milk.



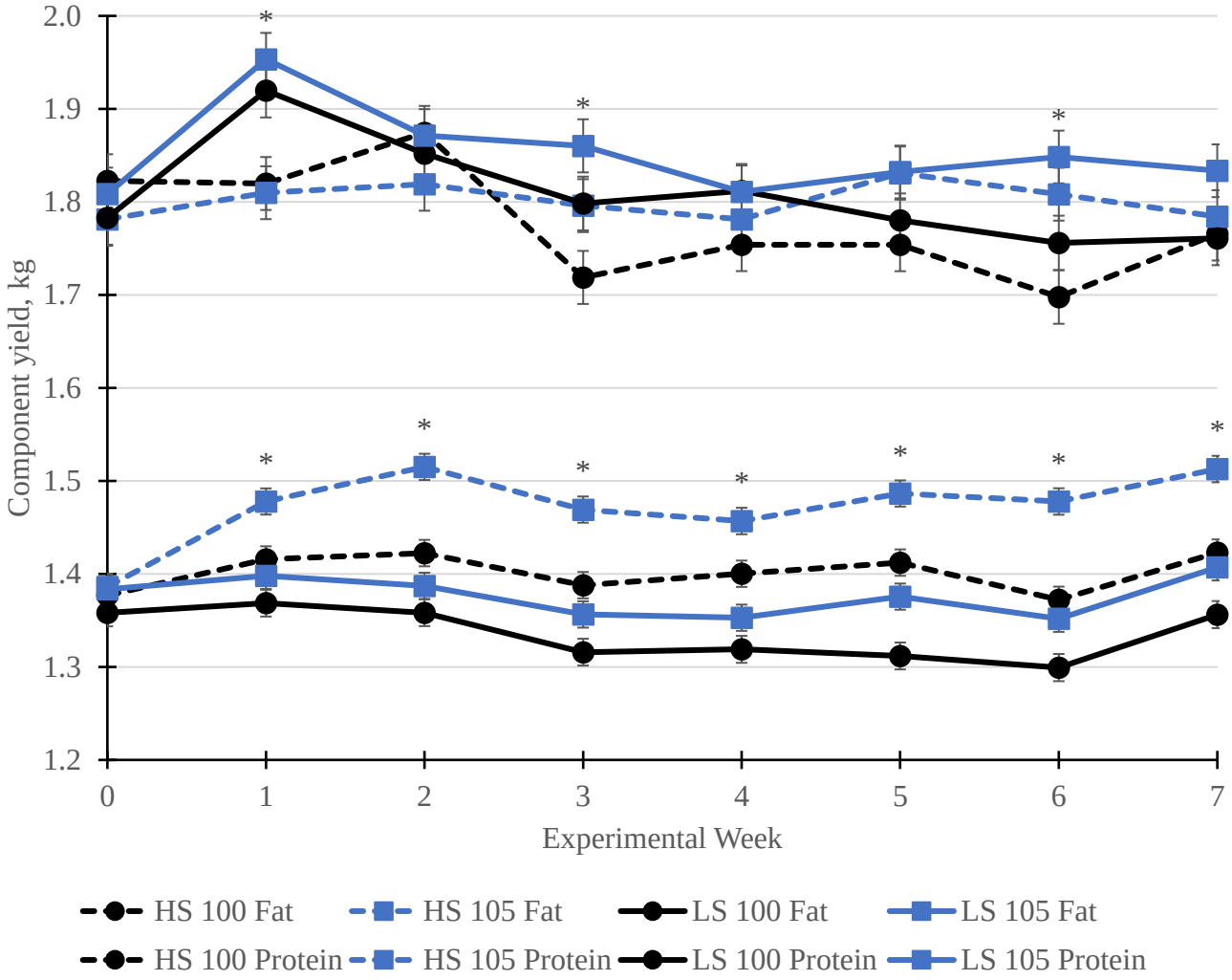
[1]Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
* Indicates differences among diets within a week ($P < 0.05$)

Figure 3.4. Effect of varying dietary starch level and essential amino acid supply relative to metabolizable energy[1] on body weight.



[1]Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
* Indicates differences among diets within a week ($P < 0.05$)

Figure 3.5. Effect of varying dietary starch level and essential amino acid supply relative to metabolizable energy[1] on true protein yield over the duration of the experiment



[1]Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
* Indicates differences among diets within a week ($P < 0.05$)

proportions of oleic acid were significantly higher ($P = 0.01$) over cattle fed the HS diets. Total daily yield of de novo fatty acids was significantly higher in cattle fed the HS diets ($P = 0.01$). Alternatively, cattle consuming LS diets yielded a greater daily amount of preformed fatty acids ($P = 0.01$). Yield of mixed fatty acids were not different among diets although cattle fed the LS 100 diet produced numerically less mixed fatty acids than any other dietary group. Mean chain fatty acid chain length tended to be greater in milk from cattle fed LS diets ($P = 0.06$) with cattle fed the HS 100 diet having the lowest mean chain length. Mean fatty acid saturation level was significantly higher in milk from cattle fed the 105 level of EAA with the HS 105 yielding the highest unsaturation level ($P < 0.01$).

Initial and final BW were not different among diets ($P = 0.95$ and $P = 0.96$, respectively; Table 3.9). Body weight change was significantly greater for cattle in enrollment 1 than in enrollment 2 ($P < 0.01$); however, no differences were observed among diets ($P = 0.40$). Mean BW by diet over the experiment are in Figure 3.4. Weight gain was the highest during wk 4-7 for all diets, with the LS 100 diet, which consistently had the lowest BW, achieving the greatest gain to match final BW of all other diets. Initial and final BCS were not different among diets ($P > 0.10$). Plasma urea N was significantly higher in cattle fed the LS 105 diet ($P < 0.01$) and was not different among enrollment periods ($P = 0.47$). Daily rumination time was significantly greater for cattle on LS diets ($P = 0.04$), ruminating nearly 20 minutes more per day.

The main effect of starch level was significant for efficiency metrics including milk yield:DMI ($P = 0.03$), ECM yield:DMI ($P < 0.01$), and 3.5 % FCM yield:DMI ($P < 0.01$; Table 3.9). With lower DMI and similar milk and component yields, the LS diets exhibited greater efficiency over the HS diets with the greatest difference in efficiency found when evaluating

3.5% FCM. Milk N to feed N was not different among diets, with the lowest NUE found in the HS 100 cattle and the highest in both the HS and LS 105 cattle.

3.4.3 Amino acid balances and model outputs

Model predicted EAA supply, expressed as grams per day and grams to megacalories of ME are in Table 3.11. Formulated targets for each EAA are also provided for reference. Because of differences in DMI, particularly with increased intakes for HS diets, the subsequent gram supply for each EAA was greater than anticipated. Improvements in EAA supply from 100 to 105 were apparent for the HS diets; however, EAA supply did not always increased from LS 100 to LS 105. It is worth noting that comparing the supply differences from 105 and 100 for both HS and LS diets to the intended formulation targets indicate that a 5% in EAA supply would have been achieved if the difference in predicted supply was applied. Methionine, lysine, and histidine, three EAA considered most limiting in dairy diets were supplied in excess to formulation targets for all diets fed. Expressing the gram supply of each EAA relative to Mcals of ME shows a significant trend for the main effect of starch, where cattle fed the HS diets had higher grams of EAA to ME. Further, the main effect of EAA supply was significant for all EAA, indicating greater EAA supply to ME for 105 diets over 100. Again, nearly all diets had a great supply of EAA to ME than was formulated for, with predicted LS 105 supplies being closest to its intended target. Predicted energy density of diets were different with cattle fed LS diets having a higher energy density ($P < 0.01$).

The feed chemical fraction and predicted ruminal digestion are in Table 3.12. As expected, the main effect of starch was significant for both starch intake ($P < 0.01$) and ruminal starch digestion ($P < 0.01$). The diets high in starch provided nearly 2.25 kg more starch intake than the LS diets. Further, CNPCS v7 predicted about 1.4 kg more starch digestion for HS diets over

Table 3.10. Effect of dietary starch level and EAA supply on milk component and fatty acid yield and composition in cattle on experiment.[1]

	Diet[2]					*P*			
Parameter	HS 100	HS 105	LS 100	LS 105	SEM	Starch	AA	S x AA	Week
ECM[3]	50.0	51.0	48.9	49.7	0.69	0.09	0.19	0.96	0.15
3.5% FCM[4]	50.2	51.5	48.6	50.3	0.82	0.10	0.08	0.87	0.10
Component yield, kg									
True protein	1.54	1.61	1.42	1.47	0.02	< 0.01	0.01	0.73	0.60
Fat	1.85	1.87	1.78	1.85	0.04	0.26	0.23	0.57	0.06
Lactose, anhydrous	2.23	2.32	2.15	2.22	0.05	0.25	0.28	0.86	0.04
Milk composition, %									
True protein	3.30	3.33	3.14	3.19	0.02	< 0.01	0.04	0.53	< 0.01
Fat	4.02	3.93	3.98	4.04	0.07	0.63	0.85	0.29	0.72
Lactose, anhydrous	4.74	4.73	4.72	4.74	0.01	0.66	0.76	0.44	< 0.01
Fatty acid yield profile and yield									
C16:0, g/100 g milk	1.64^{a}	1.51^{bx}	1.59^{aby}	1.61^{a}	0.02	0.28	0.02	< 0.01	< 0.01
C18:0, g/100 g milk	0.299	0.282	0.345	0.322	0.012	0.11	0.28	0.82	< 0.01
C18:1 *cis* 9, g/100 g milk	0.634^{x}	0.648^{xy}	0.678^{xy}	0.682^{y}	0.014	0.01	0.49	0.74	0.12
De novo[5], g/d	474^{b}	471^{b}	428^{a}	456^{b}	10.5	0.01	0.24	0.15	0.52
De novo, g/100 g milk	1.03^{b}	0.99^{ab}	0.95^{a}	1.00^{ab}	0.02	0.07	0.91	0.02	0.01
Mixed, g/d	779	765	740	765	15.9	0.22	0.73	0.22	0.01
Mixed, g/100 g milk	1.70^{y}	1.58^{x}	1.64	1.67	0.03	0.55	0.16	0.03	< 0.01
Preformed, g/d	504	533	547	548	10.70	0.01	0.17	0.20	< 0.01
Preformed, g/100 g milk	1.11	1.13	1.22	1.19	0.03	< 0.01	0.83	0.41	< 0.01
Fatty acid characteristics									
Mean chain length	14.49^{a}	14.56^{bc}	14.59^{c}	14.53^{ab}	0.02	0.06	0.96	< 0.01	0.90
Mean unsaturation level	0.229^{a}	0.246^{b}	0.236^{a}	0.234^{a}	0.002	0.38	0.01	< 0.01	0.07
Milk urea nitrogen, mg/dL	11.8	11.0	10.3^{x}	12.9^{y}	0.34	0.70	0.18	0.08	< 0.01

[1] Data collected throughout the second enrollment period on a sub sample of 16 cows per diet (n=64). Samples were analyzed by Barbano et al., Department of Food Science, Cornell University, Ithaca, NY.

[2] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).

[3] Calculated using equation published by Tyrrell and Reid (1965).

[4] Calculated using equation from NRC (2001)

[5] *De novo:* Defined as fatty acids which contain between 4 and 15 carbons; Mixed: Defined as fatty acids which contain 16 carbons; Preformed: Defined as fatty acids which contain 18 or more carbons.

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

LS diets. Nitrogen fractions had varying levels of intake across diets, with LS cattle consuming more ammonia, fiber bound N, and indigestible N ($P < 0.01$). Consumption of insoluble true N was greater in HS 100 diets ($P < 0.01$). Predicted ruminal digestion of these feed fractions followed a similar trend. Total intake and ruminal digestion of carbohydrates was significantly greater for cattle consuming the HS diets ($P < 0.01$; Data not shown). Cattle consuming the HS 105 diets had the greatest intake of N, with HS 100 and LS 105 exhibiting comparable N intakes and LS 100 consuming the least. The predicted total ruminal N digestion was not significantly different for HS 100, HS 105, and LS 105 diets with the LS 100 diet having 30 g/d less of N digested in the rumen ($P =0.03$; Data not shown).

Selected CNCPS v7 model output regarding lactation performance, rumen characteristics, and N and EAA efficiency of use are in Table 3.13. Discrepancies exist when comparing actual milk to ME and MP allowable milk, particularly when evaluating LS diets. Metabolizable energy balance was negative and significantly less for LS diets than when compared to HS diets ($P < 0.01$). Metabolizable protein balance followed the same trend for the main effect of starch ($P = 0.02$) where the model predicted a negative balance for LS diets and HS diets had a balance that averaged 9 g/d in excess. When disaggregating MP supply into feed and microbial sources, differences were observed for the main effect of starch ($P < 0.01$) and EAA supply ($P =0.03$). Undoubtedly due to intake, HS cattle had an average increase supply of nearly 250 g/d more MP from feed sources while cattle consuming the 105 EAA supply had averaged increase of 140 g/d of feed MP. The MP from microbial mass was significantly greater for HS diets ($P < 0.01$). The apparent efficiency of use for both EAA and MP supply was significantly greater ($P < 0.01$ & $P = 0.02$, respectively) for LS diets over HS due to the reduce MP supply observed for those diets. Further, predicted mean rumen ammonia content was significantly higher for LS 105 cattle (15.3

mg/dL) over all other diets (averaging 12.5 mg/dL; $P < 0.01$) and predicted bacterial growth depression was not apparent for any diets. Total NDF pool size was not different among diets regardless of DMI ($P = 0.17$). Predicted fecal N content tended to be higher for cattle consuming HS diet ($P = 0.09$), but urinary N tended to be greater for 105 diets ($P = 0.07$). Although productive N to urinary N was not statistically different among diets, numerical differences were observed where LS 105 had a lower efficiency (0.98) to all other diets.

