APPLYING EFFECTIVE ENERGY CONCEPT FOR INTAKE PREDICTION AND BALANCING RUMINAL NITROGEN AND POST-RUMINAL AMINO ACID REQUIRMENTS FOR BEEF CATTLE

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

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APPLYING EFFECTIVE ENERGY CONCEPT FOR INTAKE PREDICTION AND BALANCING RUMINAL NITROGEN AND POST-RUMINAL AMINO ACID REQUIREMENTS FOR BEEF CATTLE

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ABSTRACT

Proper animal nutrition involves balancing nutrient supply to nutrient requirement. Accurate intake prediction is fundamental to diet formulation and appears simple but has historically been challenging to accomplish. Multiple factors control intake and understanding process of intake control is complicated by a plethora of interactions among these factors. Several intake prediction equations have been developed and equation accuracy has been improved by applying various adjustment factors. Research has been conducted that allows requirements of ruminal degradable peptide and nitrogen and absorbable amino acids required for growth to be estimated. However, requirement of these compounds is based upon caloric-supported growth, or accurate intake prediction. The first experiment in this dissertation examined the validity of effective energy intake predicting equations. Accuracy of effective energy equations (EE) was compared with NRC equations based on initial body weight (NRC_{iBW}) and

dietary NE_m concentrations with ($NRC_{NEm\text{-}mon}$) and without monensin adjustment (NRC_{NEm}), and net energy equations (NE) based on net energy requirements for maintenance and gain. The EE equations more accurately predicted intake, had less variation and the greatest coefficient of determination (r^2), and smaller line bias decomposition. These findings support the conclusion that EE models were the best for predicting intake by steers. The second study implemented EE intake prediction in a diet formulation model to formulate diets with inadequate, balanced or sufficient ruminal degradable nitrogen to support microbial growth requirement *in vitro* and *in vivo*. In an *in vitro* continuous culture study, there was a cubic response ($P < 0.01$) for grams of bacterial nitrogen produced by rumen microbes and MOEFF when RDN was increased. The MOEFF was maximized when RDN requirement and supply was balanced. In an *in vivo* animal growth study, greater ($P < 0.01$) feed efficiency was found in negative RDN balance diet (-0.69% RDN balance diet), which was presumably due to recycled nitrogen supply meeting the estimated deficiency. Finally, research was done to determine the effect of post-ruminal arginine levels on animal growth and how balanced/unbalanced RDN and post-ruminal arginine diets with and without roughage would impact animal growth performance and feed efficiency. We hypothesized that there would be no further improvement in feed efficiency once RDN and post-ruminal amino acid requirements were met. Two animal growth experiments were conducted. No significant difference in ADG, but DMI and feed to gain ratio were greater ($P < 0.01$) when RDN and post-ruminal arginine requirement were met. In the second animal trial, post-ruminal arginine levels resulted in no difference in ADG during 168 days on feed; however, the balanced post-ruminal arginine diet was observed to have greater period ADG (0-28 days and 0-87

days ADG, $P \leq 0.08$) and lower feed to gain ratio (more efficiency). In summary, the effective energy equation is a better estimation for intake and beneficial to improve MOEFF and feed efficiency by formulating diet to meet ruminal degradable protein, ruminal degradable nitrogen and absorbable amino acids requirements, respectively. The implication of these experiments is feed efficiency could be maximized by formulating diet to meet ruminal nitrogen required for microbes and post-ruminal amino acid requirement. Accurate animal gain potential estimates and dietary energy densities would improve intake prediction accuracy and post-ruminal amino acids flow assessment.

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Feed cost has been reported to account for 60% of the total cost for a commercial feedlot (Elstien, 2002), and with the competitive demand for corn the proportionate cost of feed could be even more (Wallander et al., 2011). Therefore feeding animals to maximize efficiency has been proposed as a strategy to reduce cost and improve profits. Appropriate feeding contains at least two aspects: accurate energy estimates for growth and proportionately correct concentration of nutrients relative to energy consumption.

Animal feed intake can be impacted by numerous factors such as animal variations (breeds, gender, age, mature size, RFI), environmental conditions and diet formulation. NRC (1996, 2000) has published series of equations to predict average DMI for beef cattle, and other researchers have evaluated and improved upon these equations (McMeniman et al., 2009, 2010). Though research had shown that NRC equations could predict intake by feedlot cattle with reasonable accuracy, we hypothesized accuracy and precision could be improved. This chapter reviewed factors influencing animal feed intake, equations routinely used in the U. S. for intake prediction, evaluated accuracy of these intake predictions and introduced the effective energy concept (Emmans, 1994).

Appropriate diet formulation should maximize rumen microbial protein production by supplying but not exceeding ruminal degradable protein and nitrogen requirement, determined relative to rumen available carbohydrate. Microbial efficiency is affected by substrate availability and dilution rate (Meng et al., 1999). Inadequate protein supply relative to fermentable carbohydrate results in reduced fiber digestion and decreased microbial efficiency (Hoover and Stokes, 1991; Klevesahl et al., 2003). This chapter also reviewed the ruminal degradable protein (peptide) requirements and ruminal degradable nitrogen for maximal rumen microbial growth.

MECHANISMS CONTROLLING FEED INTAKE IN RUMINATS AND FACTORS INFLUENCING FEED INTAKE

Animals eat to meet their requirement under a particular circumstance; questions about what kind of "signal" and through which pathway satiety is controlled is still debated but unresolved. A hypothalamic lesion rat trial showed the energy balance control center was located in the ventromedial nuclear region of the hypothalamus (Hetherington and Ranson, 1940). Control center simulation would inhibit feeding and a lesion in this area would cause immediate overeating and become stable at a certain point. Baile and McLaughlin (1987) reported involvement of the central nervous system (CNS) in the animal feed intake control. A model for human energy homeostasis was described to delineate how CNS works (Schwartz et al., 2000). *Predicting Feed Intake of Food-Producing Animals* published by NRC in 1987 presented an overview of intake control, which listed a number of CNS and peripheral receptors and how they work. Tension receptors detect rumen distension; duodenal receptors detect VFA concentration and various metabolites (glucose, free fatty acids, and amino acids). Hormones (GH, insulin, somatomedin) and peptides in nervous pathways also act as signals in providing CNS with information about nutrient state and coordinating eating behavior (Deetz and Wangsness, 1981; Mclaughlin, 1982; Houseknecht et al., 1998; Baile and Della-Fera, 2001; Delavaud, 2002; Startin, et al., 2011).

Any factor influencing animal physiological need could affect dry matter intake. Animal individual variations such as animal breed, mature size, frame size, body composition, age, and gender would alter intake. Within these factors, body composition (especially body fat) is considered to have an important feedback role in intake control.

