UPDATING MATHEMATICAL MODELS USED IN THE DIET FORMULATION PROCESS

By

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ABSTRACT

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The hypothesis of this dissertation was that deriving statistical models using advanced statistical techniques and a database collected in recent years would yield predictions that better fit the current dairy production system than the models of the NRC (2001). In *chapter three,* we determined the effects of dry matter intake (**DMI**), body weight (**BW**), and diet characteristics on total tract digestibilities of dry matter, neutral detergent fiber, and starch (**DMD**, **NDFD**, and **StarchD**, respectively) in high-producing dairy cows using a database composed of 1,942 observations from 662 cows in 54 studies from Michigan, Ohio, and Georgia. Our results suggest that DMD decreases as intake increases but at a lower rate than the model of NRC (2001). Both NDFD and StarchD were best estimated with diet characteristics as well as intake; starch content has a much greater impact on NDFD than does intake. In *chapter four*, we modeled DMI in Holstein dairy cows based on milk energy (**MilkE**), BW, change in body weight (**ΔBW**), body condition score (**BCS**), height (**Ht**), days in milk (**DIM**), and parity (primiparous and multiparous) using a database containing 47,253 weekly observations on 3,607 cows enrolled in 57 studies from 8 states across the US. The proposed model was validated against the NRC (2001) prediction equation for DMI using an independent dataset. The proposed model outperformed the NRC (2001) model to predict DMI. Whereas both models were similar at predicting DMI during early-lactation (1 – 75 DIM), the proposed model outperformed the NRC (2001) during mid- and late-lactation (76 – 368 DIM). In *chapter five,* we performed a meta-regression using data from 5 experiments conducted at Michigan State University to

determine the effect of dietary fatty acid (**FA**) composition on digestibilities of DM, NDF, FA, 16-carbon FA, 18-carbon FA, and digestible energy (DMD, NDFD, **FAD**, **16-CD**, **18-CD**, and **EnergyD**, respectively) and on DE intake (**DEI**). The final database was composed of 423 individual observations collected on 183 lactations from 124 Holstein mid-lactation cows receiving diets that varied on FA composition. Palmitic and stearic were the FAs with greatest impacts on digestibilities and DEI; palmitic increased DEI when included in the diet up to 1.2% of DM, and stearic linearly decreased DEI. In *chapter six,* we conducted a meta-regression analysis on 129 treatment means from 26 peer-reviewed publications to predict body composition (**EBFat**, **EBProtein**, **EBAsh**, and **EBWater**) as a percentage of empty body weight (**EBW**) in Holsteins. The statistical models contained the random effect of study and fixed effects of method (direct, carcass, and dilution), stage (heifer and cow), and one of the three possible ways to express EBW (4^{th} order polynomial of EBW - **polEBW**, natural logarithm of EBW - **lnEBW**, or EBW to the power of 0.75 – **EBW^0.75**), average daily gain (**ADG**) for heifers, and BCS for cows. Additionally, the models were weighted by the inverse of the standard deviations of the studies. Compared to NRC 2001, the proposed model suggests that fat content of the gain, and thus the energy content of gain, is greater for young heifers and less for older heifers than the values predicted by NRC 2001.

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KEY TO ABBREVIATIONS

16-C	16-carbon fatty acids
16-CD	16-carbon fatty acids digestibility
18-C	18-carbon fatty acids
18-CD	18-carbon fatty acids digestibility
ADF	Acid detergent fiber
ADG	Average daily gain
ALFALFA%DM	Alfalfa as a percentage of dry matter
BCS	Body condition score
BW	Body weight
BWBCS3	Body weight adjusted to a body condition of 3
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
Ca-PFA	Calcium salts of palm FA
CCC	Concordance correlation coefficient
CORNSIL	Corn silage
CORNSIL%DM	Corn silage as a percentage of dry matter
CP	Crude protein
DE	Digestible energy

DEI	Digestible energy intake
DEIday	Digestible energy intake expressed as Mcal per day
DEIkg	Digestible energy intake expressed as Mcal per kilogram of intake
DIM	Days in milk
DMD	Digestibility of dry matter intake
DMI	Dry matter intake
DMI%BW	Dry matter intake as a percentage of body weight
DMI%BW^0.75	Dry matter intake as a percentage of metabolic body weight
EBAsh	Empty body ash
EBFat	Empty body fat
EBProtein	Empty body protein
EBW	Empty body weight
EBW^0.75	Empty body weight to the power of 0.75
EBWater	Empty body water
ED	Energy digestibility
EE	Ether extract
EQEBG	Size-scaled empty body weight gain
EQEBW	Size-scaled empty body weight
FA	Fatty acids
FAD	Fatty acids digestibility
FCM	Fat-corrected milk
FFM	Fat-free mass
fNDF	Forage NDF

GE	Gross energy
GRASS%DM	Grass as a percentage of dry matter
HFERM	Highly fermentable corn source
HFERM%DM	Highly fermentable corn source as a percentage of dry matter
HOT	Hepatic oxidation theory
HPO	Hydrogenated triacylglycerides from palm oil
Ht	Height
iNDF	Indigestible neutral detergent fiber
LIN	Linear
lnEBW	Natural logarithm of empty body weight
ltADG	Lifetime average daily gain
BW^0.75	Metabolic body weight
BW^0.75BCS3	Metabolic body weight adjusted to a body condition score of 3
MFERM	Moderately fermentable corn source
MFERM%DM	Moderately fermentable corn source as a percentage of dry matter
MI	Michigan
MidAge	Age at the mid of the experiment
MilkE	Milk energy
MM	Multiples of maintenance
MSEP	Mean square error of prediction
MSU	Michigan State University
MY	Milk yield
NDF	Neutral detergent fiber

NDFD	Neutral detergent fiber digestibility
NE_g	Net energy for growth
NE_L	Net energy for lactation
NFC	Nonfiber carbohydrate
NFFS	Nonforage fiber sources
NFFS%DM	Nonforage fiber sources as a percentage of dry matter
nfNDF	Nonforage NDF
NLIN	Nonlinear
NP_g	Net protein for growth
NRC	National Research Council
OH	Ohio
OM	Organic matter
OSU	Ohio State University
OthersFA	Others fatty acids
PC	Predictive correlation coefficients
pdNDF	Potential digestible neutral detergent fiber
PMM	Peak milk month
polEBW	4^{th} order polynomial of EBW
RE	Retained energy
RMSE	Root mean square error
RMSEP	Root mean square error of prediction
SAS	Statistical analysis system
SE	Standard error

SED	Standard error of the difference
SEM	Standard error of the mean
StarchD	Starch digestibility
SWG	Shrunk weight gain
TDN	Total digestible nutrients
TDN_{1x}	Total digestible nutrients at maintenance level
UGA	University of Georgia
US	United States
VIF	Variance inflation factors
WOL	Week of lactation
ΔBW	Change in body weight

CHAPTER 1

INTRODUCTION

Dairy farms' profit margins have steadily decreased over the last 20 years, and nowadays many farmers are struggling to financially thrive in a market with low milk prices and high production costs. To overcome this scenario, farms have undertaken two strategies: 1) increasing herd size in order to dilute fixed costs and enjoy the economic benefits of large-scale production, and 2) increasing milk production and the level of efficiency in the conversion of feed into milk.

Diet plays a critical role in the process of improving farm productivity while also controlling costs. Diet is one of the most significant costs involved in running a dairy farm; and consequently, farmers tend to be reluctant to increase spending on rations. However, diet has a direct impact on milk yield, milk composition, animal health, and reproduction, and wise investments in nutrition should improve a farm's overall profits. Thus, diet has become a major cost driver, with a direct impact on a farm's primary source of income. Given this information, a possible way to help dairy farms overcome the current challenges of low milk prices and high production costs is to formulate more precise and tailored diets to maximize nutrition and, ultimately, improve dairy farm profits.

Currently, the most commonly used and globally recognized system for formulating and evaluating dairy cattle diets is the National Research Council Nutrient Requirements of Dairy Cattle (**NRC**). Although other software have been developed to formulate dairy diets (i.e., CNCPS, Spartan Dairy, DAIR4, PCDAIRY, CPM, AMTS), the NRC still serves as the primary reference book and, more often than not, the NRC equations are applied in other software.

The NRC's publications pass through a rigorous evaluation process. Since its first publication in 1945, the NRC has undergone eight revisions. The current version, the NRC (2001), incorporates many improvements from the previous edition, including significant changes and updates in the statistical models applied to determine animals' nutrient requirements, diet digestibilities, and nutritional recommendations, as well as changes in the software itself. However, the models and prediction equations incorporated in the NRC (2001) were derived from data collected during the 1980s and 1990s. A lot has changed since this data was collected. Cows are much bigger, producing much more milk, and under a different management system (i.e., grazing vs. confined) than in the past. Also, research has helped formulate a much deeper understanding of the physiological factors underlying animal nutrition, and more data is available to derive new models. Many of today's researchers are focusing on evaluating and updating the outdated equations proposed by NRC (2001).

The hypotheses of this dissertation are that equations derived from a larger and newer database would increase the accuracy and precision of the prediction and better represent the current dairy production systems. The objectives of this dissertation share a similar focus: 1) to develop new prediction equations for dry matter, NDF, and starch digestibility at production level (chapter 3); 2) to model dry matter intake in Holstein dairy cows based on milk energy, live body weight, change in live body weight, body condition score, height, days in milk, and parity (chapter 4); 3) to evaluate the effect of palmitic, stearic, oleic, linoleic, and linolenic on dry matter, NDF, fatty acid, energy digestibility, and digestible energy intake (chapter 5), and 4) to develop prediction equations to estimate body composition in heifers and cows using data collected in Holsteins and apply these equations to determine the net energy and net protein

requirements for growth, and the net energy per kilogram of empty body weight in mature animals (chapter 6).

CHAPTER 2

LITERATURE REVIEW

DAIRY FARM PROFITABILITY

In 1997, the average milk price received by dairy farmers was $13.30/cwt, without adjusting for inflation (NASS-USDA, 2018); once the 1997 price was adjusted for inflation to 2017 values, the milk price was $20.33/cwt (BLS, 2018). In comparison, the average milk price in 2017 was $17.60/cwt (NASS -USDA, 2018), indicating a decrease of 13.4% in the inflation corrected milk price over the 20-year period from 1997 to 2017 (Figure 2.1).

This decrease in milk price can be explained by multiple factors, such as changes in global dairy markets (Hemme, 2017; Stephenson, 2017) and the increase in milk supply to the U.S. market at a higher rate than the increase in milk demand. In fact, the U.S.' average commercial milk stocks increased from 2,671 million kilograms in 1990 to 6,216 million kilograms in 2010 (NASS-USDA, 2018). Furthermore, although they represent a smaller niche market, there has also been an increase in milk alternative products, such as soy, almond, and coconut products.

To make this scenario even more difficult, the price of dairy feed has also increased. In 1997, the price of dairy feed was $3.80/cwt; once the 1997 price was adjusted for inflation to 2017 values, the dairy feed price was $6.14/cwt (BLS, 2018). In contrast, the average price of dairy feed in 2017 was $7.26/cwt. This indicates an increase of 18.7% in the price of dairy feed from 1997 to 2017 (ERS-USDA, 2018) (Figure 2.1).

Declining milk prices and increasing dairy feed prices have resulted in many modern dairy farmers hardly being able to recoup production costs. The average production cost in 2017 was $22.27/cwt (ERS-USDA, 2018), and the average milk price for the same year was $17.60 (NASS -USDA, 2017). Unfortunately, this phenomenon is not unique to 2017. Dairy farmers have been struggling with low milk prices and high dairy feed prices for many consecutive years. The average production cost from 2010 to 2017 was $23.62/cwt (ERS-USDA, 2018), and the average milk price for the same period was $18.75/cwt (NASS -USDA, 2018), indicating that the milk price covered on average only 79.4% of production costs, resulting in farmers losing money.

The cost structure of a dairy farm divides production costs into two categories: allocated overhead and operating costs. On the average US dairy farm, allocated overhead costs represented 39.4% of production costs in 2017. The most significant allocated overhead costs included the capital recovery of machinery and equipment, the opportunity cost of unpaid labor, and hired labor, representing 17.0, 11.1, and 7.37% of the production cost, respectively (ERS-USDA, 2018). On the other hand, operating costs represented 60.6% of the production cost, and the most significant costs associated with this category were feed, followed by veterinary and medicine, and custom services, representing 47.1, 3.74, and 2.60% of the production cost, respectively (ERS-USDA, 2018). The 2017 dairy farm cost structure is presented in Table 2.1.

As mentioned previously, dairy farmers have been forced to adopt specific strategies to overcome the financial challenges of high production costs and low milk prices. In order to optimize the use of facilities, machinery, labor, and capital invested into the business, dairy farmers have expanded their business via either natural growth (by investing more money and resources into the business) or via mergers and acquisitions. This expansion of dairy farms is

responsible for the decreasing number of dairy farms and increasing size of dairy farms in the last ten years (2007 to 2017). During this time period, the U.S. experienced a decrease in the number of dairy operations from 53,132 to 40,219 and an increase in the herd size per farm from 173 to 234 milking cows (NASS-USDA, 2018).

Dairy farmers have also felt significant pressure to increase both production and efficiency. Despite an observed increase in milk production per cow from 9,164 to 10,406 kg/year (NASS-USDA, 2018) during the period of 2007-2017, there is still room for improvement. In order to further increase production and efficiency, advances in herd genetics, management, health, comfort, and nutrition are crucial.

Advances in herd genetics have had a fundamental impact in allowing lasting and persistent improvements (Veerkamp, 1998; VandeHaar et al., 2016; Hardie et al., 2017; Miglior et al., 2017; Weigel et al., 2017); however, when compared to dietary changes, these effects have a more long-term effect and are not immediately apparent. In contrast, enhancements in management (Bewley et al., 2017; Roche et al., 2009), health (Risco, 2017; Overton et al., 2017), welfare (Fraser and Koralesky, 2017; von Keyserlingk and Weary, 2017), reproduction (Moore and Hasler, 2017; Stevenson and Britt, 2017), and nutrition (VandeHaar and St-Pierre, 2006; Grant and Dann, 2017; Schingoethe, 2017) have a much more immediate effect on farm profits.

Given that feed is the most significant cost involved in a dairy farm (Table 2.1) and has a direct impact on milk production and animal health (McGuffey, 2017), improvements in animal nutrition have been the primary concern of the majority of farmers, and much research has been conducted in this field. Overall, research on animal nutrition has focused on understanding the biological mechanisms involved in the control of feed intake (Ingvartsen and Andersen, 2000;

Allen, 2000; Allen et al. 2009), the conversion of feed into animal products (VandeHaar, 1998; Hall and Mertens, 2017; VandeHaar and Tempelman, 2017; Karlsson et al., 2018), nutrient partitioning (Allen and Piantoni, 2014; Baumgard et al., 2017), feed supplements that improve milk yield (Casper, 2017; Goff, 2017; Ferreira and Weiss, 2017; Schwab and Broderick, 2017; Palmquist and Jenkins, 2017), and a specific diet for each stage of lactation and level of production (Contreras-Govea et al., 2015; Kalantari et al., 2016; VandeHaar and Tempelman, 2017).

The innovations and improvements developed by animal nutrition research are translated to dairy farms through new commercial products, nutritional recommendations, and, ultimately, changes in diet formulation. In this sense, diet formulation is the link between advances in animal nutrition research and the diets used in dairy farms. In order for farmers to reap the benefits of advances in animal nutrition research and to improve their financial situation, it is fundamental that these advances are translated to dairy farmers via diet formulation. Thus, the goal of this dissertation is to help dairy farmers improve their finances by improving the statistical models that play a critical role in the diet formulation process.

DIETARY FORMULATION

The initial publication about the use of computer-based software to formulate dairy diets dates back to at least the 1960s. Bath (1966) used linear programming to test rapidly all combinations of feed ingredients, which would meet specific nutrient requirements and select the formula which met them at the lowest cost and therefore formulate the least-cost concentrate mix.

Applying the computer-based program proposed by Bath (1966), Bath et al. (1968) evaluated four concentrated mixes that varied in price from $4.49, $2.99, and $1.05 per ton less than the control diets and found that diets formulated using the least-cost method proposed by Bath (1966) could improve dairy farms' profits by reducing diet cost while maintaining production.

Given the success and the vast potential of computer-based software on diet formulation, many other researchers started to work on the improvement and development of new programs. Chandler and Walker (1972) developed statistical models that calculated animal requirements based on the Dairy NRC (1971). These statistical models included dry matter intake, and the requirements of crude protein, net energy, calcium, and phosphorus. The program proposed by Chandler and Walker (1972) gave nutritionists the ability to determine changes in nutrient requirements immediately as a result of variable productive conditions.

As an alternative to the least-cost system, Bath (1975) proposed a maximum-profit ration formulation program. The maximum-profit system maximized profit by adjusting ration composition and milk production to the feed and milk prices. Brown and Chandler (1978) incorporated equations to predict milk yield (**MY**) and dry matter intake (**DMI**) into a computer-based maximum-profit ration formulation program. By combining the maximum-profit ration formulation program with prediction equations of MY and DMI, the program proposed by Brown and Chandler (1978) had the advantage of automatically adjusting intake and milk production rates to correspond to changing economic situations.

Since these early publications, much research has been done to evaluate and improve diet formulation software (Jones et al., 1978; Black and Hlubik, 1978; Stallings and McGilliard,

1984; Colenbrander et al., 1986; Galligan et al., 1986; Weaver et al., 1988). Nowadays, the software most often used to evaluate or formulate diets are the NRC Dairy 2001 and CNCPS, with many derivations such as the Spartan Dairy, PCDAIRY, CPM, and AMTS.

The diet formulation software combines many statistical models that predict the animals' nutritional requirements, the DMI, and the nutrient digestibility of the diet required to meet the nutrient needs of the animal. Regardless of the software used by the nutritionist to formulate and/or evaluate the diet, the software capability is limited to the accuracy and precision of its statistical models. Hence, constant review and modernization of these models are required to keep the software updated with current knowledge in the field of dairy nutrition and adequately support current production systems. Additionally, information from recent research can be used to increase the accuracy of the equations that underlie nutrient requirements.

To illustrate this point, since the first edition of the NRC Dairy in 1945, there were eight revisions (1950, 1956, 1958, 1966, 1971, 1978, 1988, and 2001) to reflect the most recent knowledge in the field of dairy cattle nutrition. In its first edition, the NRC Dairy had 21 pages and focused on the discussion of nutrient deficiency symptoms. In contrast, in its current edition, the NRC 2001 had 401 pages and is a reference book on all aspects of nutrition and feeding. It is clear that as time progresses, the amount of information also grows.

Although there have been constant efforts to keep the NRC updated, the NRC (2001) equations were developed based on a 1990s database. Since the 1990s, a lot has changed, technology advanced and genetic selection allowed today's animals to produce higher volumes of milk. In recognition of the need for a revision, the NRC 2001 is undergoing an update, and most likely will issue a newly revised edition during the following year. In fact, many recent

publications have been focused on the evaluation of the NRC (2001) models and suggested new techniques and updates.

As an example, White et al. (2017a) evaluated the NRC (2001) and found substantial mean and slope bias on the NRC (2001) predictions of nutrient digestibility, and proposed new models for digestibility of fiber, fat, protein, and non-fiber carbohydrate. In a similar work, White et al. (2017b) evaluated the NRC (2001) rumen degradable and undegradable protein system and reported a poor statistical fit of the model. As an alternative, White et al. (2017b) derived a new system based on the prediction of postruminal appearance rates of A, B, and C protein fractions for different feed types. This new proposed system had a superior statistical fit than the system based on the passage and digestion rate used in the NRC (2001).

Moraes et al. (2018) evaluated the metabolizable protein system proposed on the NRC (2001) and found that metabolizable protein requirements either for maintenance plus lactation or exclusively for lactation, were slightly smaller than NRC (2001) at low yields but greater than the requirements from the current system at high yields. Other publications have focused in other areas such as microbial nitrogen flow (Roman-Garcia et al., 2016; White et al., 2016), absorbed amino acids (Estes et al., 2018), physically adjusted NDF (White et al., 2017c; White et al., 2017d), and phosphorus balance (Feng et al., 2016).

Despite the extensive efforts to update the statistical models proposed in the NRC (2001), there are still many models that need to be revised and areas to be further explored, such as the predictions of DMI [Equation 2.1], the rate of decline in digestibility with level of feeding [Equation 2.2], fat supplementation and its effect on energy digestibility and energy intake, retained energy, net protein requirements for growth [Equation 2.3 and Equation 2.4,

respectively], and tissue energy contained per kilogram of empty body weight (EBW) in mature cows. These equations are shown below.

$$DMI = (0.372 * FCM + 0.0968 * BW^{0.75}) * (1 - e^{(-0.192*(WOL+3.67))})$$

[Equation 2.1; NRC, 2001]

where FCM is 4 percent fat-corrected milk (kg/d), BW is body weight (kg), and WOL is week of lactation.

$$Discount = [(TDN_{1X} - [(0.18 * TDN_{1x}) - 10.3]) * Intake]/TDN_{1X}$$

[Equation 2.2; NRC, 2001]

where the discount is a multiplying factor to the TDN_{1X} that represents the digestibility at the production level, TDN_{1X} is the total digestible energy at maintenance level as a percent of dry matter intake and it is for the entire diet, and $Intake$ is expressed as incremental intake above maintenance.

$$Retained\ Energy = 0.0635 * EQEBW^{0.75} * EQEBG^{1.097}$$

[Equation 2.3; NRC, 2001]

where $EQEBW$ is the size-scaled empty body weight, and $EQEBG$ is the size-scaled empty body weight gain.

$$Net\ Protein = SWG * (268 - (29.4 * (RE/SWG)))$$

[Equation 2.4; NRC, 2001]

where SWG is shurnk weight gain, and RE is retained energy.

DRY MATTER INTAKE

There is a long history of studies focusing on the biological factors regulating DMI and the development of prediction equations for DMI. The earliest of these studies were published in the *Journal of Dairy Science* and *Journal of Animal Science* in the late 1950s and early 1960s (Crampton et al., 1957; McCullough, 1959; Conrad et al., 1964; Baile, C.A., 1971; Brown et al., 1977).

Based on many research observations, Brown and Chandler (1978) were the pioneers in incorporating prediction equations for DMI in computer-based software. Subsequent studies have shown that DMI is better modeled through nonlinear models; however, due to computational limitations, Brown and Chandler (1978) developed a response surface and divided the lactation into four smaller segments which were defined more adequately by linear models. In this initial model, Brown and Chandler (1978) used the equation to predict DMI per unit of milk that was proposed by Brown et al. (1977). This equation predicted DMI to produce at low, mid, and high production points of each segment, while crude fiber, concentrate, and crude protein were held constant at median values.

Nowadays, our understanding of factors regulating the DMI in dairy cows has increased. Allen (2000) discussed the effects of diet on short-term regulation of DMI and emphasized the importance of fiber content, ease of hydrolysis of starch and fiber, particle size, particle fragility, silage fermentation products, concentration and characteristics of fat, and the amount and ruminal degradation of the protein. In addition to the diet characteristics, Allen (2000) also discussed the effect of metabolic fuels on the regulation of DMI. Allen (2000) recognized that

propionate had a fundamental role in the metabolic control of DMI; however, the mechanism behind the effect of propionate in the regulation of DMI was not yet clear. This mechanism was further understood and published nine years later.

Allen et al. (2009) discussed the metabolic control of feed intake and proposed an integrated mechanism – known as hepatic oxidation theory (**HOT**) – explaining how hepatic fuel oxidation sends signals to the brain feeding centers and controls DMI. This theory explained the effects of the many fuels arriving in the liver (i.e., propionate, acetate, glucose, lactate, and NEFA) and emphasized the crucial role of propionate in regulating DMI. Furthermore, Allen et al. (2009) indicated that hepatic oxidation likely controls feed intake to a greater extent for ruminants in a feedlot setting consuming high-starch diets, for cows with low nutrient requirements (late lactation and dry cows), and for animals in a lipolytic state (periparturient cows) than for ruminants fed high-forage diets or with very high-nutrient requirements (cows at peak lactation) (Allen et al., 2009).

Animals fed high-forage diets or those with very high-nutrient requirements are likely to have their intake limited by the time required for chewing or by distension within the gastrointestinal tract (Allen, 2000). The gastrointestinal tract contains mechanoreceptors that can sense the distention of the tract. When activated, the mechanoreceptors send signals to the enteric nervous system and to the central nervous system to terminate the consumption of feed. In ruminants, the reticulorumen is the segment most likely to limit the DMI due to distention (Allen, 1996). Besides the mechanoreceptors, hormonal control – like the cholecystokinin – can also be responsive to the flow of ingesta through the gastrointestinal tract and stimulate the center of satiety to cease the DMI (Baile et al., 1983; Choi et al., 2000; Allen, 2000).

Moving from the regulation and control of DMI to the optimization and evaluation of DMI, Weiss (2015) demonstrated that DMI is a function of the cow, diet, environment, facilities, and management, and interactions among these factors. Cow factors such as milk yield, stage of lactation, and body weight will determine potential DMI. Factors associated with diet (i.e., ingredients, nutrients, physical form, digestibility), environment (i.e., temperature, humidity), and facilities and management (i.e., access to feed and water, bunk space, grouping) set actual DMI, which is never higher than the potential DMI. In this sense, the goal is to provide a proper diet and remove facility and management factors that limit intake.

Within the context of DMI prediction and its use in diet formulation, the accurate measurement and prediction of DMI are essential for the formulation of balanced, economical diets and the diagnosis of milk yield losses (Roseler et al. 1997a). Despite the efforts of many researchers to derive a prediction equation for DMI that considers factors related to animal, diet, and environment (NRC, 1989; Kertz et al., 1991; Fox et al., 1992; Holter and Urban, 1992; Rayburn and Fox, 1993; Holter et al., 1997); including all these factors in a unique statistical model is very challenging. In fact, these variables account for only about 40% of the variation in the prediction of DMI (Roseler et al., 1997a; Roseler et al., 1997b).

Today, the most used prediction equations for DMI are based only on animal factors (body weight, milk yield, and stage of lactation); hence, the prediction of DMI represents the average intake across many diets in different environments for any given animal factors. For example, the NRC (2001) predicts DMI based on metabolic body weight (**BW^.75**), four percent fat-corrected milk (**FCM**), and a week of lactation (**WOL**) [Equation 2.1].

The NRC (2001) prediction equation was derived by combining the equation of Rayburn and Fox (1993) with an adjustment for the week of lactation developed by Roseler et al. (1997a). In the study conducted by Rayburn and Fox (1993), data from 1,284 Holstein cows over 149 treatment periods were used to evaluate alternative methods of predicting DMI and derive a new DMI prediction equation. In Roseler et al. (1997a), data on complete lactation from 241 Holstein cows were used to improve the prediction for DMI of lactating cows fed highly digestible high energy diets.

Fox et al. (2004) proposed an alternative equation to estimate DMI based on body weight (**BW**), FCM, and adjustment factors for night cooling, mud depth, and month post-calving when peak milk yield (**MY**) occurred. This model combined animal factors with environmental factors and resulted in the adoption of the proposed equations by the CNCPS. However, the CNCPS has updated soon after, resulting in the inclusion of the equation utilized by NRC (2001) for lactating dairy cows (Tylutki et al., 2008) instead.

New DMI prediction equations have been derived using mid-infrared spectroscopy on milk samples (Shetty et al., 2017), incorporating the lipostatic theory (Kennedy, 1953) into a dynamic model based on dairy cow performance (Ellis et al., 2006), and adding the rumination time to the prediction of DMI (Clement et al., 2014). However, the NRC (2001) prediction equation is still the most used on diet formulation software (CNCPS, Spartan Dairy, CPM, AMTS).

Even though the DMI equation proposed by the NRC (2001) was derived from large datasets (as described above) and underwent extensive evaluations, this equation was based on data animal data from the late1980s and 1990s, and may not be accurate for current dairy cows.

Given this information, in CHAPTER 4 ("Updating predictions of dry matter intake of lactating dairy cows"), we proposed an equation to predict DMI based on milk energy, body weight, body condition score, parity, and days in milk. The proposed model was derived using data collected from 3,607 Holstein cows enrolled in 57 studies from 8 states across the US.

NUTRIENT DIGESTIBILITY

While the DMI and diet composition establishes nutrients consumed by animals, the most significant factor affecting nutrient availability in lactating dairy cows is the digestibility of nutrients in the ration (Casper, 2017). Thus, digestibility is a decisive factor in the process of diet formulation. Due to the fundamental importance of digestibility and its relationship with DMI, digestibility has been studied since the 1950s. It is well-known that digestibility decreases with intake (Crampton et al., 1957; Conrad et al., 1964; Tyrell and Moe, 1975; Colucci et al., 1982; VandeHaar, 1998; Casper and Mertens, 2008; Casper, 2017). However, the difficult part of the relationship between digestibility and DMI is accurately predicting the reduction in digestibility as intake increases.

The equation used by the NRC (2001) to estimate digestibility is composed of the dietary total digestible nutrients measured at maintenance level (**TDN_{1x}**), and the intake expressed as multiples of maintenance [Equation 2.2]. The primary concept behind the NRC (2001) equation is that digestibility declines with the level of feeding. However, the rate of decline in digestibility is associated with the digestibility of the diet at maintenance (Wagner and Loosli, 1967; NRC, 2001).

First, to use the NRC (2001) system, the TDN_{1x} of feeds must be estimated. The NRC (2001) used a summative approach to derive TDN_{1x}, in which truly digestible nutrient fractions

(nonfiber carbohydrate, crude protein for forages, crude protein for concentrate, fatty acids, and NDF) are calculated based on a set of equations using diet composition, feedstuff processing, source of protein meal, and type of fat supplement (Weiss et al., 1992; NRC, 2001). Once the truly digestible nutrients are estimated, they are summed, and the metabolic fecal total digestible nutrient is subtracted (fixed value of 7) to calculate the TDN_{1x} (NRC, 2001).

The second component in the NRC (2001) equation is the intake expressed as multiples of maintenance, where multiple of maintenance is calculated based on the energy intake above maintenance. For example, if a lactating cow is consuming 40 Mcal/d of net energy for lactation (**NE_L**, Mcal), and it is estimated that 10 Mcal/d of NE_L are for maintenance (~ 636 kg of BW; NE_L maintenance = $BW^{0.75}$ * 0.08; NRC, 2001), then the intake is at 4X the maintenance (Eastridge, 2002).

The NRC (2001) system was a significant improvement from the previous NRC (1989) system, which used a constant depression on digestibility of 4% per multiple of maintenance. However, the NRC (2001) digestibility system still faces some problems. First, it applies a linear decrease in the diet digestibility as intake increases. VandeHaar (1998) demonstrated that a curvilinear decrease better represents the depression on digestibility. Second, the equation was derived based primarily on data obtained from animals at low to moderate levels of intake and may not adequately describe digestibility of the current high-producing dairy cows, which often have intake greater than 4X the maintenance. Finally, the NRC (2001) decrease is applied to the entire diet, even though the literature already suggested that feeds should have specific discount factors (Van Soest, 1984). Furthermore, recent research suggested that the intake has a different effect on each dietary fraction (White et al., 2017a).

An alternative approach to the NRC (2001), is the CNCPS system which uses passage and degradation rates assigned to carbohydrate and protein fractions to determine digestibility (Van Amburgh et al., 2015). On the current CNCPSv6.5, each of the eight carbohydrate fractions and five protein factions has its own degradation rate (Van Amburgh et al., 2015), and passage rates were assigned to forage, concentrate, and liquid pools as a function of intake (Higgs et al., 2015).

Although the concept of dividing the carbohydrate and proteins into homogeneous fractions and assigning specific degradation rates is an appealing approach, it has many flaws. First, the two-pool structure used in the CNCPS does not account for the fractional rate of release from the non-escapable to the escapable fractions (selective retention and two sequential pools; Allen and Mertens, 1988). Second, the degradation rates assigned to each fraction were determined mostly using *in vitro* or *situ* techniques and may not truly represent degradation rates *in vivo*. Third, the models are so detailed that there is no data to parameterize them (Allen, 2011; Allen and VandeHaar, 2016). Finally, France et al. (2000) concluded that mechanistic models are less accurate than empirical models because of their increased complexity and numerous inputs.

As discussed above, both the NRC (2001) and the CNCPSv6.5 systems to determine digestibility at production level have limitations and require improvements. Accordingly, recent publications have been focused on this area. Nousaiainen et al. (2009) performed meta-analyses using 497 dietary treatment means from 92 studies to evaluate the effects of forage and concentrate factors on total diet digestibility. In this publication, the authors determined that forage quality, the proportion of concentrate, CP content, fibrous by-product, and NDF digestibility are fundamental in determining diet digestibility. Using the same database, Huhtanen et al. (2009) investigated the effect of feeding level and diet composition on

digestibility. He found that TDN for lactating cows could be calculated using organic matter (**OM**) digestibility at a maintenance level determined either in vivo in sheep or by using in vitro methods, DMI, crude protein (**CP**) content, and proportion of forage in the diet.

In regard to nutrient digestibility, White et al. (2017a) built a database composed of 550 treatment means from 192 studies and derived prediction equations for NDF, fat, protein, and nonfiber carbohydrate (**NFC**). In their paper, the authors derived independent equations for legume, corn silage, and other forages to predict NDF digestibility; separate fatty acid (**FA**) digestion coefficients for different fat supplements (animal fats, oils, and other fat types) and the basal diet; unique CP digestibility equations for forages, animal protein feeds, plant protein feeds, and other feeds; and NFC digestibility coefficients for grain-specific starch digestibilities. Finally, the authors concluded that future work should more thoroughly investigate opportunities to account for the relationships between DMI and nutrient digestibilities (White et al., 2017). Moreover, Ferrareto et al. (2013) investigated the effect of cereal grain type and corn grain harvesting and processing methods on digestion. Boerman et al. (2015) examined the intestinal digestibility of individual long-chain fatty in lactating cows. Weld and Armentano (2017) performed a meta-analysis to determine the effects of supplemental fat on fiber digestibility. However, these meta-analyses included only the treatment means of the contributing factors available in the studies summarized. When treatment means are used, the ability to quantify variability among animals within the same diet is reduced, and therefore valuable information about the effects of DMI on digestibility is lost. In CHAPTER 3 (“Predicting nutrient digestibility in high-producing dairy cows”), we further investigated the effects of intake and diet composition on the digestibility of dry matter, NDF, and starch, and proposed new prediction

equations. For this analysis, we used a database containing 1,942 individual observations from 662 cows on 195 different treatments.

FAT SUPPLEMENTATION AND ENERGY INTAKE

As discussed above, DMI and nutrient digestibility determine the amount of nutrients absorbed by the animals that can then be used for maintenance and production. However, among all the nutrients absorbed by the animal, the lack of energy is the most likely to limit production in the high-producing lactating cow (Eastridge, 2002). For example, it is well-established that most animals undergo a period of negative energy balance during the beginning period of lactation (Bauman and Currie, 1980). In light of this, fat supplements are often added to dairy cattle diets with the goal of increasing energy intake in order to increase milk energy output or energy balance (Allen and Piantoni, 2014). However, the response to fat supplementation has been inconsistent. In many experiments, fat supplements have increased energy intake; however, in other experiments, fat supplements have depressed DM digestibility and DMI (Allen, 2000).

In order to investigate these varied responses to fat supplementation, Allen (2000) constructed a database composed of treatment means from 60 studies to examine the effect of different sources of supplemental fat (oilseeds, unprocessed animal fat, hydrogenated FA & triglycerides, and calcium salts of palm FA – **Ca-PFA**) on DMI and found differences among sources. Ca-PFA statistically decreased DMI on 11 of the 24 comparisons and resulted in a numerical decrease in DMI in 22 of the 24 comparisons; in contrast, the other sources of fat seemed to have little impact on DMI (Allen, 2000).

To further investigate the effects of different fat supplements, Weiss and Wyatt (2004) compared the effects of Ca-PFA and hydrogenated triacylglycerides from palm oil (**HPO**) on

dietary digestible energy (**DE**). In agreement with Allen (2000), Weiss and Wyatt (2004) also reported that DMI was reduced when cows were fed the high-concentration of Ca-PFA. However, the digestibility of energy, dry matter, and organic matter, were higher for diets with Ca-PFA than for the diets with HPO. As a result, cows fed Ca-PFA produced more milk than cows fed the control or HPO diets. Finally, the authors concluded that supplementing diets with Ca-PFA increased DE intake. However, due to the negative effects on DMI, the relative increase was less than that for DE concentration (Weiss and Wyatt, 2004).

Another important source of variation regarding fat supplements is the degree of saturation. As discussed by Allen (2000), unsaturated FA has higher hypophagic effects than saturated FA. To investigate the effect of degree of saturation, Harvatine and Allen (2006) linearly substituted 2.5% FA from saturated fat supplements (prilled, hydrogenated free fatty acids) for partially unsaturated fat supplements (calcium soaps of long-chain FA) and reported that increasing unsaturated fat supplements decreased milk fat yield and tended to reduce intake of digestible energy. Additionally, animals receiving the diet high in unsaturated FA experienced milk fat depression with decreased milk fat yield associated with increased concentrations of *trans*-10, *cis*-12 conjugated linoleic acid, and total *trans* C18:1 FAs in milk.

In 2011, Weiss et al. investigated the effects of fat supplements that differed in FA composition (chain length and degree of saturation) and chemical form (free FA, Ca-PFA, and triacylglyceride). Weiss et al. (2011) compared a control diet (2.9% of DM as long-chain FA) to three diets with 3% added FA. The three fat supplements were mostly saturated free FA, Ca-PFA, and triacylglyceride. The authors observed a positive effect of free FA on the digestibility of FA, and concluded that free FA supplements were much more digestible than triacylglyceride supplements.

Current research on fat supplementation is focusing on the specific effects of each free FA supplement on digestibility, energy intake, nutrient partitioning, and production. Lock et al. (2013) compared a control diet without supplemental fat to a diet with palmitic (**C16:0**) enriched (~85% C16:0) fat supplement (2% of DM). The authors did not observe a difference in milk yield or milk protein yield, but the diet with C16:0-enriched supplement increased milk fat concentration and milk fat yield. Furthermore, the treatment with supplemental fat decreased DMI and increased the conversion of feed into milk. Lastly, the authors suggested that these results should be evaluated under different milk production levels.