3.5 Discussion

This study's objective was to evaluate difference in NUE when energy source was varied between glucogenic or lipogenic sources and if both improvements in lactation performance without sacrificing NUE could be realized if a larger supply of EAA to ME was fed. Findings from this study could aid in the evaluation and potential revision of an optimized supply of EAA when regressed against daily ME supply. Under the average stage of lactation as well as the duration of experimental feeding, it is reasonable to assume that these findings are applicable to high producing, post-peak cattle that are in positive energy balance and partitioning most of their nutrients to lactation requirements. Response variables including DMI, lactation performance, and N and energy balances tend to corroborate with other studies with a similar objective, including post ruminal starch and/or fatty acid infusion studies (Reynolds et al., 1994, Lemosquet et al., 2009, Nichols et al., 2019), and longitudinal feeding studies (van Knegsel et al., 2007, Boerman et al., 2015)

Higher dietary starch levels did not decrease DMI relative to LS diets with more lipogenic nutrients, contrary to what others have (Lemosquet et al., 1997, Nichols et al., 2019). These previous studies attribute depression in DMI to higher concentrations of circulating blood glucose and insulin which are known to depress hepatic gluconeogenesis through influence on

TCA cycle intermediates, causing chemostatic fill effect and a decrease in nutrient intake (Lomax et al., 1979, McGuire et al., 1995, Allen et al., 2009). Results from this experiment suggest that the generation of propionate through fermentation of glucose and starch by bacteria in the rumen was not sufficient to act as a major satiety signal to the liver, which is the major hypothesis behind the hepatic oxidation theory limiting DMI in cattle fed higher starch diets (Allen et al., 2009). Dietary starch levels for HS diets were not as high as intended at formulation, averaging 27.2 % of dietary DM. Other feeding studies which have experienced differences in DM between glucogenic and lipogenic diets have fed starch diets which far exceed 30% of dietary DM (van Knegsel et al., 2007, Boerman et al., 2015). It is more likely that the reason for this increase in intake for HS diets is due to a lack of rumen NDF fill under the observed ingredient inclusion rates and intended DMI for formulated diets. In Table 3.13 diets averaged an NDF intake of 1.24% of BW, which is within the range of maximum NDF intake according to Cotanch et al. (2014). Further, uNDF pool size for all diets is on the upper limits of ruminal uNDF capacity (Cotanch et al., 2014). Taken together, all diets, regardless of differences in DMI, did not limit aNDFom intake cattle but rather caused uNDF ruminal pool size limits to be met first.

Higher levels of dietary starch and EAA appeared to have an additive effect on milk yield and ECM yield as observed with cattle fed HS 105. These increases are likely of a combination of increased nutrient supply to the mammary and the concomitant upregulation of the mTOR and IGF-1 pathways to improve productive output (McGuire et al., 1992, Manjarin et al., 2012, Appuhamy et al., 2014). Irrespective of this outcome, cattle fed the LS diets had improved ECM output on average. The relationship between increased dietary oleic supply and improved ECM yield has been observed in high producing cattle, but not in later lactation animals

Table 3.11. Predicted essential amino acid supply, in daily metabolizable grams and grams per megacalorie of metabolizable energy for each dietary treatment fed.

	Diet[1]									*P*		
Parameter[2]	HS 100		HS 105		LS 100		LS 105		SEM	Starch	AA	S x AA
Supply, g/d	Target	Predict	Target	Predict	Target	Predict	Target	Predict				
Arginine	139.5	190.1^{ab}	146.4	198.3^{a}	139.5	166.0^{c}	146.4	175.0^{bc}	5.3	< 0.01	0.16	0.94
Histidine	62.2	78.2^{ab}	65.7	85.1^{a}	62.2	68.5^{c}	65.7	70.4^{bc}	2.2	< 0.01	0.10	0.28
Isoleucine	147.7	178.0^{abx}	155.3	182.2^{b}	147.7	157.8^{ay}	155.3	163.4^{a}	4.7	< 0.01	0.36	0.89
Leucine	233.9	280.5^{ab}	245.6	289.5^{a}	233.9	240.6^{c}	245.6	250.8^{bc}	7.6	< 0.01	0.27	0.94
Lysine	207.3	243.9^{ab}	217.5	255.7^{a}	207.3	214.9^{c}	217.5	224.0^{bc}	6.5	< 0.01	0.17	0.85
Methionine	78.0	91.9^{abx}	82.1	97.6^{a}	78.0	82.8^{by}	82.1	86.3^{b}	2.5	< 0.01	0.12	0.66
Phenylalanine	147.1	175.3^{ab}	154.6	180.2^{a}	147.1	153.5^{c}	154.6	159.9^{bc}	4.7	< 0.01	0.29	0.87
Threonine	146.4	174.8^{ab}	153.9	182.0^{a}	146.4	155.0^{c}	153.9	158.0^{bc}	4.6	< 0.01	0.33	0.65
Tryptophan	40.4	50.6^{ab}	42.4	52.3^{a}	40.4	44.6^{c}	42.4	45.7^{bc}	1.3	< 0.01	0.36	0.87
Valine	169.6	195.5^{ab}	177.8	203.5^{a}	169.6	172.9^{c}	177.8	177.5^{bc}	5.3	< 0.01	0.29	0.75
Lys:Met	2.66	2.65^{a}	2.79	2.62^{b}	2.66	2.60^{c}	2.79	2.60^{c}	0.001	< 0.01	< 0.01	< 0.01
Grams EAA:Mcal ME												
Arginine	2.04	2.62^{aby}	2.14	2.68^{ax}	2.04	2.44^{c}	2.14	2.58^{b}	0.01	< 0.01	< 0.01	0.02
Histidine	0.91	1.08^{abx}	0.96	1.15^{a}	0.91	1.01^{c}	0.96	1.04^{by}	0.01	< 0.01	< 0.01	0.01
Isoleucine	2.16	2.46^{a}	2.27	2.46^{a}	2.16	2.32^{c}	2.27	2.41^{b}	0.01	< 0.01	< 0.01	< 0.01
Leucine	3.42	3.87^{a}	3.59	3.91^{a}	3.42	3.54^{c}	3.59	3.69^{b}	0.02	< 0.01	< 0.01	0.01
Lysine	3.03	3.37^{b}	3.18	3.45^{a}	3.03	3.16^{d}	3.18	3.30^{c}	0.01	< 0.01	< 0.01	0.07
Methionine	1.14	1.27^{b}	1.20	1.32^{a}	1.14	1.22^{c}	1.20	1.27^{b}	0.005	< 0.01	< 0.01	0.76
Phenylalanine	2.15	2.42^{a}	2.26	2.43^{a}	2.15	2.26^{c}	2.26	2.36^{b}	0.01	< 0.01	< 0.01	< 0.01
Threonine	2.14	2.41^{b}	2.25	2.46^{a}	2.14	2.28^{d}	2.25	2.33^{c}	0.01	< 0.01	< 0.01	0.94
Tryptophan	0.59	0.70^{a}	0.62	0.71^{a}	0.59	0.66^{c}	0.62	0.67^{b}	0.003	< 0.01	< 0.01	0.08
Valine	2.48	2.70^{ay}	2.60	2.75^{ax}	2.48	2.55^{c}	2.60	2.61^{b}	0.01	< 0.01	< 0.01	0.41
ME, Mcal/kg	2.61	2.60	2.60	2.59	2.60	2.61	2.62	2.62	0.004	< 0.01	0.97	0.03

[1] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).

[2] Predicted parameters obtained from CNCPS v.7 model outputs.

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Table 3.12. Predicted intake and ruminal digestion of CNCPS feed fractions for diets varying in dietary starch and essential amino acid supply.

	Diet[1]					*P*		
Parameter[2]	HS 100	HS 105	LS 100	LS 105	SEM	Starch	AA	S x AA
Intake, g·d^{-1}								
CHOA4	753	828	770	782	40.1	0.71	0.33	0.45
CHOB1	8051	8236	5975	5975	187.9	< 0.01	0.65	0.63
CHOB2	1513^{bcy}	1485^{c}	1714^{a}	1641^{abx}	34.4	< 0.01	0.21	0.52
CHOB3 Fast	5164^{ab}	5507^{ax}	5010^{aby}	4926^{b}	118.3	0.02	0.33	0.11
CHOB3 Slow	1305	1287	1281	1243	28.0	0.25	0.37	0.73
CHOC	2115	2092	2218	2088	44.8	0.30	0.15	0.26
NA1	55.0^{abx}	49.5^{by}	58.4^{a}	55.0^{a}	1.2	0.01	0.01	0.42
NA2	198	201	182	198	7.4	0.23	0.26	0.41
NB1	383	374	273	282	4.7	< 0.01	0.98	0.10
NB2	40.4^{d}	82.5^{c}	105^{b}	128.3^{a}	2.2	< 0.01	< 0.01	< 0.01
NC	33.9^{c}	43.8^{b}	48.7^{a}	49.1^{a}	1.0	< 0.01	< 0.01	< 0.01
Ruminal digestion, g								
CHOA4	541	596	559	568	26.9	0.85	0.29	0.41
CHOB1	6371	6422	4973	4928	127.8	< 0.01	0.98	0.71
CHOB2	1197	1126	1388	1318	22.9	< 0.01	0.02	1.00
CHOB3 Fast	4319	4359	4376	4256	90.0	0.80	0.68	0.40
CHOB3 Slow	605	589	611	588	9.6	0.81	0.09	0.78
NA1	55.0^{ay}	49.5^{b}	58.4^{ax}	55.0^{ay}	1.2	0.01	0.01	0.42
NA2	159	155	149	160	5.0	0.53	0.55	0.18
NB1	227^{ax}	220^{ay}	169^{b}	174^{b}	1.6	< 0.01	0.51	0.01
NB2	29.4^{d}	55.0^{c}	73.0^{b}	87.9^{a}	1.3	< 0.01	< 0.01	< 0.01

[1] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).

[2] CHO A4 = Sugar, CHO B1 = Starch, CHO B2 = Soluble fiber, CHO B3 = Fast or slow degrading NDF, CHO C = Indigestible NDF, NA1 = Ammonia N, NA2 = Soluble true N, NB1 = Insoluble true N, NB2 = Fiber bound N, NC = Indigestible N; (Van Amburgh et al., 2015)

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

[xy] Within a row, means without a common superscript differ ($P < 0.10$)

Table 3.13. CNCPS v7 predicted outputs for diets varying in dietary starch and essential amino acid supply.

	Diet					*P*		
Parameter	HS 100	HS 105	LS 100	LS 105	SEM	Starch	AA	S x AA
DMI[2], kg·d^{-1}	27.9^{xy}	28.6^{x}	26.1^{y}	26.0^{y}	0.6	0.01	0.68	0.52
Actual milk[2], kg·d^{-1}	43.6^{aby}	45.6^{ax}	43.3^{b}	44.5^{ab}	0.5	0.18	0.02	0.41
ME allowable milk	45.1^{ab}	46.9^{a}	41.5^{b}	41.4^{b}	1.3	0.01	0.54	0.48
MP allowable milk	44.8^{abx}	46.7^{a}	38.8^{by}	40.7^{b}	1.4	< 0.01	0.25	1.00
ME MP first limiting	44.4^{abx}	46.2^{a}	38.7^{by}	40.5^{b}	1.4	< 0.01	0.25	1.00
ME MP allowable average	44.9^{ab}	46.8^{a}	40.1^{b}	41.0^{b}	1.3	0.01	0.37	0.73
ME Supply, Mcals ME/d	72.5^{xy}	74.1^{x}	68.0^{xy}	67.9^{y}	1.7	0.01	0.68	0.61
ME Required, Mcals ME/d	70.4^{xy}	72.2^{x}	70.0^{y}	71.3^{xy}	0.6	0.28	0.03	0.68
ME Balance, Mcals ME/d	2.07	1.86	-1.98	-3.44	1.8	0.03	0.66	0.73
MP supply, g·d^{-1}	3196^{ab}	3347^{a}	2774^{c}	2883^{bc}	88.4	< 0.01	0.20	0.82
MP required[3], g·d^{-1}	3184^{b}	3339^{a}	2998^{c}	3078^{bc}	30.5	< 0.01	0.01	0.26
MP balance, g·d^{-1}	11.6	7.4	-223.9	-195.8	70.7	0.02	0.88	0.82
MP RUP, g·d^{-1}	1488^{ab}	1633^{a}	1245^{c}	1377^{bc}	48.7	< 0.01	0.03	0.90
MP microbial, g·d^{-1}	1707	1714	1528	1506	39.9	< 0.01	0.85	0.72
MP microbial, %	50.2^{b}	48.0^{c}	51.9^{a}	49.0^{c}	0.2	< 0.01	< 0.01	0.14
Apparent efficiency of EAA[4], %	58.8	59.2	63.0	62.5	1.3	0.02	0.99	0.76
Apparent efficiency of MP[5], %	72.3	72.2	78.4	77.6	1.6	0.01	0.78	0.82
Rumen NH_3, mg·dl^{-1}	12.5^{b}	12.7^{b}	12.5^{b}	15.3^{a}	0.2	< 0.01	< 0.01	< 0.01
Bacterial growth depression, %	0.02	0.00	0.08	0.00	0.01	0.01	< 0.01	< 0.01
RDP, % DM	10.6	10.5	10.8	11.5	0.1	< 0.01	< 0.01	< 0.01
RUP, % DM	5.37	5.92	5.22	5.68	0.04	< 0.01	< 0.01	0.27
Rumen NDF pool size and fluxes								
uNDF pool size, kg·kg^{-1} BW	0.69	0.66	0.74	0.71	0.01	< 0.01	0.01	0.78
uNDF flux, (kg·d^{-1}) ·kg^{-1} BW	0.91	0.87	0.97	0.93	0.01	< 0.01	0.03	0.96
Total NDF pool size, kg·kg^{-1} BW	1.17	1.19	1.18	1.15	0.02	0.37	0.96	0.17
Total NDF flux, (kg·d^{-1}) ·kg^{-1} BW	1.24	1.28	1.25	1.20	0.03	0.24	0.95	0.23
Nitrogen accounting, grams								
Nitrogen intake	710	751	667	712	15.8	0.03	0.04	0.88
Total fecal nitrogen	257	275	247	254	7.9	0.09	0.18	0.49
Total urinary nitrogen	213	221	194	224	8.6	0.36	0.07	0.24
Productive nitrogen	222	236	209	217	2.4	< 0.01	< 0.01	0.29
Productive N to Urinary N, g·g^{-1}	1.05	1.07	1.08	0.98	0.05	0.47	0.42	0.20