DMI is suggested to decrease 2.7% for each percentage increase in body fat if body fat is within the range 21.3% to 31.5 % (Fox et al., 1988). Intake variation of animals with different breed and gender may be largely attributed to differences in mature size and frame size. At a given body weight, heifers are fatter (more mature) than steers; therefore, instead of adjusting for sex, a frame-equivalent weight adjustment was applied (Fox et al., 1988). Additionally, gender may affect intake and body weight is related: when body weight was less than 250 kg heifers had greater intake capacity than steers or bulls (Ingvartsen et al., 1992) but medium-framed heifers decreased DMI by 10% (NRC, 1984). Cattle put on feed at an older age consume more feed per unit BW than when placed on feed at a younger age (i.e. yearlings eat more than calves), which may be due to a more retarded growth before being put on feed (NRC, 1984). For beef cattle, intake difference due to breed is limited, however, Holstein and Holstein crossbred beef is an exception. Hicks et al. (1990a) observed DMI increased 12% for Holstein steers compared with beef steers; also, this adjustment was adopted in NRC model (1987, 2000). Recent research showed residual feed intake (RFI) was a better description for feed efficiency (Koch et al., 1963; Arthur et al., 1997) than feed to gain or gain to feed ratio and RFI was a moderate heritable genetic trait (Arthur et al., 1997). Low RFI steers consumed 19.1% less DMI than high RFI steers (Gomez et al., 2003). Davis (2009) reported there was a 1.83 kg/day difference on average intake of 63 feeding days, and 0.5 kg/day difference on average intake of 126 feeding days between negative RFI (-0.98) and positive RFI (1.05) crossbred steers fed the same diet.

Animal physiological stage affected feed intake. Dairy cattle is a good example: lactating cows consume 35% to 50% more than non lactating cows at the same weight on

the same diet (ARC, 1980), and for high producing cows intake increased 0.2 kg of DM per kilogram of fat-corrected milk (ARC, 1980; NRC, 1987). During last month of prepartum, intake declines by 2% per week due to rumen space is partly taken by growth calf (NRC, 1987; Ingvartsen et al., 1992), and intake increases to a peak 4 to 6 months postpartum because of maintenance and milk production energy demand (NRC, 1987).

The NRC (1987, 2000) reviewed effects of environmental factors on dry matter intake: intake would increase as temperature fell below 5˚C and decreased when temperature was higher than 25 ˚C. The shift in intake was also impacted by exposure to wind, storm, and mud (Figure 1.1). For high producing dairy cows under heat stress, intake decreased remarkably as did milk yield (West, 2003). Cold weather increases maintenance requirement to produce more heat for body activities. Delfino and Mathison (1991) found steers housed outdoors with an average temperature of -7.6 ± -6.8 ˚C was estimated to require 41% more ME for maintenance than those housed indoors with an average temperature of 16.9 ± 2.7 ˚C. Adams et al. (1986) conducted two winter grazing trials and found that forage intake was reduced at lower minimum daily temperature (ranging from -18 ˚C to 2 ˚C). Seasonal factors were studied and showed that intake would be increased 1.5 to 2% in long day months and decreased in short day months (Hicks et al., 1990b); however it is challenging to delineate a clear enough seasonal factor because temperature, weather conditions and photoperiod interact with seasonal patterns.

Dietary factors including concentrate to forage ratio, forage quality, ingredient, digestibility of grain, processing and water intake influence intake. The tension receptor "senses" the distention resulting from gut filling and slow passing of fibrous feed components, which could explain how NDF content (fiber/forage level) in diet limits

intake (Allen, 1996). In addition, since fiber digestion and passage rate vary, feed intake is related to rate of fiber disappearance. Water accounts for 85% of rumen content and is absorbed rapidly, so drinking water did not have much influence on dry matter intake (Van Soest, 1994). However, forced water intake above requirement could have a negative effect on dry matter intake (ARC, 1980). Usually, grain products are cracked, ground or steam-flaked to collapse the cell structure and fibrous feedstuffs are ground to reduce particle size and increase cell structure microbe accessibility. However, influence of feed processing on DMI differs depending on concentrate or forage based diets. Processing roughage increases passage rate and improves forage intake, while processing grain allows greater digestion of plant cell wall and increasing digestibility may reduce intake. Leonard et al. (1989) compared grinding corn vs. whole corn diets, and found no significance on intake, but grinding increased starch digestibility 15%.

Management strategies, such as use of implants and ionophores would influence dry matter intake. Feeding management, such as feeding frequency and feed delivery method can alter animal eating behavior. Estradiol/progesterone implant increased DMI from 4 to 16% depending on type of implant and when implant was administered relative to slaughter (Solis et al., 1989; Samber et al., 1996; Parr et al., 2011). Ionophores typically decrease feed take. Based on Fox and Black (1984), NRC (1987) proposed an adjustment for intake prediction that was to decrease DMI 6 to 10% when an ionophore was used. Feedlot cattle dry matter intake in other studies was reported to decrease with diets containing monensin compared with non-additive diets (4 to 8% depression in DMI; Stock et al., 1995; Lana et al., 1997; Meyer et al., 2009). Feeding twice a day and clean bunk feeding increased intake partly because offering fresh feed stimulated eating

activity. Robles et al. (2007) found feeding more than twice a day did not affect dry matter intake but increased water consumption linearly ($P = 0.08$). The DMI intake was found to decrease 12% in managed delivery feeding group compared with *ad libitum* feeding group. Bunk management could also contribute on controlling feed wastage (Pritchard and Bruns, 2003).

EVALUATION HISTORICAL PREDICTING EQUATIONS AND USING EFFECTIVE ENERGY CONCEPTS TO ESTIMATE FEED INTAKE

One of the top concerns of beef enterprises is maximizing profit, which depends upon the input for operating a feedlot and the outcome. Feed cost has been considered as the most costly proportion of production, representing two thirds of total investment (Elstien, 2002). Therefore, accurate prediction of feed intake is necessary to estimate feed cost for producers and to assist the projection. Understanding feed intake by the animal at different growth stage of animal productivity could minimize overfeeding and reduce feed waste.

DMI was a function of dietary NE_m concentration, with adjustments for frame or sex (NRC, 1984):

$$DMI = SBW^{0.75} \times (0.1493 \times NE_m - 0.046 \times NE_m{}^2 - 0.0196) \quad \text{Eq. 1-1}$$

in which DMI is expressed in kg/day, SBW is expressed in kg, and NE_m concentration is expressed as Mcal/kg dry matter. Data from experiments conducted with growing and finishing beef cattle obtained from published literature refined the relationship between

maintenance energy intake and body weight and were to develop a regression equation that accounted for 69.8% of variation in NE_m intake per unit $SBW^{0.75}$ (NRC, 2000):

$$NE_m intake = SBW^{0.75} \times (0.2435 \times NE_m - 0.0466 \times {NE_m}^2 - 0.1128) \quad \text{Eq. 1-2a}$$

where NE_m intake is expressed in Mcal/day. An intercept adjustment term was applied for both yearling steers and heifers (Eq. 1-2b):

$$NE_m intake = SBW^{0.75} \times (0.2435 \times NE_m - 0.0466 \times {NE_m}^2 - 0.0869) \quad \text{Eq. 1-2b}$$

DMI could be predicted by dividing NE_m intake (Mcal/day) by dietary NE_m concentration (Mcal/kg) (Eq. 1-3a, b).

$$DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_m - 0.0466 \times {NE_m}^2 - 0.1128)}{NE_m} \quad \text{Eq. 1-3a}$$

$$DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_m - 0.0466 \times {NE_m}^2 - 0.0869)}{NE_m} \quad \text{Eq. 1-3b}$$

In comparing these three equations (Eq.1-1 and Eq. 1-3a, b), it was found that intake predicted from Eq. 1-3 explained 10% more variation in DMI than prediction from Eq. 1-1 (Table 1.1). Researchers found initial weight on feed for cattle fed mostly high-energy diets to have predictive value (NRC, 1987). With evaluating relationship between initial body weight and DMI in historical data, predicting equations based on initial body weight were developed:

$$DMI = 1.8545 + 0.01937 \times iBW \quad \text{Eq. 1-4}$$

$$DMI = 4.54 + 0.0125 \times iBW \quad \text{Eq. 1-5}$$

Equation 1-4 was developed from animals fed diet with NE_m concentrations ranging from less than 1.0 to 2.4 Mcal/kg and intercept adjustment for size, sex and age was applied: intercept for larger-frame steer was 2.477; intercept for large-frame heifer calves and medium-frame yearling heifer was 3.212; and intercept for medium-frame yearling steer

was 3.616. Equation 1-5 was developed from cattle fed at two commercial feedlots (1661 data points) with a narrower range of diet NE_m (1.1 to 1.59 Mcal/kg).