Following the publication of these results, Piantoni et al. (2013) assessed the effect of C16:0 supplementation in dairy cows varying the level of production. Piantoni et al. (2013) compared a control diet without supplemental fat to a diet supplemented with C16:0 (2% of DM) using dairy cows ranging in milk production from 34 to 66 kg/d. The authors did not find a significant interaction between diets and preliminary milk yield; and therefore, the effect of supplemental C16:0 was independent of the level of production. In regards to the production variables' responses, the diet with supplemental C16:0 increased milk fat percentage and yields of milk, milk fat, and 3.5% fat-corrected milk compared to the control diet. With respect to the responses from the digestibility variables, the diet with supplemental C16:0 increased the total-tract digestibility of NDF and organic matter, but decreased FA digestibility compared to the control diet.

Rico et al. (2014) evaluated the effects of diets containing C16:0 or stearic (**C18:0**) fat supplements (2% of DM) on the production end efficiency of cows with a milk production ranging from 38 to 65 kg/d. The authors concluded that the results were consistent across the

level of production and C16:0 supplementation improved milk fat concentration and yield, as well as the efficiency of feed conversion into milk compared with C18:0 supplementation.

Finally, in a recent meta-analysis, Weld and Armentano (2017) used 108 fat-supplemented treatment means from 38 publications to summarize the effect of fat supplementation on the total-tract digestibility of NDF (**ttNDFd**) in lactating dairy cattle. The authors categorized the fat supplements as medium-chain FA (C12 and C14), oil, C16, animal-vegetable, tallow, Ca-PFA, calcium salts of long-chain FA, and saturated fat. In this meta-analysis, the authors found that medium-chain FA and unsaturated vegetable oil decreased ttNDFd, and adding 3% of calcium salts of long-chain FA or saturated fats increased ttNDFd. In regards to DMI and digestible energy intake, adding fats, other than those with medium-chain FA, consistently increased the DE density of the diet. However, due to reduced DMI, this increased energy density may not result in increased digestible nutrient intake (Weid and Armenano, 2017).

Despite the many research studies conducted to determine the effect of free FAs on dairy cattle production, feed efficiency, and nutrient digestibility, a comprehensive examination of the effect of specific free FA on digestibility of dry matter, NDF, fatty acid, and energy, as well their effect on digestible energy intake is still lacking. In CHAPTER 5 (“Dietary fatty acid composition and digestible energy in lactating dairy cows”), we performed a meta-regression using data from 5 experiments conducted at Michigan State University to determine the effect of dietary FA composition on dry matter, NDF, total FA, 16-carbon FA, 18-carbon FA, energy digestibilities, and digestible energy intake.

BODY COMPOSITION IN HOLSTEIN CATTLE

Whereas the previous sections focused on how to meet the nutritional requirements of dairy cows; the main focus in this section is accurately determining what these nutritional requirements are. Of particular importance, is the net energy and the net protein required for growth (**NE_g** and **NP_g**, respectively) in dairy heifers, and the energy supply/required per kilogram of change on empty body weight (**EBW**) in dairy cows.

With this in mind, NE_g and NP_g can be estimated from the energy and protein content of the tissue deposited during growth (NRC, 2001). The NRC (2001) predicts NE_g and NP_g using Equations 2.3 and 2.4, respectively. These equations were proposed and validated by Fox et al. (1999), who modified the equation proposed by the Beef NRC (1996) to compute growth requirements in dairy cattle.

The Beef NRC (1996) adopted the equations initially proposed by Garrett (1980) and Garrett (1987) and further adjusted by Fox et al. (1992) and Tylutki et al. (1994). Until today, Garrett (1980) has been one of the most extensive and comprehensive studies evaluating energy utilization in growing cattle.

Garrett (1980) derived an equation to predict retained energy (**RE**) as a function of the rate of gain and EBW. Garret (1980) used data from 72 comparative slaughter experiments that together evaluated the carcasses of 1,843 and 330 British-breed steers and heifers, respectively, and 52 Charolais steers, all of which had received hormonal implants, and 861 and 405 British-breed steers and heifers, respectively, which had not received implants. In a second paper, using this same database, Garrett (1987) derived equations to determine the fat and protein content of the gain as a function of the estimated RE. Subsequently, Fox et. (1992) used a size scale

approach to modify the proposed equations (Garrett, 1980 and Garrett, 1987) for all classes of beef and dairy cattle, which was later refined and validated by Tylutki et al. (1994).

Fox et al. (1999) also modified the Beef NRC (1996) equations used to compute growth requirements, target weights, and energy reserves to develop a growth model for dairy cattle. In the growth model proposed by Fox et al. (1999), the equation proposed in the Beef NRC (1996) were size scaled for dairy breeds, and a set of prediction equations were derived to estimate NE_g and NP_g. This model was then evaluated using data from serially slaughtered nonimplanted Holstein heifers published by Fortin et al. (1980) and Anrique et al. (1990). The authors concluded that the proposed model successfully predicted NE_g and NP_g of dairy cattle, accounting for 96% of the variation in energy retained with a 4% bias.

At that time, the development of a growth model based exclusively on data collected in dairy cattle was almost impossible due to the lack of data. The size scaling approach used by Fox et al. (1999) offered a great alternative because the original equations were derived using a large dataset (Garrett, 1980) and the predictions seemed to have a good fit to dairy cattle. However, some discrepancies still needed to be addressed. For example, the size scale approach assumes that the chemical composition of gain is similar among animals at the same proportion of mature BW (NRC, 2001), but this may not be true in remarkably different production systems, as is the case. As mentioned above, in addition to being based on beef breeds raised on a feedlot diet, most of the data came from animals with hormonal implants. In contrast, replacement dairy heifers are raised receiving diets with much more forage than feedlot diets and without hormonal implants.

Since the NRC (2001), many studies have been published reporting data on body composition in dairy cattle raised within dairy farm conditions; and therefore, data is now available to develop a growth model based on data collected from dairy cattle. For example, Moallem et al. (2004) evaluated the effect of bovine somatotropin and rumen-undegradable protein on body composition in 24 Holstein heifers. In this study the author evaluated body composition in animals at 5 and 10 months with an average body weight of 72 and 185 kg of EBW, respectively, and body composition of 66% water, 5% ash, 19% protein, and 9% fat at 5 months and 61% water, 5% ash, 19% protein, and 13% fat at 10 months.

Brown et al. (2005) evaluated carcass composition in Holstein heifer calves provided with different amounts of energy and protein intake. In this study, the average daily gain (**ADG**) for the first period (2-8 weeks) varied from 0.379 to 0.668 kg/d, and in the second period (8-14 weeks) ADG varied from 0.400 to 1.13 kg/d. The authors did not find a difference in carcass composition for the first period, but in the second period, as ADG increased, the fat content in the carcass also increased. These results suggested that the effect of ADG in body composition is small in calves from 2 to 8 weeks.

Investigating the effect of nutrient density in the milk replacement and feeding rate, Hill et al. (2008) found that empty body fat increased from 17% to 24% from the control (20% CP and 21% fat, fed at 441 g of DM/d) to the high-density treatment (27% CP and 28% fat, fed at 951 g of DM/d) within the same feeding rate, and had the maximum fat content of 27% fat on the high-density treatment and high feeding rate (27% CP and 28% fat, fed at 951 g of DM/d). However, treatments and feeding rate also affected ADG. The control treatment and the high-density and high-feeding rate had ADG of 0.368 and 0.736 kg/d, respectively. Although the

authors attributed the difference in body fat to the change in nutrient density, the ADG was likely an essential factor in the differences found in regards to body composition.

In regards to the type of FA added to the milk replacer, Mills et al. (2010) fed 36 Holstein bulls from birth to 85 kg of body weight with three diets varying in triglycerides, a control diet with no added medium-chain triglyceride, a diet with 32% medium-chain triglycerides primarily as caprylate (8 carbons), and a diet with 32% medium-chain triglycerides primarily as laurate (12 carbons). Although the diet with caprylate had lower ADG than the control diet and the diet with laurate (0.880, 0.990, and 0.940 kg/d, respectively), the authors did not observe a difference in body composition. Based on this, the authors suggested that the medium-chain triglyceride may influence body composition since the animals differed in ADG but not body composition. Nevertheless, the differences in ADG reported in this paper were very small; and therefore, if diet were to impact body composition, it likely would have much less importance than ADG.

As discussed above, there is a need to improve the accuracy of predicting NE_g and NP_g in dairy breeds, and since there is now enough data published on body composition collected from Holstein animals, it is possible to derive more specific and accurate models to predict NE_g and NP_g using data only from Holsteins. Thus, in CHAPTER 6 (“Body composition of Holstein cattle”), we performed a meta-regression using data collected from 26 studies that reported body composition in Holstein animals in order to derive prediction equations for body composition and applied them to determine NE_g and NP_g in Holstein heifers.

APPENDICES

APPENDIX A

Tables

Table 2.1. Cost structure of dairy farm per hundredweight (cwt) of milk sold in 2017.

Items	US$/cwt	% of production cost
Operating costs		
Feed	10.50	47.1%
Veterinary and medicine	0.83	3.74%
Bedding and litter	0.24	1.06%
Marketing	0.26	1.17%
Custom services	0.58	2.60%
Fuel, lube, and electricity	0.51	2.30%
Repairs	0.56	2.51%
Other operating costs	0.00	0.00%
Interest on operating capital	0.01	0.050%
Total operating costs	13.49	60.6%
Allocated overhead		
Hired labor	1.64	7.37%
Opportunity cost of unpaid labor	2.46	11.1%
Capital recovery of machinery and equipment	3.80	17.0%
Opportunity cost of land (rental rate)	0.02	0.1%
Taxes and insurance	0.22	1.0%
General farm overhead	0.64	2.9%
Total allocated overhead	8.79	39.4%

Source: ERS-USDA (https://www.ers.usda.gov/)

APPENDIX B

Figures

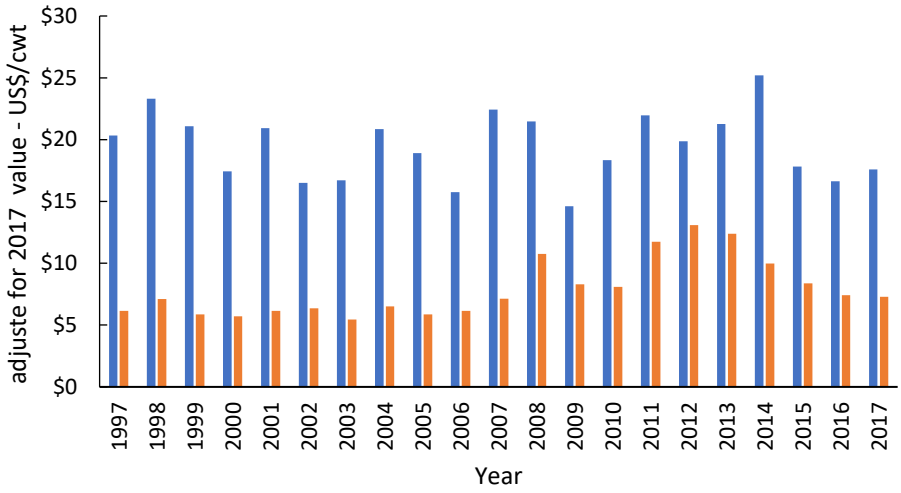

Figure 2.1. Milk (■) and feed (■) prices from 1997 to 2017 per hundredweight. The prices are adjusted to 2017 values (adjusted used the inflation for each year; LBS, 2018).
Source: ERS-USDA, 2018

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CHAPTER 3

PREDICTING NUTRIENT DIGESTIBILITY IN HIGH-PRODUCING DAIRY COWS

ABSTRACT

Our objective was to determine the effects of dry matter intake (**DMI**), body weight (**BW**), and diet characteristics on total tract digestibilities of dry matter, neutral detergent fiber, and starch (**DMD**, **NDFD**, and **StarchD**, respectively) in high-producing dairy cows. Our database was composed of 1,942 observations from 662 cows in 54 studies from Michigan, Ohio, and Georgia. On average, cows ate 23 ± 4.5 kg DM/d, weighed 669 ± 79 kg, and produced 38 ± 10 kg of milk/d. Diets were 31 ± 5% NDF, 27 ± 6% starch, 2.6 ± 1.2% fatty acids, and 17 ± 1.4% crude protein. Digestibility means were 66 ± 6, 42 ± 11 and 93 ± 5% for DMD, NDFD, and StarchD, respectively. Forage sources included corn silage, alfalfa, and grasses. Corn source was classified by its ruminal fermentability. Data were analyzed using a mixed effects model including diet chemical composition, forage source, and corn source, all expressed as a percentage of DM; DMI as a percentage of BW (**DMI%BW**); location; and 2-way interactions as fixed effects. Cow, block, period, treatment, and study were included as random effects. Best fitting candidate models were generated using backward and stepwise regression methods. Additionally, the simplest model was generated using only DMI and location as fixed effects and all random effects. Candidate models were cross-validated across studies, and the resulting predictive correlation coefficients across studies (**PC**) and root mean square error of prediction (**RMSEP**) were compared by t-test. For each nutrient, the digestibility model that resulted in the highest PC and lowest RMSEP was determined to be the best fitting model. We observed heterogeneous coefficients among the different locations, suggesting that specific location

factors influence digestibilities. The overall location-averaged best fitting prediction equations were: DMD = 69 – 0.83 x DMI%BW (PC = 0.22, RMSEP = 5.39); NDFD = 53 + 0.26 x Grass%DM - 0.59 x Starch%DM + 3.06 x DMI%BW – 0.46 x DMI%BW2 (PC=0.53, RMSEP=9.70); and StarchD = 96 + 0.19 x HFERM%DM – 0.12 x Starch%DM – 1.13 x DMI%BW (PC=0.34, RMSEP=4.77); where HFERM%DM is highly-fermentable corn source as percentage of DM. Our results confirm that digestibility is reduced as DMI increases albeit at a lower rate than that reported in NRC (2001). Furthermore, dietary starch depresses NDFD. While DMD can be predicted based on DMI only, the best predictions for NDFD and StarchD require diet characteristics in addition to DMI.

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CHAPTER 4

UPDATING PREDICTIONS OF DRY MATTER INTAKE OF LACTATING DAIRY COWS

ABSTRACT

Our objective was to model dry matter intake (**DMI**) in Holstein dairy cows based on milk energy (**MilkE**), body weight (**BW**), change in body weight (**ΔBW**), body condition score (**BCS**), height (**Ht**), days in milk (**DIM**), and parity (primiparous and multiparous). Our database included 47,253 weekly observations on 3,607 cows enrolled in 57 studies from 8 states across the US. The means ± standard deviations of these variables were 24 ± 5 kg DMI, 30 ± 6 Mcal/d MilkE, 624 ± 83 kg BW, 0.24 ± 1.50 kg/d ΔBW, 3.0 ± 0.5 BCS, 149 ± 6 cm Ht, and 102 ± 45 DIM. Data analysis was performed using a random regression model containing location, experiment within location, diet within experiment and location and cow within experiment as random effects whereas the fixed effects included the linear effects of the covariates described previously and all possible 2-way interactions between parity and the other covariates. A nonlinear (**NLIN**) mixed model analysis was developed using a two-step approach. In the first step, we used a linear model component of the NLIN model to predict DMI using only data from mid-lactation dairy cows (76 to 175 DIM) without including information on DIM. In the second step, a nonlinear adjustment for DIM using all data from 0 to 368 DIM was estimated. Additionally, the NLIN model was compared to a linear (**LIN**) model containing a fourth-order polynomial for DIM using data throughout all of the lactation (0 to 368 DIM) in order to assess the utility of an NLIN model for the prediction of DMI. In summary, a total of 8 candidate models were evaluated; i.e., four ways to express energy required for maintenance (BW, $BW^{0.75}$, BW adjusted for BCS of 3, and $BW^{0.75}$ adjusted to a BCS of 3) x 2 modeling strategies (LIN

versus NLIN). The candidate models were compared using a 100-fold across study cross-validation study, with the best fitting model chosen as the proposed model. The metrics used in the validation were the mean bias, slope bias, concordance correlation coefficient (**CCC**), and root mean square error of prediction (**RMSEP**). The proposed prediction equation was: DMI (kg/d) = ((3.7 + Parity*5.7) + 0.305 * MilkE (Mcal/d) + 0.022 * BW (kg) + (– 0.689 + Parity*(– 1.87)) * BCS) *(1 – (0.212 + Parity*0.136) * $exp^{(-0.053*DIM)}$) (Mean bias = 0.021 kg, Slope bias = 0.059, CCC = 0.72, and RMSEP = 2.89 kg), where Parity is equal to 1 if animal is multiparous and 0 otherwise. Finally, the proposed model was validated against the NRC (2001) prediction equation for DMI using an independent dataset used exclusively for the validation purpose (13,953 weekly observations on 2,005 Holstein cows) in a similar approach described above. The proposed model had smaller mean bias and RMSEP, and higher CCC than the equation suggested by NRC to predict DMI and has potential to benefit nutritionists with diet formulation.

INTRODUCTION

Actual or accurately estimated dry matter intake (**DMI**) is essential for the formulation of diets to prevent underfeeding or overfeeding of nutrients and to promote efficient nutrient use (NRC, 2001). Equations used to predict DMI of lactating dairy cows are based on existing databases that contain observed intake and predictor variables such as milk energy (**MilkE**), body weight, and days in milk (**DIM**). Consequently, the accuracy and precision of these predictions are dependent on the quality of the database and the use of appropriate statistical analysis to derive the prediction equations.

Traditionally, the prediction of DMI in lactating dairy cows is based on milk production and the energy required for maintenance, which together represents the major energy

expenditures of the lactating dairy cow (VandeHaar et al., 2016). Furthermore, due to the peculiarities of each stage of lactation (i.e., transition and early lactation dairy cows; Allen et al., 2009), it is crucial to consider the effect of DIM.

The most recent edition of the Nutrient Requirements of Dairy Cattle (NRC, 2001) includes an empirical equation to estimate DMI of lactating Holstein cows based on animal factors that could be easily measured or known, as follows:

$$DMI(kg/d) = (0.372 * FCM + 0.0968 * BW^{0.75}) * (1 - exp^{(-0.192*(WOL+3.67))}$$

where FCM = 4 percent fat-corrected milk (kg/d), BW = body weight (kg), and WOL = week of lactation; NRC, 2001. This prediction equation was based on data collected on 1,284 Holstein cows using the equation proposed by Rayburn and Fox (1993) and further modified by Fox (1999). Also, the nonlinear effect of the week of lactation was based on Roseler et al. (1997).

A more recent and much larger database of weekly DMI data from many research stations was used by Lu et al. (2017) to estimate the genetic and nongenetic components of regression of DMI on milk energy and maintenance as a function of various management and environmental factors. Similarly, Tempelman et al. (2015) developed station-specific prediction equations for DMI but without distinguishing genetic from non-genetic components as in Lu et al. (2017). Nevertheless, both studies used only data between 50 and 200 DIM (Tempelman et al., 2015; Lu et al., 2017), and therefore might not be valid for predicting DMI outside that range of DIM.

The objective of this study was to derive a prediction equation for DMI based on animal factors using a more recent database better reflective of current dairy production systems. Our

data was comprised of the updated version of the database used in Tempelman et al. (2015) that included data from animals between 1 and 368 DIM and to validate the developed prediction equation against the NRC 2001 prediction for DMI using an independent dataset. We hypothesized that an equation derived from this larger and newer database would increase the accuracy and precision of DMI predictions compared to the equation provided in NRC (2001).

MATERIALS AND METHODS

Data

The database was composed of individual weekly observations of DMI, MilkE, body weight (**BW**), metabolic BW (**BW$^{0.75}$**), body condition score (**BCS**), change on BW (**ΔBW**), height (**Ht**), DIM, and parity (primiparous and multiparous) from ten research stations across the US. Additionally, we calculated BW adjusted to a BCS of 3 (**BWBCS3**, kg) and BW$^{0.75}$ adjusted to a BCS of 3 (**BW^0.75BCS3**, kg) as a function of BW and BCS [$BWBCS3 = BW + BW * 0.084 * (3 - BCS)$, and $BW\text{^}0.75BCS3 = BWBCS3^{0.75}$]. To calculate the BWBCS3, was assumed that one-unit shift in BCS causes a 8.4% change in BW, as determined by Souza and VandeHaar (2018) using a database composed of 2,181 Holstein cows. The milk energy was calculated according to NRC (2001).

Recording frequencies of the variables included in the database varied from daily, thrice weekly, twice weekly, weekly, and biweekly depending on the variable and the research station. In order to standardize variables as weekly means across all studies, each variable (i.e., MILKE, BW, BCS, ΔBW, and Ht) on each animal on the same treatment for each experiment was analyzed using a 5th order polynomial linear regression model on days since the start of that

particular treatment. Subsequently, the predicted values from these animal-specific analyses were used to calculate weekly means for each animal on a particular treatment.

The final database was composed of 61,206 weekly observations on 4,785 Holstein dairy cows from 105 studies (Table 4.1). About 36% (22,045 weekly observations) of this data was previously described by Tempelman et al. (2015), but using only their data from US research stations (University of Florida, Gainesville; Iowa State University, Ames; Michigan State University, East Lansing; University of Wisconsin, Madison; United States Dairy Forage Research Center, Madison; and USDA Animal Genomics and Improvement Laboratory, Beltsville) to be consistent with other data added to this database. The remaining 64% (39,161 weekly observations) of the data came either from locations already used in Tempelman et al. (2015) but collected in more recent experiments or collected at DIM less than 50 or greater than 200 (50% of all data) at these same stations or from new locations (14% of the data) including the Purina Animal Nutrition Center (Gray Summit, MO), Cargill Research & Development Center (Minneapolis, MN), Miner Institute (Chazy, NY),The Ohio State University (Columbus, OH), and Virginia Tech University (Blacksburg, VA). Our final database was thereby composed of records from cows between 1 and 368 DIM and collected in research stations across the US (Florida, Iowa, Maryland, Michigan, Missouri, New York, Ohio, Virginia, and Wisconsin) from 2007 to 2016. All the data were collected from lactating Holstein dairy cows offered a total mixed ration once per day and milked two or three times per day.

Finally, the final database was subdivided into two independent datasets. The first dataset, comprised of 47,253 weekly observations from 3,743 lactations (1,769 primiparous and 1,974 multiparous) on 3,607 cows, was used in the model assessment process (referred to as the "training dataset") whereas the second dataset, comprised of 13,953 weekly observations from

2,202 lactations (1,120 primiparous and 1,082 multiparous) on 2,005 cows, was used in the independent validation process. The distribution of the number of observations in each dataset throughout the lactation is presented in Figure 1. The criteria used to determine the separation of the database into two datasets (model assessment and independent validation datasets) was based on the number of weekly observations per cow. Studies that provided 7 or more weekly observations per cow constituted the model assessment dataset, whereas the remaining smaller studies constituted the independent validation dataset. Basic summary statistics of the raw weekly data for each of the two datasets are provided in Table 4.2.

Statistical Analyses

All data analysis was performed using SAS v. 9.4 (SAS Institute Inc., Cary, NC). For numerical stability, all covariates were standardized to have a mean of zero and a standard deviation of 1 using PROC STANDARD and the functions MEAN=0 and STD=1. All covariates were jointly checked for multicollinearity using variance inflation factors (**VIF**, multicollinearity analysis).

In this paper, we will refer to the models that initiated the modeling processes as the "starting models", the models that were selected by the modeling processes and were used in cross-validation as the "candidate models", and the model selected for best fit based on cross-validation and further evaluated against the NRC (2001) DMI model as the "proposed model". These models are more carefully explained in the subsequent sections.

Modeling Process

The nonlinear (**NLIN**) modeling process was composed of two major phases. The first phase was the development of a linear model to predict DMI in mid-lactation cows (76 – 175 DIM) without the inclusion of DIM as a covariate. The second phase was the inclusion of the nonlinear effect of DIM on DMI across the entire modeling dataset including early (1-75 DIM), mid (76-175 DIM), and late-lactation (176-368 DIM).

For the first phase of the modeling process, we specified a starting model that contained the fixed effects of partial linear regressions on MilkE, the corresponding BW measure (BW, BW^0.75, BWBCS3, or BW^0.75BCS3), ΔBW, BCS, parity (primiparous and multiparous), and all possible two-way interactions between parity and the other covariates. Location, experiment within location, diet within experiment and location, and cow within experiment and location were treated as random effects. Furthermore, the model also included diet specific partial regressions of DMI on MilkE and on BW, recognizing that the partial regressions of DMI on MilkE and on BW could depend upon diet compositions. The generic starting model is presented in Equation [4.1].

$$DMI_{p,c,d,e,l} = \beta_0 + u_{0,d} + (\beta_1 + u_{1,d}) * MilkE + (\beta_2 + u_{2,d}) * BW + \beta_3 * \Delta BW + \beta_4 * BCS + \beta_5 * Parity_p + \beta_6 * MilkE * Parity_p + \beta_7 * body\ weight * Parity_p + \beta_8 * \Delta BW * Parity_p + \beta_9 * BCS * Parity_p + Cow_c(Exp_e * Location_l) + Diet_d(Exp_e * Location_l) + Exp_e(Location_l) + Location_l + \varepsilon_{p,c,d,e,l} \quad [4.1]$$

where $DMI_{p,c,d,l,s}$ (p = parity, c = cow, d = diet, e = experiment, l = location) is the observed DMI; $(\beta_0 + u_{0,d})$ is the intercept specific to diet d, $(\beta_1 + u_{1,d})$ is the partial regression

coefficient of DMI on MilkE specific to diet *d*, $(\beta_2 + u_{2,d})$ is the partial regression coefficient of DMI on BW (BW, BW^0.75, BWBCS3, or BW^0.75BCS3), specific to diet *d*, β_3 is the partial regression coefficient of DMI on ΔBW; β_4 is the partial regression coefficient of DMI on BCS; β_5 is the partial regression coefficient of DMI on $Parity$ (*p* = primiparous or multiparous); $\beta_6, \beta_7, \beta_8, \beta_9$ are the effects for the two-way interactions between the corresponding covariates and parity. All other terms were random effects including $Cow_c \sim NIID\ (0, \sigma^2_{Cow_c})$ for cow *c* = 1 to 2,805, $Diet_d \sim NIID(0, \sigma^2_{Diet_d})$ for diet *d* = 1 to 153, $Exp_e \sim NIID\ (0, \sigma^2_{Exp_e})$ for experiment *e* = 1 to 57, $Location_l \sim NIID\ (0, \sigma^2_{Location_l})$ for location *l* = Florida, Iowa, Maryland, Michigan, Minnesota, New York, Virginia, and Wisconsin with $\varepsilon_{p,c,d,e,l}$ being the error term. We assumed a multivariate normal distribution for diet effects and diet-specific partial regressions on MilkE and BW as follows.

$$\begin{bmatrix} u_{0,d} \\ u_{1,d} \\ u_{2,d} \end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \mathbf{G} = \begin{bmatrix} \sigma^2_{u_0} & \sigma_{u_0,u_1} & \sigma_{u_0,u_2} \\ \sigma_{u_0,u_1} & \sigma^2_{u_1} & \sigma_{u_1,u_2} \\ \sigma_{u_1,u_2} & \sigma_{u_1,u_2} & \sigma^2_{u_2} \end{bmatrix} \right) \quad [4.2]$$

Note that the specification in Equation [4.2] also allowed for non-zero covariances, σ_{u_0,u_1}, σ_{u_0,u_2}, and σ_{u_1,u_2} (i.e., off-diagonals of **G)** between diet-specific intercepts and slopes for MilkE, between diet-specific intercepts and slopes for BW, and between diet-specific slopes for MilkE and body weight, respectively.

The starting models described above Equation [4.1] were subjected to model selection, which was composed of two phases. During the first phase, each of the full models was subjected to a backward model selection method using the SAS procedure PROC GLMSELECT with the

option HIERARCHY = SINGLE, such that the model was forced to include the corresponding main effects if the interaction between any two effects were deemed to be significant. Since the PROC GLMSELECT procedure does not allow for the specification of random effects, the model was limited to the specification of fixed effects only in this first stage of model selection. During the second phase of model selection, the model chosen by the PROC GLMSELECT procedure was analyzed using the PROC HPMIXED procedure, in which the above-mentioned random effects were included. The fixed effects with the highest *P* values were successively removed until only significant fixed effects ($P < 0.05$) and all specified random effects remained in the model, yielding a linear mixed model as a candidate model to predict DMI for mid-lactation dairy cows. With this model selection process, we created four different candidate models (i.e., four different ways to express the covariate BW; namely, BW, BW^0.75, BWBCS3, and BW^0.75BCS3).

The NLIN model involved the estimation of the nonlinear effect of DIM on DMI using the SAS procedure NLMIXED. The nonlinear adjustment was based on the adjustment factor proposed by Roseler et al. (1997; i.e., $1 - exp^{-(\beta_1 - \beta_2 * PMM) * WOL + \beta_3)}$, where PMM is peak milk month and WOL is week of lactation) with further modifications as follows 1) key coefficients included the effect of parity to allow for parity specific effects, 2) removal of the PMM term because information was not available in our dataset, and 3) inclusion of a term which determines the maximum possible discount (β_1 in Equation [4.3] below). The generic model used during the second phase is presented in Equation [4.3].

$$DMI = \widehat{DMI}_c * (1 - (\beta_1 + \beta_2 * Parity) * exp^{((\beta_3 + \beta_4 * Parity) * DIM)}) + \varepsilon_c \quad . \qquad [4.3]$$

Here *DMI* is the observed *DMI*, $\widehat{DMI}_c$ is the predicted DMI from candidate linear submodel *c* (*c* =1 to 4) based on the four different ways to express the covariate BW, β_1 is the coefficient that determines the maximum possible discount for DIM for primiparous cows, β_2 specifies the difference for this discount between multiparous and primiparous cows, β_3 is the nonlinear regression coefficient on DIM for primiparous cows with β_4 specifying the difference for this coefficient between multiparous and primiparous cows Furthermore , *Parity* is the dummy variable (*Parity* = 0 for primiparous and *Parity* = 1 for multiparous) for parity in Equation [4.3] whereas ε_c is the error term assumed to be normally identically and independently distributed..

To verify the importance of using a nonlinear adjustment for DIM in the prediction of DMI, we refitted the 4 linear candidate models (based on model selection) to the entire training dataset by adding a fourth-order polynomial for DIM and all possible 2-way interactions with parity as fixed effects as part of a linear (**LIN**) modeling strategy. Hence our model comparison involved a total of 8 candidate models (4 different ways to express BW x NLIN vs. LIN).

Cross-validation

The eight candidate models were formally compared using a 5-fold across study cross-validation repeated 20 times using the training dataset of 47,253 weekly observations as described earlier. That is, for each of the 20 cross-validations replicates, the data was partitioned across rather than within studies into five nearly equal-sized subsets each involving all data from 20% of the studies, such that for one-fold, 4 of these subsets were used for training with the remaining subset used for validation. Hence, across five folds, each subset took a turn being the validation dataset. The candidate models were compared for fit criteria such as mean bias, slope bias, concordance correlation coefficient (**CCC**), mean square error of prediction (**MSEP**), root

mean square error of prediction (**RMSEP**), and the decomposition of MSEP in mean bias, slope bias, and random error (as % of MSEP) (Tedeschi, 2006). To determine our proposed model, the fit criteria were analyzed using the PROC GLIMMIX procedure of SAS v.9. 4 (SAS Institute Inc., Cary, NC) according to the following model (Equation [4.4]):

$$Y_{ijl} = \mu + r_i + BW_j + Strategy_l + BW * Strategy_{jl} + e_{ijl} \quad [4.4]$$

where Y_{ijl} is one of the fit statistics of interest; μ is the overall mean; r_i is the random effect of fold (i = 1 to 100); BW_j is the fixed effect of corresponding BW measure (j = BW, BW^0.75, BWBCS3, or BW^0.75BCS3), $Strategy_l$ is the fixed effect of strategy (l = LIN and NLIN), $BW * Strategy_{jl}$ is the interaction between corresponding BW and strategy, and e_{ijl} is the residual error. The candidate model that consistently had superior fit statistics was considered the best fitting model and, therefore, our proposed model.

A generic SAS code for model validation (using only linear models) and a short explanation is available in the Supplementary Material 4.1.

Independent validation process

As an independent validation process beyond the cross-validation strategy described previously, the proposed model was compared to the DMI prediction equation proposed on the NRC (2001) using the independent validation dataset of 13,953 weekly observations involving smaller studies with 6 or less weekly observations per cow. That is, for each of 20 validation replicates, the data was partitioned across studies into five nearly equal-sized subsets, each subset involving all data from 20% of the studies. For each fold, the fit statistics (CCC, MSEP,

RMSEP, and decomposition of MSEP) were computed as described previously. The major difference between the previously described cross-validation study and this independent validation study was that the same entire training dataset of 47,253 weekly observations involving all of the larger studies (>7 records per cow) was used for each validation subset such that predictions for each validation subset were based on the same fixed effects and variance components as based on the proposed model. Furthermore, to facilitate stage-specific comparisons, the validation dataset was partitioned further according to the stage of lactation (early: 1 to 75 DIM; mid: 76 to 175 DIM; and late: 176 to 368 DIM). The various fit statistics were compared using the PROC GLIMMIX procedure of SAS v.9. 4 (SAS Institute Inc., Cary, NC) according to the following model (Equation [4.5]):

$$Y_{ijk} = \mu + r_i + Model_j + Stage_k + Model * Stage_{jk} + e_{ijk} \quad [4.5]$$

where Y_{ijk} is the fit statistic of interest; μ is the overall mean; r_i is the random effect of fold (i = 1 to 100); $Model_j$ is the fixed effect of model (j = Proposed or NRC), $Stage_k$ is the fixed effect of stage of lactation (k = early, mid, or late), $Model * Stage_{jk}$ is the fixed effect of the interaction between model and stage, and e_{ijk} is the residual error.

RESULTS

Regarding the four different measures to express BW (BW, BW^0.75, BWBCS3, or BW^0.75BCS3), we did not observe any difference in their ability to predict DMI based on the cross-validation (P > 0.9, results not shown). The fact that we did not observe differences between the four ways to express BW might be because all measures were highly correlated with each other (r = 0.96 to 0.99) and all cows in our dataset were Holsteins. Thus, for the remainder

of the paper, we only show and discuss data for the models developed using BW, due to its simplicity and applicability.

During the model assessment process, the main effect of ΔBW and the interactions of Parity with MilkE, BW, and ΔBW were removed from the starting model ($P > 0.05$). With respect to the nonlinear effect of DIM in the NLIN model, parity was an important ($P < 0.01$) source of variation for the maximum discount on DMI due to DIM ($i.e., \beta_2$ in Equation [4.3]); but the effect of parity on the estimated nonlinear coefficient for DIM ($i.e., \beta_4$ in Equation [4.3]) was not significant ($P = 0.68$) and therefore removed.

The NLIN model was superior to the LIN model for all fit statistics, and therefore we selected a nonlinear relationship for the effect of DIM on DMI (Table 4.3). We propose the NLIN model with body weight expressed in BW as in Equation [4.6].

DMI (kg/d) = [(3.7 + Parity * 5.7) + 0.305 * MilkE (Mcal/d) + 0.022 * BW (kg) + (– 0.689 + Parity * (– 1.87)) * BCS] * [1 – (0.212 + Parity* 0.136) * $exp^{(-0.053*DIM)}$]

[4.6]

where Parity is equal to 1 if animal is multiparous and 0 if primiparous.

The diagonal values of the estimated ***G*** matrix, which represent the estimated variance components for the diet specific intercept, MilkE, and BW, respectively, were $\hat{\sigma}^2_{u_0} = 0.56$, $\hat{\sigma}^2_{u_1} = 0.056$, and $\hat{\sigma}^2_{u_2} = 0.000247$. The estimated DMI for multiparous animals adjusted to the mean values of MilkE, BW, BCS, and DIM as reported in Table 4.2 and using Equation [4.6] is 24.46 kg. Based on the multivariate normality assumption as invoked in Equation [4.3], this implies

that roughly 95% of the diets lead to a mean intake within 24.46 $\pm 2 * \sqrt{\hat{\sigma}^2_{u_0}}$ or [22.99kg,25.98kg] for multiparous animals adjusted to mean values of MilkE, BW, BCS, and DIM. Similarly, 95% of the diets would have a partial regression of DMI on MilkE of 0.305 (from Equation [4.6]) $\pm 2 * \sqrt{\hat{\sigma}^2_{u_1}}$ or [-0.17kg/Mcal, 0.78kg/Mcal] whereas 95% of the diets would have a partial regression of DMI on BW of 0.022 (from Equation [4.6]) $\pm 2 * \sqrt{\hat{\sigma}^2_{u_2}}$ or [-0.01kg/kg, 0.053kg/kg]. This variability across diets naturally reflects, for example, differences in the energy density across different diets whereas negative lower bounds likely reflect that these diet effects are either not precisely normally distributed or based on the relatively small number of observations.

For the comparison of the proposed model (Equation [4.6]) and the NRC (2001) DMI prediction equation, the effects of model, stage, and interaction between model and stage were highly significant ($P < 0.01$, Table 4.4) for all fit statistics. First, related to the effect of "model," the proposed NLIN model was superior to the NRC (2001) model for all fit statistics (Table 4.4). Regarding the effect of "stage," the prediction equations performed better for cows during mid-lactation (CCC, slope bias, MSEP, RMSEP), followed by early-lactation and lastly by late-lactation. The interaction between model and stage of lactation was significant, where the proposed model was slightly better than the NRC (2001) model for early lactation but much superior to NRC (2001) for mid- and late-lactation (Table 4.4). The predicted vs. observed plot for each stage of lactation is presented in Figures 4.2, 4.3, and 4.4.

On dairy farms, diets are fed to a group of animals that contains a mix of primiparous and multiparous cows. In this case, Equation [4.6] can be modified to represent the proportion

between primiparous and multiparous in the group. As an example, in our training dataset the ratio between primiparous and multiparous were 44:66, and therefore, to use the equation for the entire training dataset the estimated coefficient for parity will be multiplied by 0.66 (proportion of multiparous cows in the dataset). As a result, the prediction equation for DMI for the average cow in the training dataset is presented in Equation [4.7]. The Equation [4.7] was named as a simplified proposed model.

$$\widehat{DMI} = (6.89 + 0.305 * MilkE + 0.022 * LBW - 1.74 * BCS) * \left(1 - 0.288 * exp^{(-0.053 * \mathrm{DIM})}\right) \quad [4.7]$$

The difference in the prediction of DMI by the proposed model (Equation [4.6]) and the simplified proposed model (Equation [4.7]) is presented in Table 5. We recommend the use of the Equation [4.6] rather than the Equation [4.7]. However, when parity information is not known the Equation [4.7] could be used as an alternative.