[1] Diets formulated with two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100 % of optimized EAA supply to ME (100) and 105 % of optimized EAA supply to ME (105).
[2] Observed means across experiment.

[3] Model predicted MP requirements under the assumption of a 73% efficiency of use for MP supplied.
[4] Calculated as a weighted average using the apparent efficiency of use for each EAA.
[5] Apparent efficiency of use = MP Required/MP supply
[ab] Within a row, means without a common superscript differ ($P < 0.05$)
[xy] Within a row, means without a common superscript differ ($P < 0.10$)

(de Souza et al., 2018). Further, improved supply of stearic acid is also linked with improved ECM output (Piantoni et al., 2013, de Souza et al., 2019b). High starch diets improved milk protein output with greater effect than increased EAA supply. Improved milk protein yield is typically observed in diets with higher starch levels (Cantalapiedra-Hijar et al., 2014, Boerman et al., 2015); however, inconsistent results have been observed when only glucose or starch has been infused abomassally (Clark et al., 1977, Nichols et al., 2016). Conversely, experiments where AA or casein are infused with glucogenic nutrients showed markedly improved milk protein output (Raggio et al., 2006, Rius et al., 2010b). Under the context of this study, improved milk protein output is likely a product of increased microbial protein yield in conjunction with elevated propionate supply, likely improving hepatic glucose production for eventual use by the mammary gland (Raggio et al., 2006, Rius et al., 2010a).

Dry matter intake over the experiment was similar for cows fed both LS diets with these diets matching more closely to their formulated intake. Significant enrollment effects in intake illustrate a higher intake in enrollment 2 than enrollment 1 for LS diets, differing by about 1 kg/d between enrollments. Under this context, it's reasonable to conclude that inclusion of lipogenic nutrients did not drastically reduce DMI in the cattle which were fed LS diets. Minimal changes in DMI have been observed both in cattle supplemental saturated fatty acids through dietary inclusion (Harvatine and Allen, 2006, Boerman et al., 2015) and when infused post ruminally (Drackley and Elliott, 1993, Bremmer et al., 1998). Other literature has shown a modest (Nichols et al., 2018a) or a significant reduction in DMI (Drackley et al., 2007). It is unclear why a depression in DMI was not observed; however, model predictions of ME balance are negative for cattle fed LS (Table 3.13), and it might be that these cattle consumed as much intake as uNDF pool size would allow to try and meet ME requirements. Low starch diets also

averaged 4.5% DM for EE, which is not physiologically high relative to other literature diets (Greco et al., 2015, Palmquist and Jenkins, 2017) as well as infusion studies where a hypophagic response occurred when the dietary percentage becomes too high.

Milk fat yield was also higher in cattle fed the LS diets versus HS diets; however, cattle fed HS 105 produced similar yields of milk fat to LS cattle. Inclusion of a high palmitic acid fat supplement has been shown to improve milk fat yield irrespective of the level of milk production observed (Piantoni et al., 2013, Rico et al., 2014) and cattle within this study observed a similar effect. Both C18:0 and C18:1 *cis*-9 were greater in cattle fed the LS diets which is likely due to the direct supplementation within the diet. *De novo* milk FA synthesis was significantly lower in cattle fed the LS diets which has been noted to varying degrees in other studies which have fed a high palm supplement (Lock et al., 2013, Piantoni et al., 2013, Boerman et al., 2015). The improvement in both stearic and oleic acids for cattle fed LS diets further reinforce that the improvement of preformed FA is a directly link to the inclusion of this supplement. However, cattle fed HS 100 had the greatest proportion of palmitic acid in milk fat which could either be a result of improved *de novo* synthesis or a dilution through lower levels of preformed fat composition.

Intakes of aNDFom, pdNDFom and uNDF were similar among all diets, irrespective of the larger DMI observed in HS cattle. Digestibility of both the aNDFom and pdNDFom were numerically higher for cattle fed the LS 105 diet compared to all other diets which were not different among diets. Improvements in apparent total tract digestibility for aNDFom has been reported previously in cattle supplemented palmitic sources to those fed and non-fat control diet (Boerman et al., 2015, Rico et al., 2017). To maintain the iso-caloric nature of the LS diets while adding a fat supplement, a high inclusion of soy hulls as an NDF source was added, which tends

to have a greater digestibility than other NDF sources. Further, a greater inclusion rate of soybean meal was also added to LS diets and given the higher soluble protein pool than other protein feeds, provided the necessary N supply for fiber consuming bacteria to mee the demand of a greater fermentable aNDFom pool size.

Average and final BW and BCS were not different among diets; however, rates of BW gain were greater for LS cattle, particularly after wk 4 of the experiment. Greater partitioning of nutrients to body reserves has been observed when increasing oleic supply (de Souza et al., 2019a) and might have partially contributed to this improved rate of gain during this period. Feed efficiency parameters suggest improved efficiency for LS cattle, particularly when comparing ECM and 3.5% FCM to DMI. These improvements in efficiency are two-fold, driven by a significant improvement in milk fat yield, contributing more weight to ECM and FCM calculations, with a concurrent reduction in DMI. Improvements in EAA supply modestly increased efficiency, having only a numerical improvement for 105 diets over 100. Milk N:Feed N was not different among diets in this study, contradicting the study's hypothesis that cattle fed the HS diets with improved glucogenic nutrients would observe an improved NUE. The original hypothesis developed for this study was based on data from Lemosquet et al. (2010) and Raggio et al. (2006) who evaluated the effects of casein and propionate exclusively on the effect of protein output and NUE. Recent work has suggested that improvements in NUE can be achieved when a saturated fatty acid supplement was supplied (Nichols et al., 2018b). Further studies from this lab intend to evaluate changes when glucogenic nutrients, lipogenic nutrients, and EAA supply are all considered as it is likely that an improved supply of lipogenic nutrients could alter the optimum supply of EAA to ME.

Intake and predicted ruminal digestion, in Table 3.12, show increased starch intake and ruminal digestion, complimenting the intended dietary supply of more glucogenic nutrients being provided to cattle fed the HS diets. Intake of fast, slow, and undegraded pools of NDF were consistent for all diets, reinforcing the obligatory need to meet rumen fill of aNDFom. Model predicted outputs generated from feed chemistry and animal and lactation characteristics show an elevated EAA supply for all diets relative to intended formulation. Improvements in EAA supply from 100 to 105 diets are predicted for most EAA; however, the range when increasing each EAA supply varies from 1 to 10 grams. The reasons for this shift in supply are dependent on the level of starch provided. High starch diets were likely affected by the unintended increase in DMI, driving both the ME and MP balance into excess; whereas LS diets had a higher inclusion rate of feed ingredients which had an increased CP supply over formulated nutrients. Taken together, the variability of feed ingredients used to model these diets throughout the experiment were more variable than expected. Consideration of this variability is important as the CNCPS v7 predict negative ME balances for the LS diets, but not HS diets. Cattle fed the LS 100 diet observed a -1.98 Mcal/d deficiency; however, the SEM for diets was 1.8 Mcal/d so much of this deficit could be considered within the error of predictions. Unfortunately, the LS 105 diet was predicted to have a deficiency of 3.44 Mcal/d and is further beyond the margin of error for these predictions. The effect of saturated fatty acid supply on nutrient digestibility, particularly for NDF and FA, are not appropriately described in the CNCPS and it is likely that describing these effects would improve ME supply for cattle on these diets. The MP supply was also predicted to be negative for LS diets, regardless of the increased EAA supply. Microbial MP yield was greater for HS cattle and is attributed to increased nonstructural carbohydrates which improve fermentation and microbial proliferation in the rumen (Cantalapiedra-Hijar et al.,

2014). Cattle fed the HS diets observed an apparent efficiency of MP close to model assumption of 73% whereas LS diets had improved efficiency, averaging 78 %. The balance of MP for these diets is calculated using the 73% efficiency of use; however, shifts in efficiency of use for both AA and, by extension, MP have been shown to variable when dietary supply shifts relative to requirements (Lapierre et al., 2012). Although not significant, numerical improvements in productive N:urinary N are seen for HS diets as well as the LS 100 diet. Predicted mean rumen ammonia content is higher for the LS 105 diet and does subsequentially increase predicted urinary N output, offsetting this metric in spite of improvements in productive N over the LS 100 diet.

3.6 Conclusion

When supplied with higher levels of glucogenic nutrients, cattle were able to increase milk protein yield, but did not gain an appreciable improvement in NUE. This sequestration of efficiency is due to increased DMI, increased microbial and total MP supply, and decreased efficiency of use for those EAA suppled. On the contrary, cattle consuming the LS diet with an improved lipogenic nutrient profile realized an improvement in milk fat output without drastically increasing DMI. Collectively, the energetic and nitrogenous efficiency of cattle can be affected through different biochemical pathways. Nutrient variability due to shifts in premixes for all diets increased the variation of model supply predictions relative to observed lactation performance. Under a more commercial setting, farmers should be vigilant in testing for nutrient composition of these dietary premixes to comply with formulations and effectively maintain EAA balances relative to ME. Repetition of this study under different experimental conditions is likely warranted to confirm results. Changes would incorporate the inclusion of larger particle feed ingredients, including steam flaked corn, apart from a dietary premix to avoid

ingredient settling and nutrient drift of diets over time as well as formulating diets to achieve ruminal aNDFom fill at similar DMI which should prevent a large separation in DMI between LS and HS diets.

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CHAPTER 4: EVALUATION OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM V7.0 PREDICTIONS TO ASSESS OPTIMIZED SUPPLY OF ESSENTIAL AMINO ACIDS BASED ON METABOLIZABLE ENERGY.

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4.1 Abstract

The ability to optimize essential amino acids (EAA) and metabolizable protein (MP) supply according to cattle requirements has improved with predictions provided by the revised Cornell Net Carbohydrate and Protein System (CNCPS v7). Revisions in EAA maintenance requirements through the incorporation of endogenous N transactions along the gastrointestinal tract as well as the implementation and continued revisions made to the rumen protozoa sub model are a few of the model changes which have provided the opportunity to improve optimization of EAA supply while maintaining a balanced EAA profile to maximize animal productivity and reduce unutilized nutrient excretion. Work has been performed to predict optimum supplies of EAA and relate them to the metabolizable energy (ME) status of the animal with reasonable success; however, the need to confirm these numbers through model evaluation is warranted. Logistic models were fit to EAA supply and requirement data provided by CNCPS prediction for experimental diets fed in Chapter 2 and 3 and were used to confirm both the optimized efficiency of use and gram supply per unit of ME for all EAA recommended by Higgs and Van Amburgh (2016). Fit statistics for the models used to fit all EAA were variable, with R^2 values ranging from 0.45 for His to 0.72 for ARG, with the average R^2 equaling 0.58. Model

predicted apparent efficiencies of use, defined as the quotient between model predicted EAA requirements (AAR) to EAA supplied (EAA), showed similar results for His, Lys, Phe, Trp, and MP when compared to previously recommended optimum efficiency of use. Methionine supply, as a percentage of total EAA supply was similar to previous calculations (5.2% vs. 5.7% of total EAA), although the supply of Lys was nearly two units less the previous derivation (13.2% vs. 15.1%). The loglogistic relationship between the optimum supply of each EAA and ME also observed good fit, with the optimized supply of Met and Lys exhibiting subtle changes in the experimental dataset from previous recommendations. This suggests that the balancing of EAA based on ME status remains crucial in optimizing supply and that the majority previously optimized supplies of EAA to ME are robust under different animal and lactation requirements.