Table 1.2 was adopted from the NRC (2000) updated edition, showing prediction from equation Eq. 1-5, based on iBW, accounted for less variation (20% to 45% less than Eq. 1-1 and 1-3) with more over or under prediction. Another study showed prediction from Eq.1-5 explained similar variation with prediction from Eq. 1-3 (64% and 66%, respectively; McMeniman et al., 2009). Prediction equation based on initial weight (Eq. 1-5) seems practical since initial weight would be generally easy to get in farm, however, the prediction stability is a concern. Prediction from equations Eq. 1-1 and 1-3 had a similar result (Table 1.2) with greater bias occurring for Eq. 1-1 for Guelph data set and a poorer coefficient of determination (r^2) of intake prediction for the Alberta data set raised specific concern for all-forage fed beef cattle.

McMeniman et al. (2009) conducted studies to evaluate the equations in NRC (NRC, 1996; Eq. 1-3a, 1-3b and 1-5) and another equation with adjustment when monensin was used (Eq. 1-6a and b) was evaluated as well. The NRC suggested DMI was decreased 4% and dietary NE_m concentration was increased 12% when using monensin:

$$DMI = 0.96\times \frac{SBW^{0.75}\times[0.2435\times 1.12\times NE_m - 0.0466\times(1.12\times NE_m)^2 - 0.1128]}{1.12\times NE_m} \quad \text{Eq. 1-6a}$$

$$DMI = 0.96\times \frac{SBW^{0.75}\times[0.2435\times 1.12\times NE_m - 0.0466\times(1.12\times NE_m)^2 - 0.0869]}{1.12\times NE_m} \quad \text{Eq. 1-6b}$$

With observed DMI regressed on predicted DMI for each equation (Eq. 1-3, 1-5 and 1-6), coefficient of determination (r^2) was 0.66, 0.64 and 0.67, respectively (McMeniman et al., 2009). Although equations had significant mean and linear bias ($P<0.01$), the r^2 showed

validation confidence and improvement after adjustment. None of the equations shown above accounted for energy required for growth. Would an equation accounting for maintenance and growth improve prediction accuracy of intake?

Other discussions about increasing prediction accuracy included applying adjustment factors for body fat, equivalent weight, breed, age, feed additives, and environmental conditions (Fox, 1988). These discussions were adopted and reported in the NRC 1996 edition and other NRC publications, such as: *Predicting Feed Intake of Food-Producing Animals* (NRC, 1987). A review was presented defining how these adjustments were incorporated (Table 1.3). Other models were reported to consider specific energy requirement for pregnant and non-pregnant beef cows, and cattle fed all-forage diets. Hicks et al. (1990b) proposed addition of actual feed intake data during the early feeding period in a prediction model may increase coefficient of determination. Following Hicks's step, a study predicted average DMI from iBW, sex and average DMI of day 8 to day 28 of the feeding period. Accuracy and precision was improved with adding day 8 to day 28 DMI into prediction models (McMeniman et al., 2010).

Intake prediction is actually predicting animal energy requirements. For beef cattle maintenance and gain requirement are specified. As noted in the NRC equations (Eq.1-2, 1-3 and 1-6), average shrunk body weight (SBW) inclusion requires final body weight or ADG prediction. The NRC proposed if intake is known, the ADG could be computed by equations based on available dietary net energy for gain after subtracting feed required for maintenance from given intake. Therefore, the desired gain or hypothetical animal gain potential could help to predict feed intake by separately predicting feed required for maintenance and feed required for gain. However, animal

deposits protein tissue and fat tissue in various proportions depending on relative point to maturity. Emmans (1994) proposed the effective energy scale, reporting the heat of protein and lipid combustion was different, 23.8 and 39.6 KJ/g respectively. The second law of thermodynamics states all energy forms can be quantitatively converted to heat; in this case, energy required for deposition as protein gain and as fat gain is different. Effective energy equations consider energy needed for maintenance protein retention (PR) and lipid retention (LR), and is expressed as ($MH + 50PR + 56LR$). The PR and LR are positive protein and lipid retention in grams per day. For ruminants energy released as methane during fermentation (MTHE) is also considered. Protein retention and lipid retention could be estimated by a quadratic function of empty body weight (Simpfendorfer, 1974; NRC 2000): $Protein\ (kg) = 0.235{\times}EBW - 0.00013{\times}EBW^2 - 2.418$; $Fat\ (kg) = 0.037{\times}EBW + 0.00054{\times}EBW^2 - 0.610$; in which EBW may be estimated as $EBW = 0.917{\times}SBW - 11.39$ (Owens et al., 1995). The MTHE was predicted using a fermentation balance equation (Wolin et al., 1960) estimated when feeding concentrate diets on average 12% of intake energy was released as methane and 61.2% of the released energy was utilized to maintain animal body temperature and maintenance activities. The effective energy yielded to a ruminant animal by its diet was estimated as $EE_{diet}(MJ/kgOM) = 1.15{\times}ME - 3.84 - 4.67{\times}DCP$; where ME is the metabolizable energy concentration (kJ/kg) and DCP is the digestible crude protein content (Emmans, 1994).

Emmans (1994) also demonstrated the consistency between species on energy scale of protein retention and lipid retention and reported that MH was estimated based

on body weight. Lofgreen and Garrett (1968) proposed the California Net Energy System, defining NE_m requirement of beef cattle as $NE_m = 0.077 Mcal/EBW^{0.75}$. However, the previous NRC (1984) *Nutrient Requirements of Beef Cattle* reported that maintenance heat estimation could use either 77 Kcal/$EBW^{0.75}$ or 77 Kcal/$BW^{0.75}$.

As discussed previously, feed intake is controlled by multiple factors and makes intake prediction challenging. Intake prediction equations accounting for initial body weight and maintenance energy requirement were developed, but there were few studies comparing prediction equations accounting for maintenance energy requirement, energy required for maintenance and gain, and effective energy required for different body composition. We hypothesized equations accounting for energy required for body composition would predict more accurately and experiments in following chapter would test this hypothesis.