DISCUSSION

This study shows that the NRC 2001 model does a reasonably good job of predicting intake in Holstein cows, but the proposed model was clearly superior to the NRC 2001 model in the independent validation analysis, especially during the mid- and late-lactation. We suspect this is because we had data from more cows and included parity and BCS in our prediction.

The DIM adjustment for the prediction of DMI is necessary due to the peculiarities of different postpartum stages over and beyond the effects of MilkE or BW (Dann et al., 1999; Drackley, 1999; Chan et al., 2006; Janovick et al., 2011; Allen, 2014). For the proposed model, as in Roseler (1997), the greatest discounts in DMI are early in lactation and not important after

70 DIM (Table 4.6). Multiparous animals had proportionately greater deviance in the effect of DIM on DMI than primiparous in early lactation (Table 4.6). As an example, at the onset of the lactation (1 DIM), multiparous cows have a discount of 21% on the predicted DMI based on its MilkE, LBW, and BCS, whereas primiparous cows have a discount of 13%.

The NRC (2001) applies a more aggressive discount due to DIM on the DMI than the proposed model as shown in Table 4.6. The difference in the effect of DIM on DMI observed between the two equations may be from differences in the covariates used to derive the equation. That is, whereas the proposed model included the effect of parity and DIM expressed as days, the NRC (2001) does not account for differences in parity and use the week of lactation. Furthermore, the NRC (2001) prediction equation for DMI was derived mostly with data collected in the 1990's, such that it is likely the data were collected on animals with lower milk production and lower body size than the animals in our database (mostly data collected post-2007). Additionally, since 2000, many researchers have focused on better understanding the mechanisms that control feed intake and the development of strategies to maximize intake during early-lactation (Drackley, 1999; Allen, 2000; Grummer et al., 2004; Allen et al., 2005; Allen and Bradford, 2006; Allen et al., 2009). With the advancement in these areas, we believe that differences in management, environments, and diet formulation could explain the difference observed between the proposed and NRC (2001) models with respect to the effect of DIM.

The estimated partial regression coefficients of DMI on milk production and energy required for maintenance based on the proposed model, 0.305 kg/Mcal and 0.022 kg/kg of LBW, respectively, differed from that of the NRC (2001) model using FCM and BW^0.75, 0.372 kg/kg of FCM and 0.0968 kg/kg$^{0.75}$ of BW, respectively (which convert to 0.330 kg/Mcal and 0.018 kg/kg of LBW). In other words, the proposed model assumes a lower DMI per unit of MilkE

than the NRC (2001) model but a higher DMI required for maintenance. However, as described above, our analysis also suggested important variability across diets on these estimated partial coefficients. Therefore, the differences observed between the estimated coefficients and the NRC (2001) model likely depends on diet differences as well.

The fact that the proposed model implies a lower DMI per unit of milk can be attributed to two broad categories of factors: animal and/or diet characteristics. The database used in this analysis is composed of animals that likely have greater milk production as compared to the database utilized by the NRC (2001). With respect to diet characteristics, the diets fed to the cows in our database might have been higher in concentrates than the diets fed to the cows used in the NRC (2001) equation because they were higher producers. Additionally, Tempelman et al. (2015), using data only from mid-lactation cows, estimated coefficients for MilkE ranging from 0.30 to 0.38 and for BW^0.75 ranging from 0.10 to 0.13, for the US stations, which are more close to the estimated coefficients in this paper than the coefficients reported by the NRC (2001). Figure 4.5 illustrates the behavior of the proposed and NRC (2001) models over a lactation curve of a dairy cow using the observed DMI, MilkE, and BW of the average cow in the validation dataset.

A substantial difference between the proposed model and the NRC (2001) DMI prediction equation is that our model includes the effect of BCS. The effect of BCS was highly significant ($P < 0.01$). The estimated coefficient for BCS suggested that for each 1-unit increase on BCS the animal reduces her intake by 0.69 and 2.6 kg/d for primiparous and multiparous, respectively. This result implies that thinner animals are hungrier and consume more feed than fatter animals, which agrees with long-term mechanisms associated with the maintenance of BW (i.e., leptin; Block et al., 2003; Allen, 2014). The interaction between parity and BCS suggested

that BCS has a greater impact on DMI for multiparous cows than for primiparous cows. This fact was expected since primiparous animals are still growing while multiparous animals are generally close to mature size. We derived the Equation [4.7] to illustrate how the proposed model (Equation [4.6]) would be applied to a group of cows where the proportion of primiparous and multiparous animals are known.

CONCLUSION

The proposed model to predict DMI contains the effect of MilkE, LBW, BCS, parity, and DIM. This model differs from the NRC (2001) DMI prediction equation in regards that the NRC model contains only FCM, MBW, and WOL. Comparing both models, proposed model and NRC (2001), using an independent validation dataset, both models were similar to predict DMI during early-lactation (1 – 75 DIM), the proposed model outperformed the NRC (2001) during mid- and late-lactation (76 – 368 DIM). Finally, in regard to the estimated coefficients for milk and body weight, the proposed model suggests a lower DMI for both milk production and the energy required for maintenance.

APPENDICES

APPENDIX A

Tables

Table 4.1. Frequency of number of studies, diets, cows, and lactations by state.

Station[1]	No. of studies	No. of diets	No. of cows	No. of lactations	No. of weekly records	Average number of weekly records per lactation
AGIL	3	6	675	998	10,847	10.9
UF	17	54	523	643	6,222	9.7
ISU	3	5	955	1,021	10,457	10.2
MSU	21	76	339	540	5,922	11.0
CRDC	1	23	237	361	1,454	4.0
PANC	2	2	177	206	2,984	14.5
Miner	2	7	125	125	1,301	10.4
OSU	2	11	124	124	1,851	14.9
VT	6	18	111	112	943	8.4
UW	43	132	1,431	1,695	17,472	10.5
FRC	5	10	88	120	1,804	12.4
TOTAL	105	344	4,785	5,945	61,206	10.3

[1]AGIL = USDA Animal Genomics Improvement Laboratory, UF = University of Florida, ISU = Iowa State University, MSU = Michigan State University, CRDC = Cargill Research & Development Center, PANC = Purina Animal Nutrition Center, Miner = Miner Institute, OSU = The Ohio State University, VT = Virginia Tech, UW = University of Wisconsin-Madison, FRC = USDA Forage Research Center

Table 4.2. Number of observations (n), mean, and standard deviation (SD) of the covariates used to model (Training dataset) and validate (Validation dataset) the proposed equations for dry matter intake.

Variables[1]	Training dataset			Validation dataset		
	n	Mean	SD	n	Mean	SD
DMI, kg/d	47,253	24.4	4.62	13,953	24.1	4.53
MilkE. Mcal/d	47,253	29.4	6.30	13,953	29.1	6.47
BW, kg	47,253	625	81.7	13,953	631	86.9
BW^0.75, $kg^{0.75}$	47,253	125	12.2	13,953	126	13.0
ΔBW, kg/d	42,867	0.039	0.217	11,265	0.005	0.309
BCS (scale 1 to 5)	31,631	3.04	0.457	9,050	2.99	0.429
Ht (cm)	9,759	149	5.92	6,401	149	7.31
DIM (days)	47,253	105	50.0	13,953	114	61.7

[1]DMI = dry matter intake, MilkE = milk energy, BW = body weight, BW^0.75 = metabolic body weight, ΔBW = change in body weight, BCS = body condition score, Ht = height, and DIM = days in milk.

Table 4.3. Fit Statistics for the cross-validation across studies of the candidate models (LIN: linear, NLIN: non-linear) using the modeling dataset.

Fit Statistics[1]	LIN[2]	NLIN[2]	SEM[3]	*P*-value[4]
CCC	0.751	0.800	0.006	<0.01
Mean Bias	-0.53	0.008	0.059	<0.01
Slope Bias	0.050	0.033	0.009	0.03
MSEP	10.9	6.83	0.143	<0.01
RMSEP	3.20	2.61	0.073	<0.01
Decomposition of MSEP, %				
Mean Bias	13.6	6.90	0.616	<0.01
Slope Bias	1.96	2.28	0.184	0.09
Random Error	84.4	90.8	0.647	<0.01

[1]CCC: concordance correlation coefficient, MSEP: mean square error of prediction, RMSEP: root mean square error of prediction; [2]The two modeling strategies, where the LIN model is a linear model including a fourth-order polynomial of DIM, and the NLIN model contain the nonlinear effect of DIM; [3]Standard error of the mean common to both LIN and NLIN; [4]For the mean comparison of LIN and NLIN

Table 4.4. Fit Statistics for the across study stage of lactation (early, mid, and late) specific cross-validation performance of the proposed and NRC (2001) models using the independent validation dataset.

Fit Statistics[1]	Proposed Model			NRC (2001) Model			SEM[3]	*P*-values		
	Early[2]	Mid[2]	Late[2]	Early[2]	Mid[2]	Late[2]		Model	Stage	Model*Stage
CCC	0.717^{Aa}	0.735^{Aa}	0.688^{Ab}	0.702^{Ba}	0.681^{Ba}	0.645^{Bb}	0.010	<0.01	<0.01	0.01
Mean Bias	-0.281^{Aa}	0.058^{Ab}	0.293^{Aa}	-0.801^{Ba}	-1.54^{Bb}	-1.45^{Ba}	0.012	<0.01	<0.01	<0.01
Slope Bias	-0.031^{Aa}	0.028^{Ab}	0.068^{Ac}	-0.137^{Ba}	-0.117^{Bb}	-0.016^{Bc}	0.014	<0.01	<0.01	<0.01
MSEP	10.2Ac	7.13^{Aa}	8.42^{Ab}	11.8^{Bc}	10.5^{Ba}	11.3^{Bb}	0.339	<0.01	<0.01	<0.01
RMSEP	3.19^{Ac}	2.67^{Aa}	2.90^{Ab}	3.42^{Bc}	3.23^{Ba}	3.32^{Bb}	0.048	<0.01	<0.01	<0.01
Decomposition of MSEP, %										
Mean Bias	7.15^{Aa}	3.45^{Ab}	8.02^{Ab}	12.0^{Ba}	25.9^{Bb}	24.2^{Bb}	1.57	<0.01	<0.01	<0.01
Slope Bias	2.73^{c}	0.74^{a}	3.91^{b}	5.24^{c}	2.28^{a}	1.32^{b}	0.564	0.13	<0.01	<0.01
Random Error	90.2^{Aa}	95.8^{Ab}	88.1^{Ac}	82.7^{Ba}	71.8^{Bb}	74.5^{Bc}	1.60	<0.01	<0.01	<0.01

[1]CCC: concordance correlation coefficient, MSEP: mean square error of prediction, RMSEP: root mean square error of prediction; [2]Stage of lactation: early - 1 to 75 days in milk; mid - 76 to 175 days in milk; and late - 176 to 368 days in milk; [2]Mean bias; [3]Standard error of the mean common to all models; different capital letter within the fit statistic represent statistical differences for model; and different lower letter within the fit statistic represent statistical differences for stage

Table 4.5. Fit Statistics for the cross-validation across studies of the proposed model and simplified model using the validation dataset.

Fit Statistics[1]	Proposed[2]	Simplified[2]	SEM[3]	P-value[4]
CCC	0.721	0.714	0.006	0.52
Mean Bias	0.021	0.025	0.004	0.47
Slope Bias	0.059	0.062	0.009	0.01
MSEP	8.36	9.05	0.241	0.01
RMSEP	2.89	3.01	0.038	0.01
Decomposition of MSEP, %				
Mean Bias	5.70	6.32	0.667	0.02
Slope Bias	2.11	2.15	0.218	0.85
Random Error	92.2	91.5	0.646	0.02

[1]CCC: concordance correlation coefficient, MSEP: mean square error of prediction, RMSEP: root mean square error of prediction; [2]The proposed model with parity effect and the simplified model using the proportion of the primiparous: multiparous (44:56) cows in the training dataset; [3]Standard error of the mean common to both LIN and NLIN; [4]For the mean comparison of LIN and NLIN

Table 4.6. Nonlinear adjustment of days in milk on the predicted dry matter intake estimated by the proposed (primiparous and multiparous) and NRC (2001) models.

Days in Milk	Proposed Model Primiparous	Proposed Model Multiparous	NRC 2001
10	0.875	0.795	0.624
20	0.927	0.880	0.714
30	0.957	0.929	0.783
40	0.975	0.958	0.835
50	0.985	0.976	0.875
60	0.991	0.986	0.905
70	0.995	0.992	0.928
80	0.997	0.995	0.945
90	0.998	0.997	0.958
100	0.999	0.998	0.968
110	0.999	0.999	0.976
120	1.000	0.999	0.982
130	1.000	1.000	0.986
140	1.000	1.000	0.989
150	1.000	1.000	0.992

APPENDIX B

Figures

a)

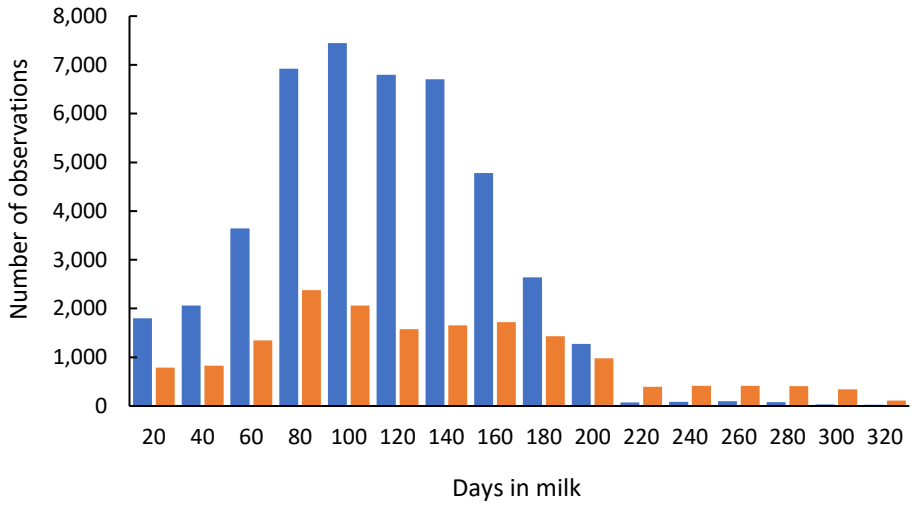


Figure 4.1. Distribution of the number of observations in the training (■) and in the validation (■) datasets.

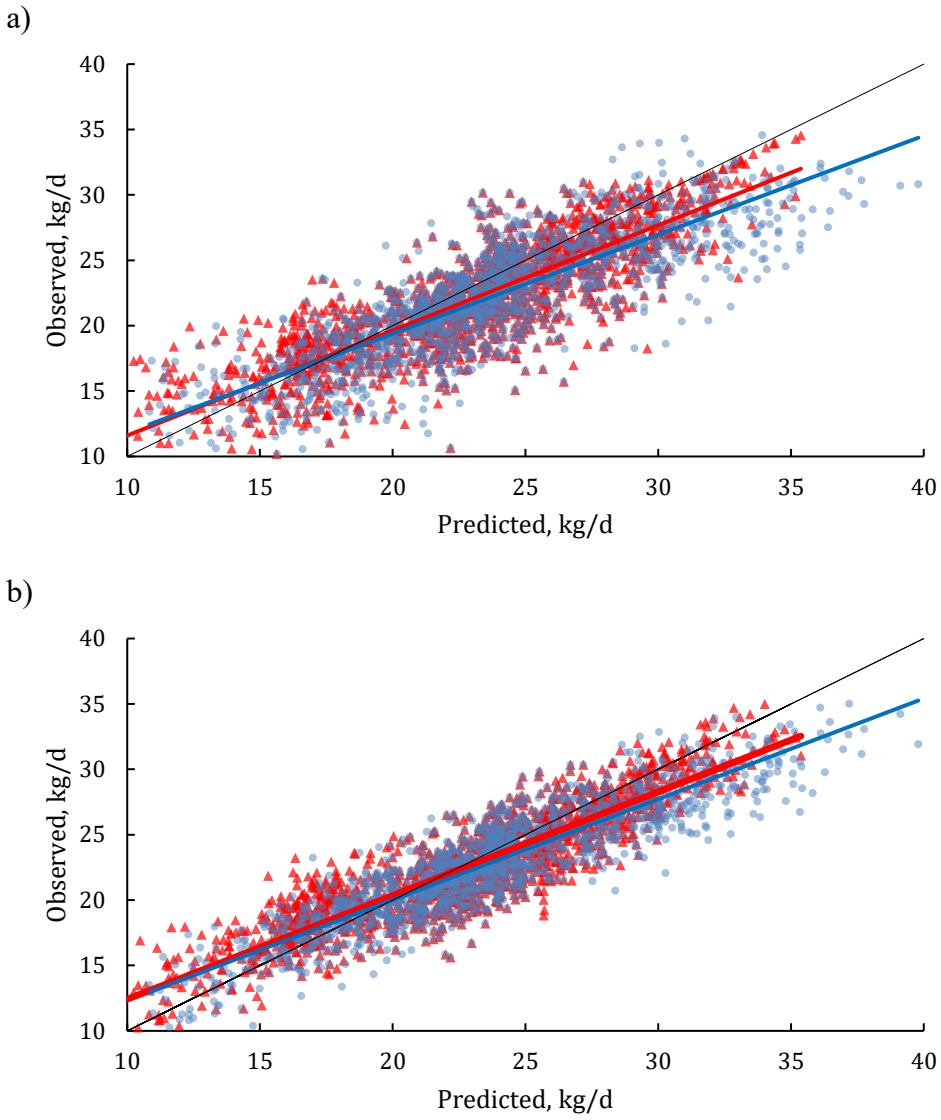
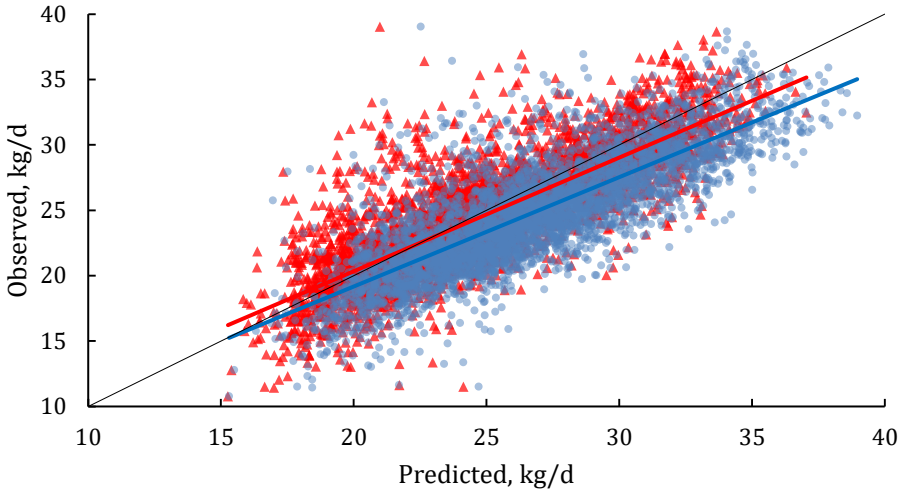


Figure 4.2. Plot of the predicted versus observed dry matter intake (DMI) using the NRC 2001 (——) and the proposed (——) models for the early-lactation period (1 to 75 days in milk). Each data point (NRC 2001 - ● , Proposed model - ▲) represents the raw (a) and the adjusted for random effects (b) individual weekly DMI.

a)



b)

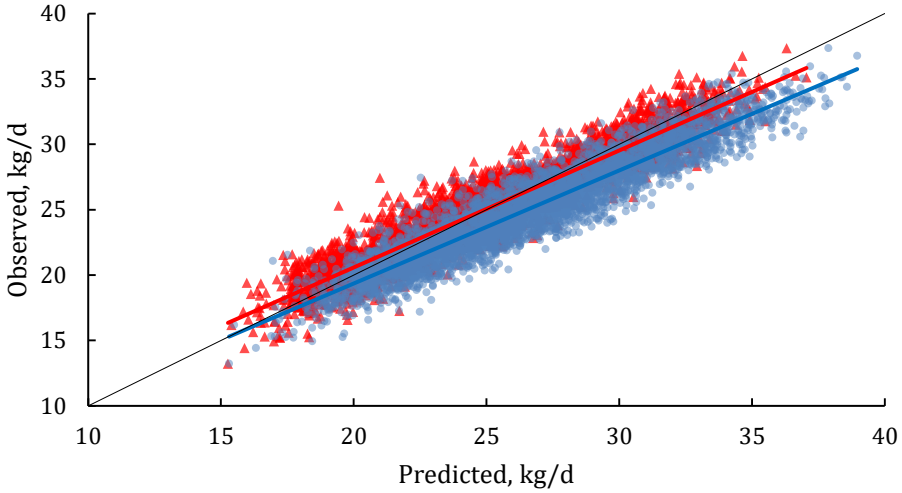
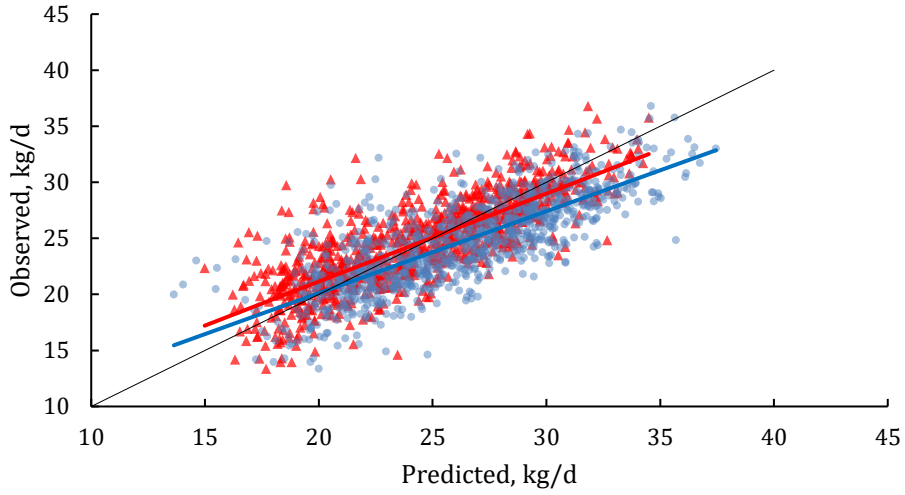


Figure 4.3. Plot of the predicted versus observed dry matter intake (DMI) using the NRC 2001 (—) and the proposed (—) models for the mid-lactation period (76 to 175 days in milk). Each data point (NRC 2001 - ● , Proposed model - ▲) represents the raw (a) and the adjusted for random effects (b) individual weekly DMI.

a)



b)

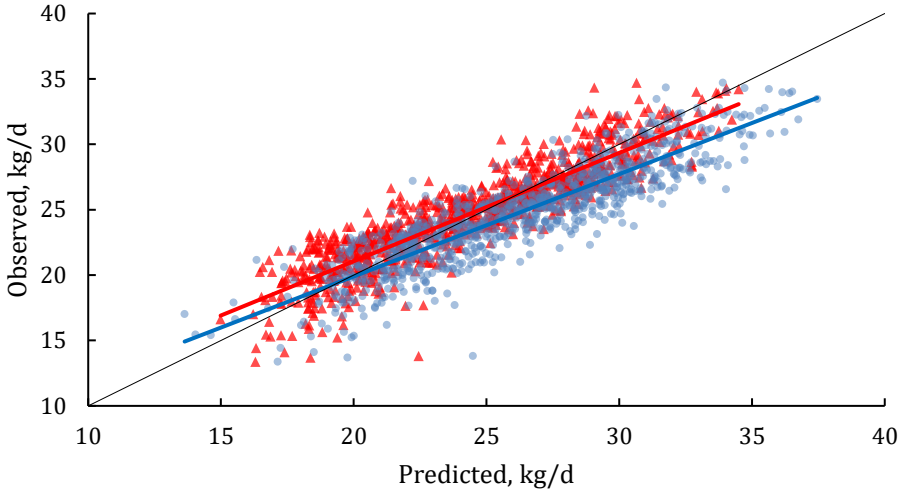


Figure 4.4. Plot of the predicted versus observed dry matter intake (DMI) using the NRC 2001 (—) and the proposed (—) models for the late-lactation period (greater than 176 days in milk). Each data point (NRC 2001 - ● , Proposed model - ▲) represents the raw (a) and the adjusted for random effects (b) individual weekly DMI.

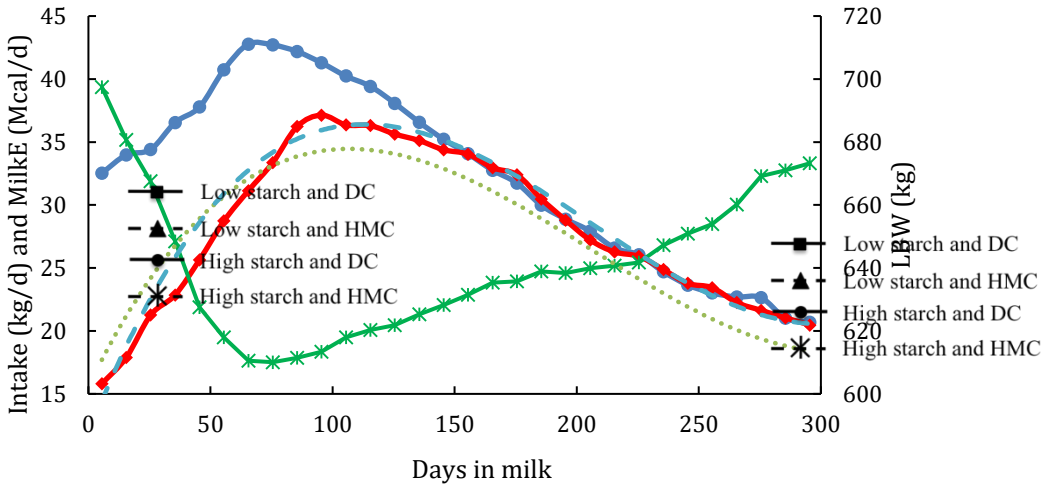


Figure 4.5. Curve of lactation on the average Holstein dairy cow. Where the solid lines represent the observed milk energy (MilkE,), observed dry matter intake (DMI,), and observed body weight (BW,), and the dashed lines represent the predicted dry matter intake by the NRC 2001 ( ) and proposed ( ) models.

APPENDIX C

Supplemental Table

Supplemental Table 4.1. Prediction of DMI using the two-step model on crossbreeds and pure Jersey dairy cows in total mixed ration and grazing system.

Study	Ref	Breed	Country	System	DMI (kg/d)	BW (kg)	DIM (d)	MilkE (Mcal/d)	BCS
Leiva et al., 2018	J. Dairy Sci. 10: 1 - 14	Gir x Holstein	Brazil	Total mixed ration	22 ± 1.6	573	105	16.5	3.41
					23 ± 1.6	587	105	17.0	3.48
Coffey et al., 2017	J. Dairy Sci. 100: 7556 - 7568	Jersey x Holstein	Ireland	Grazing system	17 ± 0.2	467	129	21.4	2.97
					16 ± 0.2	466	129	17.5	2.96
					15 ± 0.2	443	129	17.5	2.96
Al-Marashdeh et al., 2016	J. Dairy Sci. 99: 7123 - 7132	Jersey x Friesian	New Zeland	Grazing system	12 ± 0.2	465	207	10.8	3.27
					10 ± 0.2	466	207	11.6	3.35
					11 ± 0.2	466	207	12.1	3.31
Hynes et al., 2016	J. Dairy Sci. 99: 8111 - 8120	Swedish Red x Holstein	Ireland	Grazing system	20 ± 0.5	573	157	19.0	2.39
Kristensen et al., 2015	J. Dairy Sci. 98: 263 - 274	Jersey	Denmark	Total mixed or partial mixed ration	19 ± 1.2	414	191	21.4	3.00
Vance et al., 2012	J. Dairy Sci. 95: 1527 - 1544	Jersey x Holstein	Ireland	Total mixed ration	20 ± 0.7	578	150	23.0	3.00
				Grazing system	18 ± 0.7	528	150	18.4	3.00
Xue et al., 2011	J. Dairy Sci. 94: 1455 - 1464	Jersey x Holstein	United Kingdom	Total mixed ration	15 ± 0.4	466	175	15.0	2.70
					18 ± 0.4	473	175	17.3	2.75
Prendiville et al., 2009	J. Dairy Sci. 92: 6176 - 6185	Jersey	Ireland	Grazing system	15 ± 0.2	369	175	12.4	2.93
		Jersey x Holstein			16 ± 0.2	448	175	13.9	3.00
Heins et al., 2008	J. Dairy Sci. 91: 3716 - 3722	Jersey x Holstein	United States	Total mixed ration	22 ± 0.4	467	75	21.0	2.90

APPENDIX D

SAS Macro: Model Validation

```
DATA example;
INPUT A$ B$ C$ Y X1 X2 X3 X4 X5 X6;
DATALINES;
```

A1	B1	C1	23	7	11	20	25	32	39
A1	B1	C2	16	9	14	15	26	31	39
A1	B2	C1	18	9	14	20	29	34	39
A1	B2	C2	23	7	15	17	26	31	39
A1	B3	C1	18	8	14	20	28	35	38
A1	B3	C1	.	8	.	20	28	35	38
A1	B3	C2	22	8	10	15	25	34	36
A1	B4	C1	16	10	13	19	29	33	40
A1	B4	.	16	.	13	19	.	33	40
A1	B4	C2	18	10	12	16	28	32	39
A2	B1	C1	16	8	11	19	30	35	40
A2	B1	C2	16	8	14	15	28	33	37
A2	B2	C1	17	7	14	19	30	33	35
A2	B2	C2	20	9	12	16	29	32	39
A2	B3	C1	28	9	11	20	25	33	38
A2	B3	C2	11	9	10	15	27	33	39
A2	.	C1	21	5	13	15	25	.	36
A2	B4	C1	21	5	13	15	25	35	36
A2	B4	C2	12	5	14	17	30	31	36
A3	B1	C1	17	5	14	20	25	35	35
A3	B1	C2	27	10	15	17	28	32	37
.	B1	.	17	5	14	.	25	35	35
A3	B2	C1	19	9	11	15	27	30	35
A3	B2	C2	29	6	13	15	28	35	35
A3	B3	C1	26	9	11	19	30	31	37
A3	B3	C2	19	10	14	19	27	32	39
A3	B4	C1	19	8	10	17	28	30	35
A3	B4	C2	10	9	11	20	28	34	36
A4	B1	C1	20	6	11	18	30	35	37
A4	B1	C2	25	7	14	20	28	33	35
A4	B2	C1	21	5	13	16	26	35	38
A4	B2	C2	17	9	10	20	27	31	35
A4	B3	C1	12	9	15	20	27	31	38
A4	B3	.	12	.	15	20	27	31	.
A4	B3	C2	10	10	14	15	30	33	35
A4	B4	C1	23	8	12	17	26	32	35
A4	B4	C2	13	9	13	16	30	33	35
A5	B1	C1	29	6	13	17	26	32	39
A5	B1	C2	26	10	14	20	30	33	37
A5	B2	C1	28	5	14	18	30	33	36
A5	B2	C2	28	5	12	19	26	35	40
A5	.	C1	17	7	.	20	27	33	40
A5	B3	C1	17	7	11	20	27	33	40

```
A5    B3    C2    12    10    12    18    28    32    35
A5    B4    C1    10    5     12    17    27    32    38
A5    B4    C2    20    7     11    19    26    32    35
;

ODS HTML CLOSE;
ODS HTML;
TITLE 'HPMixed model analysis';
TITLE2 'Observations that need to be thrown out because of missing covariates';
PROC HPMIXED DATA = example;
  CLASS A B C; /*enter your classificatory variables*/
  MODEL Y = X1 X2 X3 X4 X5 X6 /*fit the fullest model*/
  /SOLUTION CL;
  RANDOM int /SUBJECT=A;/*enter first set of random effects here (if necessary)*/
  RANDOM int /SUBJECT=B;/*enter second set of random effects here (if necessary)*/
  RANDOM int /SUBJECT=C TYPE = un;/*enter third set of random effects here (if
necessary)*/
  OUTPUT OUT=output1 PRED=predm RESID=residm;
RUN;
TITLE; TITLE2; TITLE3;
DATA exampleclean (DROP=predm residm);
  SET output1;
  IF predm eq . THEN DELETE;
  IF Y eq . THEN DELETE; /* Y is the response variable*/
run;

DATA exampleclean;
  SET exampleclean;
  record = _n_;
RUN;

DATA _null;
  SET exampleclean;
  CALL symput('total',_n_)/* Number of records in cleaned-up data*/;
RUN;

***** Create the cross-validation folds for each several random partitions of the data *****;

%MACRO cvfold(npartition,nfold,n_size);
  %DO partition = 1 %TO &npartition %BY 1;
          DATA temp;
                DO record = 1 TO &total;
                random_num = ranuni(-1);
        OUTPUT;
           END;
          RUN;
```

```
        PROC SORT DATA=temp;
                BY random_num;
        RUN;
        DATA partition&partition (DROP=counter random_num);
                SET temp;
                counter = _n_;
                fold&partition = floor(&nfold*(counter-1)/&n_size)+ 1;
        RUN;
        PROC SORT DATA=partition&partition;
                BY record;
        RUN;
 %END;
%MACRO append2;
 %DO i = 1 %TO &npartition ;
 partition&i
 %END;
%MEND append2;
DATA finalpartition;
 MERGE %append2;
 BY record;
RUN;
TITLE "Double check on balance of folds within each partition";
PROC FREQ DATA=finalpartition ;
 TABLE fold1-fold&npartition;
RUN;
%MEND cvfold;

***** Macro to conduct the cross-validation *****;

%MACRO cvrandom(npartition,nfold,datasource,model);
PROC DATASETS LIBRARY=work NOLIST;
 DELETE CVsummary&model;
RUN;
%DO partition = 1 %TO &npartition %BY 1;
 %DO fold = 1 %TO &nfold  %BY 1;
 DATA partition&partition.fold&fold;
   MERGE &datasource finalpartition(keep=fold&partition record);
         BY record;
   IF fold&partition =&fold THEN
    DO;
     partition = 'validation';
                Y = .; /* Y is the response variable*/
         END;
        ELSE partition = 'training';
 RUN;
 ODS EXCLUDE ALL;
```

```
PROC HPMIXED DATA= partition&partition.fold&fold;
  ID record partition;
  CLASS A B C; /*enter your classificatory variables*/
  MODEL Y = %fixedeffects; /* Y is the response variable*/
  %randomeffects;
        OUTPUT OUT=p&partition&fold PRED=pred;
RUN;
ODS EXCLUDE NONE;
DATA pcheck&partition&fold;
  UPDATE p&partition&fold &datasource;
  BY record;
RUN;
ODS EXCLUDE ALL;
PROC CORR DATA=pcheck&partition&fold COV OUTP=COV&partition&fold ;
  WHERE partition = 'validation';
  VAR Y Pred; /* Y is the response variable*/
RUN;
ODS EXCLUDE NONE;
** Save key statistics;
DATA null;
  SET cov&partition&fold;
        **variance for obs;
        IF _TYPE_="COV" and _NAME_ = "Y" THEN CALL symput('var1',Y); /* Y is the
response variable*/
        **variance for pred;
        IF _TYPE_="COV" and _NAME_ = "pred" THEN CALL symput('var2',pred);
        **covariance between obs and pred;
        IF _TYPE_="COV" and _NAME_ = "Y" THEN CALL symput('cov12',pred);
        **mean for obs;
        IF _TYPE_="MEAN"  THEN CALL symput('mean1',Y); /* Y is the response variable*/
        **mean for pred;
        IF _TYPE_="MEAN"  THEN CALL symput('mean2',pred);
        **correlation between obs and pred;
        IF _TYPE_="CORR" and _NAME_ = "Y" THEN CALL symput('cor12',pred);
RUN;
DATA ccc&partition&fold;
  **compute CCC and correlation;
  ccc = 2*&cov12/(&var1+&var2+(&mean1-&mean2)**2);
        corr = &cor12;
        fold = &fold;
        partition = &partition;
RUN;
DATA SSEP&partition&fold;
  **compute MSEP and RMSEP and its components;
  SET pcheck&partition&fold ;
        WHERE partition = 'validation';
```

```
        msep = (Y-pred)**2;
        odev = (Y-&mean1);
        pdev = (pred-&mean2);
RUN;
PROC MEANS DATA=SSEP&partition&fold SUM N NOPRINT;
  VAR msep odev pdev;
        OUTPUT OUT=msep&partition&fold (drop=_type_ _freq_) SUM= N=  /AUTONAME;
RUN;
DATA msep&partition&fold (KEEP=msep rmsep ECT ECR ED fold partition);
  SET msep&partition&fold;
        msep = msep_Sum/msep_N;
        rmsep = sqrt(msep);
        s2p = (pdev_N-1)*&var2/pdev_N;
        s2o = (odev_N-1)*&var1/odev_N;
        **compute mean bias as % of MSEP;
  ECT = (((&mean2-&mean1)**2)*100)/MSEP;
        **compute slope bias % of MSEP;
        ECR = (((sqrt(s2p) - &cor12*sqrt(s2o))**2)*100)/MSEP;
        **compute random error % of MSEP;
  ED = (((1-&cor12**2)*s2o)*100)/MSEP;
        fold = &fold;
        partition = &partition;
RUN;
DATA slopebias&partition&fold;
  SET pcheck&partition&fold;
        WHERE partition = 'validation';
        residual = Y-pred;
        **compute slope bias for residuals on centered predictions;
        pred_dev = pred-&mean2;
RUN;
ODS EXCLUDE ALL;
PROC REG DATA=slopebias&partition&fold  ;
 MODEL residual=pred_dev;
       ODS OUTPUT ParameterEstimates = ParameterEstimates&Partition&Fold;
RUN;
ODS EXCLUDE NONE;
PROC TRANSPOSE DATA=ParameterEstimates&Partition&Fold
OUT=slopebiasestimates&partition&fold ;
RUN;
DATA slopebiasestimates&partition&fold (KEEP = fold partition intercept_res slope_res);
  SET slopebiasestimates&partition&fold;
  IF _NAME_ = "Estimate";
        fold = &fold;
        partition = &partition;
        intercept_res = COL1;
        slope_res = COL2;
```

```
  RUN;
  DATA catchall&partition&fold;
    MERGE ccc&partition&fold msep&partition&fold slopebiasestimates&partition&fold;
          BY partition fold;
  RUN;
  PROC APPEND BASE=CVsummary&model DATA=catchall&partition&fold FORCE;
  RUN;
  PROC DATASETS LIBRARY=work NOLIST;
    DELETE pearson&partition&fold msep&partition&fold cov&partition&fold
partition&partition.fold&fold slopebiasestimates&partition&fold;
  RUN;
  %END;
%END;
  DATA CVsummary&model;
    SET CVsummary&model;
          model = &model;
    fixed = "%fixedeffects";
    random = "%randomeffects";
  RUN;
  ODS results ON;
  TITLE "Final summary statistics for Model &model";
  PROC PRINT DATA=CVsummary&model;
  RUN;
%MEND cvrandom;

***** Validation structure *****;

%LET npartition=2;  /* specify number of partitions */
%LET nfold = 5;     /* specify number of folds per partition */

%cvfold(&npartition,&nfold,&total);

** Model Description;

* Model 1;
%LET model = 1;
  %MACRO fixedeffects;
    X1 X2 /* define the fixed effects for Model 1*/
  %MEND fixedeffects;
  %MACRO randomeffects;
    RANDOM int /SUBJECT=A; /* define the random effects for Model 1*/
    RANDOM int /SUBJECT=B; /* define the random effects for Model 1*/
    RANDOM int /SUBJECT=C TYPE=un; /* define the random effects for Model 1*/
  %MEND randomeffects;
  %cvrandom(&npartition,&nfold,exampleclean,&model);
```

```
* Model 2;
%LET model = 2;
  %MACRO fixedeffects;
    X3 X4 /* define the fixed effects for Model 2*/
  %MEND fixedeffects;
  %MACRO randomeffects;
    RANDOM int /SUBJECT=A; /* define the random effects for Model 2*/
    RANDOM int /SUBJECT=B; /* define the random effects for Model 2*/
    RANDOM int /SUBJECT=C TYPE=un; /* define the random effects for Model 2*/
  %MEND randomeffects;
  %cvrandom(&npartition,&nfold,exampleclean,&model);

* Model 3;
%LET model = 3;
  %MACRO fixedeffects;
    X5 X6 /* define the fixed effects for Model 3*/
  %MEND fixedeffects;
  %MACRO randomeffects;
    RANDOM int /SUBJECT=A; /* define the random effects for model 3*/
    RANDOM int /SUBJECT=B; /* define the random effects for model 3*/
    RANDOM int /SUBJECT=C TYPE=un; /* define the random effects for model 3*/
  %MEND randomeffects;
  %cvrandom(&npartition,&nfold, exampleclean,&model);

** Models comparisons;

DATA CVsummary;
  SET CVsummary1 CVsummary2 CVsummary3;
  block = compress(partition||fold);
RUN;
%MACRO comparemodels(response);
ODS EXCLUDE ALL;
TITLE "Summary for comparing models for &response";
PROC MIXED DATA=CVsummary;
  CLASS block model;
  MODEL &response = model;
  LSMEANS model /diff;
  RANDOM block;
  ODS OUTPUT lsmeans=lsmeans&response;
  ODS OUTPUT diffs=diffs&response;
RUN;
ODS EXCLUDE NONE;
PROC PRINT DATA=lsmeans&response;
RUN;
PROC PRINT DATA=diffs&response;
RUN;
```

```
PROC DATASETS LIBRARY=work NOLIST;
  DELETE lsmeans&response diffs&response;
RUN;
%MEND comparemodels;
%comparemodels(corr)
%comparemodels(ccc)
%comparemodels(intercept_res)
%comparemodels(slope_res);
%comparemodels(msep);
%comparemodels(ECT);
%comparemodels(ECR);
%comparemodels(ED);
%comparemodels(rmsep);
QUIT;
```

Instructions on how to use the SAS macro

The provided code is a generic SAS macro for model validation. We intend to provide a starting code for researchers that want to perform model validation in SAS; adaptations in the code are needed to fit the specificity of each database and the researcher goals. Given that, each researcher is in charge to develop his codes, and he can use as a start point the proposed generic SAS code.