4.2 Introduction

Accurate predictions for the efficiency of use of each EAA requires a robust understanding of both their supply and requirements relative to the animal being described. In the CNCPS v7, digestible EAA supply for ruminants is the summation of EAA flows from rumen microbial mass, feed protein and EAA which has escaped ruminal degradation, and endogenous losses which are recycled for productive use (Lapierre et al., 2008a, Ouellet et al., 2010). The EAA requirements predicted in the model account for maintenance requirements, including scurf, endogenous losses in the gut, and metabolic urinary and fecal losses according to Fox et al. (2004) and Higgs and Van Amburgh (2016), and productive requirements, including lactation, pregnancy, and growth requirements (Tylutki et al., 2008). The ability to describe EAA requirements for all metabolic functions, including functions such as oxidation for synthesis of NEAA and gluconeogenesis, within a nutritional model can be tedious and might be beyond the described model boundaries, as is with the CNCPS. To account for this, determination of true

EAA requirements in the CNCPS requires the knowledge of net requirements for individual processes and the 'efficiency of use' of supplied EAA to meet the net requirements for that biological process (O'Connor et al., 1993). Any EAA requirements that are not described in the model should be considered as part of the difference between the observed nutrient supply and model predicted requirements, and this quantitative difference is used to calculate the efficiency of use for EAA under the current model description (Hanigan et al., 1998, Doepel et al., 2004, Moraes et al., 2018). The calculation between net requirements and efficiency of use can be difficult to optimize as the efficiency of use for each EAA is dependent on several factors, including the identification of other limiting nutrients which might alter productive use of EAA supply (Higgs et al., 2014) or homeorhetic changes in metabolism which might cause EAA nutrients to be partitioned in ways deemed inefficient for desired productivity (Bauman, 2000). Once described, the appropriate efficiency of use can lead to the ability to balance and optimize the supply of each EAA, allowing for a reduction in total N feeding while maintaining the high levels of milk and milk component yields (Haque et al., 2015). The consideration of each EAA supply with regards to the energy status of the animal also plays a crucial role in improving the efficiency of use for these nutrients. Research exploring the relationships between protein, energy, and metabolic flexibility (Lobley, 2007) in ruminant animals has lead to recommendations of supplying EAA based on the ME status of the animal.

Providing an optimized supply of EAA relative to predicted ME requirements is predicated on accurate predictions of EAA supply. And, because microbial protein provides a large percentage of MP supplied to cattle, inaccurate predictions of this supply will result in the inability to appropriately balance EAA supply and make use of the optimized efficiency of use for these

nutrients. Microbial protein yield is primarily composed of fiber and non-fiber carbohydrate digesting bacteria (Russell et al., 1992, Tylutki et al., 2008); however, literature has suggested that the rumen protozoa population contributes substantially to microbial AA supply (Firkins et al., 2007, Dineen et al., 2020). The CNCPS v7 utilizes a protozoal sub-model for the predictions of microbial AA supply but initial parameters which described the metabolism of those species in the rumen created biases in microbial and total AA supply. Work done by Dineen (2020) reparametrized this sub-model to correct these biases and improve the microbial AA supply. These changes have yet to be tested on the experimental diets fed in Chapter 2 and 3 to evaluate shifts in microbial MP, total MP, and EAA supply.

The objective of this study was to evaluate the efficiency of use of each EAA relative to total EAA requirements and the assess the optimized supply of EAA relative to ME using CNCPS model predictions for experimental diets described in previous chapters. Results from this evaluation will provide confirmation of previously described optimum supplies or will suggest revisions for these numbers. A secondary objective was to evaluate CNCPS outputs from the same experimental dataset using the reparametrized protozoal sub-model. Results from this assessment will provide insight on the appropriateness of these sub-model revisions.

4.3 Materials and methods

4.3.1 Predictions of essential amino acid efficiency of use

Efforts by Higgs et al. (2014) have previously described the efficiency of use for each EAA using the predictive capabilities of the CNCPS v.7. From this work, a collection of EAA infusion experiments were used to generate logistic models which described the relationship between AA supplied (AAS) and CNCPS predicted AA requirements (AAR) for cattle over a

wide range of supplied EAA. This relationship was also tested on the combined data described in Chapter 2 and 3, which also fed a range of EAA supply to cattle, but at a supply that was higher than most infusion studies used in the original dataset generated by Higgs et al. (2014). Descriptive statistics for this dataset are in Table 4.1. The model approach used to estimate optimum efficiency of use for the dataset is similar to the approach described by Doepel et al. (2004) and adapted by Higgs et al. (2014). Briefly, AAS and AAR of each EAA were estimated for all treatment by week observations from experiments in chapter 2 and 3. The following logistic model was considered to give the best fit describing the relationship between AAS and AAR:

$$y = \frac{\theta_1}{1+ \theta_2 e^{\theta_3(x)}} \quad [1]$$

Where y is the AAR and x is the AAS, both in grams per day, and $\theta_{1\text{-}3}$ are the model parameters which were used to describe the relationship between the two variables. Parameters were estimated using PROC NLMIXED in SAS v9.4 (Cary, NC). The Marquardt iterative method was selected, given its use by Higgs and Van Amburgh (2016) when previously optimizing for EAA efficiency of use, to find best parameters for the non-linear model. Determination of the optimal supply of each EAA was determined to be the where the upper critical limit was observed for the fit model (Doepel et al., 2004). Alternatively, this can be considered the point on the curve where the rate of change in AAS to AAR was most rapid, indicating a change in how the model appropriates AAS to AAR. Solving for the upper critical limit for each EAA

model can be achieved by evaluating the third derivative of Eq. 1, which measures the rate of change in the concavity of the model. This model takes the form:

$$\frac{d^3y}{dx^3} = -\theta_1\theta_2{\theta_3}^3 e^{\theta_3(x)} \frac{1-4\theta_2 e^{\theta_3(x)} + {\theta_2}^2 e^{2\theta_3(x)}}{(1+\theta_2 e^{\theta_3(x)})^4} \quad [2]$$

And can be solved for zero be reducing Eq. 2 and solving for x:

$$x = \frac{1}{\theta_3}\log\left(\frac{2-\sqrt{3}}{\theta_2}\right) \quad [3]$$

where x is the optimum AAS under the dataset used to fit the logistic model. Once solved, the known value for AAS in Eq.3 can be substituted for x in Eq.1 and used to solve for y, or AAR. The quotient of optimized AAR and AAS will yield the optimal efficiency of use as described by the model and, by extension, will give information on what optimal level of supply for each EAA should be fed above model predicted requirements to account for biological functions not described within the model.

4.3.2 Calculations for optimal grams of EAA relative to energy

In addition to the determination of optimal efficiency of use for each EAA using the dataset from Chapter 2 and 3, calculations were also made to assess the optimal supply of EAA relative to ME. This approach allowed comparisons to be made to optimal EAA supplies suggested by Higgs et al. (2014). Similar methods described by Higgs et al. (2014) were also used to calculate

the optimal supply of EAA. Briefly, the efficiency of use for all EAA was calculated for each diet by week predictions made by the CNCPS. The relationship between efficiency of use and the gram supply of each EAA relative to ME was evaluated using a log-logistic model with three parameters for model fit:

$$y = \theta_1 - \log\left(1 + \theta_2 e^{-\theta_3(x)}\right) \quad [4]$$

where *y* is the ratio of AAR to AAS, *x* is AA supply expressed relative to Mcals of ME and $\theta_{1\text{-}3}$ are the model parameters used to describe the shape of the curve. Parameters for each EAA were also calculated using PROC NLMIXED in SAS with the Gauss-Newton method for optimization selected. Rearrangement of Eq. 4 and solving for x will by substituting the optimal efficiency of use for each EAA in place of y allows for the optimized gram of EAA per unit of ME to be determined.

$$x = \frac{-1}{\theta_3} log\left(\frac{e^{\theta_1 - y} - 1}{\theta_2}\right) \quad [5]$$

4.3.3 Metabolizable energy and amino acid supply under revised protozoal model.

Recent reparameterization of the protozoal sub model has improved the predictive accuracy of both bacterial and protozoal yields from the rumen and refined the predictive AA flows made by the CNCPS v.7 (RP v7) (Dineen, 2020). Specific changes to protozoal characteristics include

revisions in passage rate, decreased lysis (Wells and Russell, 1996) and microbial predation rates (Coleman and Sandford, 1979), and revisions towards the AA profile of microbes and protozoa (Dineen, 2020). Improvements for most model predicted EAA flow were observed after the revisions to the protozoal sub model were implemented; however, biases do still exist for predicted Met and Lys flows, where the model underpredicts total digestible flows for both AA.

Diets described in Chapter 2 and 3 were formulated using CNCPS v7 but these corrections in the protozoal sub-model were not available at the time of formulation. It was known that the previous version of the protozoal sub-model would cause protozoa to excessively predate the rumen bacterial population and depress total microbial protein yield below measured values. This was due, in part, due to the assignment of the solid phase passage rate to the protozoal passage rate, as demonstrated in previous investigations (Craig et al., 1987). To account for this bias, two parallel model runs on the same formulation were conduct, one run with the protozoal model active and one without. The MP and EAA flows were averaged from the two runs and used as the supply of EAA from the formulated diet. With these updates to the protozoal sub-model implemented, diets were reran through the RP v7 and predictions of nutrient supply were compared to previous model outcomes.

4.4 Results and Discussion

4.4.1 Essential amino acid efficiency of use

Descriptive statistics in Table 4.1 provide an overview of animal and feed inputs used to make predictions for an optimized EAA and MP supply. Both experiments where data were sourced from fed total mixed ration (TMR) diets to Holstein dairy cattle weighing an average 707 kg and producing an average of 41.3 kg of milk. Average animal inputs from the Higgs et al. (2014)

dataset are nearly 150 kg and 15 kg less for body weight (BW) and milk yield, respectively (Data not shown), and have a substantially lower requirement for MP and EAA. Although DMI is higher for this dataset over the previous dataset, all experiments used for the previous dataset were infusion studies which typically restrict feed intake to maintain more control over total EAA supply (Metcalf et al., 1996, Raggio et al., 2006, Doepel and Lapierre, 2011). The CP content of the diets analyzed through CNCPS v7 and RP v7 averaged 15.5% and ranged from 13.4% to 17.6% (Table 4.1) which is a similar level of CP to most North American TMR based diets (NRC, 2001, Tebbe and Weiss, 2020).

Predictions for the logistic model parameters, goodness of fit, optimized EAA and MP efficiency of use and corresponding supply, expressed in g/d and as a percent of total EAA are in Table 4.2. This data indicates the predicted optimum efficiency of use for most EAA are not remarkably different than what was observed by Higgs et al. (2014). A mean shift in efficiency of use for all EAA was -0.3% of previously derived values, ranging from a -14% to 8% shift in predicted optimum efficiency of use. Under these dataset values, the predicted optimum efficiency of use for Met and Lys improved by an average of 6%, suggesting that these two EAA were supplied at a lower, but not limiting, level relative to model predicted requirements which is known to increase nutrient efficiency of use (Hanigan et al., 1998). Improvements in our understanding of these EAA requirements allow for the opportunity to balance for the EAA supplies, thereby reducing AAS without sacrificing milk volume, component yield, and other forms of productive responses. The apparent efficiency of use for MP was similar to previously predicted efficiencies, improving from 73% to 74%. Data from literature suggest that the apparent efficiency of use for MP can vary from 62% in cattle fed a diet formulated for 70% of

MP requirements and abomassally infused with 695 g/d of AA to match the profile of casein and meet 100% of MP requirements to 94% in cattle which were only fed at a rate of 70% MP requirements (Omphalius et al., 2020). The MP apparent efficiency of use for the experimental dataset showed a similar range, where an apparent efficiency of use at 68.5% corresponded to an MP supply which was 105% of requirements and an apparent efficiency of use at 92.8% corresponded to an MP supply which was 78% of MP requirements. This range describing the variable efficiency for MP does have biological relevance; however, the improvement of efficiency at the expense of drastically reducing MP supply relative to requirements is impractical when the goal of balancing EAA and MP supply is to optimize ECM and productive efficiency (Higgs and Van Amburgh, 2016)

Visual representation of model fit and determination of optimum supply for the three EAA commonly designated as most limiting in cattle diets, Met, Lys, and His, and MP are in Figures 4.1 to Figure 4.4, respectively. Model fit for curves describing Met and MP supply to predicted requirements agree with model fit observed in Higgs et al. (2014). Digestible supply of Lys and His for most of the experimental dataset appeared higher than what was calculated as the optimum supply relative to predicted requirements for those EAA (Figure 4.2 and Figure 4.3). Results from Chapter 2 and 3 illustrated higher predicted Lys supply for nearly all experimental diets relative to formulated supply. Further, model predicted Lys requirements did not increase when the digestible supply of Lys increased. This suggests that either the profile of Lys from microbial sources, feed sources, or both are over estimating Lys supply for these diets or that diets which did not increase their model predicted requirements of Lys relative to Lys supply had other limiting nutrients which reduced productive output. Model predicted His requirements and

Table 4.1. Descriptive statistics of dataset[1] used for prediction of EAA efficiency of use and optimal supply of EAA relative to ME.