RUMINAL DEGRADABLE PROTEIN AND NITROGEN, AND MICROBIAL EFFICIENCY

The current edition of *Nutrient Requirements of Beef Cattle* (NRC, 2000) maintained the metabolizable protein (MP) concept as presented in 1985 (NRC, 1985) and MP was adopted in *Nutrient Requirements of Dairy Cattle* in 1989 and its subsequent editions (NRC, 1989, 2001). MP is defined as the true protein available for intestinal absorption, which includes microbial protein and ruminal undegradable protein (RUP). Using MP raised interests of increasing microbial protein production since dietary protein is typically the most expensive nutrient in diets and microbial protein could account for

60% on average of the MP required (Firkins, 1996; Koenig et al., 2000). Microbial efficiency (MOEFF) is used to measure rumen microbial growth and is equal to grams of nitrogen produced per kilogram of organic matter truly fermented (Demeyer and Van Nevel, 1986). MOEFF had been shown to be a function of solid dilution rate (Meng et al., 1999). Also, multiple factors can influence MOEFF, including substrate supply, energy cost for bacterial maintenance and growth, energetic uncoupling, pH and etc. (McAllister et al., 1994; Firkins, 1996; Russell, 1998). Dilution rate determines MOEFF by impacting 1) microbial maintenance energy requirements through controlling microbial growth rate and energetic uncoupling, 2) substrate availability by controlling amount of time a feedstuff is rumen available, and 3) nitrogen recycling in the rumen through controlling protozoal predation (Mueller, 2004).

Rumen microbial protein synthesis requires nitrogen, which can be derived from diet protein hydrolysis or non-protein nitrogen. Pittman and Bryant (1964) tested the nitrogen sources used for growth by *Bacteroides Ruminicola* under rumen-similar condition and found that high molecular weight peptide nitrogen or ammonia nitrogen were needed rather than free amino acids or small peptides. Bryant and Robinson (1962) suggested amino acids were incorporated to some extent by all rumen bacterial species, a big proportion of rumen bacterial population would rather incorporate ammonia nitrogen into its cellular constituents other than amino acids nitrogen. Soto et al. (1994) conducted *in vivo* infusion and *in vitro* cultural studies with peptide, amino acids and ammonia enriched media. They found no benefit comparing peptide and amino acids on growth of celluloytic bacteria (*Ruminococcus Albus*, *Ruminococcus Flavefaciens* and *Fibrobacter Succinogenes*), whereas non-cellulolytic bacteria were stimulated by more than 70% by

peptides compared to supplemented amino acids. The bacterial growth limitation was dependent on amino acids/peptides amount rather than profile or specific limiting amino acids (Argyle and Baldwin, 1989). Russell et al. (1983) suggested that at least two-thirds of nitrogen for non-structural carbohydrate fermentation should come from peptide nitrogen, and the other one-third could either be peptide, amino acids, or ammonia nitrogen. Fu (2000) reviewed ruminal degradable peptide requirements and effect of peptide levels and supplementation on microbial growth and efficiency. They concluded rumen degradable protein level in current feedlot diets could meet rumen microbial requirements for obtaining optimal MOEFF, and, current-feeding practices could supply adequate to excessing RDP for growing calves and finishing steers.

Hume et al. (1970) fed sheep with increasing urea levels as the only nitrogen source and found ruminal protein production kept increasing until dietary nitrogen concentration reached 3.29%. Non-protein nitrogen could be utilized for bacteria growth and enabled rumen bacteria to yield significant amount of protein to meet animal maintenance and growth requirement. Mehrez and Øskov (1977) in an *in situ* digestion study suggested ammonia required for optimal digestion was 19.4 mg/dl. Other research studying ammonia nitrogen requirements of rumen bacteria for maximized microbial growth ranged from 1 to 18 mg/dl (Hume et al., 1970; Satter and Slyter, 1974; Slyter et al., 1979; Wallace, 1979; Kang-Meznarich and Broderick, 1980; Erdman et al., 1986; Hoover et al., 1991). An *in vitro* study conducted by Satter and Slyter (1974) reported that 5 mg/dl ammonia nitrogen concentration was enough to support maximal microbial growth, which agreed with another nitrogen utilization experiment using rumen fistulated steers (Slyter et al., 1979). In all-concentrate diets lower levels of ammonia nitrogen (2

mg/dl) were found to be able to allow rumen bacteria to grow maximally (Satter and Roffler, 1975), however, 5 mg/dl ammonia was reported to maximize MOEFF (Satter and Slyter, 1974; Hespell and Bryant, 1979).

BALANCE FOR RUMINAL DEGRADABLE PEPTIDE AND RUMINAL DEGRADABLE NITROGEN FOR BEEF CATTLE

Current feedlot diets formulating approaches result in excessive RDP supplied to rumen bacteria (Fu et al., 1999). Since RDP and RDN requirements for maximal rumen bacterial growth are dependent on non-structural and structural carbohydrate mass and degradability. Synchronization between available protein and available carbohydrate is dependent upon supplying the appropriate ratio of RDP and RDN as well as matching the rate peptides and ammonia are released. Brooks (2010) determined the protein and carbohydrate degradation rate from several feedstuffs using *in vitro* methods. Briefly, he and co-workers found the degradation rate of potentially digestible protein, NDF and NSC were similar in various feedstuffs: 2.92% and 4.79% h^{-1} for NDF and NSC, respectively, and 2.2% h^{-1} for corn and bloodmeal protein, 2.8% h^{-1} for SoyPlus protein and 3.8% h^{-1} for soybean meal protein (Brooks et al., 2012). Dilution rate had been discussed and shown to influence MOEFF (Hoover et al., 1982; Firkins et al., 1996; Meng et al., 1999; Mueller, 2004). The relationship between dilution rate and MOEFF followed a quadratic function for protein, NSC and NDF (Meng et al., 1999): $MOEFF_{starch} = 7.1 + 341.6\times D - 965.3\times D^2$, $MOEFF_{NFC} = 1.7 + 368.7\times D - 586.9\times D^2$, and $MOEFF_{PR} = 9.3 + 599.2\times D - 1445.6\times D^2$. These equations can be

used to calculate the mass of microbial protein produced from rumen fermentation. Based upon microbial protein produced, RDP required by rumen microbes and RDP supplied in diet can be balanced. Brooks (2010) conducted *in vitro* and *in vivo* studies to compare four diets (115, 95, 85. 70% RDP supplied of RDP requirement) and validate that RDP peptide requirement could be explained by this approach. Once RDP required and RDP supplied are estimated, surplus RDP calculated by subtracting RDP required from RDP supplied is going to be accounted as available RDN. Using the same approach, RDN coming from surplus RDP plus RDN provided in non protein-nitrogen if urea is added is balanced with RDN required by rumen microbes. Brooks et al. (2010, 2012) suggested with further research on enhancing RDN estimation, properly balanced diets may prevent energy spilling, increase microbial protein production and improve feed efficiency.

CONCLUSION

In summary, feeding animals properly to maximize feed efficiency involves feeding appropriate nutrient amount and proportion. These two aspects cannot be isolated from each other. Intake prediction has always been a challenge, especially in a practical field with limiting information. Over the years, there have been several different equations developed and adjustments made to improve prediction accuracy. Effective energy implementation into intake prediction model for ruminants is presenting a potential advantage in improving accuracy and implementation of effective energy into diet formulation shows benefit to optimize diet. The optimal diet needs to provide nutrients in proper ratios to promote microbial efficiency and growth performance. Any imbalance in ruminal or post-ruminal system would lead to negative effect on digestion, efficiency and growth due to VFA overproduction, metabolism excessive nitrogen, compensation the shortage of absorbable amino acids. Accurate intake projection helps to understand requirements of RDP and RDN for maximal microbial efficiency. Diets for feedlot cattle can be formulated with balanced protein and energy needed for rumen microbes growth and animal growth based on estimated RDN, RDP and post-ruminal amino acid requirements and intake projection.