To use the generic SAS code for model evaluation, the user will need to enter the following information: observed, fixed, and random variables. In the generic SAS code, the observed variable is “X,” the fixed variables are “Y1 to Y6”, and the random variables are “A, B, and C.” All random variables are classificatory variables. By default, the code allows comparison of 3 models using two partitions and five folds.

In the code, we included several comments to facilitate the adaptation of the generic SAS code to the case of each researcher. Please, follow the comments and pay attention if your variables have a different name from the variables used in the example.

Finally, the output is composed of 4 parts: (1) HPMixed model analysis, (2) Double check on the balance of folds within each partitioning, (3) Final summary statistics, and (4) Summary for comparing models.

(1) HPMixed model analysis: check if the code is reading the database properly.

(2) Double check on the balance of folds within each partitioning: here you can check how the database is partitioned.

(3) Final summary statistics: here is an overview of the fit statistics parameters and the fixed and random effects included in the model. By default, the code allows three models, so in this part of the output will be generated three tables, each one for one model. The fit statistic parameters are coded as:

ccc: concordance coefficient correlation;

corr: correlation between observed and predicted;

msep: mean square error of prediction;

rmsep: root mean square error of prediction;

ECT: mean bias (as % of MSEP, decomposition of the MSEP);

ECR: slope bias (as % of MSEP, decomposition of the MSEP);

ED: random error (as % of MSEP, decomposition of the MSEP);

intercept_res = mean bias;

slope_bias= slope bias;

(4) Summary for comparing models: in this part of the report each fit statistic parameter has two tables. The first table is testing if the estimated coefficient is different from zero, and in the second table is the comparison between the three models.

REFERENCES

REFERENCES

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CHAPTER 5

DIETARY FATTY ACID COMPOSITION AND DIGESTIBLE ENERGY IN LACTATING DAIRY COWS

ABSTRACT

The objective of this study was to determine the effect of dietary FA composition on dry matter (**DM**), neutral detergent fiber (**NDF**), FA, 16-carbon FA, 18-carbon FA digestibilities, and digestible energy intake per kilogram of DMI (**DMD**, **NDFD**, **FAD**, **16-CD**, **18-CD**, **EnergyD**, and **DEIkg** respectively). The database for this study was composed of 423 individual observations collected on 183 lactations from 124 Holstein mid-lactation cows receiving diets that varied in FA composition in 5 experiments conducted at Michigan State University. The ranges of C16:0, C18:0, C18:1, C18:2, and C18:3 were 0.33 to 1.9, 0.04 to 0.88, 0.42 to 1.0, 1.05 to 2.0, and 0.17 to 0.30 % of DM, respectively. Starch and forage NDF (**fNDF**) content varied from 26 to 29 and 19 to 21 % of DM, respectively, and DMI varied from 14 to 40 kg/d. The meta-regression was performed using the following fixed effects: all the FA variables described above, the FA variables' respective quadratic terms, 2-way interactions between each FA category and starch, fNDF, and DMI. The following were random effects and allowed for study-specific intercepts and slopes on DMI; cow nested within block and experiment, period nested within the experiment, and block nested within the experiment. The following effects were observed: C16:0 had a quadratic effect and C18:0 had a negative linear effect for DMD. Positive linear effects of C16:0, C18:1, and C18:2 were observed for NDFD, a quadratic effect of C18:0 was also observed for NDFD. C16:0 and C18:0 decreased linearly FAD. Quadratic effects of C16:0 and a negative linear effect of C18:0 were observed on the 16-CD. A positive linear effect of C16:0 and a negative linear effect of C18:0, C18:1, and C18:2 were observed on

18-CD. Quadratic effects of C16:0 and C18:0, and a positive linear effect of C18:3 were observed on EnergyD. Furthermore, quadratic effects of C16:0, C18:1, C18:2, and C18:3, as well as a negative linear effect of C18:0 were observed on DEIkg. To summarize, C16:0 and C18:0 were the FAs with the greatest impact on digestibility and DEIkg. It is worth mentioning that C18:2 also caused an impact; however, the impact occurred at lower magnitudes than C16:0 and C18:0. Lastly, C16:0 increased DEIkg when included in the diet up to 1.21% of DM, and C18:0 linearly decreased DEIkg. Similarly, to C16:0, when C18:2 was included in the diet up to 1.60% of DM, and DEIkg increased.

INTRODUCTION

Traditionally, fat supplements are added to dairy cow diets to increase dietary energy density and therefore increase energy intake. However, the effect of supplemental fat on energy intake is dependent on its potential impacts on dry matter intake (**DMI**) (Palmquist and Jenkins, 2017; Allen, 2000) and digestibility of fatty acids and other nutrients (Boerman et al., 2015; Weld and Armentano, 2017). In this regard, the effect of fat supplements on DMI is variable and usually depends on the type of fat being fed (Rabiee et al., 2012). For instance, in a meta-regression of 29 treatment means compiled from the literature, Allen (2000) observed that saturated fatty acid (**FA**) supplements did not affect DMI. In contrast, the hypophagic effect of feeding fat seems to be more pronounced for unsaturated than saturated FA supplements (Harvatine and Allen, 2006); with DMI decreasing linearly as the degree of unsaturation increases (Pantoja et al., 1994) and the chain length of FA infused into the abomasum increases (Drackley et al., 1992). With highly enriched (≥ 85%) FA supplements, some studies reported that palmitic acid (**C16:0**) and stearic acid (**C18:0)** decreased DMI (Lock et al., 2013; Rico et al., 2014a), while most of the studies reported that DMI was not affected when the animals were fed

at 1.5-2.3% of diet DM compared with a nonfat control diet (Piantoni et al., 2013; Piantoni et al., 2015; Boerman et al., 2017).

Regarding FA digestibility (**FAD**), previous studies have indicated differences in intestinal digestibility among different FA, including C16:0, C18:0, and oleic acid (**C18:1**) (Boerman et al., 2015; Glasser et al., 2008). Recently, Boerman et al. (2017) fed increasing levels of a C18:0-enriched supplement (~93% C18:0) to dairy cows and observed no positive effect on production responses. This was likely associated with the pronounced decrease in total FAD as FA intake increased. In contrast, Rico et al. (2017) fed increasing levels of a C16:0-enriched supplement (~89% C16:0) to dairy cows and observed a slight decrease in total FAD as FA intake increased, as well as a positive effect on production response when C16:0-enriched supplement was included up to 1.5% diet DM in the diet.

Although there are differences among specific free FAs digestibilities, the amount of FA included in the diet is relatively small for lactating dairy cattle when compared with other nutrients; and therefore, changes in FAD may have minimal effects on animal performance. However, different responses to specific free FAs supplements may be due to their impact on overall DM digestibility (**DMD**) and digestible energy intake (**DEI**). While this consideration was necessary, we were not aware of any research that has determined the impact of dietary FA profile and digestibility on energy digestibility (**EnergyD**) and DEI. Furthermore, potentially of greater importance was the fact that fat supplements may affect the digestibility of other nutrients, such as fiber (Palmquist, 1991).

A recent meta-analysis highlighted that the FA profile of supplemental fat might affect NDF digestibility (**NDFD**; Weld and Armentano, 2017). Weld and Armentano (2017) observed

that Ca-salts of palm FA did not affect NDFD while feeding saturated FA supplements (C16:0 + C18:0) increased NDFD. Additionally, recent studies feeding highly enriched C16:0 supplements have shown increases in NDFD (Piantoni et al., 2013; de Souza et al., 2016; Rico et al., 2017). Therefore, changes in the intake and digestibility of other nutrients due to fat supplementation may, in turn, affect the digestible energy available for milk production, body reserves, or both.

Studies that investigated effects of dietary FA composition on dairy cows' performance and nutrient digestibility have consistently shown that FA profile has a crucial impact on intake, nutrient digestibility, and energy partitioning (de Souza et al., 2017; Boerman et al., 2017). However, no study has yet been reported with a comprehensive analysis of the effect of dietary FA profile and other dietary factors on the DEI. With that in mind, our goal was to evaluate how dietary FA composition, in conjunction with other dietary factors, affects DEI. We hypothesized that fat supplements with a higher concentration of C16:0 would increase the digestible dietary energy because of the positive effect of FA on NDFD.

MATERIALS AND METHODS

Database

Our database was composed of the individual data points from 5 experiments performed between 2015 and 2017 at Michigan State University (**MSU**) that were designed to investigate the effect of dietary FA profile on animal performance, energy balance, and metabolism of lactating (25 to 180 DIM) Holstein dairy cows. In summary, the experiments used either a block, a Latin-square, or a split-plot design. Cows were housed in individual tie stalls and fed 115% of expected daily intake.

Additionally, cows were milked twice a day. The data regarding the milk yield, the feed offered, and the feed refused by the cows were recorded daily. The final database contained 423 observations from 183 lactations of 124 Holstein dairy cows with 20 treatments from 5 experiments. Two of these experiments were published in a peer-reviewed journal (de Souza et al., 2018; de Souza and Lock, 2018a), and three studies are still in the writing stage.

Samples of all diet ingredients (0.5 kg) and orts from each cow (~12.5%) were collected daily for five consecutive days to determine the apparent total-tract digestibility of nutrients. During the same week, fecal samples (500g) were collected every 15 hours during five days, representing every 3 hours of a 24-hour period to account for diurnal variations. Diet ingredients, orts, and fecal samples were dried at 55ºC in a forced-air oven for 72 hours for dry matter (**DM**) determination. Dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Feed ingredients, orts, and feces were analyzed for neutral detergent fiber (**NDF**), crude protein (**CP**), and starch concentration as described by Boerman et al. (2015). Fatty acid concentrations in feed ingredients, orts, and feces were determined as described by Lock et al. (2013). Gross energy was assayed by bomb calorimeter (Parr Instrument Inc., Moline, IL). Indigestible NDF was used as an internal marker to estimate fecal output to determine apparent total tract digestibility of nutrients (Cochran et al., 1986). Indigestible NDF was evaluated as NDF after a 240-h in vitro fermentation (Van Soest et al., 1991).

The variables included in the database were dietary starch (% of DM), NDF (% of DM), forage NDF (**fNDF**, % of DM), non-forage NDF (**nfNDF**, % of DM), total FA (% of DM), C16:0 (% of DM), C18:0 (% of DM), C18:1 (% of DM), linoleic acid (**C18:2**, % of DM), linolenic acid (**C18:3**, % of DM), others FA (**OthersFA**, % of DM; OthersFA = total FA – C16:0 – C18:0 – *cis-9* C18:1 – C18:2 – C18:3), gross energy (**GE,** Mcal/kg), nutrient

digestibility (NDF, total FA, 16-carbon FA, 18-carbon FA, and energy, all expressed in percentage), DEI expressed as Mcal/d and Mcal/kg of DM (**DEIday** and **DEIkg**, respectively), DMI (kg/d), and variables related to experimental design (period, block, and square). The description of the variables mentioned above is presented in Table 5.1.

Statistical Analysis

All the statistical analyses were performed using SAS v. 9.4 (SAS Institute Inc., Cary, NC). The first step to complete the statistical analyses was to standardize the covariates to have a mean of zero and a standard deviation of 1 using SAS PROC STANDARD. This step was necessary to provide numerical stability for the following analyses.

Based on the standardized database, a random regression model using SAS PROC HPMIXED was fitted to each of the six nutrient digestibilities (DMD, NDFD, FAD, 16-CD, 18-CD, and EnergyD) and DEI (DEIday and DEIkg) according to the following model [Equation 5.1]:

$$Y_{ijbk} = \left(\mu + Study_{0,k}\right) + \left(b_1 + Study_{1,k}\right)DM + b_2 Starch + b_3 fNDF + b_4 nfNDF + b_5 C16{:}\,0 + b_6 C18{:}\,0 + b_7 C18{:}\,1 + b_8 C18{:}\,2 + b_9 C18{:}\,3 + b_{10} OthersFA + \sum_{l=11}^{30}(b_l INT) + \sum_{m=31}^{40}(b_m Quad) + Cow_i(Block_b * Study_k) + Period_j\,(Study_k) + Block_b\,(Study_k) + Study_k + \varepsilon_{ijbk} \quad [5.1]$$

where Y_{ijbk} is one of the six nutrient digestibilities (DMD, NDFD, FAD, 16-CD, 18-CD, and EnergyD) and DEI (DEIday and DEIkg) of the *i*th cow within the *j*th period, *b*th block, and *k*th

the study; μ is the overall mean; $b_1, b_2, b_3, b_4, b_5, b_6, b_7, b_8, b_9,$ and b_{10}, are the partial regression coefficients of y_{ijbsk} on DMI with study-specific slope, starch, fNDF, nfNDF, C16:0, C18:0, C18:1, C18:2, and C18:3, respectively; b_l are the partial regression coefficients of y_{ijbk} on the 2-way interactions between the covariates; b_m are the partial regression coefficients of y_{ijbk} on the quadratic effect of the covariates; $Cow_i(Block_b * Study_k)$ is the random effect of the *i*th cow nested within the *j*th period, *b*th block, and *k*th study; $Period_j\ (Study_k)$ is the random effect of the *j*th period nested within the *k*th study; $Block_b\ (Study_k)$ is the random effect of the *b*th block nested within the *k*th study; $Study_k$ is the random effect of the *k*th study; and ε_{ijbk} is residual.

The distribution of the residuals was checked with normal probability and studentized residuals. Data points with a studentized residual greater than 3.5 ± SD were considered outliers. Main effects were declared significant at $P \leq 0.05$ and interactions were declared significant at $P \leq 0.10$. Finally, a model containing only the main effects described above was tested for multicollinearity using the variance inflation factor (**VIF**) test.

As part of defining our working model for each of the six nutrient digestibilities and the 2 DEI expressions, the variables with the highest *P*-values were successively removed until only significant variables (main effect: $P < 0.05$; interactions $P < 0.15$) remained in the model.

RESULTS

The model containing all the main effects showed multicollinearity problems (VIF > 10, for main effects). To remove the multicollinearity problems, the variables of OtherFA and nfNDF, with their respective quadratic and interaction terms were removed from the Equation

[5.1]. The OtherFA variable was removed from the model due to its calculation methodology which was highly correlated with other variables (it was calculated based on the difference between the total FA in the diet and the sum of C16:0, C18:0, C18:1, C18:2, and C18:3). In the case of nfNDF, all diets were formulated to have a target of NDF of 30% of DM, and so the sum of fNDF and nfNDF was close to 30% in all diets; with that fNDF and nfNDF were highly correlated. We removed the nfNDF because it represented a much smaller fraction of the diet than the fNDF.

The final models and the estimated coefficients from DMD, NDFD, FAD, 16-CD, 18-CD, EnergyD, DEIday, and DEIkg, are presented in Tables 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, and 5.9, respectively. Figures containing the FAs that had a non-significant effect ($P > 0.05$) are presented in the Supplementary Material (Figures Supplemental Figure 5.1, Supplemental Figure 5.2, Supplemental Figure 5.3, Supplemental Figure 5.4, Supplemental Figure 5.5, and Supplemental Figure 5.6).

Dry matter digestibility (DMD)

Dietary C16:0 affected DMD linearly and quadratically (estimated coefficients – linear: 3.02 ± 0.30, quadratic: -5.66 ± 1.08; $P < 0.01$, Figure 5.1) and interacted with starch content (estimated coefficient: -1.26 ± 0.46; $P = 0.01$). Dietary C18:0 decreased DMD linearly (estimated coefficient: -6.48 ± 1.19; $P < 0.01$, Figure 5.1), and its interaction with starch content was also significant (estimated coefficient: 9.37 ± 2.67; $P < 0.01$). None of the other FA had a significant impact on DMD (C18:1: $P = 0.79$, C18:2: $P = 0.15$, and C18:3: $P = 0.61$).

With respect to DMI, fNDF, and starch content, only DMI was significant and it had a linear and quadratic effect on DMD (estimated coefficients – linear: -0.01 ± 0.06, quadratic: -

0.02 ± 0.01; P = 0.01, Table 5.2) and interacted with fNDF content (estimated coefficient: 0.20 ± 0.08; P = 0.01). The main effects of fNDF and starch content on DMD were not significant (estimated coefficients – fNDF: -0.76 ± 1.16; P = 0.51; starch: 1.58 ± 1.03; P = 0.13), but both remained in the model due to their interactions with other covariates, as described above.

NDF digestibility (NDFD)

Increasing dietary C16:0 and C18:1 increased NDFD linearly (estimated coefficient - palmitic: 1.71 ± 0.67; P = 0.01, oleic: 5.12 ± 2.11; P = 0.02, Figure 5.2). Also, C16:0 interacted with fNDF (estimated coefficient: 1.38 ± 0.65). Dietary C18:0 had a linear and quadratic effect on NDFD (estimated coefficients – linear: 16.4 ± 5.40; quadratic: -34.3 ± 10.6; P = 0.01, Figure 5.2). The C18:2 dietary concentration decreased NDFD linearly (estimated coefficient: -4.87 ± 1.24; P < 0.01; Figure 5.2), and C18:3 was not significant (P = 0.72).

Although the main effects of DMI and fNDF on NDFD were not significant (P = 0.58 and P = 0.34, respectively), they remained in the model because of the significant interaction between DMI and fNDF (estimated coefficient: 0.27 ± 0.13; P = 0.03) and the fNDF interaction with C16:0 (as shown above). Starch content was not significant (P = 0.42).

Total FA digestibility (FAD)

Both C16:0 and C18:0 linearly reduced FAD (estimated coefficient for C16:0: -1.71 ± 0.51; P = 0.01; for C18:0: -11.0 ± 1.10; P = 0.01; Figure 5.3). The dietary concentration of C18:1, C18:2, and C18:3 did not affect FAD (C18:1: P = 0.06, C18:2: P = 0.11, C18:3: P = 0.71).

Dry matter intake had a linear and quadratic effect on FAD (estimated coefficients – linear: -0.34 ± 0.12; quadratic: -0.03 ± 0.01; $P = 0.03$) and fNDF content had a negative linear effect on FAD (estimated coefficient: -1.93 ± 0.53; $P = 0.01$) on FAD. Starch content was not significant ($P = 0.86$).

16-carbon FA digestibility (16-CD)

Palmitic had a linear and quadratic effect on 16-CD (estimated coefficients – linear: -9.41 ± 0.60; quadratic: -8.04 ± 2.27; $P = 0.01$, Figure 5.4). Interestingly, increasing dietary C18:0 linearly decreased 16-CD (estimated coefficient: -8.18 ± 1.66; $P < 0.01$, Figure 5.4), but it interacted with DMI (estimated coefficient: 0.69 ± 0.42; $P = 0.11$) and fNDF content (estimated coefficient: 11.7 ± 6.22; $P = 0.06$). The dietary concentrations of C18:1, C18:2, and C18:3 did not affect 16-CD (C18:1: $P = 0.07$, C18:2: $P = 0.11$, C18:3: $P = 0.33$).

Although the main effects of DMI and fNDF on 16-CD were not significant ($P = 0.51$ and $P = 0.65$, respectively), they remained in the model because of the significant interaction between DMI and fNDF (estimated coefficient: 0.39 ± 0.18; $P = 0.03$), and their interactions with C18:0 (as described above). Starch content did not affect 16-CD ($P = 0.41$).

18-carbon FA digestibility (18-CD)

Increasing dietary C16:0 linearly increased 18-CD (estimated coefficient: 1.57 ± 0.52, $P = 0.01$; Figure 5.5), whereas increasing dietary C18:0, C18:1, and C18:2 linearly decreased 18-CD (estimated coefficient – C18:0: -14.0 ± 1.03, $P < 0.01$; C18:1: -3.80 ± 1.89, $P = 0.04$; and C18:2: -7.44 ± 2.30, $P = 0.01$; Figure 5.5). Additionally, C18:0 interacted with fNDF content

(estimated coefficient: 17.4 ± 5.37, P = 0.01). The dietary concentration of C18:3 did not impact 18-CD (P = 0.34).

Dry matter intake had a linear and quadratic effect (estimated coefficients – linear: -0.37 ± 0.12 ; quadratic: -0.04 ± 0.01; P = 0.01), starch content had a negative linear effect on 18-CD (estimated coefficient: -3.87 ± 0.80, P < 0.01), and both interacted with each other (estimated coefficient: -0.32 ± 0.14, P = 0.02). Finally, increasing dietary fNDF content linearly increased 18-CD (estimated coefficient: 1.92 ± 0.85, P = 0.02).

Energy digestibility (EnergyD)

The dietary concentrations of C16:0 and C18:0 had a linear and quadratic effect on EnergyD (estimated coefficients for C16:0 – linear: 1.15 ± 0.49; quadratic: -6.65 ± 1.49; P < 0.01; estimated coefficients for C18:0 – linear: 7.30 ± 3.77; quadratic: -24.8 ± 7.19; P = 0.01; Figure 5.6). The dietary concentration of C18:3 did not impact EnergyD (P = 0.16), but it interacted with starch content (estimated coefficient: -51.3 ± 28.2, P = 0.07). The concentration of C18:1 and C18:2 did not impact energy digestibility (C18:1: P = 0.80, C18:2: P = 0.46).

Dry matter intake and fNDF content had a linear and a quadratic effect on EnergyD (estimated coefficients for DMI – linear: -0.09 ± 0.10, quadratic: -0.02 ± 0.01, P = 0.01; estimated coefficients for fNDF – linear: -1.59 ± 1.14, quadratic: -4.92 ± 1.69, P = 0.01). Starch content was not significant (P = 0.30) but remained in the model because of its interaction with C18:3 (as described above).

Digestible energy intake expressed in Mcal/d and Mcal/kg of DM (DEIday and DEIkg, respectively)

The FA had the same main effect regardless of whether DEI was expressed as Mcal/d or Mcal/kg of DM, except for C18:2. The concentration of C16:0, C18:1, and C18:3 linearly and quadratically affected DEIday (estimated coefficients for DEIday for C16:0 - linear: 0.39 ± 0.85, quadratic: -31.6 ± 3.72, $P < 0.01$; for C18:1 - linear: 15.2 ± 5.45, quadratic: -33.9 ± 14.1, $P = 0.01$; for C18:3 - linear: 325 ± 45.6, quadratic: -4,527 ± 608; $P < 0.01$, Figure 5.7; estimated coefficients for DEIkg for C16:0 - linear: 0.03 ± 0.03, quadratic: -0.51 ± 0.09, $P < 0.01$; for C18:1 - linear: 0.04 ± 0.14, quadratic: -0.90 ± 0.47, $P = 0.05$; for C18:3 - linear: 5.88 ± 0.91, quadratic: -60.0 ± 14.6, $P < 0.01$; Figure 5.8), and increasing dietary C18:0 linearly decreased DEIday and DEIkg (estimated coefficients for DEIday: -16.7 ± 2.01, $P < 0.01$; Figure 5.7; estimated coefficients for DEIkg: -0.39 ± 0.06, $P < 0.01$; Figure 5.8).

Increasing dietary C18:2 linearly increased DEIday (estimated coefficient: 21.4 ± 2.86, $P < 0.01$; Figure 5.7), while it linearly and quadratically affected DEIkg (estimated coefficients – linear: 0.16 ± 0.11, quadratic: -1.85 ± 0.45, $P < 0.01$; Figure 5.8).

For DEIday two interactions occurred - C16:0 and C18:0 interacted with starch content (estimated coefficient for the interaction with C16:0: -3.60 ± 0.80, $P < 0.01$; and interaction with C18:0: -18.1 ± 5.95, $P = 0.01$). For DEIkg, only C18:1 interacted with starch content (estimated coefficient: -0.74 ± 0.21, $P = 0.01$).

Dry matter intake and starch content had a positive linear effect on DEIday (estimated coefficients for DMI: 2.83 ± 0.07, $P < 0.01$; Starch: 3.86 ± 0.94, $P < 0.01$). The main effect of

fNDF content on DEIday was not significant ($P = 0.72$), but it remained in the model because of its interaction with DMI (estimated coefficient: 0.25 ± 0.08, $P = 0.01$).

For DEIkg, DMI had a quadratic effect (estimated coefficients – linear: -0.003 ± 0.005, quadratic: -0.001 ± 0.0003, $P = 0.01$) and interacted with fNDF content (estimated coefficient: 0.01 ± 0.001, $P = 0.09$). The main effect of starch content on DEIkg was a positive linear effect (estimated coefficient: 0.14 ± 0.04, $P = 0.01$).

DISCUSSION

As documented by Palmquist and Jenkins (2017), the initial studies with fat supplementation on dairy cows were based on feeding high levels of tallow and oilseeds. As research in the field of fat supplementation advanced, there was a shift from tallow and oilseeds to rumen-protected sources, and a focus on the degree of saturation of fat supplements was developed to overcome the problems of fat supplementation. These problems were associated with fiber digestibility, rumen fermentation, and milk composition (Jenkins and Palmiquist, 1984; Palmiquist and Jenkins, 2017).

Importantly, research has evolved from feeding the traditional animal- and plant-based fats to a new interest in the effects of feeding individual FAs, extending beyond their energy contribution to include potentially structural, metabolic, and physiological effects (Palmquist and Jenkins, 2017). Nowadays, we recognize that the FA profile of supplemental fat has a significant impact on nutrient digestibility, energy intake, and milk composition (Allen, 2000; Mosley et al., 2007; Lock et al., 2013; Piantoni et al., 2013; De Souza et al., 2017; Boerman et al., 2017; Weld and Armentano, 2017).

In accordance with the most recent research related to FA profile, our results showed that the FA included in our analysis (C16:0, C18:0, C18:1, C18:2, and C18:3) had a specific effect on the DMD, NDFD, FAD, EnergyD, and DEI. In addition to their main effects, in some cases (as shown in the results) FA interacted with dietary starch and fNDF. An essential point to consider in our analysis was that all of the five studies were designed to investigate the effect of dietary FA on the nutrient digestibility and production responses of dairy cows. Consequently, the diets were formulated to have similar starch and fNDF content across treatments. That being said, the variations in starch and fNDF content in the diets used in this analysis were minimal (starch – lower quartile: 27.4%, upper quartile: 28.5%; fNDF – lower quartile: 19%, upper quartile: 21%; Table 5.1), allowing us to investigate mainly the effect of FA profile. However, this also limited our ability to examine the impact of starch and fNDF content and potentially the interaction between these factors with dietary FA.

The fact that we had small variations on starch, fNDF, and DMI resulted in a more modest effect of these covariates on the dependent variables than the covariates associated with FA profile, as shown by the estimated coefficients when expressed in standard deviations units (Supplemental Tables 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, and 5.7). Accordingly, our discussion focused on the effect of the FA profile.

Another essential characteristic of our database was that the majority of the treatments had levels of C18:0 ranging from 0.04 to 0.23% of the DM (only three treatments with higher levels of C18:0 - 0.67, 0.72, and 0.88% of DM) and levels of C18:3 ranging from 0.17 to 0.22% of the DM (only two treatments with higher levels of C18:3 – 0.25 and 0.30% of DM). In this situation, when there was a gap in the distribution of the covariates, we were prone to the risk of

misinterpretation of the data because there was no continuous information along the range of the covariates (C18:0 and C18:3).

In the following discussion, for practical purposes, we discussed the effect of the FA profile on the dependent variables using the estimated coefficients on the original scale (% of DM, Tables 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, and 5.9). However, when comparing the relative importance of each estimated coefficient for each FA, we used the standardized coefficients (1 STD, Supplemental Tables 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, and 5.8).

Dry matter digestibility (DMD)

The dietary concentration of C16:0 had a quadratic effect on DMD, due to DMD increasing from 60 to 67% when C16:0 increased from 0.33 to 1.45% of DM (Figure 5.1a). Once 1.45% of DM was reached, the DMD had a slight decrease (Figure 5.1a). These results corroborate with Piantoni et al. (2013), which observed an increase in DMD when comparing a control diet (0.44% of DM of C16:0) to a high diet (2.41% of DM of C16:0). Additionally, there was a significant interaction between C16:0 and starch content. This interaction suggested that C16:0 had a more positive effect on DMD in diets with lower levels of starch as opposed to diets with a high level of starch. In normal conditions, starch was one of the most digestible fractions of the diet (93% digestible, de Souza et al., 2018). Hence, C16:0 would have a lower impact on DMD in diets with high starch content. Furthermore, although this interaction was significant, it had a minimal biological effect.

Whereas C16:0 had a positive effect on DMD, C18:0 linearly decreased DMD by 6.5% per 1% increase of C18:0 in the diet. Similarly, Boerman et al. (2017) reported a tendency for a linear decrease in DM digestibility when dietary levels of C18:0 increased from 0.07 to 2.19% of

diet DM. Chamberlain and DePeters (2017) also reported a tendency for decreasing the organic matter digestibility when increasing the C18:0 content from 0.01 to 0.11% of DM. Additionally, C18:0 interacted with starch content, whereas the negative effect of C18:0 on DMD was associated with diets containing high levels of starch.

NDF digestibility (NDFD)

Palmitic and C18:1 increased the NDFD by 1.71 and 5.12% per 1% increase in the diet, respectively (Figure 5.2). Piantoni et al. (2013) reported a similar positive effect of C16:0 on NDFD, where increasing C16:0 from 0.46 to 2.41% of DM increased NDFD from 35.7 to 39.0% of NDFD. Rico et al. (2014) observed interaction between C16:0 and the level of production, where on high producing cows (~ 42 kg/d) the NDFD increased from 31.2 to 33.4% when C16:0 increased from 0.58 to 2.12% of DM. de Souza and Lock (2018b) reported an increase in NDF digestibility when feeding a Ca-salts of palm FA (45% C16:0 and 38% C8:1) compared with a nonfat control diet, which was attributed to a reduction in DMI caused by the supplemental fat. In a recent meta-analysis, Weld and Armentano (2017) observed that calcium salts of palm FA did not affect NDF digestibility, while saturated prilled fat containing a mixture of C16:0 and C18:0 increased NDF digestibility.

Stearic had a quadratic effect, increasing the NDFD from 39 to 44% when C18:0 increased from 0.04 to 0.44% of DM (Figure 5.2b). Piantoni et al. (2015) reported a tendency for an increase in NDFD when feeding a C18:0 enriched supplement (diet C18:0 increased from 0.09% to 1.96% of DM). Contrary to our results, Chamberlain and DePeters (2017) did not observe a decrease in NDFD when adding C18:0. However, Chamberlain and DePeters (2017) included C18:0 up to 0.11% of DM. At this level of inclusion, we also observed a small effect of

C18:0 on NDFD. In this sense, our results were corroborated because C18:0 had an adverse impact on NDFD only at high levels of inclusion.

Total, 16-carbon, and 18-carbon FAs digestibility (FAD, 16-CD, and 18-CD, respectively)

Both C16:0 and C18:0 were the only FA that affected FAD (Figures 5.3a and 5.3b). Research shows that FA digestibility usually decreases as FA intake increases (Palmquist, 1991). When comparing the standardized coefficients, the effect of C18:0 on FAD was 2.6 times greater than the effect of C16:0. Feeding C18:0-enriched FA supplements have been shown to decrease FA digestibility in dairy cows (Piantoni et al., 2015; Boerman et al., 2017) and sheep (Toral et al., 2016).

Additionally, Chamberlain and DePeters (2017) observed that increasing the proportion of C18:0 to C16:0 in supplemental fat (2% diet DM) reduced total FA digestibility in dairy cows. By our results, Boerman et al. (2015) also reported a decrease in FAD with the increase of the total FA intake and duodenal flow. Potential reasons for the reduction in FA digestibility includes a limitation in lysolecithin secretion reducing the flux of FA into micelles (Boerman et al., 2015a), and possible saturation of absorptive sites in the intestine (Glasser et al., 2008).

Similarly, to FAD, only C16:0 and C18:0 affected 16-CD. At low levels of C16:0 (0.3 to 0.9% of DM), there was a minimal effect of C16:0 on 16-CD, which was reasonably stable at around 76%. However, at high levels of C16:0, from 1.1 to 1.91% of DM, the 16-CD was dropped to 63% (Figure 5.4a). In a meta-regression performed by Boerman et al. (2015), the authors also reported a decrease in 16-CD with the increase of the duodenal flow of C16:0; however, on their meta-regression the effect of C16:0 was much smaller than the one reported in our study. Piantoni et al. (2013) also observed a decrease in 16-CD when increasing C16:0 from

0.46 to 2.21% of DM. Interestingly, we found that increasing C18:0 also decreased 16-CD (Figure 5.4b). These results suggested that the profile of FA reaching the intestine is most likely the primary factor that impacts digestibility, rather than the total amount of FA reaching the intestine.

On the 18-CD, all the FA included in the analysis had a significant effect, except C18:3. Increasing C16:0 increased 18-CD linearly (Figure 5.5a). Stearic, C18:1, and C18:2 linearly decreased 18-CD (Figures 5.5b, 5.5c, and 5.5d). Based on the standardized coefficients, C18:0 was the most critical FA, followed by C18:2 and C18:1. Boerman et al. (2015) reported the same effect of C18:0 on 18-CD, where 18-CD was dramatically decreased with the increase of C18:0.

Energy digestibility (EnergyD)

As expected, EnergyD had a very similar pattern to DMD and NDFD. Palmitic had a linear and quadratic effect on EnergyD. When C16:0 was included from 0.33 to 1.27% of DM, it increased EnergyD from 63.3 to 69.3%. After 1.27% of C16:0 was reached, EnergyD decreased to 66.6% until the maximum level of C16:0 inclusion in our database was achieved (1.91% of DM) (Figure 5.6a). Conversely, C18:0 had a small effect on EnergyD at low levels of inclusion and an adverse impact when included at high levels. Energy digestibility increased from 67.3 to 69.7% as C18:0 increased from 0.04 to 0.35% of DM. However, after 0.35% of C18:0 was reached, EnergyD drastically dropped to 62.8% when C18:0 level reached 0.88% of DM (Figure 5.6b).