Cattle characteristics	Mean	Median	Std Dev	Min	Max
DMI, kg/d	26.7	26.6	1.58	22.9	32.0
DIM, d	139.8	134.4	31.7	84.4	214.1
Age, months	48.2	48.0	2.54	43.4	54.8
Body weight, kg	706.5	704.0	20.7	668.1	763.5
Milk yield, kg/d	41.3	41.6	3.88	31.9	48.0
Milk fat, %	4.15	4.15	0.22	3.56	5.56
Milk true protein, %	3.15	3.16	0.16	2.67	3.56
Diet characteristics, % DM					
Volatile fatty acids	4.61	4.05	1.45	2.84	8.04
Ethanol soluble carbohydrates	3.64	3.83	0.67	2.28	4.71
Starch	28.7	29.4	3.25	20.9	33.1
Soluble Fiber	5.80	5.79	0.65	4.12	7.50
aNDFom	32.0	31.8	1.59	28.5	35.8
Crude protein	15.5	15.9	1.17	13.4	17.6
Ether Extract	3.49	3.33	0.45	2.92	4.54
Ash	7.01	6.75	0.74	5.80	8.54

[1] n = 210; describing all diets by week predictions for experiments described in Chapter 2 and 3.

digestible His supply exhibited a linear trend when the digestible supply of His was below 80 g/d, after which increased variation is observed. Dietary formulation of His supply for all diets described in the experimental dataset was meant to exceed the previously described optimum under the consideration of literature which suggests an improved His supply improve milk protein response (Lapierre et al., 2008b, Lee et al., 2012). Similar to Lys, it is likely that other nutrients might have become first limiting as His increased in digestible supply. Recent work published by (Lapierre et al., 2021) suggests that optimum efficiency of use for His when evaluating milk true protein output is 77% whereas the evaluation from this dataset predicted the optimum efficiency of use at 71%. Under a balanced EAA diet, it is reasonable to assume that model predicted His requirements, as a result of improved ECM, would increase at more linear rate when presented with an increased His supply. Under these conditions, more of the variation in model predicted His requirements would be accounted for by the digestible His supply thus the AAR:AAS, or efficiency of use, would be improved to match more closely with results from Lapierre et al. (2021).

4.4.2 Essential amino acid supply relative to metabolizable energy

Model parameter estimates, goodness of fit measurements, and optimum supply of each EAA per Mcal of ME are in Table 4.3. Further, visual representation of model fit describing the gram supply of EAA per Mcal of ME and the efficiency of use for Met and Lys are in Figure 4.5 and Figure 4.6, respectively. Similar to the predicted optimum efficiency of use for Lys, the optimum supply of Lys to ME is lower than what was fed to most observations in the experimental dataset and is likely attributed to an oversupply of Lys in some of the experimental diets. Regardless of this discrepancy in supply, the predicted optimum supply of both Lys and Met are similar to previously predicted values. The predicted optimum supply of His relative to

Table 4.2. Model parameters, goodness of fit assessment, and model predictions for logistic model fit between EAA supply and requirements.

EAA	Model parameters θ_1	θ_2	θ_3	RMSE	R^2	Optimum AAR:AAS[1]	Higgs, 2014 AAR:AAS[2]	g/d[3]	% EAA	Higgs, 2014 % EAA	Lapierre, 2007 % EAA
Arg	106.9	2.98	-0.01	2.38	0.72	0.47	0.55	179	12.2%	10.2%	9.6%
His	50.9	162.50	-0.11	2.63	0.45	0.71	0.70	56	3.9%	4.5%	5.1%
Ile	148.7	4.36	-0.01	4.36	0.69	0.57	0.61	207	14.1%	10.8%	11.1%
Leu	188.0	49.56	-0.02	9.23	0.49	0.70	0.67	210	14.4%	17.1%	18.5%
Lys	159.3	33.46	-0.03	7.71	0.50	0.65	0.62	193	13.2%	15.1%	15.0%
Met	55.3	17.43	-0.05	2.12	0.68	0.57	0.53	76	5.2%	5.7%	5.3%
Phe	95.6	41.72	-0.04	4.87	0.45	0.54	0.53	139	9.5%	10.7%	11.4%
Thr	122.2	4.45	-0.01	3.51	0.69	0.49	0.53	198	13.5%	10.7%	10.4%
Trp	31.5	15.43	-0.09	1.54	0.45	0.56	0.58	44	3.0%	2.9%	--
Val	133.9	12.77	-0.02	5.88	0.56	0.67	0.62	158	10.8%	12.4%	13.6%
MP[4]	2776.2	4.33	0.00	92.49	0.70	0.74	0.73	2961	--	--	

[1] Designated as the optimum efficiency of use using CNCPS v7 predictions for the current dataset.
[2] Optimum efficiency of use described by Higgs et al. (2014)
[3] Optimum AA supply for current dataset
[4] MP = Metabolizable protein flow

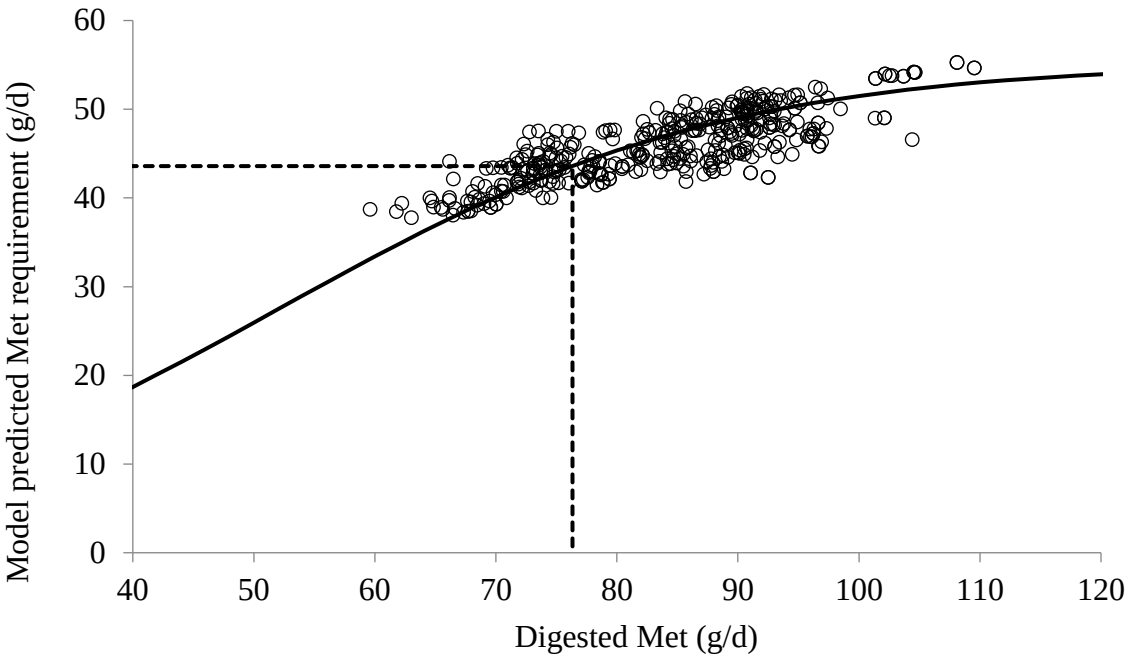

Figure 4.1. Logistic fit of Met supply and model predicted Met requirements. Optimum efficiency of use for Met, as defined by Doepel et al. (2004), is designated by dashed line.

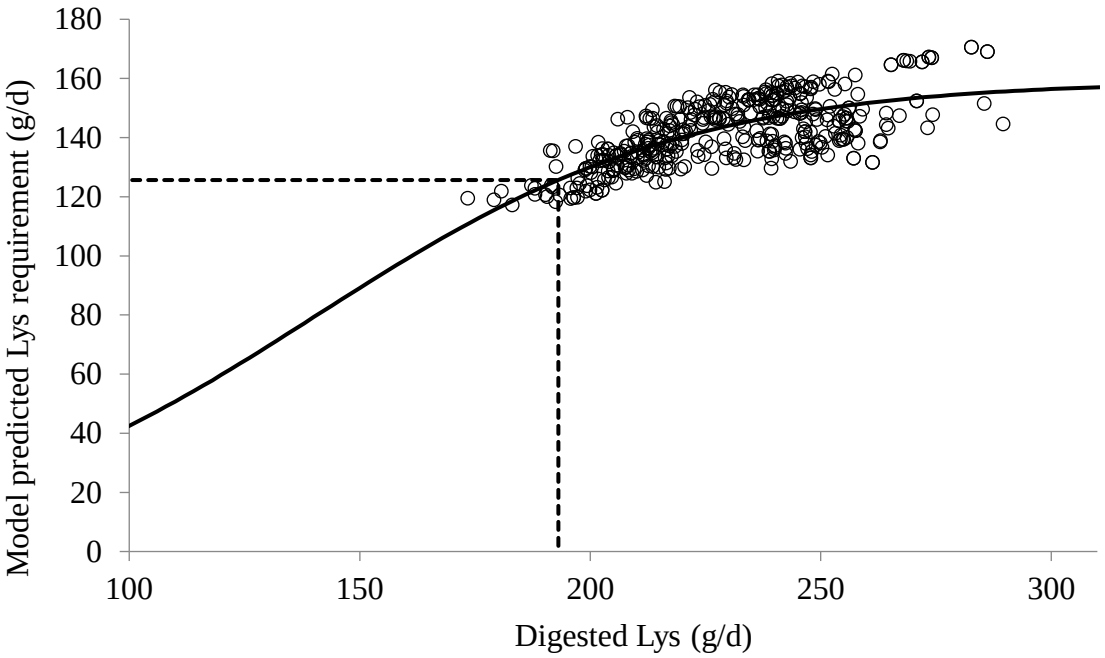

Figure 4.2. Logistic fit of Lys supply and model predicted Lys requirements. Optimum efficiency of use for Lys, as defined by Doepel et al. (2004), is designated by dashed line.

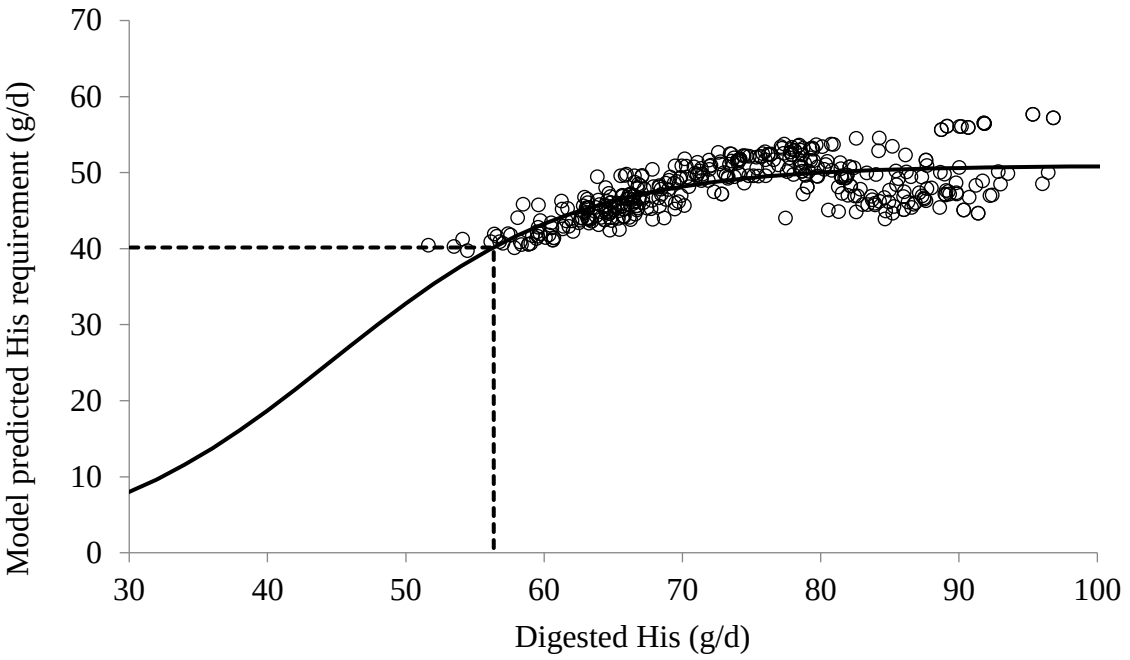

Figure 4.3. Logistic fit of His supply and model predicted His requirements. Optimum efficiency of use for His, as defined by Doepel et al. (2004), is designated by dashed line.