Table 1.1 Results of regressing predicted dry matter intake by NRC equations on actual dry matter intake for growing and finishing beef cattle[a]

Data Set[b]	Equations[c]	Observations, n	r^2	Bias, %[d]
J. Anim. Sci	Eq. 1-1	185	0.62	-1.86
	Eq. 1-3	185	0.73	-2.20

[a]Data in this table was adopted from NRC (2000 updated edition).
[b]Data set originally comes from publication of J. Anim. Sci. between 1980 to 1992; each of 185 data points represented a treatment mean of DMI.
[c]Equations are:
Eq. 1-1: $DMI = SBW^{0.75} \times (0.1493 \times NE_m - 0.046 \times {NE_m}^2 - 0.0196)$
Eq. 1-3 $DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_m - 0.0466 \times {NE_m}^2 - 0.1128)}{NE_m}$ or with intercept adjustment using 0.0869 instead of 0.1128 for cattle more than 320 kg;
[d]Bias was calculated as percentage deviation of the slope from a theoretical value of 1.0 when the predicted DMI was regressed on actual DMI with a zero intercept model.

Table 1.2 Results of regressing predicted dry matter intake on actual dry matter intake by growing and finishing beef cattle for three datasets[a]

Data Set[b]	Equations[c]	Observations, n	r^2	Bias, %[d]
Cornell	Eq. 1-1	54	0.7624	+5.88
	Eq. 1-3	54	0.7647	+0.16
	Eq. 1-5	54	0.5481	-6.49
Guelph	Eq. 1-1	38	0.7827	+8.34
	Eq. 1-3	38	0.7930	-0.49
	Eq. 1-5	38	0.3529	+4.54
Alberta	Eq. 1-1	139	0.3102	-7.90
	Eq. 1-3	139	0.3078	-8.40

[a]Table was adopted from NRC (2000 updated edition) .

[b]Cornell data was from Cornell University, including 54 data points, small, medium, and large-framed steers and heifers, NE_m = 1.4 to 2.1Mcal/kg, DOF = 100 or longer; the second data set was from University of Guelph, including 38 data points, medium and large-framed steers and heifers, alfalfa/grass silage-based diet, NE_m = 1.12 to 1.95 Mcal/kg, DOF = 16 to 24 weeks; the third data set was from University of Alberta, including 139 data points, all-forage diets with grasses, legumes and mixture of both and crop residues, NE_m = 0.69 to 1.71 Mcal/kg.

[c]Equations are:

Eq. 1-1: $DMI = SBW^{0.75} \times (0.1493 \times NE_m - 0.046 \times {NE_m}^2 - 0.0196)$;

Eq. 1-3: $DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_m - 0.0466 \times {NE_m}^2 - 0.1128)}{NE_m}$ or with intercept adjustment using 0.0869 instead of 0.1128 for cattle more than 320 kg;

Eq. 1-5: $\mathrm{DMI} = 4.54 + 0.0125 \times \mathrm{iBW}$.

[d]Bias was calculated as percentage deviation of the slope from a theoretical value of 1.0 when the predicted DMI was regressed on actual DMI with a zero intercept model.

Table 1.3 Adjustment factors for dry matter intake for cattle[a]

Adjustment factor[b]	Multiplier[c]
Breed (BI)	
Holstein	1.08
Holstein Beef	1.04
Empty body fat effect (BFAF)	
21.3 (to 350 kg EQW[C])	1.00
23.8 (400kg EQW)	0.97
26.5 (450kg EQW)	0.90
29.0 (500kg EQW)	0.82
31.5 (550kg EQW)	0.73
Anabolic Implant (ADTV1)	
With anabolic stimulant	1.00
No anabolic stimulant	0.94
Additive (ADTV2)	
Monensin 33g/kg of diet	0.90
Monensin 22g/kg of diet	0.96
Lasalocid in diet	0.98
Temperature, ˚C (TEMP)	
> 35, no night cooling	0.65
>35, with night cooling	0.90
25 to 35	0.90
15 to 25	1.00
5 to 15	1.03
-5 to 5	1.05
-15 to -5	1.07
< -15	1.16
Mud (MUD)	
None	1.00
Mild (10-20cm)	0.85
Severe (30-60cm)	0.70

[a]Table was adopted from NRC 1987.

[b]Adjustment factors are applied as: $DMI = \left[SBW^{0.75} * (0.1493 * NE_m - 0.0466 * {NE_m}^2 - 0.0196)\right] * (BFAF * BI * ADTV1 * ADTV2 * TEMP * MUD)$

[c]Corresponding equivalent weights are ≤ 350kg, 400kg, 450kg, 500kg, and 550kg.

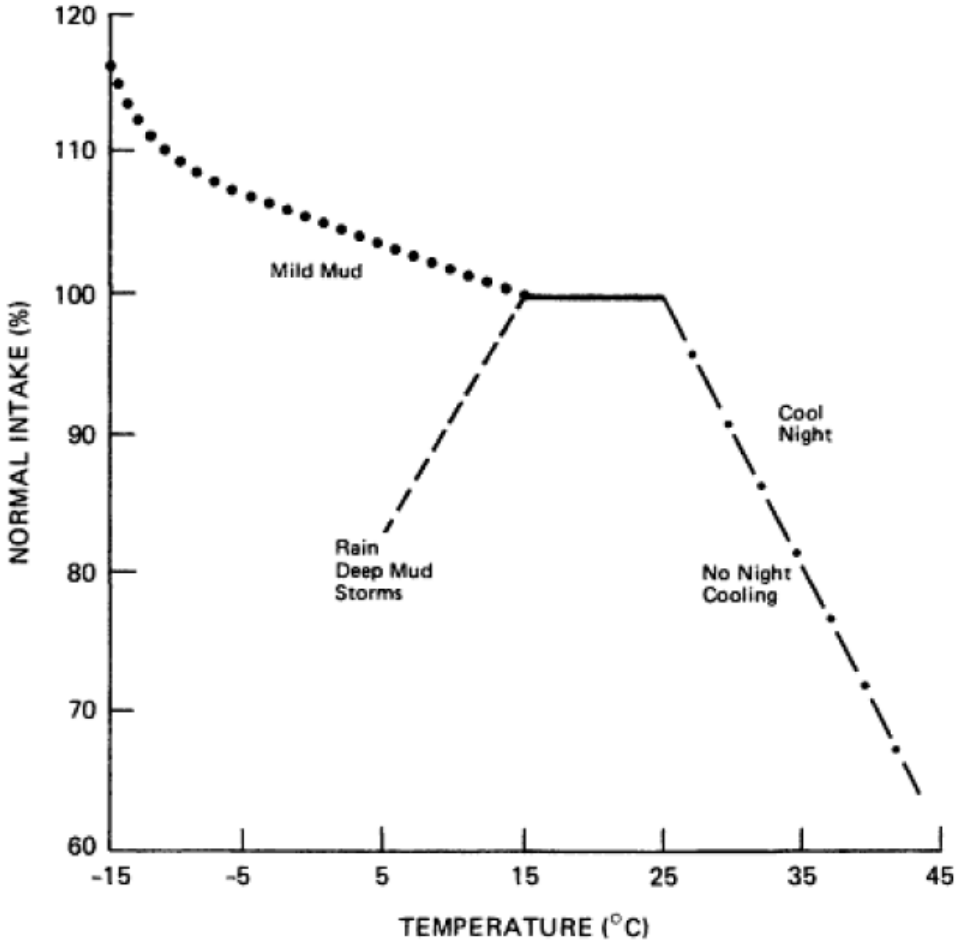

Figure 1.1 Effect of environment on dry matter intake[a]

[a]Figure was adopted from *Predicting Feed Intake of Food-Producing Animals* (NRC, 1987).