Weiss and Wyatt (2004) also reported an increase in EnergyD when increasing the ratio C16:0:C18:0. In their study, treatments with a high level of C18:0 had an EnergyD of 66.4% and the treatments high on C16:0 had an EnergyD of 69.5%. In contrast, Weiss et al. (2011) did not

detect differences in EnergyD when evaluating different types of supplemental fat. However, it is important to point out what kinds of supplemental fat vary not only according to the FA profile but also to the method of protection and degree of esterification.

Linolenic increased EnergyD by 22.6% when increased by 1-unit in the diet. However, C18:3 interacted with starch content, and the positive effect of C18:3 was associated with diets with a low level of starch (Figure 5.6c). When comparing the standardized coefficients, C18:0 had the most impact on EnergyD followed by C16:0 and C18:3. The C18:0 effect was associated with the decrease in EnergyD when included in levels higher than 0.35% of DM, which is likely associated with the reduction in FA digestibility reported for this FA. In contrast, C16:0 increased EnergyD, probably due to its effect on fiber digestibility.

Digestible energy intake (DEI)

Palmitic increased DEIday from 72.5 to 96.1 Mcal/d when it was increased from 0.33 to 1.19% of DM. Once levels higher than 1.19% of DM were achieved, C16:0 dropped DEIday down to 79.8 Mcal/d at 1.91% of DM. Additionally, the interaction between C16:0 and starch content was statistically significant and suggested that C16:0 had a more positive effect on DEIday in diets with low starch content. Although the interaction was statistically significant, it had a minimal biological impact. Similarly, to the DEIday, C16:0 increased DEIkg from 2.7 to 3.08 Mcal/kg of DM when it was increased from 0.33 to 1.21% of DM (Figure 5.8a). Similar to our results, Weiss et al. (2011), also reported an increase in DEIkg when they increased C16:0 from 0.44 to 2.09% of DM.

In contrast to C16:0, which increased DEIday and DEIkg in low to mid-levels, C18:0 linearly decreased DEIday by 16.7 Mcal/d (Figure 5.7b) and DEIkg by 0.39 Mcal/kg of DM

(Figure 5.8b) per 1-unit increase. For DEIday, there was an essential interaction between C18:0 and starch content, indicating that the adverse effect of C18:0 on DEIday was associated with increased levels of starch in the diet (Figure 5.8b). These results are in agreement with the negative effects of this FA on DM digestibility and mainly FA digestibility.

When oleic was increased from 0.42 to 0.92% of DM, it increased DEIday from 89.2 to 97.7 Mcal/d. After 0.92% was reached, DEIday had a slight drop to 97.5 Mcal/d at the maximum level of C18:1 inclusion (1.00% of DM) (Figure 5.7c). Although the main effect of C18:1 was significant for DEIday, its main effect on DEIkg was minimal, and its biological importance was dependent on the starch content. At low levels of starch, C18:1 increased DEIkg; and at high levels of starch, C18:1 decreased DEIkg (Figure 5.8c).

CONCLUSIONS

In conclusion, C16:0 and C18:0 were the FAs with the most significant impact on digestibility and EnergyD. The primary effect of C16:0 on EnergyD was associated with the increase on EnergyD when C16:0 was increased in the diet from 0.33 to 1.27% of DM. In contrast, the primary effect of C18:0 on EnergyD was associated with the decrease on EnergyD when C18:0 was increased from 0.35 to 0.88% of DM. C16:0 increased DEI when included in the diet up to 1.20% of DM, and C18:0 linearly decreased DEI.

APPENDICES

APPENDIX A

Tables

Table 5.1. Descriptive summary of the variables included in the database.

Variable[1]	Mean	SD	Lower Quartile	Median	Upper Quartile
DMI, kg/d	29.9	3.45	27.7	29.8	32.1
Dietary Composition, % of DM					
Starch	27.9	0.736	27.4	28.0	28.5
NDF	29.8	0.776	29.0	29.7	30.8
fNDF	20.2	0.776	19.0	20.3	21.0
nfNDF	9.67	0.880	8.70	9.80	10.8
Fatty Acids					
Palmitic	1.19	0.528	0.610	1.26	1.68
Stearic	0.203	0.220	0.070	0.130	0.230
Oleic	0.696	0.145	0.630	0.660	0.790
Linoleic	1.56	0.278	1.29	1.67	1.81
Linolenic	0.202	0.023	0.180	0.200	0.210
OthersFA	0.227	0.059	0.220	0.230	0.250
Digestibilities, %					
Dry matter	64.4	4.13	61.5	64.7	67.4
NDF	41.5	5.30	38.1	41.6	44.9
Fatty Acids	76.1	6.38	71.8	76.7	80.7
16-Carbon	72.7	8.02	66.9	73.6	79.0
18-Carbon	79.8	7.02	75.6	81.6	85.2
Energy	65.8	5.18	62.1	65.9	69.3
Digestible Energy Intake					
Mcal/d	88.8	11.0	82.5	89.2	95.8
Mcal/kg of DM	2.97	0.211	2.83	2.99	3.11

[1]DMI = dry matter intake, fNDF = forage NDF, nfNDF = non-forage NDF, OthersFA = total fatty acids - (palmitic + stearic + oleic + linoleic + linolenic)

Table 5.2. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on dry matter digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	66.4	0.980	<0.01
DMI (linear)	-0.014	0.064	0.83
DMI (quadratic)	-0.018	0.007	0.01
Starch	1.58	1.03	0.13
fNDF	-0.756	1.16	0.52
Palmitic (linear)	3.02	0.303	<0.01
Palmitic (quadratic)	-5.66	1.08	<0.01
Stearic	-6.48	1.19	<0.01
DMI*fNDF	0.203	0.078	0.01
Starch*Palmitic	-1.26	0.459	0.01
Starch*Stearic	9.37	2.67	<0.01

[1]fNDF = forage NDF,[2]Standard error

Table 5.3. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on NDF digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	42.5	0.857	<0.01
DMI	0.057	0.103	0.58
fNDF	0.960	1.00	0.34
Palmitic	1.71	0.667	0.01
Stearic (linear)	16.4	5.40	<0.01
Stearic (quadratic)	-34.3	10.6	<0.01
Oleic	5.12	2.11	0.02
Linoleic	-4.87	1.24	<0.01
DMI*fNDF	0.270	0.126	0.03
fNDF*Palmitic	1.38	0.653	0.03

[1]fNDF = forage NDF,[2]Standard error

Table 5.4. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on total fatty acids digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	76.5	0.601	<0.01
DMI (linear)	-0.374	0.133	0.01
DMI (quadratic)	-0.029	0.013	0.02
fNDF	-1.49	0.694	0.03
Palmitic	-1.71	0.508	<0.01
Stearic	-11.0	1.10	<0.01

[1]fNDF = forage NDF,[2]Standard error

Table 5.5. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on 16-carbon fatty acids digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	74.5	1.24	<0.01
DMI	-0.096	0.145	0.51
fNDF	-0.605	1.34	0.65
Palmitic (linear)	-9.41	0.596	<0.01
Palmitic (quadratic)	-8.04	2.27	<0.01
Stearic	-8.18	1.66	<0.01
DMI*fNDF	0.394	0.178	0.03
DMI*Stearic	0.688	0.425	0.11
fNDF*Stearic	11.76	6.22	0.06

[1]fNDF = forage NDF,[2]Standard error

Table 5.6. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on 18-carbon fatty acids digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	79.9	0.570	<0.01
DMI (linear)	-0.375	0.117	<0.01
DMI (quadratic)	-0.038	0.012	<0.01
Starch	-3.87	0.797	<0.01
fNDF	1.92	0.846	0.02
Palmitic	1.57	0.520	<0.01
Stearic	-14.0	1.03	<0.01
Oleic	-3.80	1.89	0.04
Linoleic	-7.44	2.30	<0.01
DMI*Starch	-0.317	0.139	0.02
fNDF*Stearic	17.4	5.37	<0.01

[1]fNDF = forage NDF,[2]Standard error

Table 5.7. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on energy digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	69.2	1.59	<0.01
DMI (linear)	-0.092	0.098	0.35
DMI (quadratic)	-0.022	0.008	<0.01
Starch	1.09	1.05	0.30
fNDF (linear)	-1.59	1.14	0.16
fNDF (quadratic)	-4.92	1.69	<0.01
Palmitic (linear)	1.15	0.491	0.02
Palmitic (quadratic)	-6.65	1.49	<0.01
Stearic (linear)	7.30	3.77	0.05
Stearic (quadratic)	-24.7	7.19	<0.01
Linolenic	22.6	16.0	0.16
Starch*Linolenic	-51.3	28.2	0.07

[1]fNDF = forage NDF,[2]Standard error

Table 5.8. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on digestible energy intake (Mcal/d).

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	96.0	1.47	<0.01
DMI	2.83	0.067	<0.01
Starch	3.86	0.936	<0.01
fNDF	0.358	1.02	0.72
Palmitic (linear)	0.388	0.851	0.65
Palmitic (quadratic)	-31.6	3.72	<0.01
Stearic	-16.7	2.01	<0.01
Oleic (linear)	15.2	5.45	<0.01
Oleic (quadratic)	-33.9	14.1	<0.01
Linoleic	21.4	2.86	<0.01
Linolenic (linear)	325	45.6	<0.01
Linolenic (quadratic)	-4527	608	<0.01
DMI*fNDF	0.245	0.079	<0.01
Starch*Palmitic	-3.60	0.801	<0.01
Starch*Stearic	-18.1	5.95	<0.01

[1]fNDF = forage NDF,[2]Standard error

Table 5.9. Effect of dry matter intake (DMI, kg/d) and diet composition (% of DM) on digestible energy concentration (Mcal/kg of DM).

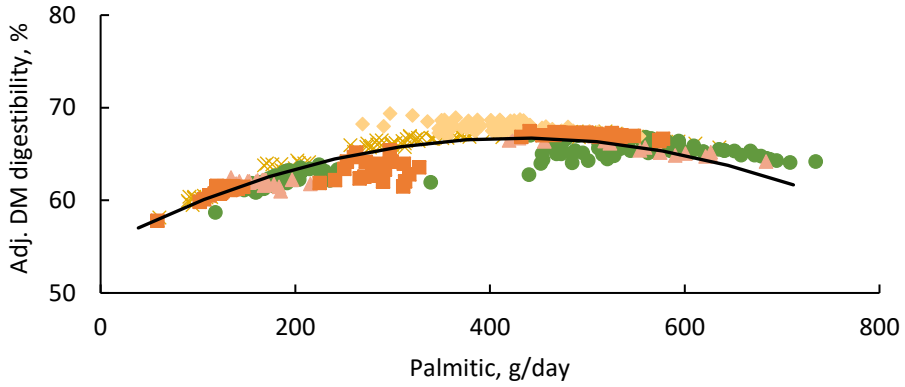
Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	3.08	0.056	<0.01
DMI (linear)	-0.003	0.005	0.46
DMI (quadratic)	-0.001	0.000	<0.01
Starch	0.144	0.038	<0.01
fNDF	0.003	0.058	0.95
Palmitic (linear)	0.027	0.029	0.35
Palmitic (quadratic)	-0.508	0.091	<0.01
Stearic	-0.386	0.064	<0.01
Oleic (linear)	0.045	0.143	0.75
Oleic (quadratic)	-0.899	0.465	0.05
Linoleic (linear)	0.159	0.108	0.14
Linoleic (quadratic)	-1.85	0.447	<0.01
Linolenic (linear)	5.88	0.912	<0.01
Linolenic (quadratic)	-60.1	14.6	<0.01
DMI*fNDF	0.009	0.001	0.09
Starch*Oleic	-0.738	0.207	<0.01

[1]fNDF = forage NDF,[2]Standard error

APPENDIX B

Figures

a)



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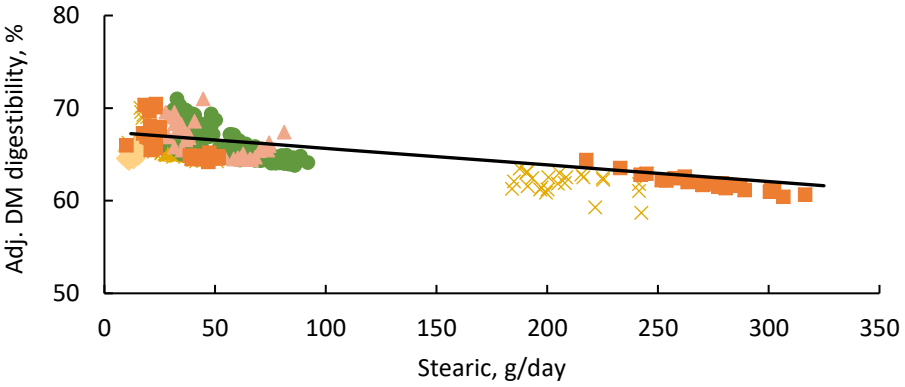
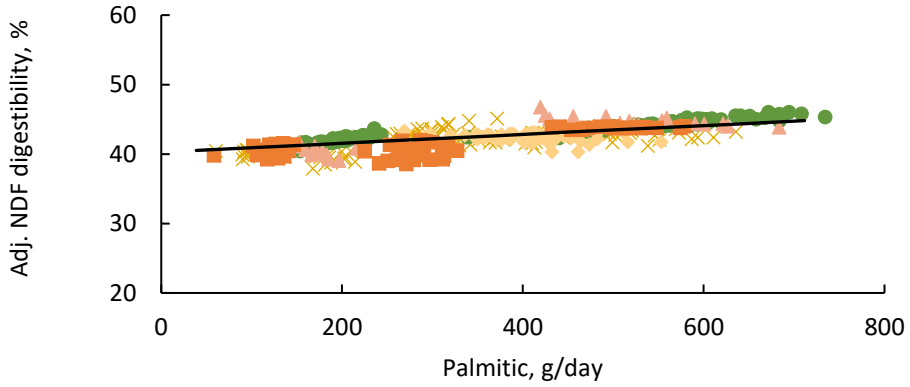


Figure 5.1. Effect of the inclusion of dietary palmitic (a) and stearic (b) on dry matter digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).

a)



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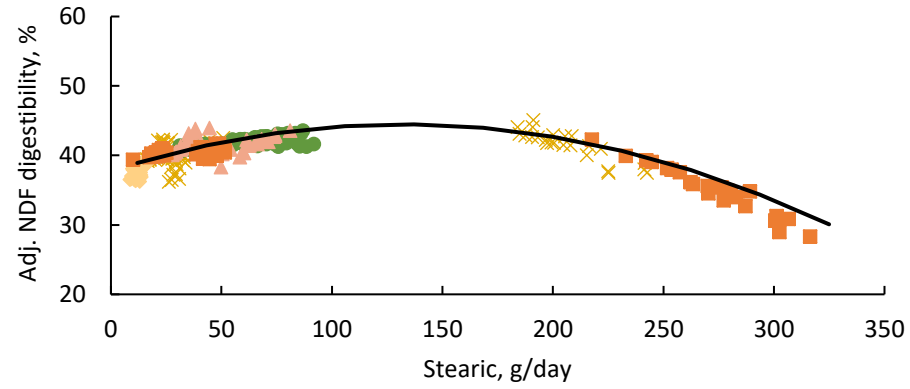
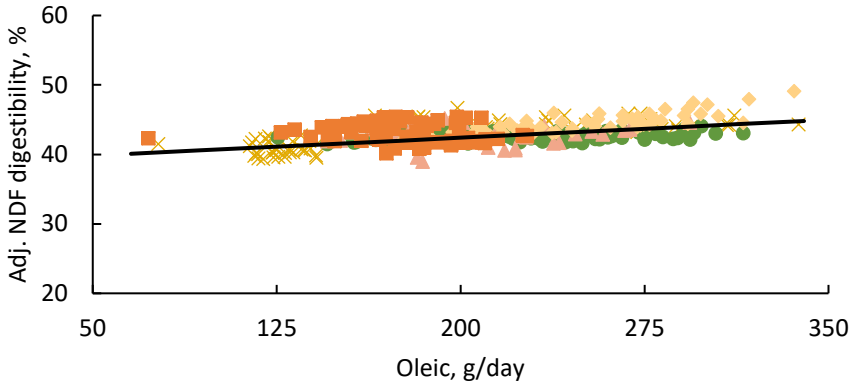


Figure 5.2. Effect of the inclusion of dietary palmitic (a), stearic (b), oleic (c), and linoleic (d) on NDF digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).

Figure 5.2 (cont'd)

c)



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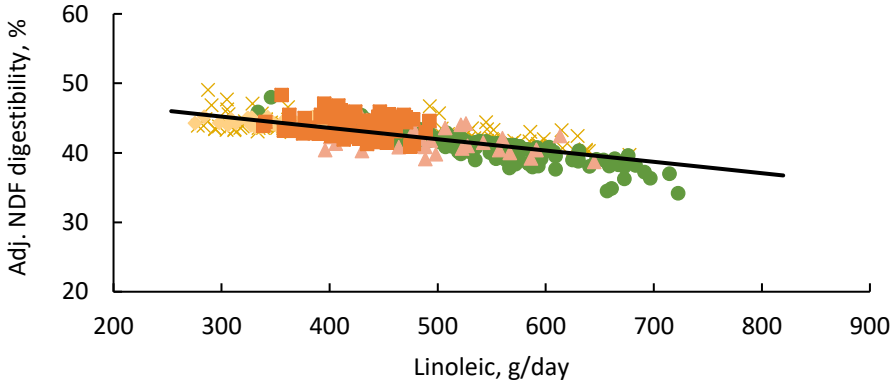

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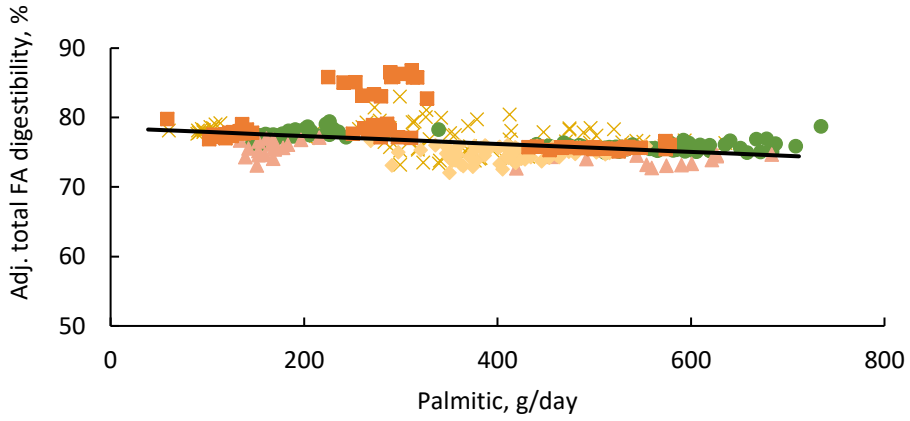

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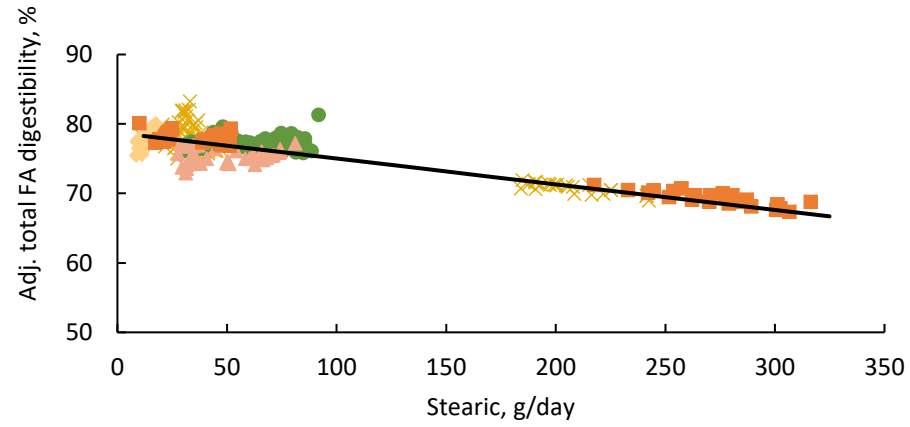
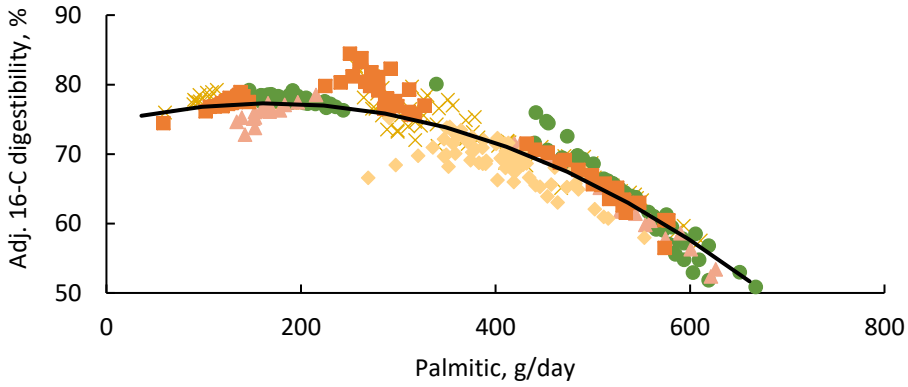


Figure 5.3. Effect of the inclusion of dietary palmitic (a) and stearic (b) on total fatty acid digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).

a)



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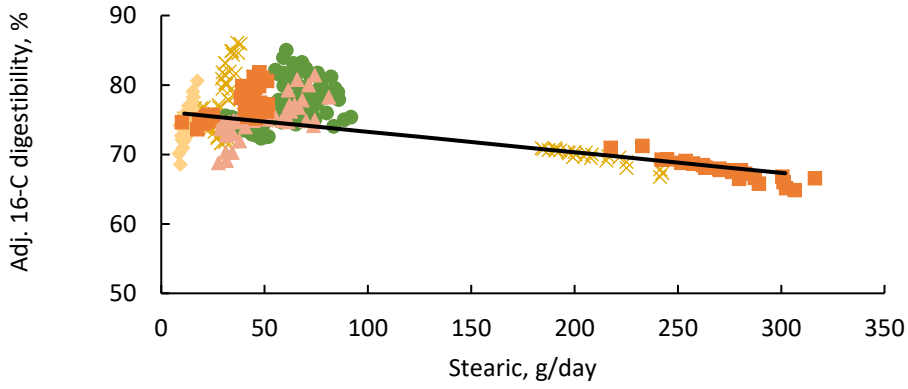

Figure 5.4. Effect of the inclusion of dietary palmitic (a) and stearic (b) on 16-carbon fatty acids digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).

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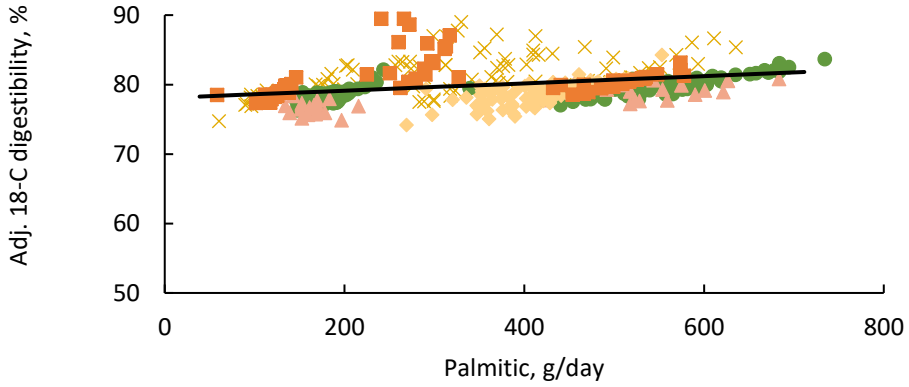

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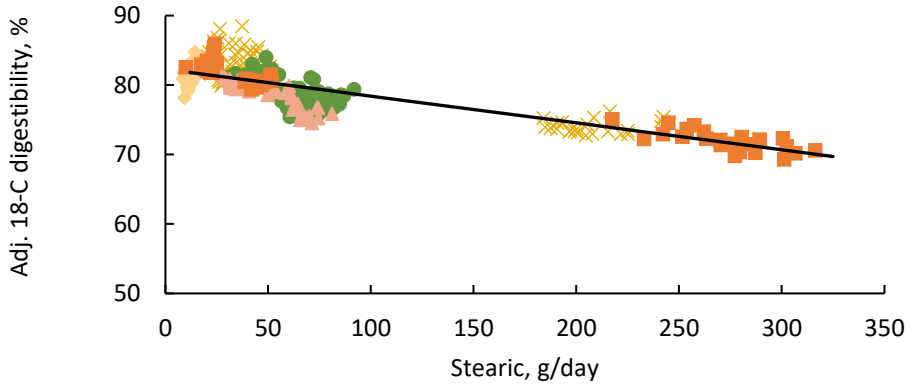
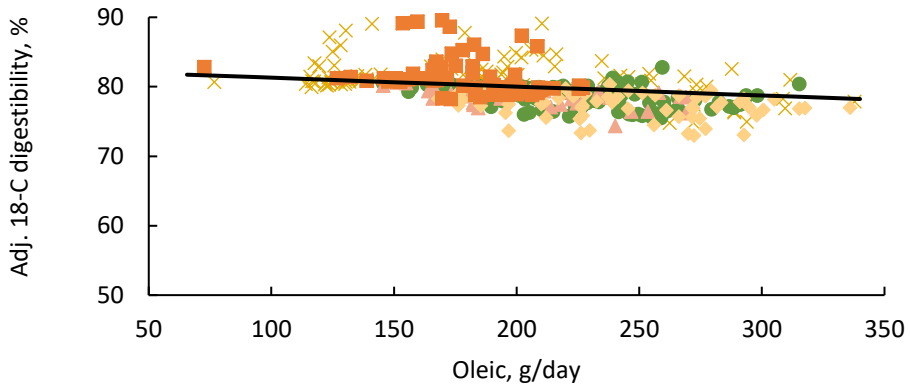


Figure 5.5. Effect of the inclusion of dietary palmitic (a), stearic (b), oleic (c), and linoleic (d) on 18-carbon fatty acids digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).

Figure 5.5 (cont'd)

c)



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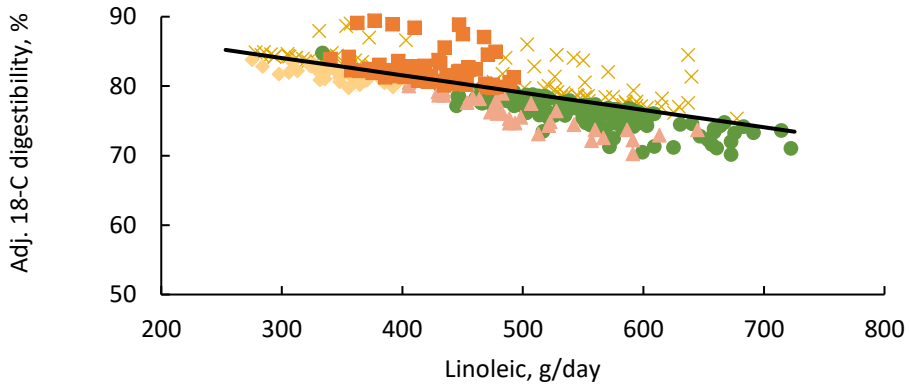

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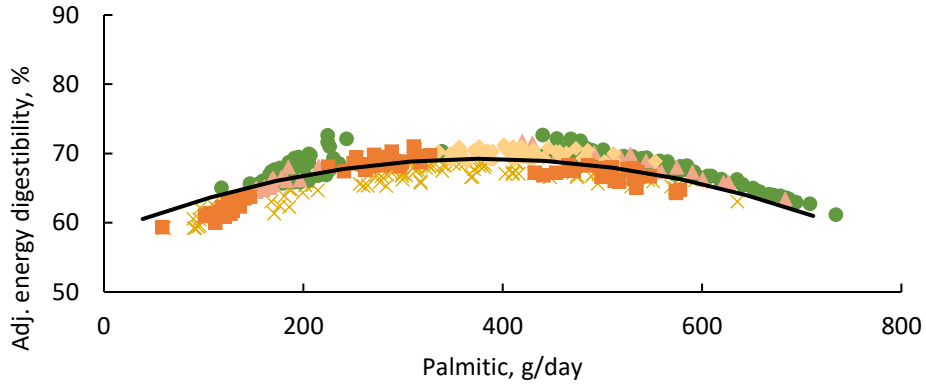

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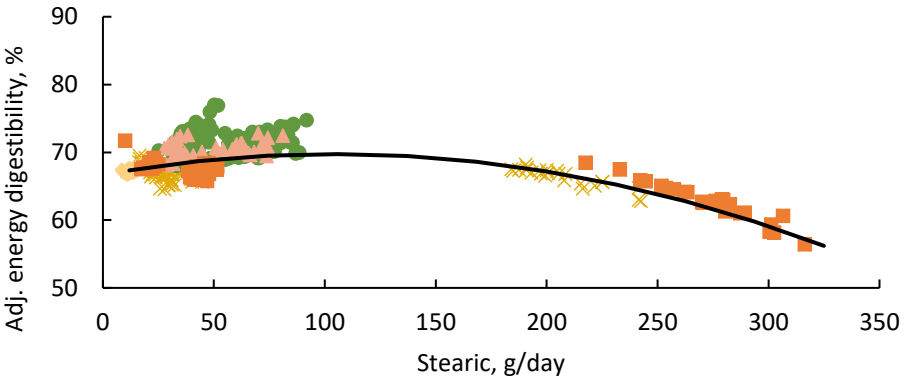

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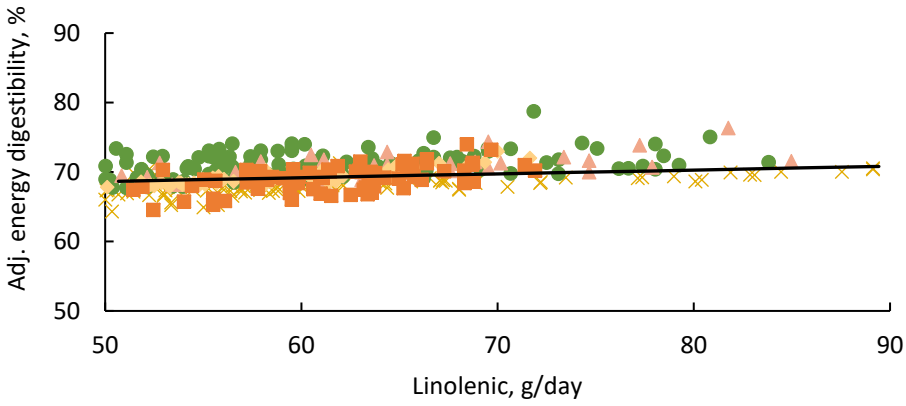

Figure 5.6. Effect of the inclusion of dietary palmitic (a), stearic (b), and linolenic on energy digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).

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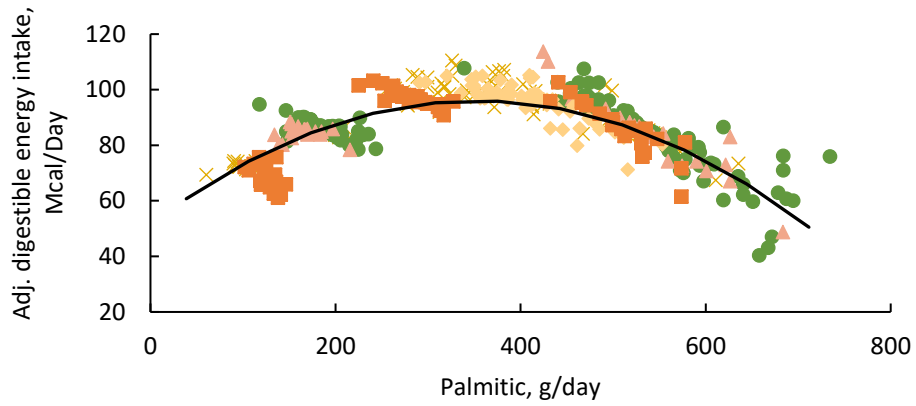

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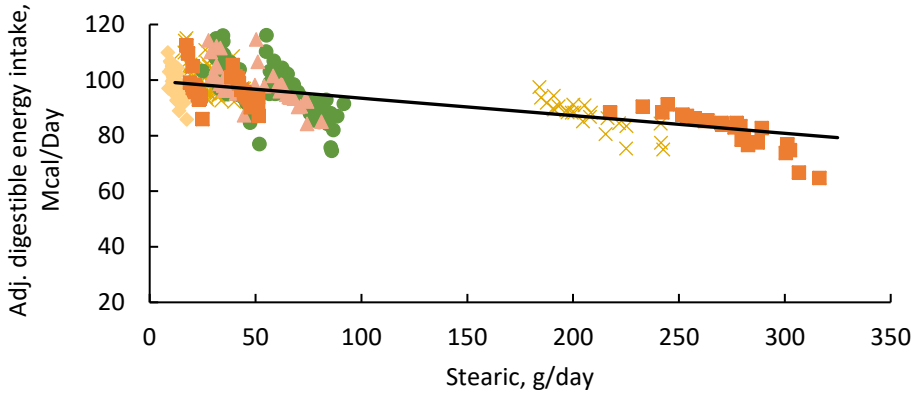
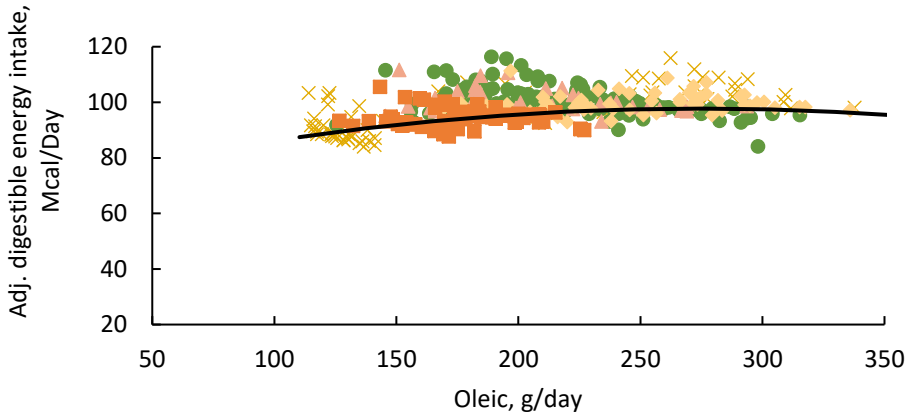


Figure 5.7. Effect of the inclusion of dietary palmitic (a), stearic (b), oleic (c), and linolenic (d) on digestible energy intake (DEI, Mcal/d). Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).

Figure 5.7 (cont'd)
c)



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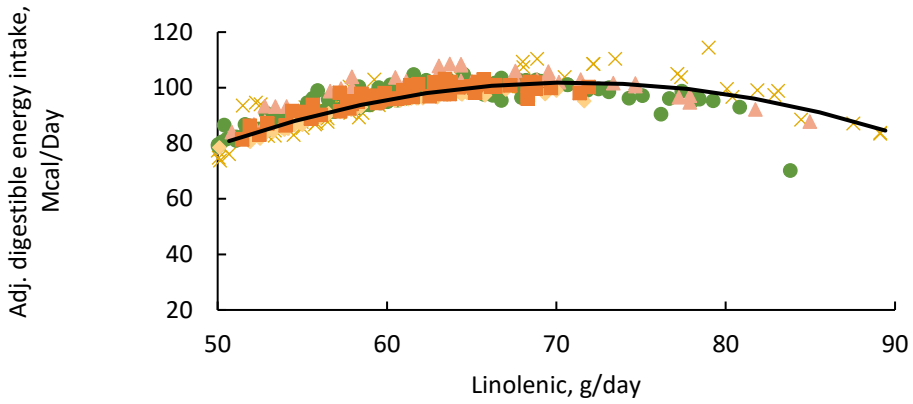

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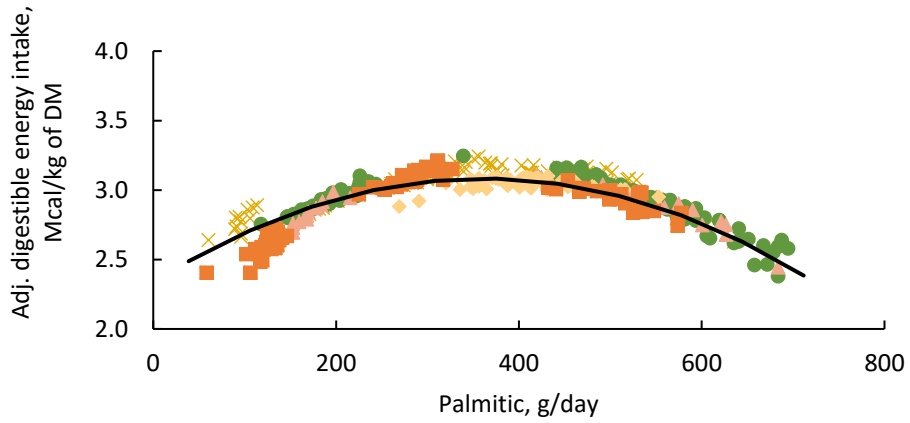

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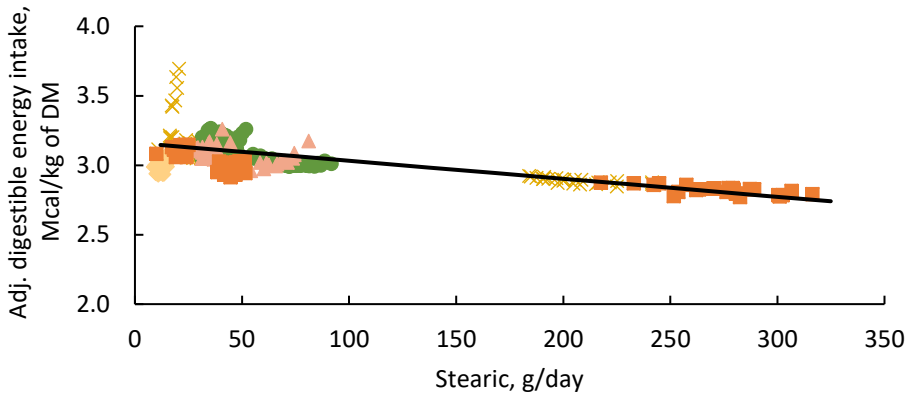
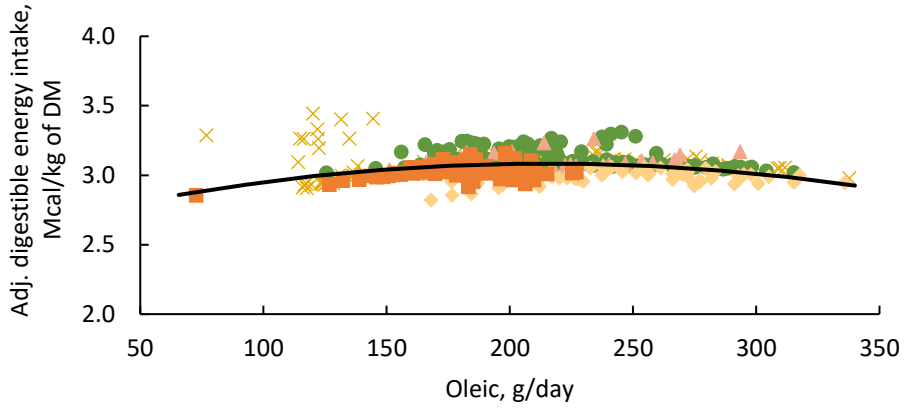


Figure 5.8. Effect of the inclusion of dietary palmitic (a), stearic (b), oleic (c), and linolenic (d) on digestible energy intake (DEI, Mcal/d). Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).