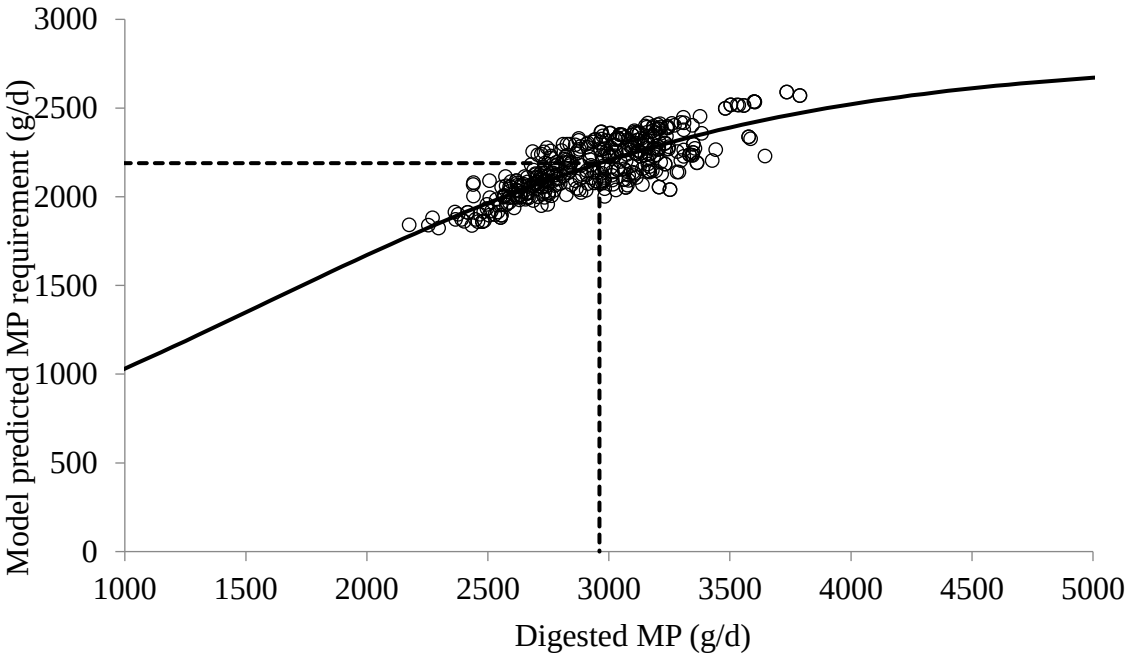

Figure 4.4. Logistic fit of Metabolizable protein supply and model predicted Metabolizable protein requirements. Optimum efficiency of use for metabolizable protein, as defined by Doepel et al. (2004), is designated by dashed line.

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ME is slightly higher than what was predicted by Higgs and Van Amburgh (2016); however, this increase in His supply is warranted given previous literature findings on improvements in lactation performance when His supply is improved. The predicted optimum supply for Ile, Thr, and Trp are considerably higher than previously calculated; however, the coefficient of determination for all three of these models is below 0.5, indicating a weaker relationship between the optimum efficiency of use and gram supply relative to ME for all three EAA. The proportion of Ile as a percent of total EAA is higher (14.1% of total EAA) than previous predictions (10.8%) and is likely the reason for the increase in predicted Ile supply per unit of ME. Conversely, Phe was 9.5% of total EAA which is 1.2 units lower than what was described by Higgs and Van Amburgh (2016). More work is needed to evaluate and test the robustness of predictions for both Phe and Trp supply relative to ME.

4.4.3 Metabolizable energy and amino acid supply under revised protozoal model.

Predictions of ME supply for the CNCPS v7 and RP v7 (Dineen, 2020) are shown in Figure 4.7. Energy prediction from RP v7 depressed ME supply by an average 0.5 Mcals over CNCPS v7 (Data not shown). This difference is subtle and should be considered within the margin of error for predictions. Predictions for total MP supply (Figure 4.8) indicates a bias where most data points show an increase in predicted supply under the adjusted protozoal model. Disaggregating total MP supply into microbial (Figure 4.9) and feed MP (Figure 4.10) sources shows an average increase of 170 g of MP from microbial sources when the revised protozoal sub model is compared to the original model. This increase in MP is due to a substantial increase in protozoal MP supply (120 g vs 292 g) when comparing predictions from CNCPS v.7 and RP v.7, respectively. Predictions for bacterial MP (Data not shown) were not different among CNCPS v.7 (1507 g) and RP v.7 (1505 g) runs, indicating that the changes made in RP

Table 4.2. Model parameters, goodness of fit for the loglogistic relationship between EAA predicted requirements and supply. Optimized supply relative to ME and Lys for each EAA is also provided.

	Model parameters					Optimum	Higgs, 2014	Lys:AA	Lys:AA
EAA	θ_1	θ_2	θ_3	R^2	RMSE	g AA/ Mcal ME	g AA/ Mcal ME[1]	Dairy[2]	Swine[3]
Arg	0.12	-0.95	0.48	0.77	0.10	2.42	2.04	1.26	1.85
His	-0.08	-1.06	0.68	0.75	0.06	0.98	0.91	3.14	2.50
Ile	-0.35	-0.89	0.16	0.49	0.12	2.47	2.16	1.24	1.78
Leu	-0.29	-0.92	0.11	0.55	0.09	3.47	3.42	0.88	0.89
Lys	0.29	-1.07	0.41	0.56	0.17	3.06	3.03	1.00	1.00
Met	0.18	-0.96	0.98	0.71	0.07	1.11	1.14	2.75	3.71
Phe	-0.02	-1.05	0.40	0.48	0.11	2.24	2.15	1.37	1.82
Thr	-0.02	-0.84	0.30	0.52	0.11	2.44	2.14	1.26	1.49
Trp	-0.21	-0.85	0.70	0.42	0.06	0.65	0.59	4.69	5.33
Val	-0.06	-1.02	0.28	0.55	0.13	2.49	2.48	1.23	1.15

[1] Optimum supply of EAA per unit of ME of use described by Higgs et al. (2014).
[2] Optimum Lys:EAA ratio for the experimental dataset used.
[2] Standardized ileal digestible Lys:EAA for a lactating sow (NRC, 2012)
undegraded feed sources.

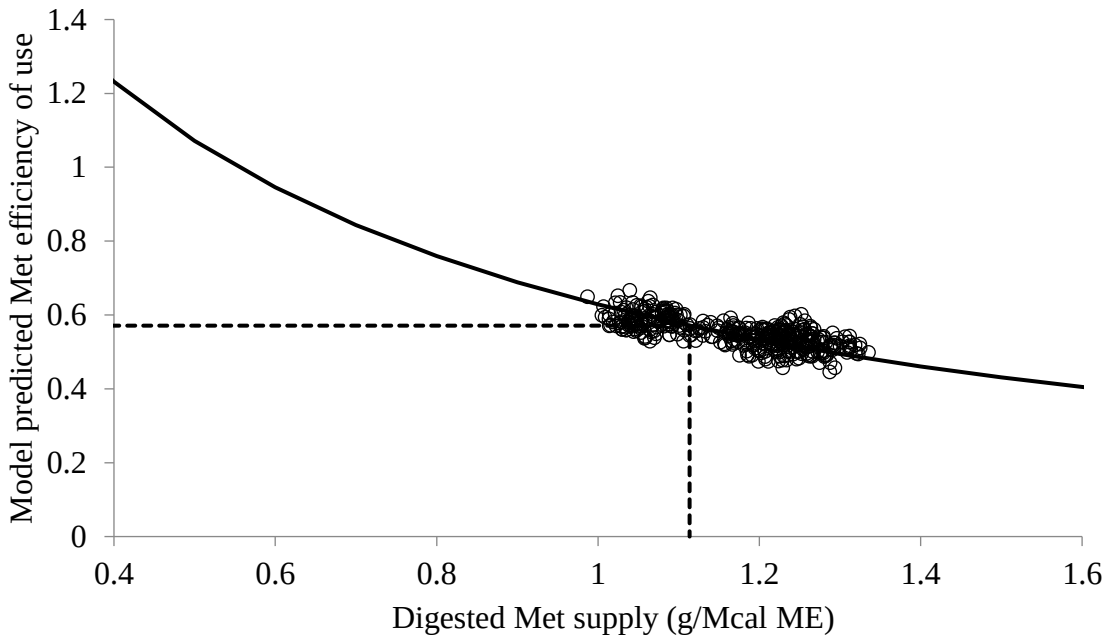

Figure 4.5. Relationship between Met optimum efficiency of use and Met supply relative to ME. The dashed line corresponds with the optimize efficiency of use for Met and subsequent supply relative to ME.

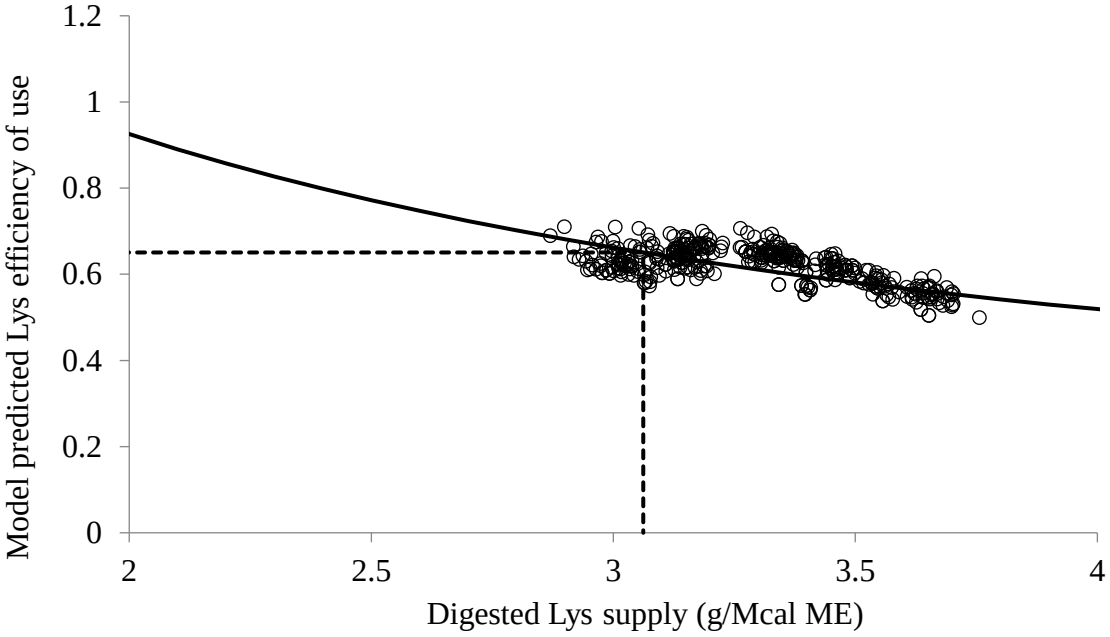

Figure 4.6. Relationship between Met optimum efficiency of use and Met supply relative to ME. The dashed line corresponds with the optimize efficiency of use for Met and subsequent supply relative to ME.

v.7 to protozoal characteristics were sufficient to avoid a bacterial MP depression when the protozoal sub-model is active. Feed MP (Figure 4.10) was only improved by 12 g when comparing the two versions of the model; however, a subset of data shows the original version of the CNCPS to predicts 50 g more MP from rumen undegraded feed sources. Most of this subset of data is associated with the POS diet from Chapter 2, which was the only diet that was fed an appreciable level of blood meal with heated treated soy and rumen protected Met and Lys. This combination of ingredients increased the level of dietary rumen undegraded protein (RUP), simultaneously improving the proportion of total MP supply which originated from rumen undigested feed. Collectively, the total predicted MP supply for all diets ran through CNCPS v.7 averaged 2,913 g and was considered deficient relative to predicted MP requirements of 2,972 g and although this deficiency represents only 2% of predicted requirements, the same diets ran through RP v.7 had an average MP supply of 3,017 g and would not be limited in supply relative to model requirements.