CHAPTER 2

EVALUATION OF INTAKE PREDICTION BY NRC (2000), NET ENERGY, AND EFFECTIVE ENERGY EQUATIONS

ABSTRACT

Three intake prediction models were compared for accuracy to predict intake using a database containing 15 animals groups including 562 steers, 291 bulls and 517 heifers. Equations evaluated were: (1) NRC models based on initial body weight (NRC_{iBW}) and dietary NE_m concentrations with ($NRC_{NEm\text{-}mon}$) and without monensin adjustment (NRC_{NEm}), (2) net energy model (NE) based on net energy required for maintenance and net energy required for gain as proposed by NRC, and (3) effective energy models ($EERQ(kJ/g) = MH + 50PR + 56LR + MTHE$; MH=maintenance heat, PR=protein retention, LR=lipid retention and MTHE=energy lost for methane) using either 77Kcal per kilogram of empty body weight ($EE_{77KcalEBW}$) or 77Kcal per kilogram of body weight ($EE_{77KcalBW}$) to estimate maintenance heat. Ratio of measured intake to predicted intake ranged from 0.8 to 1.3, with underprediction occurring in all 15 groups by NE and EE models and in most groups (11 of 15) by NRC models. Underprediction was evidenced by mean proportion bias being lower than 1. Coefficient of determination (r^2) from regression between measured and predicted intake followed the same ranking through all groups. The r^2 from greatest to smallest was $EE_{77KcalBW}$ > $EE_{77KcalEBW}$ > NE >

$NRC_{NEm\text{-}mon} \geq NRC_{NEm} > NRC_{iBW}$. The best relationship between measured and predicted intake occurred for EE models. Root mean square error (RMSE) as a measure of precision followed a similar trend ($EE_{77KcalBW} < EE_{77KcalEBW} < NE < NRC_{NEm\text{-}mon} \leq NRC_{NEm} < NRC_{iBW}$) across all animal groups. Means bias was the major cause of inaccuracy in EE predictions, evidenced by a greater deviation of measured to predicted intake ratio from 1 and greater mean absolute error (MAE), followed by random variation. Line bias component accounted for less than 10% of mean square prediction error (MSPE) of EE models. Overall relative prediction error (RPE) of EE equations being less than 20% indicated EE model was robust and most accurate. Intake predicted by EE models had more desirable measured intake to predicted intake ratios (close to 1), less variation (smaller ratio SD), greatest coefficient of determination (r^2), lower RMSE, lower RPE, and smaller line bias decomposition, indicating EE models best predicted steer intake. The better fit of EE models may have resulted due to more accurate partition of energy expenditure to lipid and protein retention. However, since variation in central tendency and random variation (accounting for more than 90% of MSPE) in EE model was substantial, an accurate method to estimate dietary ME needs to be further developed.

INTRODUCTION

Feed cost accounts for 60% of total feedlot production cost (Elstien, 2002), and with increasing competitive demands for corn feed cost could account for greater production costs percentage (Wallander et al., 2011). Accurate DMI prediction is essential for diet formulation to maximize efficiency of feed conversion to growth.

Equations to predict beef cattle DMI were referenced in NRC (1984, 1996, 2000). One equation uses initial body weight and a linear relationship between intake and cattle body weight to predict feed intake ($DMI = 4.54 + 0.0125 \times iBW$). This equation may be superior in practice because initial body weight is usually known at beginning of feeding period (McMeniman et al., 2009). However, lack of frame size adjustment and growth stage adjustment limited equation applicability. Two other prediction equations use shrunk body weight ($SBW^{0.75}$) and dietary NE_m concentration ($NE_{m\text{-}diet}$) to estimate intake ($DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_{m-diet} - 0.0466 \times NE_{m-diet}{}^2 - 0.1128)}{NE_{m-diet}}$, $and\ DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_{m-diet} - 0.0466 \times NE_{m-diet}{}^2 - 0.0869)}{NE_{m-diet}}$) showing acceptable prediction when predicting intake for commercial feedlot cattle (McMeniman et al., 2009). Limitation that no adjustments are given for any effect of growth rate and body composition restricts accuracy of these two equations (NRC, 1987). Net energy required for maintenance and gain for beef cattle at a given body weight could be estimated $NE_m = 77Kcal/EBW^{0.75}$ where EBW is average empty body weight in kilogram; and $NE_g = \sqrt[0.9116]{SWG/(13.91 \times SBW^{-0.6837})}$ where SWG is shrunk weight gain (NRC, 1996, 2000). Intake accounting for maintenance and growth is predictable by dividing energy requirement by dietary energy density.

Emmans (1994) proposed the effective energy (EE) concept for both ruminants and non-ruminants, in which energy requirement for tissue maintenance, protein retention, lipid retention and energy released as methane (ruminant only) is estimated. The primary difference between EE and NRC equations is the partition of energy for growth into protein and lipid accretion fractions. Little is known about evaluating and comparing EE equations with NRC equations. The research objective was to determine the accuracy of EE and NRC equations, and to determine if EE better estimated feed intake than NE/NRC equations presently used.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved use of animals in this research.

Experimental Data

Data used to evaluate equations were from 15 experiments conducted at the University of Missouri Beef Teaching and Research Farm and Southwest Center Research Farm from 2007 to 2011. These groups represented 109 pen records containing 1370 cattle. Fifty-four pens contained steers, 8 pens contained bulls and 47 contained heifers. Steer breeds included Hereford, crossbred Hereford, Angus, crossbred Angus, and Braunvieh; bulls were Gelbvieh, Balancer and Angus; heifers were Gelbvieh, Balancer and Simmental. Information available for each pen included diet, on and off test weight (initial body weight and final body weight), days on feed (DOF), ADG and DMI of each individual animal. Energy concentration of diets was calculated from ingredient

ME, NE_m and NE_g values published in NRC (2000). Table 2.1 presents a description of 15 groups of cattle and other information associated with intake prediction.

Equations and Models

The first set of equations evaluated in this study was based on initial body weight (NRC_{iBW}): $DMI = 4.54 + 0.0125 \times iBW$; or metabolic shrunk body weight ($SBW^{0.75}$) and dietary NE_m concentration ($NE_{m\text{-}diet}$):

$DMI = \frac{SBW^{0.75} \times (0.2435 \times NE_{m-diet} - 0.0466 \times NE_{m-diet}{}^2 - 0.1128)}{NE_{m-diet}}$. Both equations were reported in NRC (1996, 2000). An intercept of 0.0869 instead of 0.1128 was used for yearling heifers and steers. In the case there is no indication of animal age, animal with iBW less than 320 kg would be considered as calves since 320 kg was approximately 1 SD from mean iBW of both calf and yearling in reviewed dataset (McMeniman et al., 2009). An adjustment was made when monensin was fed. Dietary NE_m increased by 12% and predicted DMI decreased by 4% (McMeniman et al., 2009; 2010):

$DMI = 0.96 \times \frac{SBW^{0.75} \times [0.2435 \times 1.12 \times NE_{m-diet} - 0.0466 \times (1.12 \times NE_{m-diet})^2 - 0.1128]}{1.12 \times NE_{m-diet}}$.