Figure 5.8 (cont'd)

c)



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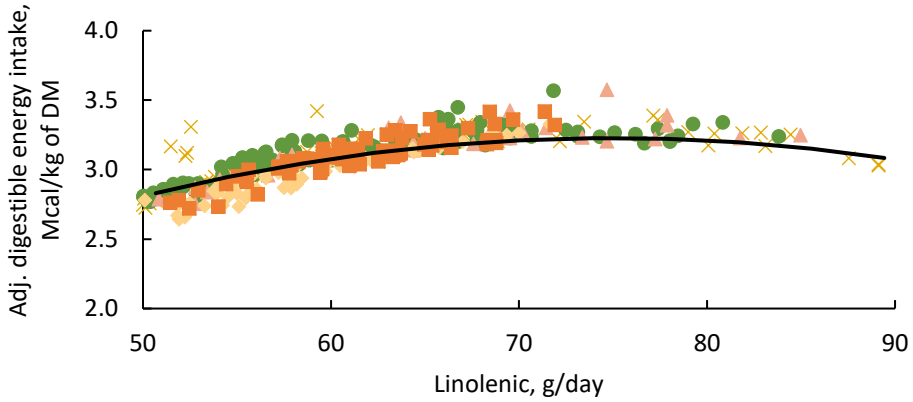


APPENDIX C

Supplemental Tables

Supplemental Table 5.1. Effect of dry matter intake (SD) and diet composition (SD) on dry matter digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	66.4	0.980	<0.01
DMI (linear)	-0.047	0.222	0.83
DMI (quadratic)	-0.216	0.081	0.01
Starch	1.16	0.755	0.13
fNDF	-0.587	0.897	0.52
Palmitic (linear)	1.59	0.160	<0.01
Palmitic (quadratic)	-1.58	0.301	<0.01
Stearic	-1.43	0.263	<0.01
DMI*fNDF	0.543	0.209	0.01
Starch*Palmitic	-0.489	0.179	0.01
Starch*Stearic	1.52	0.433	<0.01

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.2. Effect of dry matter intake (SD) and diet composition (SD) on NDF digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	42.5	0.857	<0.01
DMI	0.198	0.356	0.58
fNDF	0.745	0.773	0.34
Palmitic	0.902	0.352	0.01
Stearic (linear)	3.61	1.19	<0.01
Stearic (quadratic)	-1.67	0.515	<0.01
Oleic	0.743	0.307	0.02
Linoleic	-1.35	0.344	<0.01
DMI*fNDF	0.722	0.337	0.03
fNDF*Palmitic	0.566	0.268	0.03

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.3. Effect of dry matter intake (SD) and diet composition (SD) on total fatty acids digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	76.5	0.601	<0.01
DMI (linear)	-1.29	0.457	0.01
DMI (quadratic)	-0.350	0.152	0.02
fNDF	-1.16	0.539	0.03
Palmitic	-0.904	0.268	<0.01
Stearic	-2.43	0.243	<0.01

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.4. Effect of dry matter intake (SD) and diet composition (SD) on 16-carbon fatty acids digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	74.5	1.24	<0.01
DMI	-0.333	0.499	0.51
fNDF	-0.470	1.04	0.65
Palmitic (linear)	-4.97	0.315	<0.01
Palmitic (quadratic)	-2.24	0.635	<0.01
Stearic	-1.80	0.366	<0.01
DMI*fNDF	1.05	0.475	0.03
DMI*Stearic	0.522	0.323	0.11
fNDF*Stearic	2.01	1.06	0.06

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.5. Effect of dry matter intake (SD) and diet composition (SD) on 18-carbon fatty acids digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	79.9	0.570	<0.01
DMI (linear)	-1.29	0.404	<0.01
DMI (quadratic)	-0.448	0.144	<0.01
Starch	-2.85	0.586	<0.01
fNDF	1.49	0.656	0.02
Palmitic	0.829	0.275	<0.01
Stearic	-3.09	0.226	<0.01
Oleic	-0.551	0.274	0.04
Linoleic	-2.07	0.639	<0.01
DMI*Starch	-0.804	0.353	0.02
fNDF*Stearic	2.98	0.919	<0.01

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.6. Effect of dry matter intake (SD) and diet composition (SD) on energy digestibility.

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	69.2	1.59	<0.01
DMI (linear)	-0.317	0.339	0.35
DMI (quadratic)	-0.267	0.091	<0.01
Starch	0.798	0.775	0.30
fNDF (linear)	-1.23	0.883	0.16
fNDF (quadratic)	-2.96	1.02	<0.01
Palmitic (linear)	0.609	0.259	0.02
Palmitic (quadratic)	-1.86	0.416	<0.01
Stearic (linear)	1.61	0.831	0.05
Stearic (quadratic)	-1.20	0.349	<0.01
Linolenic	0.517	0.365	0.16
Starch*Linolenic	-0.86	0.475	0.07

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.7. Effect of dry matter intake (SD) and diet composition (SD) on digestible energy intake (Mcal/d).

Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	96.0	1.47	<0.01
DMI	9.77	0.232	<0.01
Starch	2.84	0.688	<0.01
fNDF	0.278	0.788	0.72
Palmitic (linear)	0.205	0.449	0.65
Palmitic (quadratic)	-8.81	1.04	<0.01
Stearic	-3.68	0.444	<0.01
Oleic (linear)	5.11	0.791	<0.01
Oleic (quadratic)	-0.923	0.297	<0.01
Linoleic	5.93	0.793	<0.01
Linolenic (linear)	7.45	1.04	<0.01
Linolenic (quadratic)	-2.37	0.319	<0.01
DMI*fNDF	0.656	0.212	<0.01
Starch*Palmitic	-1.40	0.311	<0.01
Starch*Stearic	-2.93	0.965	<0.01

[1]fNDF = forage NDF,[2]Standard error

Supplemental Table 5.8. Effect of dry matter intake (SD) and diet composition (SD) on digestible energy concentration (Mcal/kg of DM).

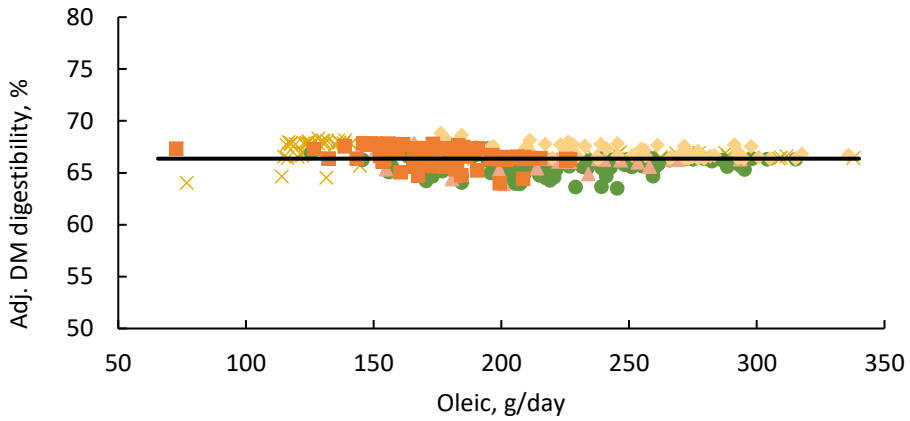
Effect[1]	Estimated Coefficient	SE[2]	*P*-value
Intercept	3.08	0.056	<0.01
DMI (linear)	-0.012	0.016	0.46
DMI (quadratic)	-0.014	0.004	<0.01
Starch	0.106	0.028	<0.01
fNDF	0.002	0.045	0.95
Palmitic (linear)	0.014	0.015	0.35
Palmitic (quadratic)	-0.142	0.025	<0.01
Stearic	-0.085	0.014	<0.01
Oleic (linear)	0.006	0.021	0.75
Oleic (quadratic)	-0.019	0.010	0.05
Linoleic (linear)	0.044	0.030	0.14
Linoleic (quadratic)	-0.142	0.034	<0.01
Linolenic (linear)	0.135	0.021	<0.01
Linolenic (quadratic)	-0.031	0.008	<0.01
DMI*fNDF	0.025	0.001	0.09
Starch*Oleic	-0.079	0.022	<0.01

[1]fNDF = forage NDF,[2]Standard error

APPENDIX D

Supplemental Figures

a)



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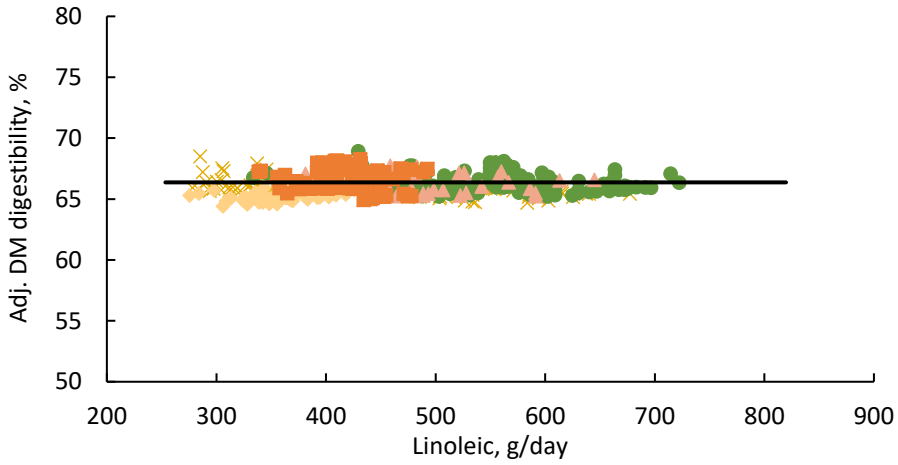

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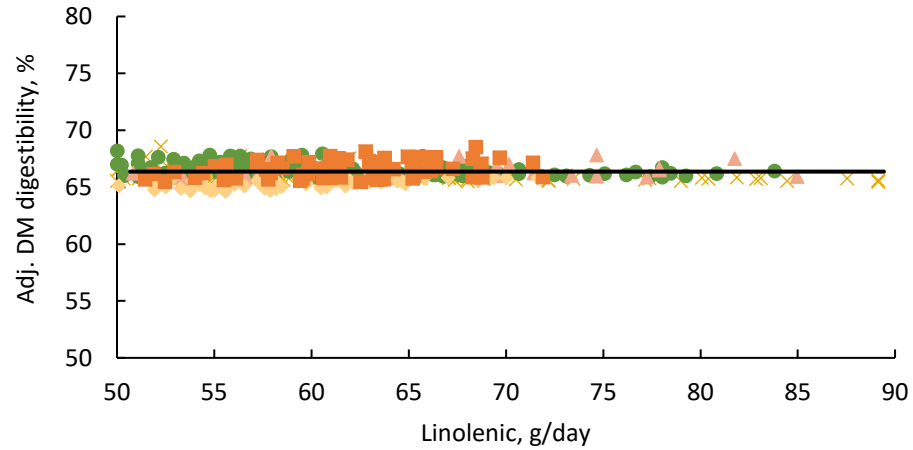
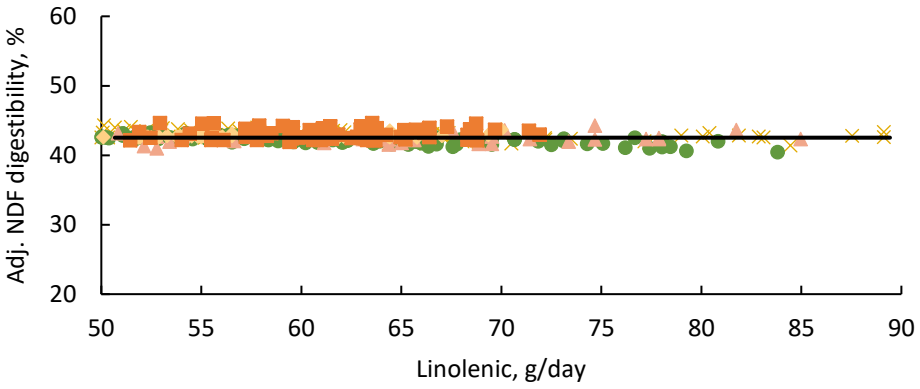


Supplemental Figure 5.1. Effect of the inclusion of dietary oleic (a), linoleic (b), and linolenic (c) on dry matter digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).



Supplemental Figure 5.2. Effect of the inclusion of dietary linolenic on NDF digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).

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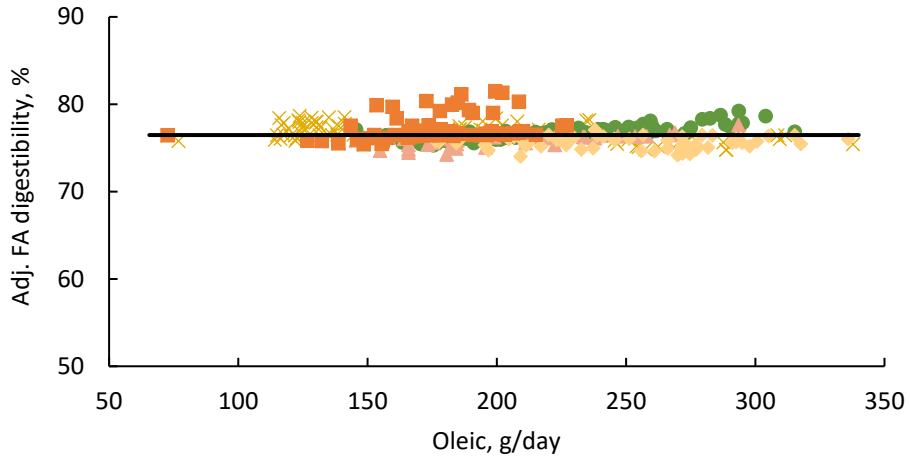

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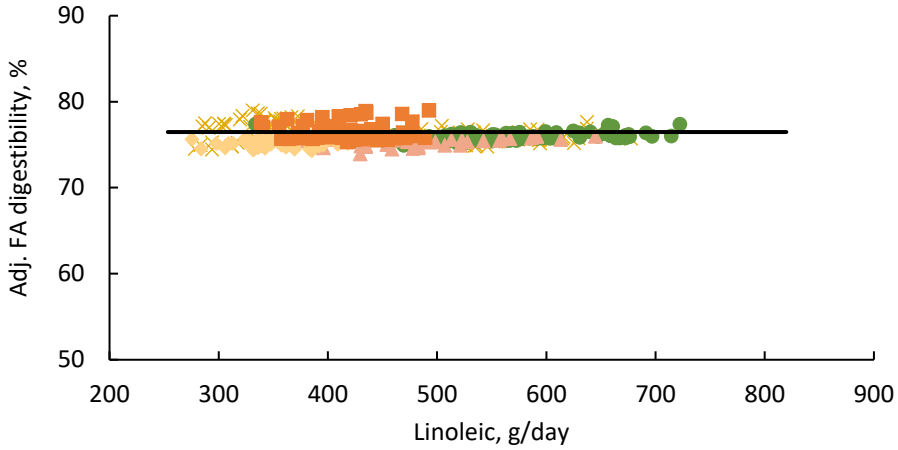

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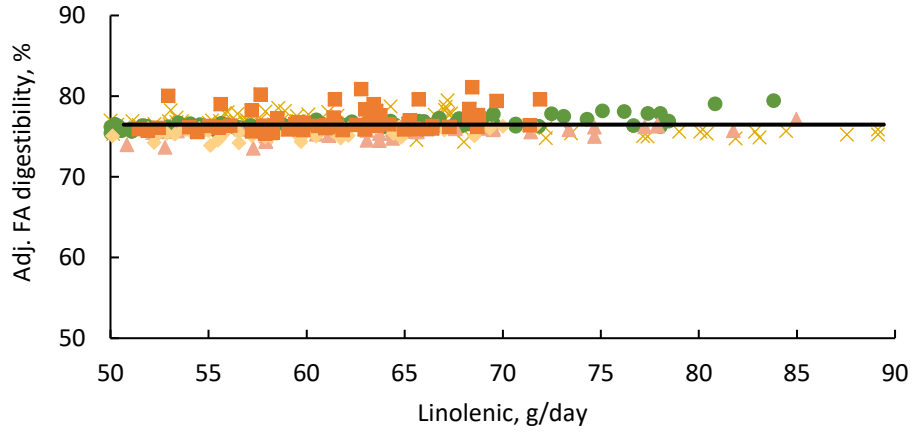

Supplemental Figure 5.3. Effect of the inclusion of dietary oleic (a), linoleic (b), and linolenic (c) on FA digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).

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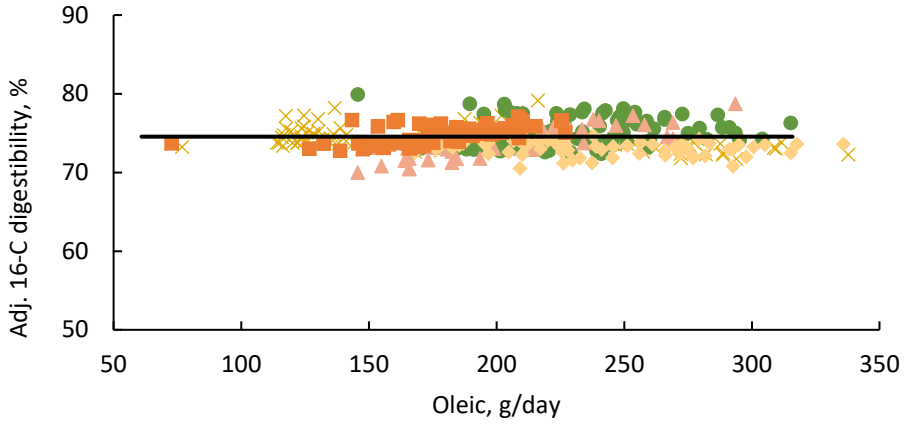

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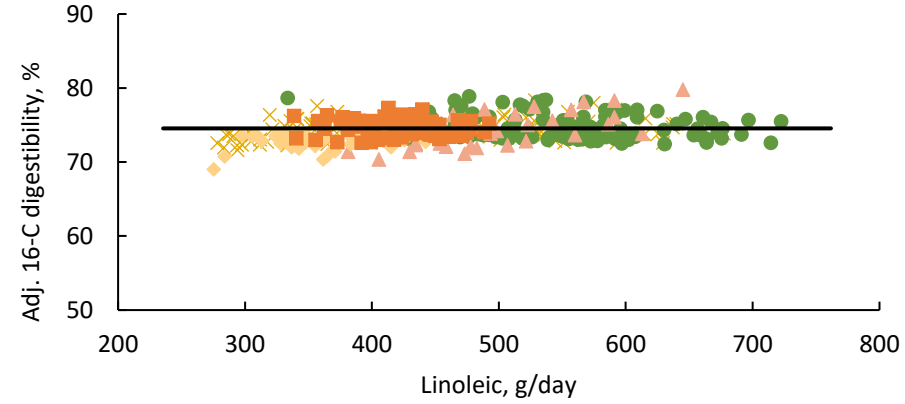

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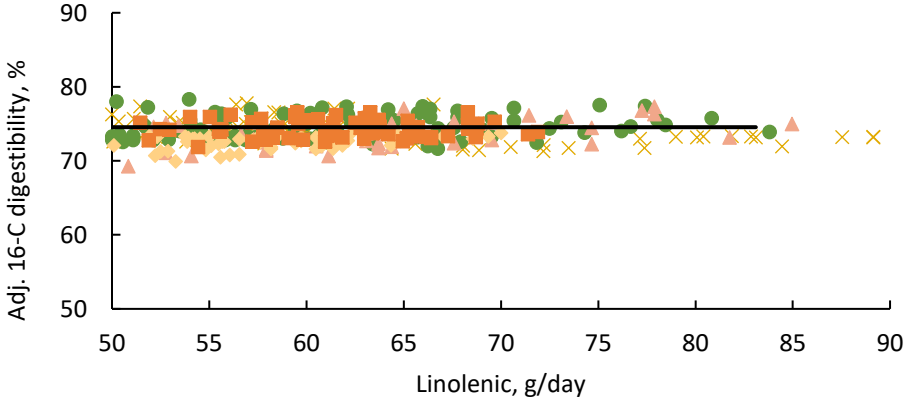
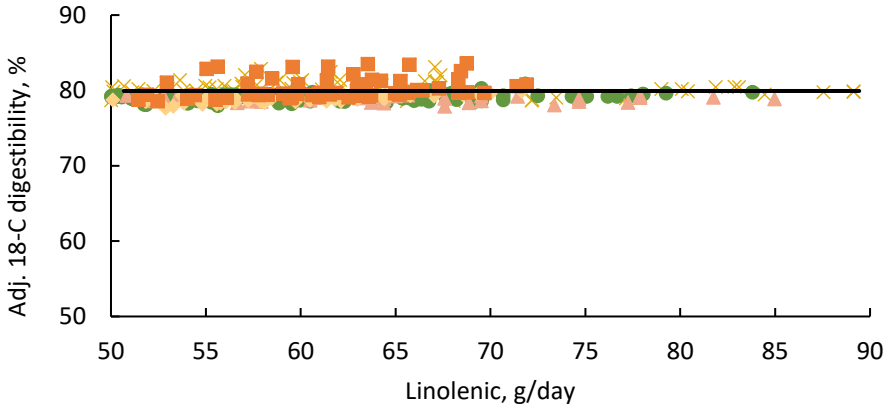


Supplemental Figure 5.4. Effect of the inclusion of dietary oleic (a), linoleic (b), and linolenic (c) on 16-carbon FA digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).



Supplemental Figure 5.5. Effect of the inclusion of dietary linolenic on 18-carbon FA digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆, and study 5: ■).

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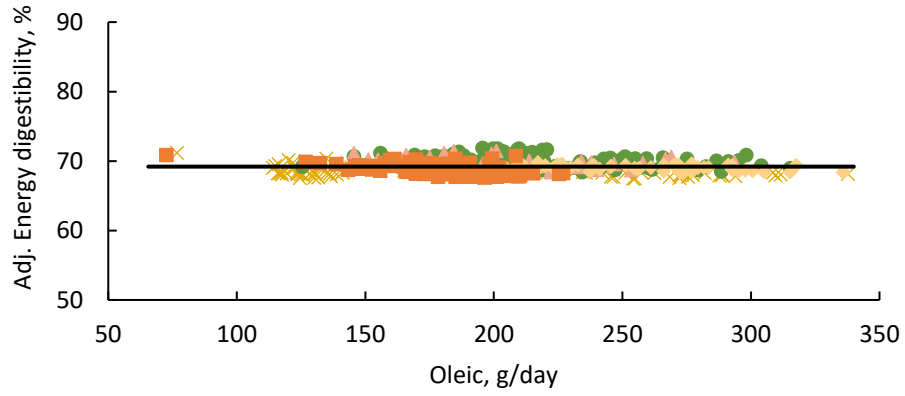

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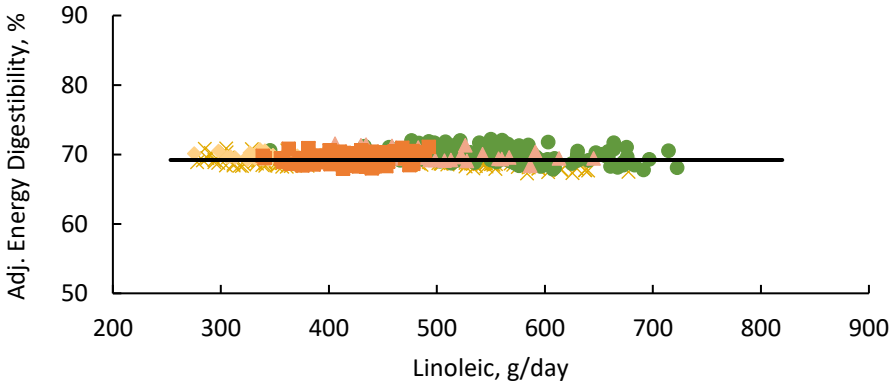
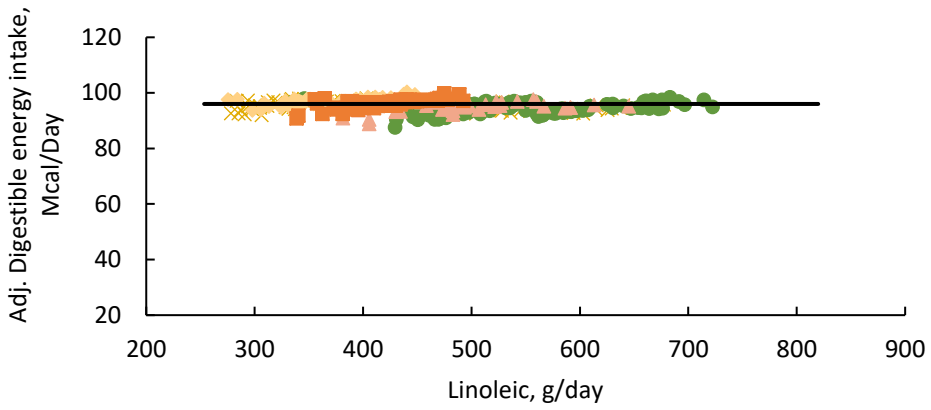
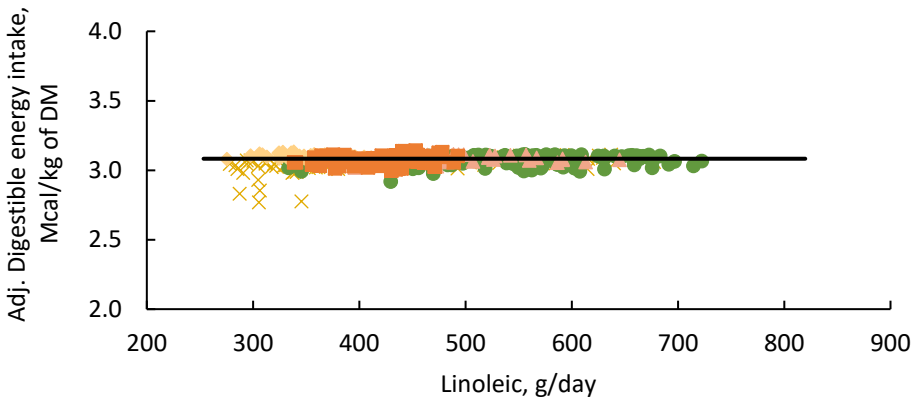


Supplemental Figure 5.6. Effect of the inclusion of dietary oleic (a) and linoleic (b) on energy digestibility. Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).



Supplemental Figure 5.7. Effect of the inclusion of dietary linoleic on digestible energy intake (DEI, Mcal/d). Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).



Supplemental Figure 5.8. Effect of the inclusion of dietary linoleic on digestible energy intake (DEI, Mcal/kg of DM). Data points represent the adjusted for study effect individual observations and are coded by the study (study 1: × ; study 2: ● ; study 3: ▲ ; study 4: ◆ , and study 5: ■).

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CHAPTER 6

BODY COMPOSITION OF HOLSTEIN CATTLE

ABSTRACT

Our objective was to predict body fat, protein, ash, and water composition (**EBFat**, **EBProtein**, **EBAsh**, and **EBWater**) as a percentage of empty body weight (**EBW**) in heifers and cows using only data from Holsteins cattle. In order to accomplish our objective, we conducted a meta-regression analysis of 129 treatment means from 26 peer-reviewed publications. Methods to determine composition included direct analysis of EBW, analysis of carcass or rib, and dilution of deuterium oxide or urea. Means and standard deviations for the variables included in the analysis were 11 ± 5 % EBFat, 19 ± 2 % EBProtein, 4 ± 1 % EBAsh, 66 ± 4 % EBWater, 158 ± 122 kg EBW, and 0.77 ± 0.24 kg average daily gain (**ADG**) for heifers, and 22 ± 6 % EBFat, 16 ± 1 % EBProtein, 4 ± 1 % EBAsh, 60 ± 5 % EBWater, 479 ± 54 kg EBW, and 2.9 ± 0.4 body condition score (**BCS**) for cows. The first step was a mixed model with a random effect of study, fixed effects of method (direct, carcass, and dilution), stage (heifer and cow), and one of three possible ways to express EBW (4^{th} order polynomial of EBW - **polEBW**, natural logarithm of EBW - **lnEBW**, or EBW to the power of 0.75 – **EBW^0.75**). Additionally, the models were weighted by the inverse of the standard deviation of each respective study. The models derived during the first step were named baseline. The second step involved adding the effects of ADG for heifers and BCS for cows to the baseline models. To evaluate these models, we performed cross-validations within the dataset to select the best fitting models based on the concordance correlation coefficient (**CCC**), mean square error of prediction (**MSEP)**, its decomposition, and root MSEP (**RMSEP**). Based on the cross-validation, the best fitting models were the ones developed using lnEBW. The proposed heifer models for EBFat and EBProtein were: EBFat = -

6.0 + 1.37*lnEBW – 15.3*ADG + 5.23*lnEBW*ADG (CCC=0.88, RMSEP=0.99); EBProtein = 23 – 1.12*lnEBW (CCC=0.59, RMSEP=1.43). The proposed cow models for EBFat and EBProtein were: EBFat = 1.6 + 6.9*BCS (CCC=0.92, RMSEP=2.1); EBProtein = 21 – 1.9*BCS (CCC=0.88, RMSEP=0.54). The proposed models were further used to determine energy and protein requirements for growth and for EBW change in mature animals. The proposed models suggest that the energy requirement for growth is less for older heifers and more for younger heifers than what the NRC 2001 predicted.

INTRODUCTION

The literature contains many papers showing the importance of having a well-designed growth program for replacement heifers due to its impact on future milk production and the economics associated with milk production (Van Amburgh, 2017; Bach, Khan, and Miller-Cushon, 2017; Hoffman, 2017; Overton and Dhuyvetter, 2017). McCandlish (1922) was one of the first to publish a review showing the effects of age of cow, gestation period, the season of freshening, birth weight of calves, and rate of growth on growth and nutrition of dairy calves. In that paper, the author emphasized the relationship between nutrition and average daily gain (**ADG**). Since then, many studies have focused on better understanding the relationship between nutrition and ADG, as well as their effect on mammary gland development and future milk production (Radcliff *et al*, 2000; Lammers, Heinrichs, and Kensinger, 1999; Radcliff *et al.*, 2000; NRC, 2001; Moallen *et al*., 2004; Shamay *et al.*, 2005; Davis Rincker *et al*, 2008).

Two requirements must be considered in diet formulation for growing animals: requirements for maintenance and requirements for growth (NRC, 2001). Requirements for maintenance can be determined experimentally using metabolic chambers, while requirements

for growth can be determined based on body composition during growth. Accordingly, different rates of growth can be achieved by supplying the energy and protein required for each specific rate of growth. As animals achieve maturity, changes in body composition primarily reflect depletion or repletion of tissues when diets provide insufficient or excess energy (NRC, 2001). By appropriately predicting the changes in body composition with changes in body condition score, we can better account for the amount of energy associated with changes in the body weight of mature animals. Thus, the prediction of body composition is a crucial step in determining the energy and protein requirements for growing animals and deposition of energy in mature animals. However, there are no prediction models for body composition in dairy breeds derived primarily with data collected in dairy breeds.

The NRC (2001) model for energy and protein requirements for growth of replacement calves and heifers, and body composition at different body condition scores (**BCS**) for mature animals was developed based on Fox *et al.* (1999), which adjusted the Beef NRC (1996) body composition model to dairy breeds by using a size scale approach. The size scale approach assumes that the chemical composition of gain is similar among animals at the same proportion of mature BW (NRC, 2001), but this may not be true in dairy and beef cattle. In the past 20 years new data collected in Holstein animals have become available.

We hypothesized that new prediction models for body composition derived from more recent data collected using Holstein cattle would better fit the current dairy production system and provide better predictions of energy and protein retention during growth. Our objective was to derive prediction models for body composition by performing a meta-regression of data from Holstein cattle so that we can use these models to determine net energy and net protein

requirements for growth of replacement heifers and the energy retained or lost with changes in empty body weight (**EBW**) in mature animals.

MATERIALS AND METHODS

Literature search and selection of peer-reviewed manuscripts

We used Google Scholar (https://scholar.google.com/), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), and Science Direct (http://www.sciencedirect.com/) search engines to search for peer-reviewed manuscripts that contained data for body composition in dairy cattle. The search terms included "body composition," "urea dilution," "deuterium oxide," "potassium-40 liquid," "composition of growth," and "carcass composition." The search for manuscripts was performed during August 2017 to March 2018 and included manuscripts published until February 2018. Additionally, we used publications cited in NRC (2001).

The study inclusion criteria were (1) peer-reviewed manuscript published in English, (2) body composition measured in dairy cattle breeds, (3) reported body weight (**BW**) or EBW, (4) reported ADG and BCS for growing and mature animals, respectively, and (5) at least one of the following: body water composition, body fat composition, body protein composition, and body ash composition (with respective measurement of the error around the mean). Only manuscripts that satisfied the inclusion criteria were used. We also included data from a PhD dissertation (Meyer, 2007) because it contains relevant data.

Database

In total, we found 31 studies that published data on dairy breeds including Holstein or European Friesian (26 studies), Jersey (2 studies), and Holstein cross-breeds (1 study) animals

published between 1986 and 2017. Due to the lack of data available in regards to breeds other than Holstein, we decided to limit this study to Holstein cattle and removed the three studies performed with Jersey and cross-breed cattle. After further narrowing the database only to include studies that reported the variables needed to perform the meta-regression, 26 studies remained that together reported 129 treatment means.

The body composition was determined using carcass composition (*n* = 3 studies), deuterium oxide (*n* = 7 studies), EBW composition (*n* = 18 studies), potassium-40 liquid scintillation detection (*n* = 1 study), rib composition (*n* = 1 study), and urea dilution (*n* = 5 studies). These techniques are explained at Radcliff *et al*. (1997), Martin and Ehle (1986), Donnelly and Hutton (1976), Belyea *et al*. (1978), Davis Rincker *et al*. (2008), Swartz *et al*. (1991), respectively. These methods were categorized as: direct (EBW composition), carcass (carcass composition and rib composition), and dilution (deuterium oxide, potassium-40 liquid, and urea).

The variables included in the database were: study, treatment, method used to determine body composition, statistical method used in each corresponding study (fixed effect or mixed model), stage (growth or mature), year of publication (from 1986 to 2017), length of the study (days), sex, BW, EBW, BCS, ADG, age (days), EBWater, EBFat, EBProtein, EBAsh, and whether standard error of the mean (**SEM**), standard error of the difference (**SED**), or root mean square error (**RMSE**) were reported. In addition to these variables, we calculated the fat-free mass (**FFM**) as a percentage of EBW and lifetime ADG (**ltADG**) as kilograms per day. Fat-free mass as % of EBW was calculated according to the following formula: FFM = 100 – EBFat; the SEM for the computed FFM was assumed to be equal to the SEM of EBFat adjusting to the same coefficient of variance for both variables. Lifetime ADG was calculated according to the

following formula: ltADG = (EBW-40)/age at the end of the experiment, assuming an average birth EBW of 40 kg (Hickson *et al.*, 2015). A summary of the variables included in the database is provided in Table 6.1.

To have a standard measurement of the error around the mean across all 26 studies, when necessary, we adjusted the reported error to be comparable to the SEM as suggested on Roman-Garcia *et al.* (2016). Then, for the statistical analysis, the SEM was truncated at half of the mean SEM (Firkins *et al*., 2001). Finally, since fixed effect models tend to have higher SEM (Littell *et al.*, 1998), within each statistical method (fixed effect and mixed model), the SEM was standardized to the mean of their respective distributions (Roman-Garcia *et al.*, 2016).

Model derivation

To derive the prediction equations for body composition in Holstein animals, a 2-step meta-regression using SAS PROC MIXED (SAS Institute inc., Cary, NC) was performed.

During the first step, we developed three possible baseline models that used three different ways to express the covariate EBW (4th order polynomial of EBW - **polEBW**, natural logarithm of EBW – **lnEBW**, or metabolic EBW – **EBW^0.75**) is presented on [Equation 6.1]. Also, the models were weighted by the inverse of the standardized SEM as explained above.

$$Y_{ijk} = (\beta_0 + Study_{0,k}) + \beta_1 * Method_i + \beta_2 * Stage_j + (\beta_3 + Study_{1,k}) * EBW + \beta_4 * Method_i * Stage_j + \beta_5 * Method_i * EBW + \beta_6 * Stage_j * EBW + \sum_{a=0}^{4} \beta_{7+a} * Year^a + Study_k + e_{ijk}$$

[6.1, baseline]

where Y_{ijk} (i = method, j = stage, and k = study) is the observed EBWater, EBFat, EBProtein, EBAsh, or FFM; $(\beta_0 + Study_{0,k})$, β_1, β_2, and $(\beta_3 + Study_{1,k})$ are the partial coefficients corresponding to the intercept, method (i = direct, carcass, or dilution), stage (j = growing or mature), EBW (polEBW, lnEBW, or EBW^0.75), respectively; β_4, β_5, and β_6 are the partial coefficents corresponding to the 2-way interactions between the covariates; $\sum_{a=0}^{4} \beta_{7+a} * Year^a$ are the fourth-order polynomial terms for the effect of Year; $Study_k$ is the random effect of study (k = 1 to 26); and e_{ijk} is the error term.