Contrary to the improved predictions of total MP supply, Met and Lys supply (Figure 4.11 and Figure 4.12, respectively) were considerably less for RP v.7 predictions than CNCPS v.7 for the experimental dataset. In addition to changes made on the parameters which describe the protozoal sub-model, RP v.7 also utilized a revised AA profile for microbes and protozoa suggested by Dineen (2020). Microbial AA composition data from Clark et al. (1992) was previously used in the CNCPS v.7 runs and the changes in composition is likely altering the supply of all EAA. For instance, Fessenden et al. (2019) suggests that the Lys composition described by Clark et al. (1992) overestimates digestible Lys supply due to a lack results which make use of multiple hydrolysis time points to accurately account for all AA recovery in

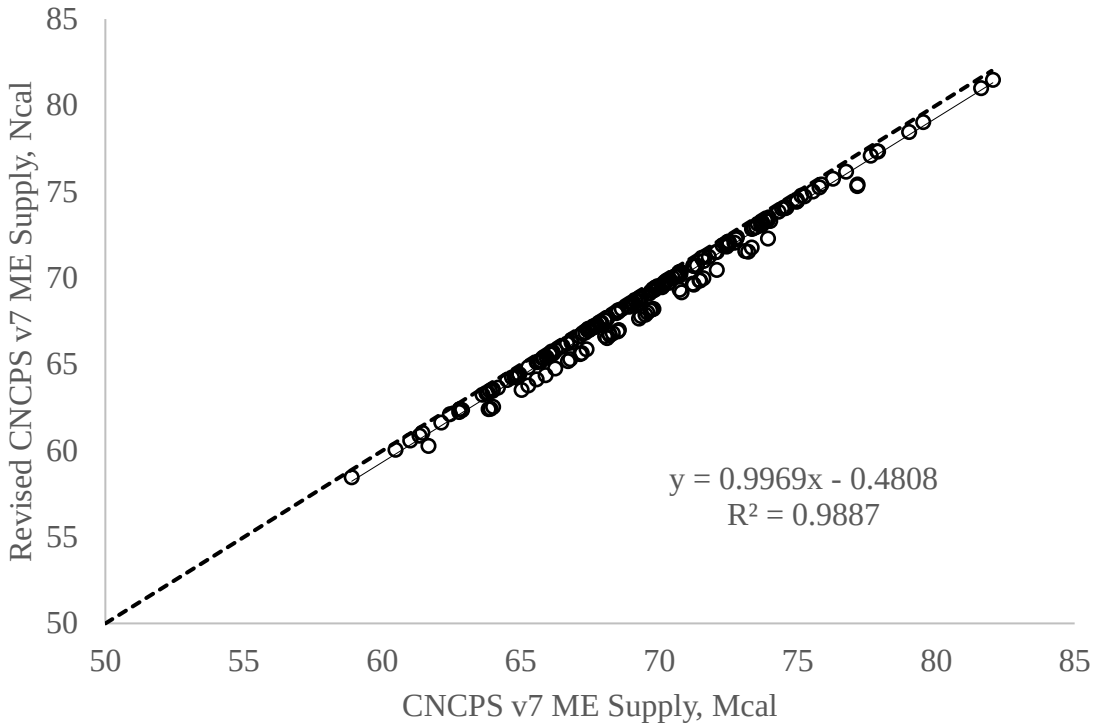

Figure 4.7. Comparison of predicted metabolizable energy (ME) using the CNCPS v7 described by Higgs et al. (2014) and a revised CNCPS v7 which use a reparametrized protozoal sub-model described by Dineen (2020). The dashed line represents unity (x = y).

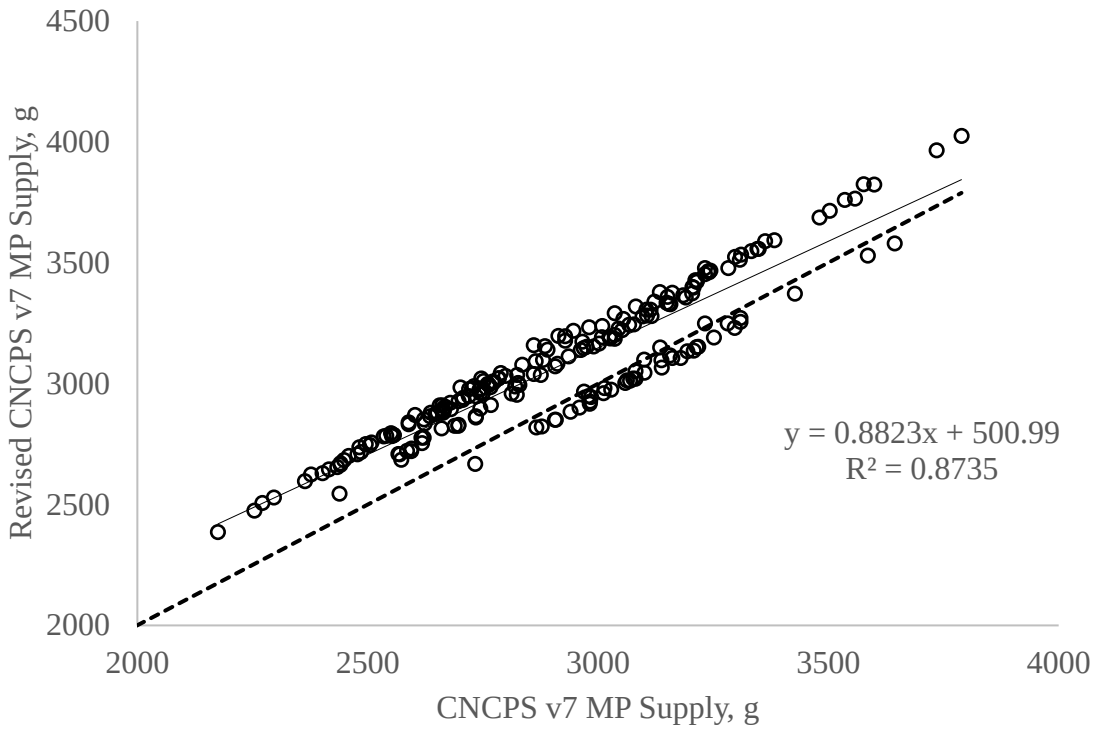

Figure 4.8. Comparison of predicted metabolizable protein (MP) using the CNCPS v7 described by Higgs et al. (2014) and a revised CNCPS v7 which use a reparametrized protozoal sub-model described by Dineen (2020). The dashed line represents unity (x = y).

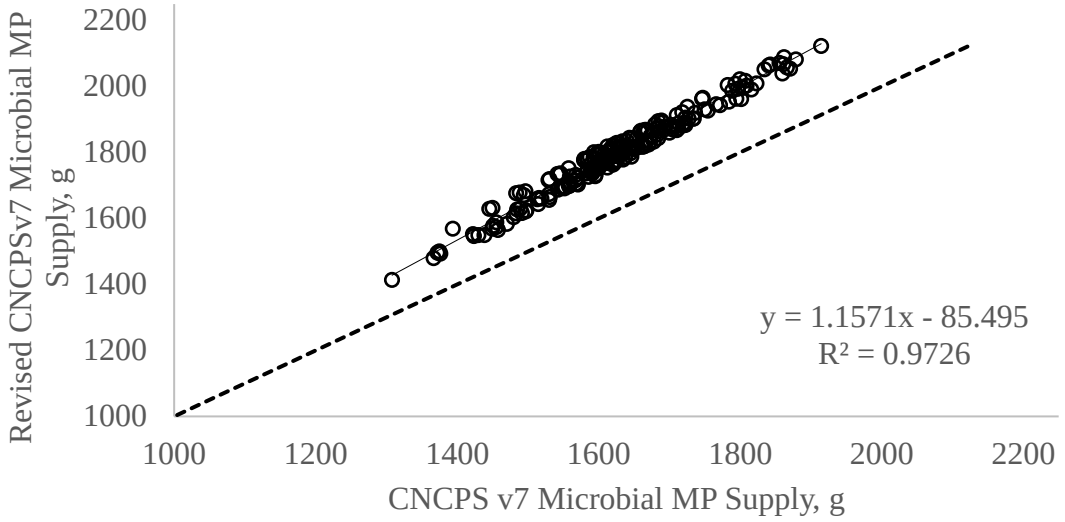

Figure 4.9. Comparison of predicted MP from rumen microbial mass using the CNCPS v7 described by Higgs et al. (2014) and a revised CNCPS v7 which use a reparametrized protozoal sub-model described by Dineen (2020). The dashed line represents unity (x = y).

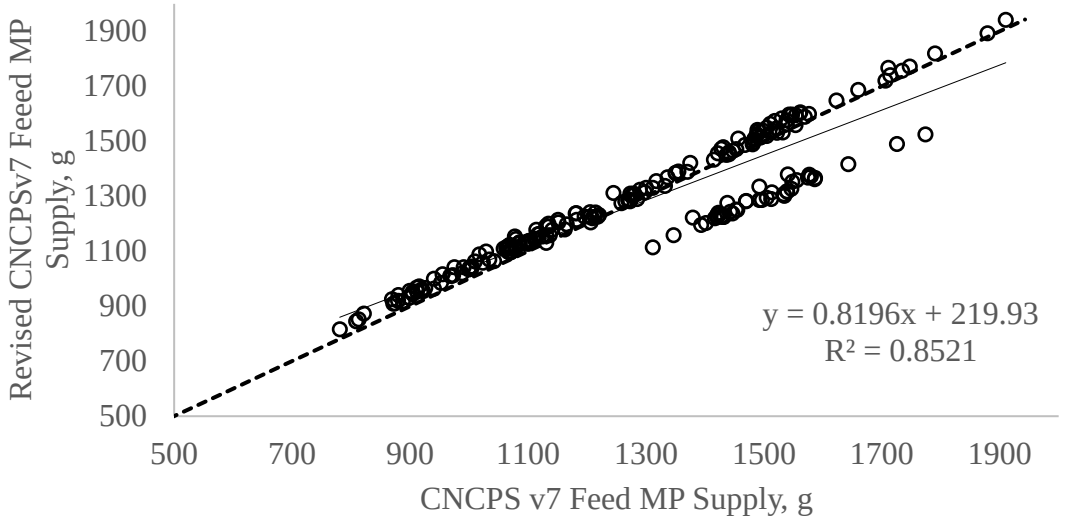

Figure 4.10. Comparison of predicted MP from undegraded feed using the CNCPS v7 described by Higgs et al. (2014) and a revised CNCPS v7 which use a reparametrized protozoal sub-model described by Dineen (2020). The dashed line represents unity (x = y).

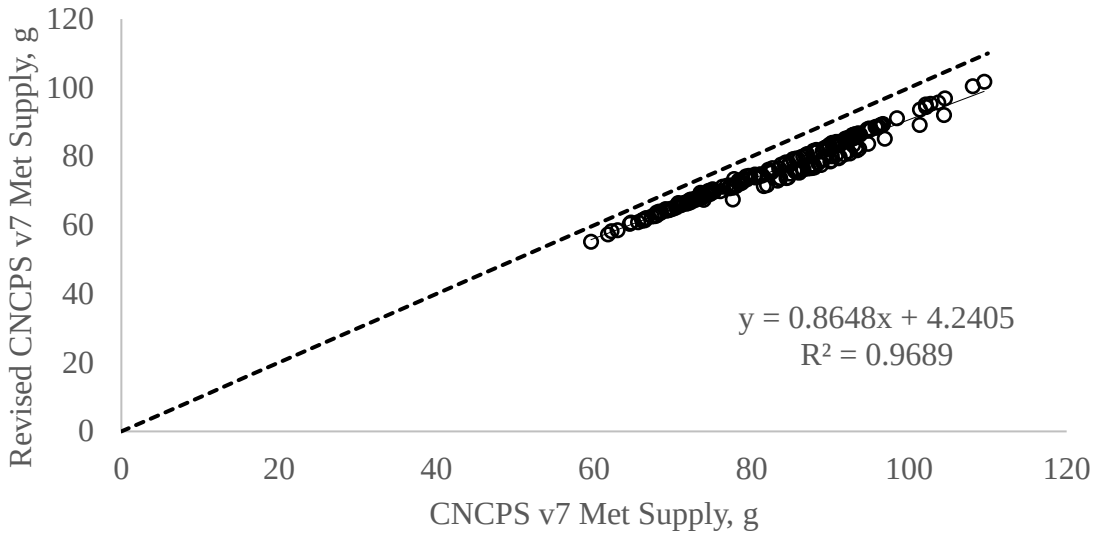

Figure 4.11. Comparison of predicted MP from rumen microbial mass using the CNCPS v7 described by Higgs et al. (2014) and a revised CNCPS v7 which use a reparametrized protozoal sub-model described by Dineen (2020). The dashed line represents unity (x = y).

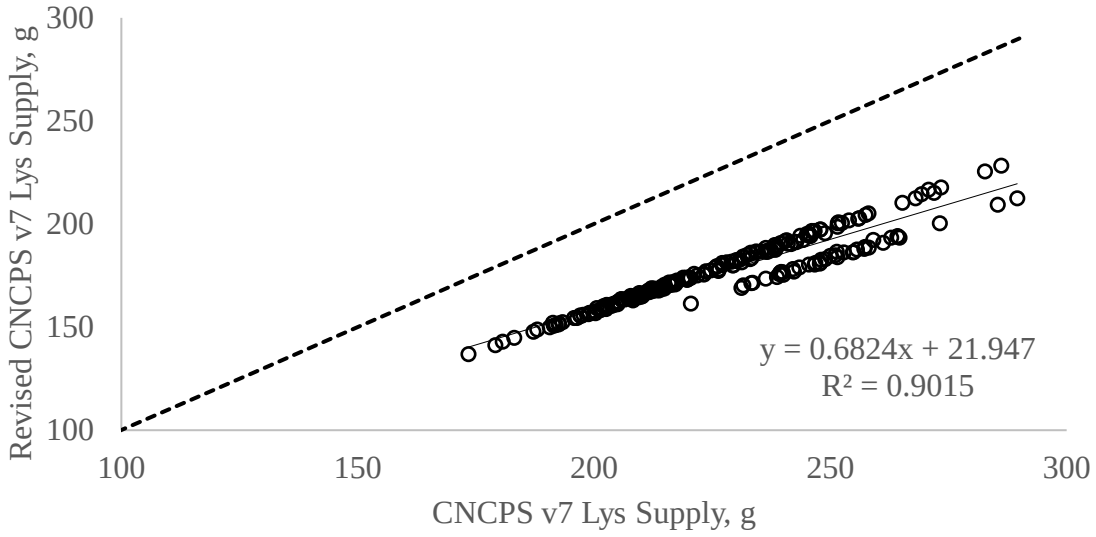

Figure 4.12. Comparison of predicted MP from rumen microbial mass using the CNCPS v7 described by Higgs et al. (2014) and a revised CNCPS v7 which use a reparametrized protozoal sub-model described by Dineen (2020). The dashed line represents unity (x = y).

microbial and feed sources. The inclusion of AA correction factors, similar to what has been described by Lapierre et al. (2019) into RP v.7 predictions would improve the supply of all EAA and help to reconcile differences in EAA supply predictions between CNCPS v.7 and RP v.7.