The second equation was based on animal energy requirement for maintenance and energy requirement for gain. NRC (1996, 2000) reported the NE_m requirement of beef cattle was $NE_m = 77Kcal/EBW^{0.75}$ where EBW is the average empty body weight in kilogram calculated as $EBW = 0.891 \times SBW$ (NRC, 2000). The relationship between NE_g and shrunk weight gain (SWG) was: $SWG = 13.91 \times {NE_g}^{0.9116} \times SBW^{-0.6837}$. When ADG was known, NE_g required could be calculated as

$NE_g = \sqrt[0.9116]{\frac{SWG}{13.91 \times SBW^{-0.6837}}}$ where SWG and SBW could be computed from measured ADG or estimated gain potential.

The third set of equations were based on effective energy (EE) requirement proposed by Emmans (1994). Effective energy required was expressed as: $EERQ(kJ/d) = MH + 50PR + 56LR + MTHE$; in which MH is the maintenance heart production equaling to 0.96 fasting heat (kJ/d), PR and LR are the rates of positive protein retention and lipid retention (g/d) and MTHE is the energy released as methane during fermentation (kJ/g). Emmans (1994) demonstrated consistency between species using this energy scale of protein and lipid accretion. The previous NRC (1984) *Nutrient Requirements of Beef Cattle* reported that maintenance heat estimation could use either 77 Kcal per unit of $EBW^{0.75}$ or 77 Kcal per unit of $BW^{0.75}$ (NRC, 1984). In this study, empty body weight was chosen to estimate maintenance heat production following the NRC (1984) recommendation ($MH_{77KcalEBW} = 77Kcal/EBW^{0.75}$), and estimates using body weight ($MH_{77KcalBW} = 77Kcal/BW^{0.75}$) were compared. Protein retention and lipid retention were estimated by quadratic function of empty body weight (Simpfendorfer, 1974; NRC, 2000): $Protein\ (kg) = 0.235 \times EBW - 0.00013 \times EBW^2 - 2.418$; $Fat\ (kg) = 0.037 \times EBW + 0.00054 \times EBW^2 - 0.610$. Empty body weight was estimated according to Owens et al. (1995): $EBW = 0.917 \times SBW - 11.39$. MTHE was predicted using a fermentation balance equation (Wolin et al., 1960) and assumed 12% of intake energy was lost. Diet effective energy density was estimated as $EE_{diet}(MJ/kgOM) = 1.15ME - 3.84 - 4.67DCP$; where ME is the metabolizable

energy concentration (kJ/kg) and DCP is the digestible crude protein content. Table 2.2 summarizes the equations used in this chapter.

Statistical Criteria for Model Validation and Evaluation

Both residual (difference between measured and predicted) vs. predicted plots and measured vs. predicted plots are used widely in statistical diagnostics (Shah and Murphy, 2006). Measured vs. predicted DMI was plotted for each cattle group as Mayer and Butler (1993) recommended. The average of measured DMI to predicted DMI ratio ($\frac{M_i}{P_i}$) and standard deviation (SD) were one of several methods to describe predictions accuracy.

Other model validation methods commonly used in feed intake prediction studies were applied besides of $\frac{M_i}{P_i}$ ratio. To evaluate the accuracy and precision deviance measurements included mean absolute error (MAE), relative prediction error (RPE), mean square prediction error (MSPE) and root mean square error (RMSE) were applied. The MAE was defined as: $\sum \frac{|M_i - P_i|}{n}$ where n equals the number of paired measured (M_i) and predicted (P_i) DMI (Schaeffer, 1980; Shah and Murphy, 2006; McMeniman et al., 2010). Relative prediction error (RPE), also known as mean absolute percent error (Mayer and Bulter, 1993) was defined as proportion of MAE of measured intake (Fuentes-Pila et al., 2003; Shah and Murphy, 2006) and was an indicator of prediction precision and reproducibility. The MSPE was defined as: $\sum \frac{(M_i - P_i)^2}{n}$, and could be regarded as the sum of three components. The first is variation in central tendency (due to

mean bias), calculated by squaring mean bias (the difference between mean of measured and mean of predicted intake) of prediction [$(\overline{M} - \overline{P})^2$]. The second is the systematic bias or line bias, which is variation from the regression, calculated as a product of variance of predicted DMI ($S_P{}^2$) and square of the deviation from one of the slope of measured DMI regressed on predicted DMI [$S_P{}^2 \times (1 - b)^2$]. The third component is the random variation around the regression line, calculated as a product of variance of measured DMI ($S_M{}^2$) and deviation from one of the coefficient of determination of the regression between measured and predicted DMI ([$S_M{}^2 \times (1 - r^2)$]; Roseler et al., 1997a, 1997b; Keady et al., 2004, Shah and Murphy, 2006; McMeniman et al., 2010). Mean bias can be used to test robustness of the model and line bias can test underlying inadequacies in model structure (Shah and Murphy, 2006).

Widely used statistical test in model validation also includes regression analysis of measured DMI vs. predicted DMI. RMSE was obtained from the linear regression of measured on predicted DMI. Other useful statistics were produced in regression: coefficient of determination (r^2) used as an indicator of degree of fit; slope and variations can be used to estimate three components of MSPE. The slope of regression of the predicted DMI on measured DMI when forcing intercept to zero is considered as mean proportional bias. A regression slope less than 1 implies an underprediction across the range of measured values, while a slope greater than 1 implies an overprediction (Roseler et al., 1997a; Shah and Murphy, 2006). Table 2.3 provides a summary of the descriptive statistics used for equations evaluation as mentioned above.

RESULTS AND DISCUSSION

Table 2.4 shows the average ratio of measured DMI to predicted DMI and SD for each prediction equation. For the all steer groups, EE and NE equations had ratios greater than one, indicating equation underprediction. Ratios greater than one were found in 9 of 15 groups for all three NRC equations and ratios less than 1 occurred for the remaining groups. This was interpreted as both underprediction and overprediction occurring by NRC models. Underprediction and overprediction was also indicated, respectively, by mean proportion bias values being lower than 1 and greater than 1 (Table 2.5). The relationship between measured DMI and predicted DMI and bias were presented graphically in Figure 2.1 through Figure 2.3. Poor fitting occurred in $NRC_{NEm\text{-}mon}$ prediction in steer group A (Figure 2.1.a-2), and NRC_{ibw} and $NRC_{NEm\text{-}mon}$ predictions in steer group C (Figure 2.1.c-2) even though $\frac{M_i}{P_i}$ ratios were close to one (1.01, 1.00 and 1.03, respectively, Table 2.4). The average measured intake to predicted intake ratio equaling one proves that the average of prediction was well fit to the average of intake measured, but not descriptive of individual intake within the population tested. Standard deviation of $\frac{M_i}{P_i}$ ratio was smaller for $EE_{77KcalBW}$ prediction than $EE_{77KcalEBW}$ prediction and NE prediction (Table 2.4). Plots (Figure 2.1 to 2.3) of measured vs. predicted intake demonstrated that all three sets of models could achieve acceptable intake prediction for steers, however, EE models could better explain individual intake variation than NE models. In prediction of heifers, $\frac{M_i}{P_i}$ ratios being greater than one indicated all equations

were underpredicted. Heifers were found consuming 3% more intake than steers (Koknaroglu et al., 2008) and Klosterman and Parker (1976) reported the intake of heifers fed the same finishing diet as steers was 5% higher. Also, Figure 2.3 shows greater variations between predicted DMI and measured DMI were found in heifers, interpreted as that intake and/or growth models used in this analysis may not be appropriate for heifers. This most likely error was inaccuracy of growth composition, and therefore estimated energy required for growth of heifers.