During the second step, the baseline was adjusted to the fixed effect of ADG and age at the midpoint of each experiment (**MidAge**) for heifers and adjusted to the fixed effect of BCS for cows. The ADG, MidAge, and BCS were included using a 4th order polynomial and possible 2-way interactions. As described in step one, models contained the random effect of study and were weighted by the inverse of the standardized SEM. The models used on the second step for heifers and cows are presented in [Equation 6.2] and [Equation 6.3], respectively.

$$Y_k = (\beta_0 + Study_{0,k}) + \beta_1 * \hat{Y}_b + \sum_{n=1}^{4}(\beta_{n+1} + Study_{n+1,k}) * ADG^n + \sum_{n=1}^{4}(\beta_{n+5} + Study_{n+5,k}) * MidAge^n + \beta_{10} * \hat{Y}_b * ADG + \beta_{11} * \hat{Y}_b * MidAge + \beta_{12} * ADG * MidAge + Study_k + e_k$$

[6.2, heifers]

where Y_k (k = study) is the observed EBWater, EBFat, EBProtein, EBAsh, or FFM; $(\beta_0 + Study_{0,k})$, β_1, $(\beta_{n+1} + Study_{n+1,k})$, and $(\beta_{n+5} + Study_{n+5,k})$ are the partial coefficients corresponding to the intercept, predicted body composition using the baseline (Equation 6.1, b = polEBW, lnEBW, and EBW^0.75), 4th order polynomial on ADG, and the 4th

order polynomial on MidAge, respectively; β_{10}, β_{11}, and β_{12} are the partial coefficents corresponting to the 2-way interactions between the covaviates; $Study_k$ is the random effect of study (k = 1 to 19); and e_k is the error term.

$$Y_k = (\beta_0 + Study_{0,k}) + \beta_1 * \hat{Y}_b + \sum_{n=1}^{4}(\beta_{n+1} + Study_{n+1,k}) * BCS^n + \beta_6 * \hat{Y}_b * BCS + Study(Year)_k + e_k$$

[6.3, cows]

where Y_k (k = study) is the observed EBWater, EBFat, EBProtein, EBAsh, or FFM; $(\beta_0 + Study_{0,k})$, β_1, $(\beta_{n+1} + Study_{n+1,k})$, and are the partial coefficients corresponding to the intercept, predicted body composition using the baseline (Equation 6.1, b = polEBW, lnEBW, and EBW^0.75), and the 4th order polynomial on BCS, respectively; β_6 is the partial coefficents corresponting to the interaction between the covaviates; $Study_k$ is the random effect of study (k = 1 to 7); and e_k is the error term.

Finally, models were subjected to backward elimination to remove nonsignificant variables. During this process, the covariate with the highest nonsignificant *P*-value was removed during successive runs until only significant effects remained in the model ($P < 0.05$). Nonsignificant terms that had significant interaction or higher polynomial order were not removed from the model. Once the final models were defined, we checked for multicollinearity among variables using the variation inflation factor (**VIF**) test; the cutoff used to determine collinearity for main effects was a VIF greater than 10 and for interactions was VIF greater than 100.

For all the models, residuals were analyzed using the SAS PROC UNIVARIATE (SAS

Institute inc., Cary, NC); normal quantiles plots, distribution and probability plots, and studentized residuals were considered for detecting outliers. Data points were only removed if they were considered outliers in at least two of these methods analyses described above, where the cutoff for studentized residuals was ± 3.5 SD.

Model evaluation

Finally, the precision and accuracy of the final models were assessed by 5-fold cross-validation with 20 random repetitions as described in De Souza *et al*. (2018). The fit statistic parameters calculated were: mean bias, slope bias, concordance correlation coefficient (**CCC**), the mean square error of prediction (**MSEP**), root mean square error of prediction (**RMSEP**), and decomposition of MSEP (mean bias, slope bias, and random error).

The fit statistics parameters were analyzed to determine the best way to express EBW (polEBW, lnEBW, expEBW), using the PROC GLIMMIX procedure of SAS v.9. 4 (SAS Institute Inc., Cary, NC) according to the following model [Equation 6.4]:

$$Y_{isj} = \mu + r_i + Stage_s + EBW_j + Stage_s * EBW_j + e_{isj} \quad [6.4]$$

where Y_{ij} is one of the fit statistics of interest; μ is the overall mean; r_i is the random effect of fold (i = 1 to 100); $Stage_s$ is the fixed effect of stage (s = growing or mature); EBW_j is the fixed effect of corresponding EBW (j = polEBW, lnEBW, or expEBW), and e_{isj} is the residual error.

Similarly, to the test of [Equation 6.4], we compared the models using ADG and ltADG. However, in this model, there was no effect of stage because only the models developed for heifers had the effect of ADG or ltADG.

Energy and protein requirements for growth in heifers and net energy provided by the change in body condition score in cows

Once chosen, the proposed models EBFat and EBProtein were used to determine net energy and net protein requirements for growth (**NE_g** and **NP_g**, respectively) in heifers and net energy associated with the change in BCS of cows.

For energy calculations we assumed that fat and protein contain 9.29 and 5.55 Mcal of energy per kilogram, respectively, as their chemical energy composition (NRC, 2001). The NP_g was calculated as the protein content of the gain.

RESULTS

Model derivation

Step 1 – Baseline

The effects of body composition measurement method (direct, carcass, and dilution) were not significant ($P > 0.15$) for EBFat, EBProtein, EBAsh, and FFM regardless of BW expression. For EBWater, significance of measurement method was $P < 0.01$, $P < 0.01$, and $P = 0.03$ for the 3 ways of expressing EBW (polEBW, lnEBW, and EBW^0.75).

The effect of stage was not statistically significant as a covariate for body composition except for two cases. Stage was significant in models that used polEBW. Stage also was significant for EBProtein ($P < 0.01$) regardless of EBW expression, and, with stage in the model, EBW was not significant ($P > 0.3$). However, without stage in the model, EBW was significant ($P < 0.02$ for all expressions of EBW). When the residual plots from both models for EBProtein

(with stage or with EBW) were compared, the model using stage consistently overpredicted EBProtein in older heifers, whereas the model using EBW had an unbiased residual. Thus, we decided to use the model with EBW and not with the stage. For EBWater, EBFat and FFM, there was no effect of the stage ($P > 0.05$).

Without stage in the model, EBW was a significant covariate for body composition regardless of expression method (polEBW, lnEBW, and EBW^0.75) except for EBash ($P > 0.08$ for all models). Models that contained polEBW used the quadratic term for EBW to determine EBWater, EBFat, and FFM ($P < 0.02$ for all linear and quadratic terms), whereas only the linear term was used for EBProtein ($P = 0.01$). Both lnEBW and EBW^0.75 were significant covariates for EBW on EBWater, EBFat, EBProtein, and FFM ($P < 0.02$ for all terms).

The effect of year on body composition was not significant in any of the models. However, animals increased in body size throughout the years.

Step 2

Using the term for MidAge along with ADG or ltADG and the predicted value from the baseline caused multicollinearity problems (VIF greater than 10 for all models). MidAge was the variable with greatest VIF, after removing MidAge, the multicollinearity problem was resolved, and the VIF for the remaining variables was less than 10. For both ADG and ltADG, none of the models used higher order polynomials. When the effect of ADG or ltADG was significant, it interacted linearly with the predicted value from the baseline.

The effects of ADG and ltADG were similar for most models and differed only for EBWater when using polEBW. For EBWater, using ADG yielded a significant effect of the

interaction between predicted values from the baseline and ADG ($P < 0.01$). Using ltADG resulted in an interaction that was not significant ($P = 0.18$).

The interaction between ADG or ltADG and the baseline predictions was significant for EBFat and FFM regardless of EBW expression. This interaction was also significant for EBProtein and EBWater except for the model using polEBW and ltADG. For the EBAsh models, none of the fixed effects (predicted values from the baseline, ADG or ltADG, and interaction) were significant ($P > 0.05$).

The effect of BCS was linearly significant for EBWater, EBFat, EBProtein, and FFM (all with $P < 0.01$) but not EBAsh, and none of the models had higher order polynomials. In addition, the interactions between the baseline predicted value from the baseline and BCS were significant.

For the models developed using lnEBW, neither the main effect of lnEBW or the interactions with the baseline predictions were significant. However, for models developed with polEBW and EBW^0.75 interactions of BCS and the baseline predictions were significant.

Model evaluation

The strategy used to evaluate the proposed models was to compare the main effect of the three possible ways to express EBW (polEBW, lnEBW, and EBW^0.75) to the stage-specific (heifers and cows) performance using the interaction between stage and EBW. Although the main effect of the stage was significant ($P < 0.05$) for most of the fit statistics for all body compositions. The comparison is unfair because of the substantial difference between the

number of observations between both stages (Table 6.1); and therefore, we will not discuss the main effect of the stage.

First, analyzing the fit statistics of the baseline models, the models using lnEBW outperformed the models using polEBW and EBW^0.75 for all body composition terms (EBWater, EBFat, EBProtein, EBAsh, and FFM) on the majority of the fit statistics analyzed (mean bias, slope bias, CCC, MSEP, decomposition of MSEP, and RMSEP – data not shown). Although the final decision on which expression of EBW yielded best predictions of body composition was based on the models created on step 2, this indicates that EBW should be expressed using lnEBW.

On the evaluation of the models generated on step 2, EBWater, EBFat, EBProtein, EBAsh, and FFM (Tables 6.2, 6.3, 6.4, 6.5, and 6.6, respectively), there were smaller effect of the expressions of EBW than those observed in the baseline analyses. However, comparing the models generated on step 2 for heifers, the model using lnEBW outperformed the EBW^0.75 on EBWater, EBFat, EBProtein, and FFM. As an alternative, the polEBW was between the other two models. Since there were no effects of EBW on EBAsh (lnEBW, polEBW, and EBW^0.75, $P > 0.05$ for all), the covariates representing EBW were removed from the EBAsh models. For the cow data, the effect of expression of EBW were not significant for all body composition terms regardless of the expression used (lnEBW, polEBW, and EBW^0.75, $P > 0.05$ for all), and, therefore, the effects of EBW were removed from all cow' models.

Furthermore, our ultimate goal was to calculate the NE_g and NP_g which use only the models developed for EBFat and EBProtein. For EBFat, models derived using lnEBW and polEBW had similar performance on the model evaluation analysis. However, lnEBW

outperformed EBW^0.75 for CCC (0.88 vs 0.86, $P = 0.04$; respectively), MSEP (0.99 vs 1.66, $P < 0.01$, respectively), and RMSEP (0.99 vs 1.29, $P < 0.01$; respectively); whereas polEBW and EBW^0.75 did not differ ($P > 0.05$, for all fit statistics). For EBProtein, lnEBW was the only model to have a slope bias not different from zero (0.089, $P = 0.17$), whereas polEBW and EBW^0.75 had significant slope bias (-0.73 and -0.97, $P < 0.02$; respectively). Additionally, polEBW had lower MSEP than polEBW and EBW^0.75 (2.11, 3.14, and 4.23, $P < 0.05$ for all comparisons; respectively) and lower RMSEP than polEBW and EBW^0.75 (1.43, 1.75, and 2.03, $P < 0.05$ for all comparisons; respectively). Because lnEBW was overall as good or better than the alternatives in model fit, and because lnEBW results in equations for body composition that can be easily converted to equations for determining the composition of gain, we selected the models developed using lnEBW for our proposed models.

Finally, we found no difference in the 100-fold cross-validation model fit when we compared ADG with ltADG in the body composition model for heifers (data not shown). Because ADG and ltADG had similar value as covariates, and because we did not know the actual birth weight of the animals used in the analysis, we selected the models using reported ADG for our proposed models.

Proposed models for body composition.

As described previously, we used lnEBW and reported ADG in our final proposed models of body composition and retained energy associated with growth or body condition change. Retained energy and protein are equivalent to the Net Energy and Net Protein requirements for growth or body condition change.

The EBWater model was the only model for which method to determine body composition was significant. The estimated partial regression coefficients for direct, carcass and dilution were 0.00 ± 0.00, 0.442 ± 0.408, and -3.79 ± 1.93, respectively, and these coefficients were added to the intercept using a weighted average based on the number of observations generated by each method (direct = 61, carcass = 24, and dilution = 43).

Following are the proposed models for body composition in heifers (EBWater, EBFat, EBProtein, EBAsh, and FFM; Equations 6.5, 6.6, 6.7, 6.8, and 6.9, respectively) and the proposed models for body composition in cows (EBWater, EBFat, EBProtein, EBAsh, and FFM; Equations 6.10, 6.11, 6.12, 6.13, and 6.14, respectively). The baseline models developed on the first step of the modeling process are presented in Table 6.7 and figures are presented in Supplementary Material 6.1, 6.2, 6.3, 6.4, and 6.5.

$$EBWater = 78.3\ (\pm 2.04) - 0.391\ (\pm 0.023) * lnEBW + 14.5(\pm 2.24) * ADG - 5.41(\pm 0.280) * lnEBW * ADG$$

[6.5, heifers]

$$EBFat = -6.05\ (\pm 0.467) + 1.37\ (\pm 0.026) * lnEBW - 15.3(\pm 0.438) * ADG + 5.23(\pm 0.034) * lnEBW * ADG$$

[6.6, heifers]

$$EBProtein = 22.6\ (\pm 0.307) - 1.12\ (\pm 0.275) * lnEBW$$

[6.7, heifers]

$$EBAsh = 3.81\ (\pm 0.303)$$

[6.8, heifers]

$$FFM = 113\ (\pm 2.90) - 3.04\ (\pm 0.027) * lnEBW + 8.67(\pm 3.25) * ADG - 3.52(\pm 0.030) * lnEBW * ADG$$

[6.9, heifers]

$$EBWater = 72.9\ (\pm 1.54) - 4.98(\pm 0.312) * BCS$$

[6.10, cows]

$$EBFat = 1.57\ (\pm 0.216) + 6.91(\pm 0.117) * BCS$$

[6.11, cows]

$$EBProtein = 21.0\ (\pm 0.861) - 1.89(\pm 0.266) * BCS$$

[6.12, cows]

$$EBAsh = 4.49\ (\pm 0.167)$$

[6.13, cows]

$$FFM = 98.8\ (\pm 3.59) - 7.02(\pm 1.09) * BCS$$

[6.14, cows]

where $lnEBW$ is the natural logarithm of empty body weight (kg), ADG is the average daily gain (kg/d), and BCS is body condition score (scale 1 – 5).

Based on the proposed models, we calculated the NE_g and NP_g (Table 6.8 and 6.9, respectively) and net energy provided per kilogram of EBW change as a function of BCS (Table 6.10). Additionally, the NE_g and NP_g calculated using the base model are presented on Supplementary Material (Supplemental Table 6.1 and Supplemental Table 6.2, respectively).

DISCUSSION

Most systems to determine body composition in dairy cattle assume that cattle from different breeds have similar body composition at a given level of maturity; and therefore, they use a size-scaling approach to adjust body composition across breeds (NRC, 2001; CSIRO, 2007; INRA, 2007; CNCPS – Fox and Van Amburgh, 2003). Because data on body composition of dairy cattle was limited, the size-scaling approach was a reasonable method to predict their body composition. However, data used to develop the size-scaling approach came mostly from steers of beef breeds being fed typical high grain feedlot diets (Beef NRC, 1996); based on our results, we suggest that even after size-scaling, these systems do not accurately represent body composition in dairy heifers. Cattle fed high grain diets in feedlots deposit more fat per unit BW than the typical dairy heifer fed a higher forage diet or a diet at restricted intake for slower growth. Our proposed models for growing animals are considerably different from the system suggested in the NRC (2001).

Body composition in heifers and net energy and net protein requirements for growth

The EBW and ADG for heifers ranged from 25 kg and 0.150 kg/d to 350 kg and 1.27 kg/day, respectively. Within this range, our proposed models suggested that EBWater (Equation 6.5, Figure 6.1) decreases as lnEBW (estimated coefficient: 0.391 ± 0.023, $P < 0.01$) and ADG

(estimated coefficient: 14.5 ± 2.24, $P < 0.01$) increase, with a negative interaction (estimated coefficient: -5.41 ± 0.280, $P < 0.01$) between the covariates.

In contrast, EBFat (Equation 6.6, Figure 6.2) increases as lnEBW (estimated coefficient: 1.37 ± 0.026, $P < 0.01$) and ADG (estimated coefficient: -15.3 ± 0.438, $P < 0.01$) increase, with a positive interaction (estimated coefficient: 5.23 ± 0.034, $P < 0.01$) between the covariates.

Whereas, EBProtein (Equation 6.7, Figure 6. 3) decreases as lnEBW (estimated coefficient: -1.12 ± 0.275, $P < 0.01$) increase. The EBAsh (Equation 6.8, Figure 6.4) were fixed at 3.81% ± 0.303 ($P < 0.01$), respectively.

Finally, the FFM had a very similar pattern to EBWater. The FFM (Equation 6.9, Figure 6.5) decreases as lnEBW (estimated coefficient: -3.04 ± 0.027, $P < 0.01$) and ADG (estimated coefficient: 8.67 ± 3.25, $P < 0.01$) increase, with a negative interaction (estimated coefficient: -3.52 ± 0.030, $P < 0.01$) between the covariates.

Analyzing the fit statistics parameters, the EBWater, EBFat, and FFM – models with the effect of both lnEBW and ADG – had CCC values of 0.85, 0.88, and 0.87, respectively; RMSEP values of 2.02 (3.05% of the observed mean), 0.99 (9.12% of the observed mean), and 2.27 (2.55% of the observed mean), respectively; and most of the MSEP associated with the random error (87, 92, and 92%, respectively). The EBProtein with only the effect of lnEBW had CCC of 0.59, RMSEP of 1.43 (7.73% of the observed mean), and 89% of the MSEP associated with the random error. The EBAsh was a fixed value and had CCC of 0.41, respectively; RMSEP of 0.93 (24.3% of the observed mean), respectively; and 82% of the MSEP associated with the random error.

Although, to the best of our knowledge, there are no formal recommendations for the model evaluation parameters in the literature, our goal was to have CCC > 0.85, RMSEP < 10% of the observed mean and MSEP associated with the random error > 90% (personal communication – Dr. Eremias Kebreab). Within these goals, the EBWater, EBFat, and FFM surpassed our thresholds and are considered proper prediction models.

The EBProtein had CCC lower than our threshold. However, this was expected because of the small effect of lnEBW on EBProtein, and the proposed model seems appropriate considering the low RMSEP.

The EBAsh was the model with the worst fit statistics parameters. In this regard, the EBAsh did not achieve the thresholds established for the fit statistic parameters. However, since the EBAsh is not used in the calculations to determine the NE_g and NP_g, this did not impact the application of the proposed model in the calculation of NE_g and NP_g.

With respect to the requirements, we used the proposed models to determine the composition of the tissue deposited during the growth (Figure 6.6) and based on its composition we calculated the NE_g and NP_g. The NE_g and NP_g are dependable on the EBW and ADG (Table 6.8 and 6.9). However, the effect of EBW is much greater for NE_g than NP_g. In order to compare the requirements calculated using the proposed model and the requirements suggested in the NRC 2001, we created three growth programs for replacement heifers based on the ADG and EBW observed in our database (Figure 6.7 and 6.8). The three scenarios were restricted, normal, and elevated. These scenarios allow for different ADG according to the EBW, wherein all scenarios the EBW varied from 40 to 400kg and the ADG ranged from 0.164 to 0.817 kg, 0.215

to 0.990 kg, and 0.250 to 1.188 kg for the scenarios restricted, normal, and elevated, respectively. The assigned ADG for each scenario was based on the ADG in our database.

After comparing the proposed models to the NRC (2001), the proposed model starts with higher NE_g and lower NP_g than the NRC (2001). In contrast, as the animals grow the proposed model suggests lower NE_g and higher NP_g than the NRC (2001). Whereas, for NE_g, differences between models were even higher in the scenarios restricted and normal (Figure 6.7). Regarding the NP_g, the NRC (2001) requirements become flat after 200 kg EBW, and after 400 kg EBW achieves a plateau. On the proposed model, since lnEBW had a small impact on EBProtein the calculated NP_g using the proposed model increases as we increased the ADG and the EBW (Figure 6.8).

The fact that NRC (2001) suggested a much faster increase in the NE_g than the proposed model did implies that, on the NRC (2001) system, heifers were depositing more fat and less protein than on the proposed model at any given ADG and EBW levels. This difference may be due to the NRC's (2001) roots in the Beef NRC (1996), in which it is expected that steers from beef breeds fed feedlot diets will gain more fat than dairy heifers fed typical dairy-replacement diets. This is supported by the fact that, as we increased the ADG, both systems become more similar – in this case, dairy heifers fed to have high ADG become more similar to steers in a feedlot.

Body composition in cows and net energy per kilogram of empty body weight change

Analyzing the models derived for cows that started with the baseline using lnEBW indicates that the effect of predicted value from the baseline was not significant for any of the body composition variables (EBWater, EBFat, EBProtein, EBAsh, and FFM). The natural

logarithm function used in the baseline suggests a minimal effect of EBW on body composition for animals with EBW greater than 450 kg. Since the majority of our cow data is above 450 kg EBW we expected that the baseline derived using lnEBW would not be significant for cows – as shown by the proposed models.

Our data supported the NRC (2001) finding that body composition in cows is related only with BCS, and not with EBW. According to our data, for a 1-unit increase in BCS the EBWater, EBProtein, and FFM decrease 4.98 (± 0.312), 1.89 (± 0.266), and 7.02 (± 1.09), respectively; whereas EBFat increases 6.91 (± 0.117) and EBAsh is fixed at 4.49 (± 0.167) (Figures 6.9, 6.10, 6.11, 6.12, and 6.13). These changes are very similar to the ones proposed in the NRC (2001); except for EBAsh, where the proposed model does not change the EBAsh content, and the NRC (2001) decreases EBAsh by 0.44% per 1-unit increase on BCS.

Although the changes in body composition associated with the change in BCS on both the NRC (2001) and the proposed model are similar, the initial values (BCS equals to 1) for body composition are different on both models, especially for EBFat and EBProtein. The proposed model starts with 8.48 EBFat and 19.1% EBProtein, and the NRC (2001) starts with 3.77 EBFat and 19.4% EBProtein (Table 6.10).

Accordingly, the models differ on the energy content per kilogram of EBW change. The proposed model suggested that the energy content ranged from 6.67 to 8.16 Mcal/kg of EBW change, assuming that a cow with BCS of 3 has a BW of 700 kg and the BW change 9.7% per 1-unit change in BCS (calculated based on the data used to derive the equations) (Table 6.10). The NRC (2001) ranged from 5.14 to 9.59 Mcal/kg of EBW change.

Finally, it is essential to consider the limitation of our database on cows. First, from the six studies used in our analysis, four studies used dilution techniques to determine body composition; and therefore, our analysis is prone to errors associated with dilution techniques. Second, although the BCS ranged from 2.1 to 3.9 – which is likely the range of BCS observed in dairy farms – the majority of the data came from cows with BCS between 2.5 and 3.0 (lower and upper quartiles); and therefore, the variation on BCS was very small. Given this information, the proposed models must be used with caution, and additional data is required to derive prediction equations for body composition in cows correctly.

CONCLUSIONS

Our analysis supports that the fat content of gain, and thus the energy content of gain, is higher for young heifers and less for older heifers than that suggested by NRC 2001. The differences between the proposed and NRC (2001) models for heifers may be because NRC (2001) used the size-scaling method to adjust a model derived for beef cattle to dairy cattle. In contrast, the proposed model was derived exclusively from data collected in Holsteins.

Concerning the composition of cows, we founded similar changes in body composition per 1-unit change in BCS to the NRC (2001). However, the starting body composition (BCS 1) differ between both models. By starting with higher EBFat, the proposed models suggest higher energy content per kilogram of EBW change at low BCS than the NRC (2001). However, as BCS increase, the NRC (2001) implies greater energy content per kilogram of EBW change than the proposed model.

APPENDICES

APPENDIX A

Tables

Table 6.1. Descriptive summary of the quantitative variables included in the database.

	Heifers			Cows		
	count	mean	SD[1]	count	mean	SD[1]
Body Composition, % of EBW[2]						
Water	79	66.2	4.09	21	60.2	4.85
Fat	88	10.9	5.00	36	21.8	6.01
Protein	91	18.5	1.58	37	15.9	1.28
Ash	81	3.81	1.13	21	4.49	1.07
Fat-free mass	88	89.0	5.00	36	78.2	6.02
EBW[2], kg	91	158	122	37	479	54
ADG[3], kg	91	0.775	0.235	-	-	-
ltADG[4], kg	91	0.593	0.286	-	-	-
MidAge[5], d	91	258	243	-	-	-
BCS[6], 1 to 5	-	-	-	37	2.87	0.44

[1]Standard deviation,[2]Empty body weight,[3]Average daily gain reported in the experiment,[4]Lifetime average daily gain,[5]Age at the middle of the experiment,[6]Body condition score

Table 6.2. Model evaluation through 100-fold cross-validation for water composition by stage (heifers and cows) and model (polEBW, lnEBW, and EBW^0.75).

Fit Statistic	Heifers			Cows			SEM[4]	*P*-value		
	polEBW[1]	lnEBW[2]	EBW^0.75[3]	polEBW[1]	lnEBW[2]	EBW^0.75[3]		Model	Stage	Model*Stage
Mean observed, %		66.2			60.2		-	-	-	-
Mean predicted, %	66.4	66.4	66.4	60.3	60.2	59.9	0.165	0.35	<0.01	0.55
Mean bias	-0.283^{bB}	-0.236^{aB}	-0.296^{bA}	-0.101^{aB}	-0.041^{aB}	0.286^{bA}	0.059	<0.01	<0.01	0.01
Slope bias	-0.048^{aB}	-0.014^{aA}	-0.079^{aC}	0.120^{cB}	-0.098^{bA}	-0.234^{aC}	0.035	<0.01	0.40	<0.01
CCC[5]	0.86^{aA}	0.85^{aA}	0.82^{aB}	0.77^{bA}	0.85^{aA}	0.71^{cB}	0.020	<0.01	<0.01	0.02
MSEP[6]	4.26^{aA}	4.21^{aA}	5.36^{bB}	2.26^{bA}	1.54^{aA}	3.77^{cB}	0.166	<0.01	<0.01	<0.01
Decomposition of MSEP, % of MSEP										
Mean bias	6.75	6.58	5.72	20.9	20	23.2	1.85	0.82	<0.01	0.50
Slope bias	8.34^{AB}	6.44^{A}	10.4^{B}	27.9^{AB}	23.1^{A}	32.6^{B}	1.99	<0.01	<0.01	0.38
Random error	84.9^{aAB}	87.0^{aA}	83.9^{aB}	51.2^{aAB}	56.8^{aA}	44.2^{bAB}	2.24	<0.01	<0.01	0.09
RMSEP[7]	2.04^{aB}	2.02^{aA}	2.27^{bC}	1.44^{bB}	1.20^{aA}	1.79^{cC}	0.045	<0.01	<0.01	<0.01

[1]Empty body weight expressed as a polynomial,[2]Empty body weight expressed as natural logarithm,[3]Empty body weight expressed to the power of 0.75,[4]Standard error of the mean across all models,[5]Concordance correlation coefficient,[6]Mean square error of prediction,[7]Square root of the mean square error of prediction,[8]Different uppercase letters in the same row represent differences among models across stages (main effect of model),[9]Different lowercase letters in the same row represent difference within stage (interaction between model and stage)

Table 6.3. Model evaluation through 100-fold cross-validation for fat composition by stage (heifers and cows) and model (polEBW, lnEBW, and EBW^0.75).

Fit Statistic	Heifers			Cows			SEM[4]	*P*-value		
	polEBW[1]	lnEBW[2]	EBW^0.75[3]	polEBW[1]	lnEBW[2]	EBW^0.75[3]		Model	Stage	Model*Stage
Mean observed, %	10.9			21.8			-	-	-	-
Mean predicted, %	11.1[a]	11.1[a]	11.2[a]	20.1[a]	20.1[a]	19.5[b]	0.167	0.14	<0.01	0.11
Mean bias	0.158[aA]	0.136[aA]	0.158[aB]	-0.020[aA]	-0.021[aA]	0.573[bB]	0.061	<0.01	0.60	<0.01
Slope bias	-0.010[aA]	0.006[abAB]	0.039[bB]	0.026[aA]	0.021[aAB]	-0.049[bB]	0.014	<0.01	0.28	<0.01
CCC[5]	0.87[abA]	0.88[aA]	0.86[bB]	0.92[aA]	0.92[aA]	0.87[bB]	0.007	<0.01	<0.01	<0.01
MSEP[6]	1.28[abA]	0.99[aA]	1.66[bB]	4.68[aA]	4.47[aA]	8.12[bB]	0.208	<0.01	<0.01	<0.01
Decomposition of MSEP, % of MSEP										
Mean bias	3.92[a]	3.34[a]	3.59[a]	10.5[a]	10.41[a]	13.7[b]	1.02	0.27	<0.01	0.11
Slope bias	4.74	4.61	5.32	13.2	12.6	12.5	1.27	0.94	<0.01	0.87
Random error	91.3	92.0	91.3	76.3	77.1	73.8	1.46	0.44	<0.01	0.58
RMSEP[7]	1.13[abA]	0.99[aA]	1.29[bB]	2.11[aA]	2.06[aA]	2.79[bB]	0.044	<0.01	0.31	<0.01

[1]Empty body weight expressed as a polynomial,[2]Empty body weight expressed as natural logarithm,[3]Empty body weight expressed to the power of 0.75,[4]Standard error of the mean across all models, [5]Concordance correlation coefficient,[6]Mean square error of prediction,[7]Square root of the mean square error of prediction,[8]Different uppercase letters in the same row represent differences among models across stages (main effect of model),[9]Different lowercase letters in the same row represent difference within stage (interaction between model and stage)

Table 6.4. Model evaluation through 100-fold cross-validation for protein composition by stage (heifers and cows) and model (polEBW, lnEBW, and EBW^0.75).

Fit Statistic	heifers			cows			SEM[4]	*P*-value		
	polEBW[1]	lnEBW[2]	EBW^0.75[3]	polEBW[1]	lnEBW[2]	EBW^0.75[3]		Model	Stage	Model*Stage
Mean observed, %		18.5			15.9		-	-	-	-
Mean predicted, %	18.5	18.5	18.7	16.0	16.0	15.9	0.0.45	0.06	<0.01	0.74
Mean bias	0.060^{A}	0.088^{A}	0.135^{B}	-0.097^{A}	-0.006^{A}	0.038^{B}	0.036	0.02	<0.01	0.63
Slope bias	-0.727^{bB}	0.089^{aA}	-0.969^{cC}	-0.460^{bB}	0.011^{aA}	-0.456^{bC}	0.043	<0.01	0.33	<0.01
CCC[5]	0.56^{aB}	0.59^{aA}	0.66^{bB}	0.59^{cB}	0.88^{aA}	0.68^{bB}	0.021	<0.01	<0.01	<0.01
MSEP[6]	3.14^{bB}	2.11^{aA}	4.23^{cC}	1.48^{bB}	0.32^{aA}	1.60^{bC}	0.081	<0.01	<0.01	<0.01
Decomposition of MSEP, % of MSEP										
Mean bias	5.68	5.11	4.72	10.8	11.4	9.91	1.06	0.59	<0.01	0.83
Slope bias	17.7^{B}	5.81^{A}	21.4^{C}	22.5^{B}	11.7^{A}	25.8^{C}	1.67	0.03	<0.01	0.17
Random error	76.6^{B}	89.1^{A}	73.9^{C}	66.7^{B}	76.9^{A}	64.3^{C}	1.71	<0.01	<0.01	0.27
RMSEP[7]	1.75^{bB}	1.43^{aA}	2.03^{cC}	1.19^{bB}	0.54^{aA}	1.23^{bC}	0.025	<0.01	<0.01	<0.01

[1]Empty body weight expressed as a polynomial,[2]Empty body weight expressed as natural logarithm,[3]Standard error of the mean across all models,[4]Empty body weight expressed to the power of 0.75,[5]Concordance correlation coefficient,[6]Mean square error of prediction,[7]Square root of the mean square error of prediction,[8]Different uppercase letters in the same row represent differences among models across stages (main effect of model),[9]Different lowercase letters in the same row represent difference within stage (interaction between model and stage)

Table 6.5. Model evaluation through 100-fold cross-validation for ash composition by stage (heifers and cows).

Fit Statistic	Heifers	Cows	SEM[1]
Mean observed, %	3.81	4.49	-
Mean predicted, %	3.81	4.49	0.031
Mean bias	-0.013	-0.048	0.0030
Slope bias	0.365	-0.426	0.126
CCC[2]	0.41	0.42	0.037
MSEP[3]	0.881	0.2578	0.063
Decomposition of MSEP, % of MSEP			
Mean bias	5.14	23.6	1.66
Slope bias	12.8	23.7	2.44
Random error	82.1	52.7	2.27
RMSEP[4]	0.928	0.494	0.033

[1]Standard error of the mean across both stages,[2]Concordance correlation coefficient,[3]Mean square error of prediction,[4]Square root of the mean square error of prediction

Table 6.6. Model evaluation through 100-fold cross-validation for fat-free mass composition by stage (heifers and cows) and model (polEBW, lnEBW, and EBW^0.75).

Fit Statistic	Heifers			Cows			SEM[4]	*P*-value		
	polEBW[1]	lnEBW[2]	EBW^0.75[3]	polEBW[1]	lnEBW[2]	EBW^0.75[3]		Model	Stage	Model*Stage
Mean observed, %		89.0			78.2		-	-	-	-
Mean predicted, %	88.6	88.9	88.9	79.6	79.9	80.2	0.173	0.07	<0.01	0.76
Mean bias	0.106^{B}	-0.159^{A}	-0.181^{A}	0.267^{B}	0.023^{A}	-0.232^{A}	0.072	<0.01	0.10	0.20
Slope bias	-0.181^{bB}	0.039^{aA}	-0.170^{bC}	-0.020^{bB}	0.021^{aA}	-0.112^{cC}	0.013	<0.01	<0.01	<0.01
CCC[5]	0.81^{cB}	0.87^{aA}	0.84^{bC}	0.93^{aB}	0.92^{aA}	0.83^{bC}	0.009	<0.01	<0.01	<0.01
MSEP[6]	8.71^{cB}	5.27^{aA}	7.81^{bC}	4.70^{aB}	4.72^{aA}	10.2^{bC}	0.274	<0.01	<0.01	<0.01
Decomposition of MSEP, % of MSEP										
Mean bias	5.00^{a}	3.59^{a}	4.23^{a}	9.48^{a}	10.3^{ab}	12.8^{b}	0.997	0.25	<0.01	0.12
Slope bias	11.9^{bB}	4.61^{aA}	12.5^{bB}	9.46^{aB}	12.6^{aA}	12.4^{aB}	1.23	<0.01	0.07	<0.01
Radom error	83.1^{bB}	91.8^{aA}	83.3^{bC}	81.1^{aB}	77.0^{bA}	74.8^{bC}	1.51	<0.01	<0.01	<0.01
RMSEP[7]	2.91^{cB}	2.27^{aA}	2.76^{bC}	2.07^{aB}	2.12^{aA}	3.16^{bC}	0.051	<0.01	<0.01	<0.01

[1]Empty body weight expressed as a polynomial,[2]Empty body weight expressed as natural logarithm, [3]Empty body weight expressed to the power of 0.75, [4]Standard error of the mean across all models,[5]Concordance correlation coefficient,[6]Mean square error of prediction,[7]Square root of the mean square error of prediction,[8]Different uppercase letters in the same row represent differences among models across stages (main effect of model),[9]Different lowercase letters in the same row represent difference within stage (interaction between model and stage)

Table 6.7. Estimated coefficient for the models derived on the first step of the modeling process (named as baseline) using the natural logarithm for empty body weight (lnEBW).

Empty body water, % of EBW			
Effect	Coeff.[1]	SE[2]	*P*-value
Intercept[3]	86.6	4.72	<0.01
lnEBW	-4.14	0.910	<0.01

Empty body fat, % of EBW			
Effect	Coeff.[1]	SE[2]	*P*-value
Intercept	-18.5	4.50	<0.01
lnEBW	6.09	0.848	<0.01

Empty body protein, % of EBW			
Effect	Coeff.[1]	SE[2]	*P*-value
Intercept	27.1	1.46	<0.01
lnEBW	-1.87	0.275	<0.01

Empty body ash, % of EBW			
Effect	Coeff.[1]	SE[2]	*P*-value
Intercept	4.20	0.431	<0.01
lnEBW	-	-	-

Fat-free mass, % of EBW			
Effect	Coeff.[1]	SE[2]	*P*-value
Intercept	118	4.50	<0.01
lnEBW	-6.09	0.847	<0.01

[1]Estimated coefficient, [2]tandard error,[3]Intercept contains the effect of the method used to determine body composition (direct composition of EBW, estimated based on carcass and rib composition, or dilution techniques). The estimated coefficient for each method were 0.0, 0.44, and -3.79 for direct, carcass, and dilution, respectively.

Table 6.8. Relationship between empty body weight and average daily gain (ADG, kg/d) on the energy required for growth calculated based on the proposed model and the NRC (2001).