4.5 Conclusion

Predictions for the optimum efficiency of use and supply relative to ME of each EAA were performed using an experimental dataset from diets fed in Chapter 2 and 3. The optimum efficiency of use for most EAA and MP are similar to results observed by Higgs and Van Amburgh (2016). Differences in the optimum EAA composition do exist between the two evaluations, particularly with regards to the supply of Leu and Ile relative to total EAA supply. The incorporation of other diets formulated with CNCPS v.7 could provide revisions to the these observed supplies. Loglogistic relationships were observed between efficiency of use and the gram supply of each EAA relative ME. The optimum supply of Met and Lys using the experimental dataset are 1.11 g/Mcal ME and 3.06 g/Mcal ME, respectively. These optimum supplies, along with the optimum supply of His, Leu, Phe, and Val show similar results to previously obtained optimum supplies. Further work is needed to confirm the optimum supply of Arg, Ile, Thr, and Trp given discrepancies between the two dataset evaluations. Model predictions using the revised protozoal sub-model improved MP supply to move the original CNCPS v7 balance out of a deficit relative to MP requirements. Little impact in predicted ME supply was observed between CNCPS v.7 and RP v.7 indicating a robustness in the prediction of available energy. Continued work is required to validate the EAA supply predicted by RP v.7, including the use of updated analytical techniques and correction factors to appropriately describe AA composition.

4.6 Acknowledgements

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CHAPTER 5: SUMMARY

5.1 Summary and Future Work

In the face of changing environmental policies and volatile milk prices, farmers continue to improve their productive output and environmental stewardship to meet the needs of their consumers. Refinement and adoption of nutritional strategies, including amino acid (AA) balancing, continue to be a vital tool in meeting essential nutrient requirements, reducing oversupply of unnecessary nutrients, and improving the productive efficiency of cattle. Proper use of AA balancing requires robust predictions of all AA requirements for varying physiological conditions all while accurately describing total AA flows provided by various sources of supply. This dissertation aims to describe the capabilities of AA balancing in high producing dairy cattle when both AA requirements and supply are accurately described. Further, this work was meant to assess previously described relationships between AA and metabolizable energy (ME) supplies and provide either confirmation or suggestions for revisions which could lead to improvements in productivity. This assessment was preformed through two different feeding studies and modeling exercise to rederive the optimum supply of EAA to ME as previously performed.

The first of two feeding studies, described in Chapter 2, evaluated animal performance when the supplied grams of each EAA relative to ME are varied around the optimized value for those EAA. Cattle were fed one of three diets: Neutral (NEU) which was formulated to contain the optimized supply of each EAA relative to ME, or two diets formulated one standard deviation below, Negative (NEG), and one standard deviation above, Positive (POS), the optimized supply according to the range in the dataset used to create the optimized supply fed in the NEU diet. The hypothesis for this study stated that cattle fed NEU diet would maintain high level of milk

and component production while improving their nitrogen use efficiency (NUE) because this diet was formulated to feed any EAA in excess relative to ME supply. The results from this study indicated that cattle consuming the NEU and POS diets produced similar volumes of milk and milk true protein despite the POS cattle consuming nearly 70 grams more nitrogen (N). Milk N output to feed N consumed was highest in NEU cattle (0.343) over other diets fed. The ratio of productive N to CNCPS predicted urinary N was also highest for NEU cattle, further implying that these cattle were more efficient with the N they consumed. Conclusions from this study support the given hypothesis and suggest that under these experimental conditions, the EAA supply relative to ME recommended by Higgs and Van Amburgh (2016) should be considered the optimum supply.

In the second feeding study, described in Chapter 3, the efficiency of use of EAA was tested under varying feeding conditions. Previous research suggested that improved levels of glucogenic nutrients improve the efficiency of use of AA and that shifts in the optimum supply of EAA relative to ME might be realized in the presence of more glucogenic nutrients. This experiment fed two levels of dietary starch, high starch (HS) and low starch (LS), and two levels of EAA supply, 100% or 105% of optimal supply of each EAA relative to ME according to Higgs and Van Amburgh (2016). Energy-corrected milk (ECM) yield and NUE were the variables of interest for this study, and it was hypothesized that cattle fed the HS diets would have a slightly improved ECM output and have a higher NUE than cattle fed the LS diets. It was also hypothesized that the increase in EAA supply, particularly when fed with the HS level, would further improve ECM output, and could have the potential to improve NUE despite the added EAA supply. All diets fed in this experiment were formulated to be iso-caloric and to meet the caloric density observed in the HS diets, the LS diets received a fatty acid supplement

which boosted the lipogenic nutrients within those diets. Inclusion of these lipogenic nutrients in the LS diets created several unforeseen responses in productive outputs and NUE. Milk yield was not different for all diets except for HS 105 which also showed improvements in ECM yield. Cattle fed the HS diets showed improved milk true protein yield; however, milk fat yield was greatest in cattle fed the LS diets. Considerably higher DMI for HS diets caused a reduction in feed efficiency variables over LS diets. Further, no differences in NUE were observed among diets and is partially attributed to these differences in DMI. It was determined that the differences in DMI were not a result of differences in glucogenic and lipogenic nutrient supply but rather because the uNDF rumen fill limit was achieved sooner in the LS and more intake of the HS was required to achieve this aNDFom fill. Updates within the CNCPS v.7 allow for the estimation of rumen fill and flux of aNDFom and uNDF by making use of the two digestible pool model to more accurately describe the rate and extent of digestion for aNDFom fed (Raffrenato et al., 2019) Results from this study did partially confirm the stated hypothesis by demonstrating higher milk protein output in cattle fed the HS diet which provided more glucogenic substrates, although NUE was not higher in these diets. Predictions of EAA supply relative to ME using the CNCPS v.7 were considerably higher than intended targets for HS diets and is also likely a result of higher DMI. For this reason, comparisons of the efficiency of use for all EAA supply relative to ME between HS and LS is not warranted; however, a wider range of predicted EAA supply relative to ME would be useful in a model evaluation setting which aims to reevaluate the optimum efficiency of use and supply of EAA.

Because of the large differences in DMI as well as higher than anticipated variability in starch and CP supply, demonstrated in Figure 3.2, this study should be repeated using a slightly revised set of dietary parameters. Ethanol soluble carbohydrates (ESC) was formulated to be consistent

for all diets and averaged 4.4% DM throughout the experiment. Changes in dietary sugar supply should be considered and work from De Ondarza et al. (2017) suggests that the supply of dietary sugar should range between 5-7% for improved fat corrected milk (FCM) response in high producing dairy cattle. Target dietary starch levels could be reduced to accommodate for the increased supply of sugar and these shifts in carbohydrate pool sizes would ensure rapidly degradable carbohydrates are available for improved rumen fermentation and microbial protein supply, while maintaining similar production of propionate and insulin signaling, leading to increases in anabolic function and improvements in milk protein output (Relling and Reynolds, 2008, Lemosquet et al., 2009, Cantalapiedra-Hijar et al., 2014). Dietary aNDFom should be formulated so that the similar fill effects would be observed for all diets. This requires a proper accounting of both fast and slow pool intake of aNDFom according to the two digestible pool method described by Raffrenato et al. (2019). Further, diets should be formulated so that limitations in intake will be dictated by aNDFom or uNDFom fill limits using body weight guidelines described by Cotanch et al. (2014). Lastly, the effect of varying dietary fatty acid supplies on nutrient partitioning and lactation performance has been described by many (Rico et al., 2014, Boerman et al., 2015, Nichols et al., 2018, de Souza et al., 2019). The nonnutritive effects of varying these fatty acids are not appropriately described within the CNCPS v.7 and it's recommended that these relationships be appropriately described and implemented for dietary formulation targets to properly assess the effect lipogenic nutrients have on the efficiency of use for all EAA and, by extension, calculate the optimized supply of EAA relative to ME.

Diets fed during these two experiments exhibited a wide range of EAA supply according to the objectives of both studies. This provided the basis for an experimental dataset which was used to reevaluate the optimum efficiency of use for EAA as well make predictions for the

optimum supply of EAA relative to ME. All pen by week observations for both studies (n= 210) were used to build the dataset and make the necessary evaluations. The CNCPS v7 was used to make predictions on ME and EAA supply as well as EAA requirements. A logistic model was used to fit the relationship between EAA supply and model predicted requirements and calculate the optimized efficiency of use for the EAA being described according to methods described by Doepel et al. (2004). The optimum efficiency of use for most EAA, particularly for Met and Lys, show reasonable agreement with results presented by Higgs and Van Amburgh (2016). The efficiency of use for other EAA, including Arg and Thr, are lower than previously reported due to an overestimation in their supply. After the optimal efficiency of use was determined for all EAA, a loglogistic model was fit to describe the relationship between the efficiency of use for each EAA and their corresponding supply relative to ME. Optimal supply relative to ME was solved for by using the optimized efficiency of use. Findings using this model analysis for most EAA are also in agreement with Higgs and Van Amburgh (2016), where both the optimized supply of Met and Lys relative to ME differ from previous findings by only 0.03g/Mcal ME. Differences do exist for Ile, Phe, and Trp optimized supplies when compared to previous findings; however, model fit for these EAA is weaker than other EAA analyzed.

Lastly the experimental dataset described previously was used to evaluate a reparametrized protozoal sub-model described by Dineen (2020). Comparisons between model outputs of ME, MP, and EAA supply provided by the original CNCPS v7 and the reparametrized version (RP v.7) were made. Findings indicate that the model changes done to RP v.7 have little impact of ME supply but do increase MP supply more than requirements. This change in supply is largely driven by an increase in protozoal MP supply, as predictions for bacterial and rumen undegraded feed MP are similar among model versions. Predicted supply of EAA is considerably less in the

RP v.7 predictions than CNCPS v.7 predictions. Part of this difference can be explained by the use of an updated microbial AA composition described by Dineen (2020) and implemented in RP v.7. Additionally, the use of improved AA analysis techniques (Fleming et al., 2019) coupled with correction factors which accurately describe the maximum recovery of all AA within a given feed or microbial matrix (Lapierre et al., 2019) can yield a more accurate supply prediction for RP v.7 which might account for the remaining difference observed from the predicted EAA supply from CNCPS v.7.

Collectively, this work provides a robust understanding toward the use of AA balancing to improve milk volume and component yield while allowing for improvement in NUE by formulating diets to meet the optimal supply of EAA relative to ME. Future work should be considered as not all EAA were confirmed to have the same or similar optimized supply when comparing predictions from the experimental dataset to what was demonstrated by Higgs and Van Amburgh (2016). Implementation of AA correction factors described by the literature (Lapierre et al., 2019) and internal lab data (Ortega, Unpublished) can be immediately implemented within the CNCPS v.7 to make refinements in AA supply predictions. Previous efforts within this lab have begun with the construction of an AA feed library for commonly fed feeds; however, the number of samples which populates this library is limited. Opportunities exist within this lab to continue analyzing feedstuffs using a liquid-chromatography mass spectrometry approach which is known to be more sensitive in the quantification of analytes over a traditional High Pressure Liquid Chromatography method.

Applied research into using AA balancing in ruminants should continue to be a focus to increase the precision with which we feed cattle. Comparisons between the optimized supply of EAA within CNCPS v.7 and CNCPS v. 6.55, the program used to feed ~65% of dairy cows in

North America, have already been performed (Table 5.1); however, the opportunity to improve these predictions by way of making more comparisons between these two versions of the model might prove useful while CNCPS v.6.55 is still commonplace. Recommendations from these comparisons suggest the Met supply should supplied at 1.19 g/Mcal ME (Table 5.1) and that the optimum Lys supply can be determined by multiplying Met supply by 2.7 (data not shown). All other EAA supply relative to ME should follow recommendations provide in Table 5.1.

Lastly, studies which evaluate the application of balancing all, several, or individual EAA could prove useful to nutritionists who may be looking to optimize nutrient supply in the face of increased feed costs, limited availability of feedstuffs which may cause imbalances in EAA supply, and a volatile source of revenue. Further, adoption of AA balancing techniques is often not considered because it implies a higher feed cost per animal fed. Additional information on the financial rewards of AA balancing could prove useful for producers who are indecisive about this technique and may require financial justification, in addition to the previously mention biological and environmental benefits, to pursue EAA balancing.

Table 5.1. CNCPS v.7 and CNCPS v. 6.55 comparisons for the optimum supply of essential amino acids per unit of metabolizable energy.

Grams AA/Mcal ME	CNCPS v.7	CNCPS v6.55
Arginine	2.04	2.59
Histidine	0.91	1.06
Isoleucine	2.16	2.08
Leucine	3.42	3.11
Lysine	3.03	2.76
Methionine	1.14	1.19
Phenylalanine	2.15	2.11
Threonine	2.14	1.94
Tryptophan	0.59	0.70
Valine	2.48	2.28

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