		Empty body weight during growth (kg)									
	ADG	Proposed[1]					NRC 2001 (Mature weight 700kg)				
		60	120	240	360	*480*	60	120	240	360	480
Net Energy, Mcal/d	0.40	0.57	0.65	0.72	*0.76*	*0.79*	0.33	0.55	0.93	1.26	1.56
	0.60	0.99	1.13	1.28	1.37	*1.43*	0.51	0.86	1.45	1.96	2.44
	0.80	1.49	1.74	1.99	2.14	*2.24*	0.70	1.18	1.99	2.69	3.34
	1.00	*2.07*	2.45	2.83	3.06	*3.21*	0.90	1.51	2.54	3.44	4.27
	1.20	*2.74*	*3.27*	*3.81*	*4.13*	*4.35*	1.10	1.84	3.10	4.20	5.22
Net Energy, Mcal/kg	0.40	1.43	1.61	1.79	*1.90*	*1.97*	0.82	1.38	2.32	3.15	3.91
	0.60	1.64	1.89	2.14	2.28	*2.39*	0.85	1.44	2.42	3.27	4.06
	0.80	1.86	2.17	2.49	2.67	*2.80*	0.88	1.48	2.48	3.37	4.18
	1.00	*2.07*	2.45	2.83	3.06	*3.21*	0.90	1.51	2.54	3.44	4.27
	1.20	*2.28*	*2.73*	*3.18*	*3.44*	*3.63*	0.91	1.54	2.58	3.50	4.35

[1]Empty body fat (%) = – 6.05 + 1.37*lnEBW – 15.3*ADG + 5.23*lnEBW*ADG and Empty body protein (%) = 22.6 – 1.12*lnEBW, where lnEBW = natural logarithm of empty body weight and ADG = average daily gain assuming using empty body gain (EBG) as ADG = EBG/0.85; values in italic are outside the range of the database used to derive the proposed model

Table 6.9. Relationship between empty body weight and average daily gain (ADG, kg/d) on the protein required for growth calculated based on the proposed model and the NRC (2001).

		Empty body weight during growth (kg)									
	ADG	Proposed[1]					NRC 2001 (Mature weight 700kg)				
		60	120	240	360	*480*	60	120	240	360	480
Net Protein, g/d	0.40	67	64	61	*59*	*58*	98	91	80	70	61
	0.60	101	96	92	89	*87*	146	135	118	103	89
	0.80	135	129	122	119	*116*	194	180	156	135	116
	1.00	*169*	161	153	148	*145*	242	224	193	167	142
	1.20	*202*	*193*	*184*	*178*	*174*	289	267	230	198	168
Net Protein, g/kg	0.40	169	161	153	*149*	*145*	244	227	200	175	153
	0.60	169	161	153	149	*145*	243	226	197	172	149
	0.80	169	161	153	148	*145*	242	225	195	169	145
	1.00	*169*	161	153	148	*145*	242	224	193	167	142
	1.20	*168*	*161*	*153*	*148*	*145*	241	223	192	165	140

[1]Empty body fat (%) = – 6.05 + 1.37*lnEBW – 15.3*ADG + 5.23*lnEBW*ADG and Empty body protein (%) = 22.6 – 1.12*lnEBW, where lnEBW = natural logarithm of empty body weight and ADG = average daily gain assuming using empty body gain (EBG) as ADG = EBG/0.85; values in italic are outside the range of the database used to derive the proposed model

Table 6.10. Relationship between body condition score (BCS) and empty body weight composition (% of EBW) on net energy content per kilogram of EBW[1] change estimated using the proposed models[1] and the NRC (2001).

BCS	Proposed					NRC (2001)				
	Body composition				Energy, Mcal/kg of EBW change[2]	Body composition				Energy, Mcal/kg of EBW change[2]
	Water	Fat	Protein	Ash		Water	Fat	Protein	Ash	
1.0	67.9	8.48	19.1	4.48	6.62	69.4	3.77	19.4	7.46	5.14
1.5	65.4	11.9	18.1	4.48	6.84 (6.62)	66.7	7.5	18.8	7.02	5.72 (5.14)
2.0	62.9	15.4	17.2	4.48	7.06 (6.84)	64.0	11.3	18.1	6.58	6.41 (5.72)
2.5	60.4	18.8	16.2	4.48	7.28 (7.06)	61.4	15.1	17.4	6.15	6.98 (6.41)
3.0	57.9	22.3	15.3	4.48	7.50 (7.28)	58.7	18.8	16.8	5.71	7.61 (6.98)
3.5	55.4	25.8	14.3	4.48	7.72 (7.50)	56.0	22.6	16.1	5.27	8.32 (7.61)
4.0	52.9	29.2	13.4	4.48	7.94 (7.72)	53.4	26.4	15.4	4.83	8.88 (8.32)
4.5	50.4	32.7	12.4	4.48	8.16 (7.94)	50.7	30.2	14.8	4.43	9.59 (8.88)
5.0	48.0	36.1	11.5	4.48	(8.16)	48.1	33.9	14.1	3.96	(9.59)

[1]Assuming that an average cow with BCS 3 has 700 kg of BW, and 1-unit change in BCS is associated with 9.7% change in BW (average of the database used to derive the equations; [2]Tissue energy contained in 1 kg of EBW gain (loss) going to the next higher (lower) 0.5 BCS

APPENDIX B

Figures

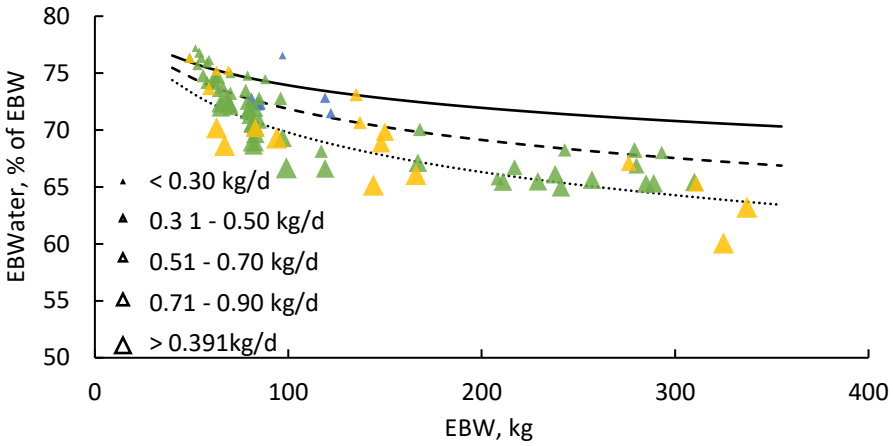


Figure 6.1. Empty body water (EBWater) composition in heifers for the rates of growth of 0.600 (——), 0.800 (– – –), and 1.000 (.....) kg/d as a function of empty body weight (EBW). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques), and the size of the points represents the average daily gain.

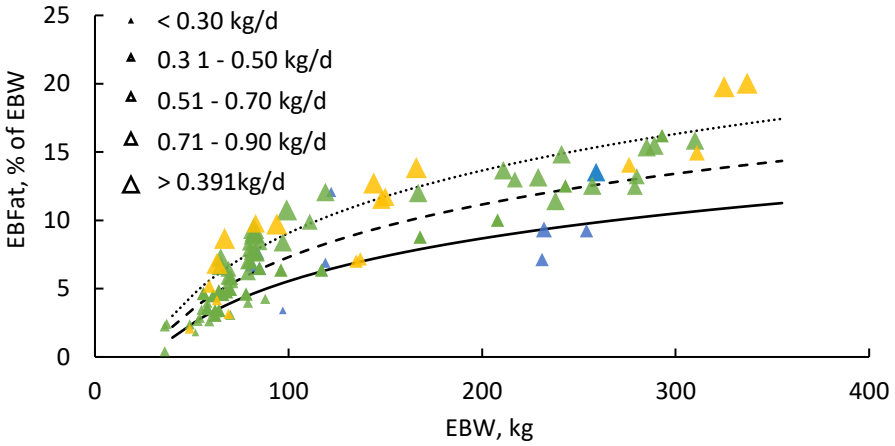


Figure 6.2. Empty body fat (EBFat) composition in heifers for the rates of growth of 0.600 (——), 0.800 (– – –), and 1.000 (·····) kg/d as a function of empty body weight (EBW). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques), and the size of the points represents the average daily gain.

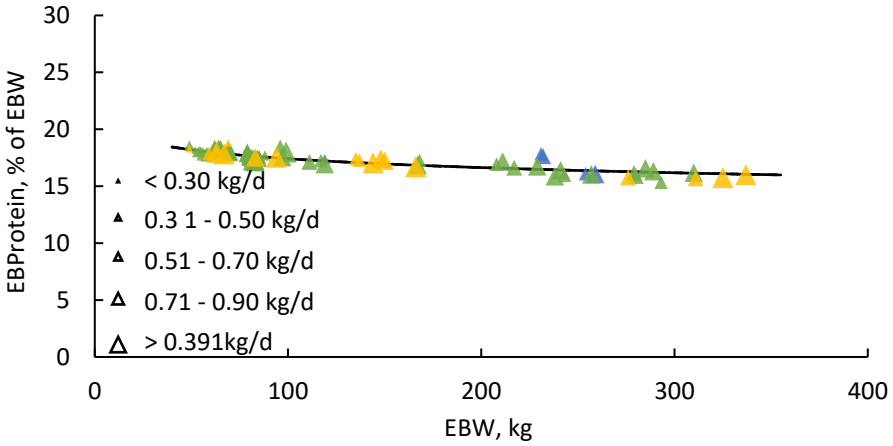


Figure 6.3. Empty body protein (EBProtein) composition in heifers as a function of empty body weight (EBW). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques), and the size of the points represents the average daily gain.

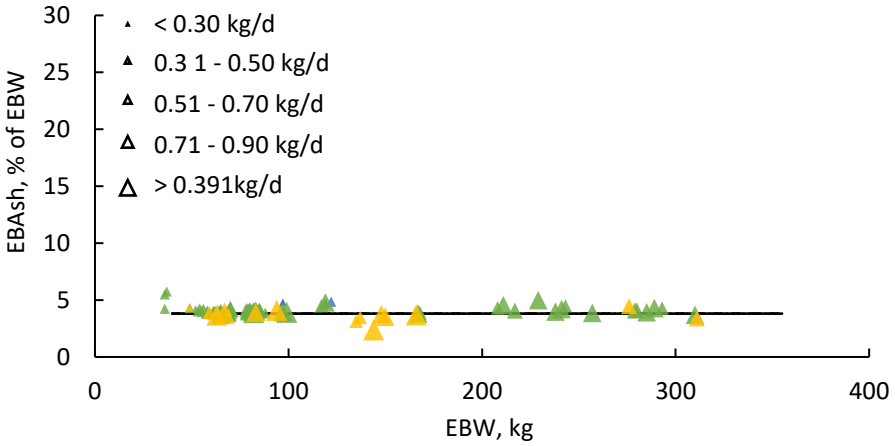


Figure 6.4. Empty body ash (EBAsh) composition in heifers as a function of empty body weight (EBW). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques), and the size of the points represents the average daily gain.

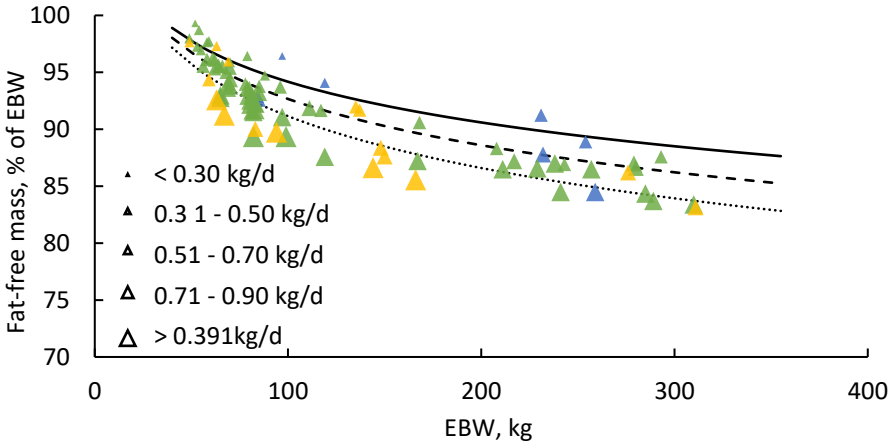
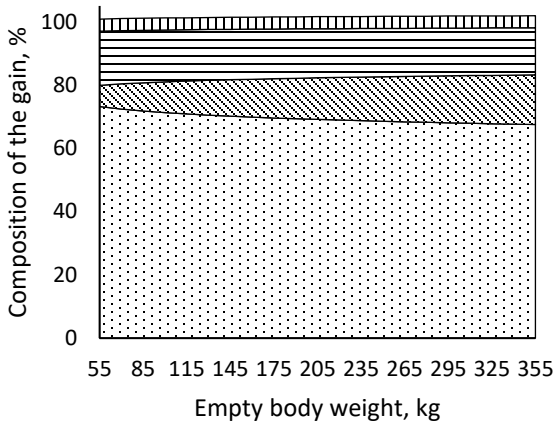
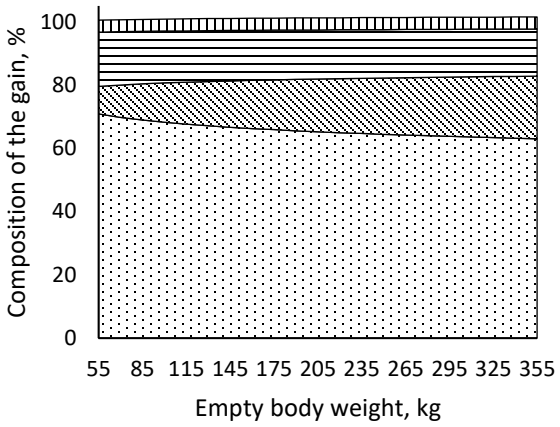


Figure 6.5. Fat-free mass (FFM) composition in heifers for the rates of growth of 0.600 (——), 0.800 (– – –), and 1.000 (.....) kg/d as a function of empty body weight (EBW). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques), and the size of the points represents the average daily gain.

a)



b)



c)

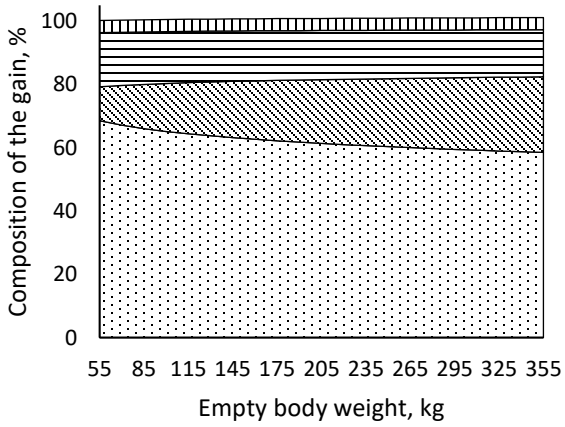


Figure 6.6. Water (□), fat (▨), protein (▭), and ash (▥) of the tissue deposited during growth at an average daily gain of 0.600 (a), 0.800 (b), and 1.00 (c) kg/d.

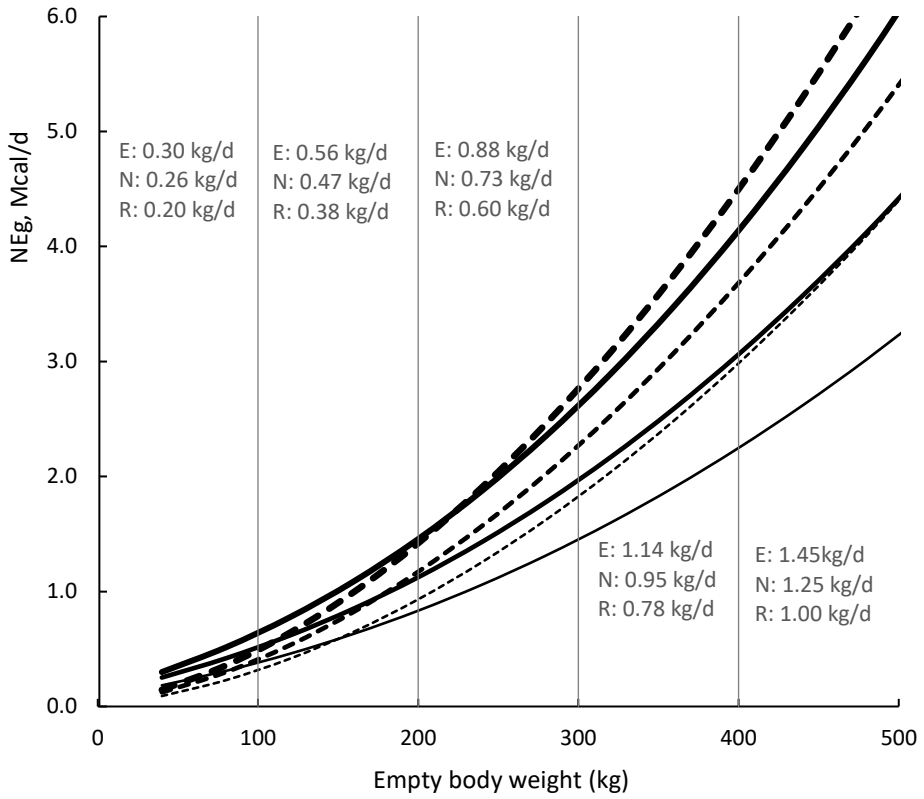


Figure 6.7. The net energy requirements for growth estimated using the proposed models (solid lines) and the NRC 2001 (dashed lines) for three rates of growth are represented by the thickness of each corresponding line. The average daily gain for each division is represented by three scenarios: restricted (R), normal (N), and elevated (E).

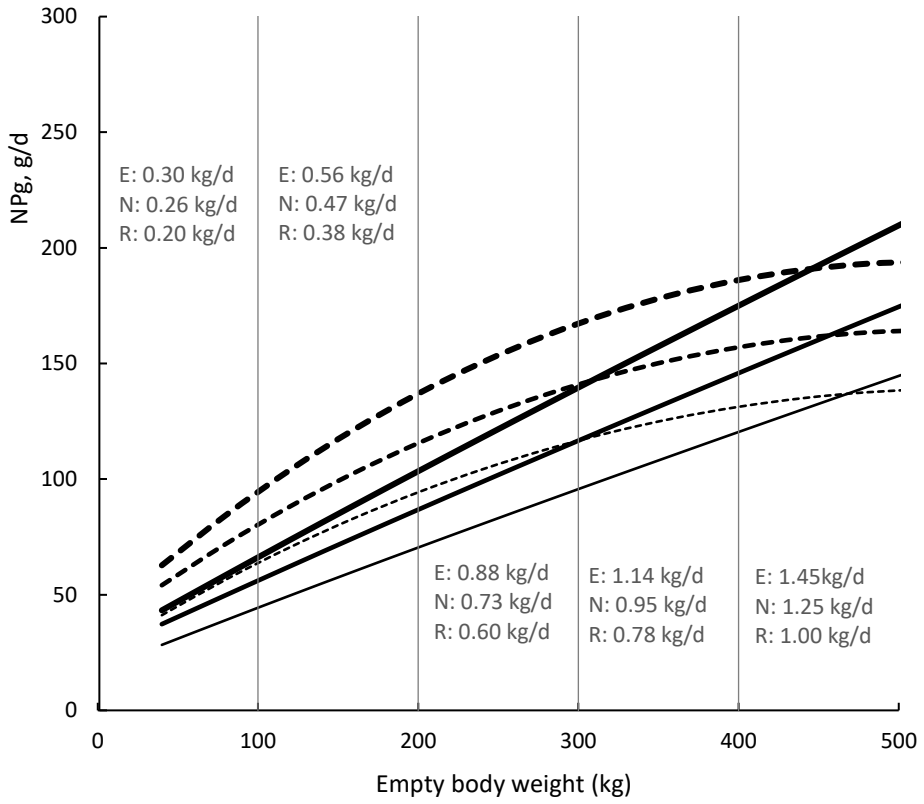


Figure 6.8. The net protein requirements for growth estimated using the proposed models (solid lines) and the NRC 2001 (dashed lines) for three rates of growth are represented by the thickness of each corresponding line. The average daily gain for each division is represented by three scenarios: restricted (R), normal (N), and elevated (E).

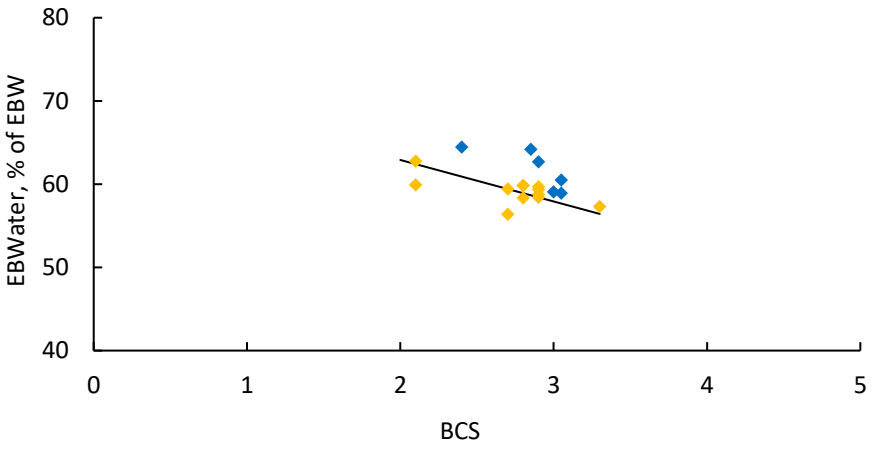


Figure 6.9. Empty body water (EBWater) composition in cows as a function of body condition score (BCS). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (◆- direct measurement of EBW composition; and ◆- dilution techniques), and the solid line represents the proposed model.

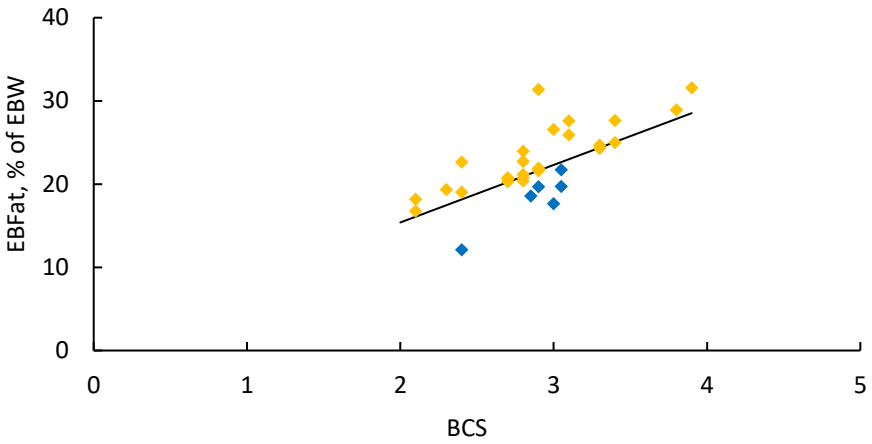


Figure 6.10. Empty body fat (EBFat) composition in cows as a function of body condition score (BCS). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (◆- direct measurement of EBW composition; and ◆- dilution techniques), and the solid line represents the proposed model.

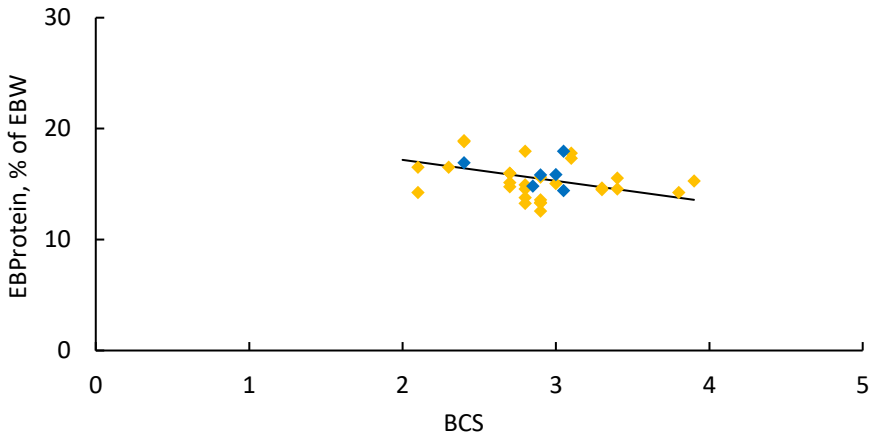


Figure 6.11. Empty body protein (EBProtein) composition in cows as a function of body condition score (BCS). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (◆- direct measurement of EBW composition; and ◆- dilution techniques), and the solid line represents the proposed model.

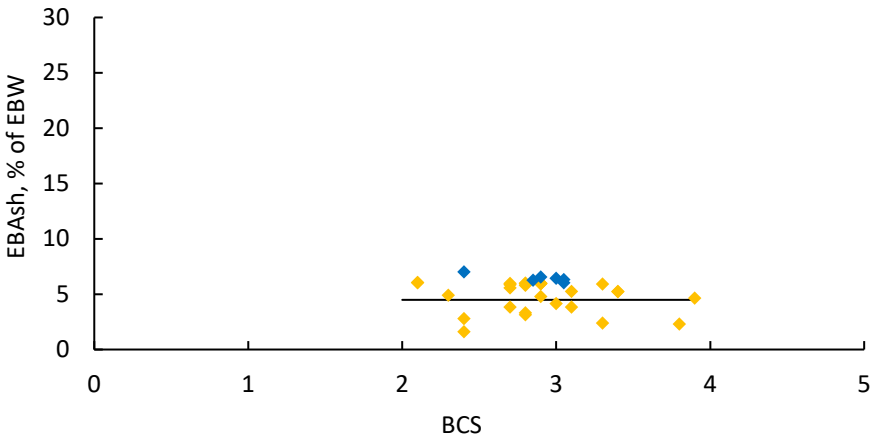


Figure 6.12. Empty body ash (EBAsh) composition in cows as a function of body condition score (BCS). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (◆- direct measurement of EBW composition; and ◆- dilution techniques), and the solid line represents the proposed model.

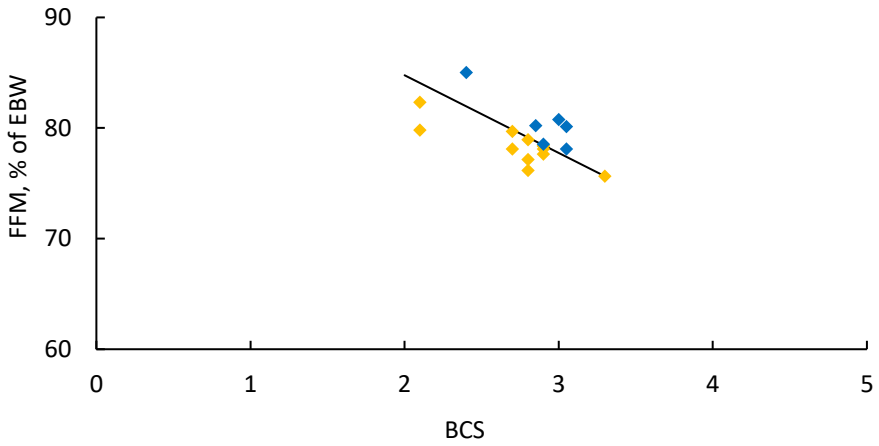


Figure 6.13. Fat-free mass (FFM) composition in cows as a function of body condition score (BCS). Each point represents the adjusted value for the study effect. The color represents the method used to determine the body composition (◆- direct measurement of EBW composition; and ◆- dilution techniques), and the solid line represents the proposed model.

APPENDIX C

Supplemental Tables

Supplemental Table 6.1. Relationship between empty body weight on the energy required for growth calculated based on the baseline model and the NRC (2001).

		Empty body weight during growth (kg)									
	ADG[1]	Proposed[2]					NRC 2001 (Mature weight 700kg)				
		60	120	240	360	*480*	60	120	240	360	480
Retained Energy, Mcal/day	0.40	0.85	0.98	1.11	1.18	*1.24*	0.33	0.55	0.93	1.26	1.56
	0.60	1.28	1.47	1.66	1.77	*1.85*	0.51	0.86	1.45	1.96	2.44
	0.80	1.70	1.96	2.21	2.36	*2.47*	0.70	1.18	1.99	2.69	3.34
	1.00	2.13	2.45	2.77	2.96	*3.09*	0.90	1.51	2.54	3.44	4.27
	1.20	*2.56*	*2.94*	*3.32*	*3.55*	*3.71*	1.10	1.84	3.10	4.20	5.22
Retained Energy, Mcal/kg of gain	-	2.13	2.45	2.77	2.96	*3.09*	0.90	1.51	2.54	3.44	4.27

[1]Average daily gain on body weight gain; [2]Values in italic are outside the range of the database used to derive the proposed model

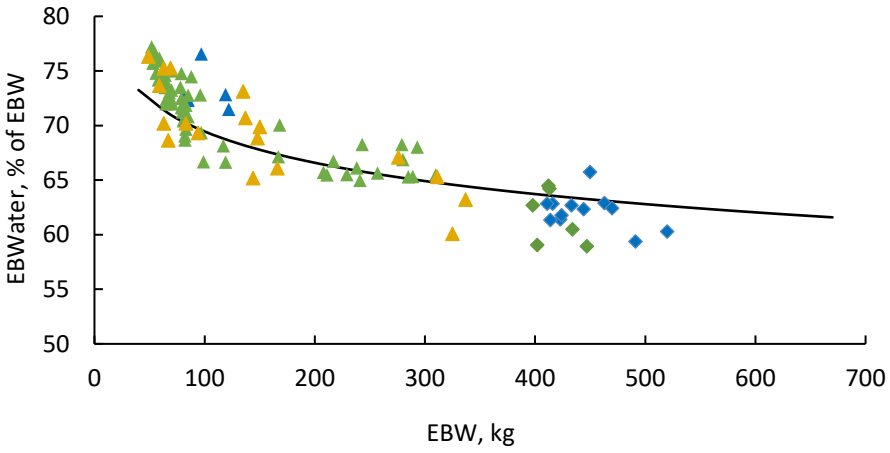
Supplemental Table 6.2. Relationship between empty body weight on the protein required for growth calculated based on the baseline model and the NRC (2001).

		Empty body weight during growth (kg)									
	ADG[1]	Proposed[2]					NRC 2001				
		60	120	240	360	*480*	60	120	240	360	480
Retained Protein, g/day	0.40	70	65	60	57	*55*	98	91	80	70	61
	0.60	105	98	90	85	*82*	146	135	118	103	89
	0.80	140	130	120	114	*109*	194	180	156	135	116
	1.00	176	163	150	142	*137*	242	224	193	167	142
	1.20	*211*	*195*	*180*	*171*	*164*	289	267	230	198	168
Retained Protein, g/kg of gain	-	176	163	150	142	*137*	242	224	193	167	142

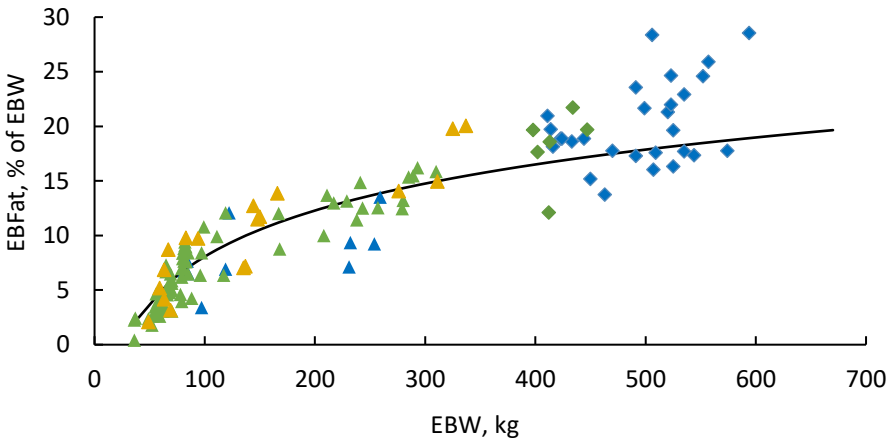
[1]Average daily gain on body weight gain; [2]Values in italic are outside the range of the database used to derive the proposed model

APPENDIX D

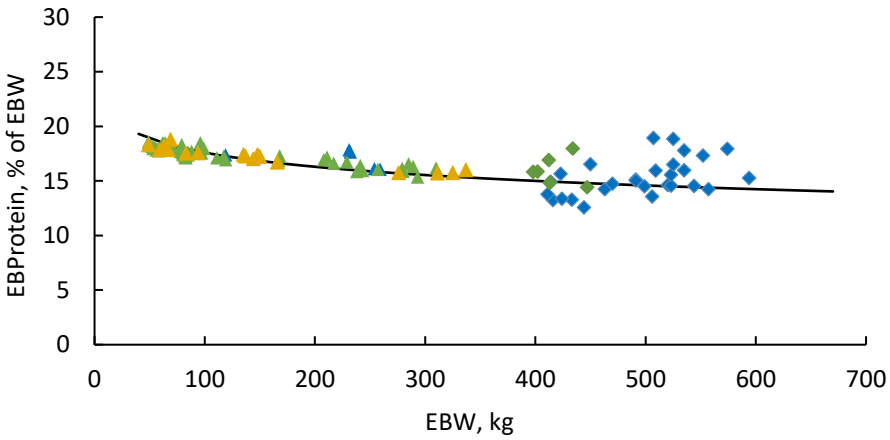
Supplemental Figures



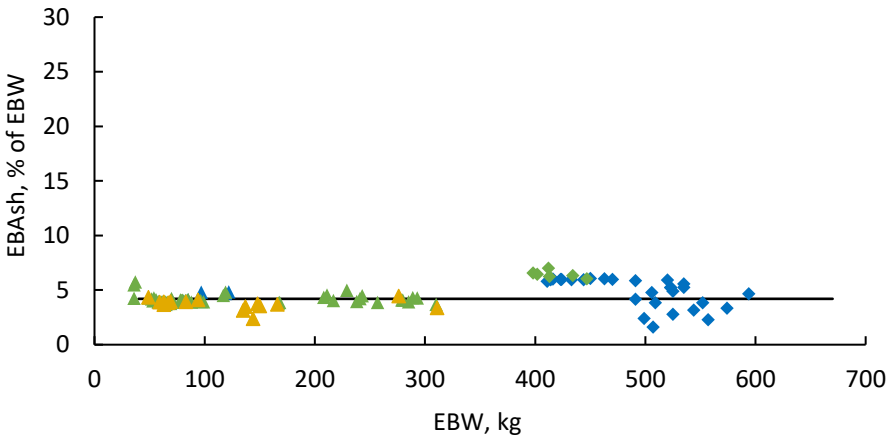
Supplemental Figure 6.1. Empty body water (EBWater) composition in heifers (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques) and cows (◆- direct measurement of EBW composition; and ◆- dilution techniques) predicted by the baseline.



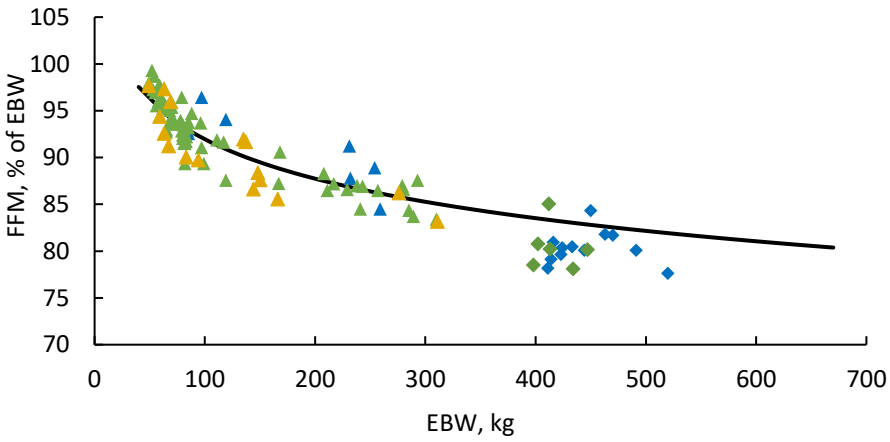
Supplemental Figure 6.2. Empty body fat (EBFat) composition in heifers (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques) and cows (◆- direct measurement of EBW composition; and ◆- dilution techniques) predicted by the baseline.



Supplemental Figure 6.3. Empty body protein (EBProtein) composition in heifers (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques) and cows (◆- direct measurement of EBW composition; and ◆- dilution techniques) predicted by the baseline.



Supplemental Figure 6.4. Empty body ash (EBAsh) composition in heifers (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques) and cows (◆- direct measurement of EBW composition; and ◆- dilution techniques) predicted by the baseline.



Supplemental Figure 6.5. Fat-free mass (FFM) composition in heifers (▲ - direct measurement of EBW composition; ▲ - carcass and rib composition; and ▲ - dilution techniques) and cows (◆- direct measurement of EBW composition; and ◆- dilution techniques) predicted by the baseline.

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CHAPTER 7

CONCLUSION

As discussed in the previous chapters, feed plays an essential role on the modern dairy farm. Feed represents the major cost within dairy operations, and it is the main input allowing the increase of milk production. Hence, by optimizing feed utilization, farms can greatly improve profitability.

Much research on dairy nutrition has focused on understanding the mechanisms associated with the conversion of feed into milk and the development of strategies to improve the efficiency of this process. Ultimately, dairy farmers benefit from advances in the field of dairy nutrition research by using new research recommendations and technologies to improve diets. These transfers of technology from the research centers to commercial farms are made through diet formulation.

Diet formulation is performed by dairy nutritionists using diet formulation software, which uses a set of prediction equations to match animal requirements with the nutrients available through a diet. Finally, the prediction equations used in diet formulation software must constantly be evaluated and updated to incorporate the discoveries generated by research and to represent the current and advanced production systems.

In this dissertation we proposed updates to the prediction equations for digestibility at production level (chapter 3) and for dry matter intake (chapter 4), investigated the effect of free FA on digestible energy (chapter 5), and developed a model to predict net energy and net protein requirements for growth (chapter 6). The proposed models represent a significant improvement on the accuracy of predictions when compared to the models proposed by the NRC (2001) and

have the potential to be incorporated in diet formulation software. By incorporating the proposed models in the diet formulation software, dairy nutritionists will have a more precise and accurate model for formulating diets.

An important area that should be further investigated and incorporated into the diet formulation software is the impact of free FA on digestible energy (chapter 5). The current diet formulation software does not account for the effect of specific free FAs and, as demonstrated in this dissertation, there is a great potential to explore the benefits of specific free FAs in regard to nutrient digestibility and digestible energy intake.

Finally, although improvements in the accuracy and precision of statistical models used in the diet formulation process are essential for dairy farmers to enjoy the benefits of advances in the dairy nutrition field, grouping strategies is a major management factor that limits the usefulness of more precise diet formulation software.

Farm managers need to adopt grouping strategies based on the nutritional needs of each dairy category. It is impossible to have a tailored diet if the diet is offered to a heterogeneous group of animals. Therefore, it is necessary to generate recommendations and extension material focusing on nutritional grouping strategies. Dairy farmers will benefit the most from work performed in this dissertation by combining precision in diet formulation with nutritional grouping strategies.