Measured DMI regressed on predicted DMI by each equation for steers, bulls and heifers are reported in Table 2.5. Through all of the groups, coefficient of determination (r^2) of each equation followed the same ranking: $EE_{77KcalBW} > EE_{77KcalEBW} > NE > NRC_{NEm\text{-}mon} \geq NRC_{NEm} > NRC_{iBW}$. The strongest relationship between measured intake and predicted intake occurred in EE models and NE models were intermediate. NRC equations presented the weakest relationship with measured intake, in which r^2 of $NRC_{NEm\text{-}mon}$ equation was slightly greater than or equivalent to NRC_{NEm} equation and both had greater r^2 than NRC_{iBW} equation. Deviance measurement that indicates prediction precision and accuracy are shown in Table 2.6, Table 2.7 and Table 2.8, respectively. Due to poor coefficient of determination rated in some heifers groups (Table 2.5), deviance measurement for part of heifer groups were deleted from the table.

MAE, which measures how close predictions are to actual measurements, consistently showed smaller values in $EE_{77KcalBW}$ equation when just comparing two EE equations ($EE_{77KcalEBW}$ and $EE_{77KcalBW}$). There was agreement between MAE and measured intake to predicted intake ratio. MAE together with measured intake to predicted intake ratio suggested that in some animal groups bias was found in EE models,

even so, EE ($EE_{77KcalBW}$) models had a better fit than other models since they had greatest r^2, lower MAE and $\frac{M_i}{P_i}$ ratio was closer to 1.

RPE of EE prediction was lower than 10% in 4 of 7 steer groups, and between 10 to 20% in the other three. RPE < 10% was considered as acceptable or satisfactory for predicted DMI (Kleijnen, 1987; Fuentes-Pila et al., 1996), between 10 to 20% as relatively acceptable for predicted DMI, and > 20% as lacking in robustness. A model is considered as robust and less risky for practical use when RPE of prediction is relatively good across all datasets (overall RPE was ≤ 20%), rather than high accuracy for some datasets and poor accuracy (RPE > 20%) for others (Fuentes-Plia et al., 1996). Root mean square error (RMSE) as a measure of precision, in 10 of 11 groups ranked as follows: $EE_{77KcalBW} < EE_{77KcalEBW} < NE < NRC_{NEm\text{-}mon} \leq NRC_{NEm} < NRC_{iBW}$ (Table 2.6, 2.7 and 2.8). The remaining group followed the same ranking as above but was not statistically different (Table 2.6, group E). The magnitude of the RMSE for EE models being lower than NRC models was 18% on average. Based upon measures of r^2, MAE, RPE, $\frac{M_i}{P_i}$ ratio and RMSE we concluded that EE was superior to other models to predict intake for steers.

The primary difference between EE and NE intake equations is an accounting for retentive tissue. The NE system applies the value to energy cost for growth, using a quadratic function to account for greater energy requirement of lipid accretion. The EE equations separate growth energy requirement into protein and lipid accretion. A general conclusion drawn from graphic presentation of data was that NE equations underpredicted energy cost of lipid accretion, evidenced by ratio slope greater than 1.

Concluded from ratio graph of EE was that slope was more similar to 1, but bias existed. Since we used published energy value of dietary ingredients, this was most likely cause for overestimation of diet ME.

The three components (mean bias, systematic bias and random variation) decomposed from MSPE showed that within all 66 predictions listed in Table 2.5, 2.6 and 2.7, mean bias accounted for more than 50% of the decomposition, from which we concluded that means bias was the major cause of inaccuracy in intake prediction, followed by random variation. In steer groups, mean bias together with random variation accounted for approximately 90% of MSPE for EE models. The mean bias component of MSPE is computed by squaring mean bias (equivalent to squaring MAE), and random variation component of the MSPE is a function of r^2 and variance of measured intake $\left[(1-r^2)\times {S_M}^2\right]$. It is considered to be able to explain a large proportion of actual variation of measured DMI when r^2 is larger than 0.5 (Yungblut et al., 1981). Greater random variation (more than 20%) shown in EE models may be associated with greater variance of measured intake (S_M^2), since the majority of r^2 in EE models were greater than 0.5, and considered not to contribute much to the large random variation decomposition. The greater random variation decomposition shown in NE and NRC models may be attributed to comparative lower r^2 and greater S_M^2 at the same time. Line bias, which is used to demonstrate the adequacy of the model, was shown to be consistently lower than 10% in all EE model predictions. However, in NRC and NE models for steers, line bias showed greater variations (ranged from 3.2 to 25.1% for NE model, and 0 to 27.7% for NRC models), the NRC and NE models were not able to offer

a consistently reliable prediction for either steer or bull groups similar to EE models. Coefficient of determination (r^2) showed EE models with an average of 54.5% explanation of intake variation for steers and 55.7% for bulls. The NE models explained an average of 50.7% variation for steers and 49.7% for bulls; the NRC models averaged 33.2% for steers and 36.6% for bulls (Table 2.5, 2.6 and 2.7). Prediction is considered as acceptable when r^2 is greater than 50%. McMeniman et al. (2009) reported r^2 being 0.64, 0.66 and 0.67 for NRC_{ibw}, NRC_{NEm} and $NRC_{NEm\text{-}mon}$ equations, respectively. Weak r^2 found in current dataset was attributed to poor regression between measured and predicted intake in certain group (e. g. Table 2.5, steer group F).

CONCLUSION

Effective energy separates energy requirement into maintenance heat, heat as methane production, and energy requirement for protein and lipid gain. Intake predicted by effective energy model had the strongest correlation with measured intake and the lowest root mean square error (RMSE), indicating the EE model would be a better steer intake predicting model than NE and NRC.

Table 2-1 Description of 15 groups of cattle used in the equation evaluation database.

Group[1]	n	Breed[2]	DOF[3]	iBw, kg	fBW, kg	ADG, kg/d	DMI, kg/d	F:G	Dietary Composition[4]			
									DM, %	CP, %	NE_m, Mcal/kg	ME, Mcal/kg
Steer												
A	92	Herf X	63	372.1	480.02	1.69	8.30	4.99	87.16	11.63	2.11	3.10
B	117	Ang/Brv/Her	62	377.4	470.57	1.50	10.86	7.77	78.49	13.30	1.90	2.79
C	87	Angus	167	338.5	548.44	1.32	8.80	6.76	85.89	12.72	2.07	2.96
D	23	Braunivieh	78	428.7	510.49	1.48	8.26	5.70	85.19	13.43	2.15	3.09
E	45	Angus/X	149	312.7	518.43	1.50	8.90	5.97	86.21	15.94	2.01	2.87
F	80	Angus/X	142	365.6	592.49	1.60	9.14	5.80	88.62	15.53	2.08	3.04
G	118	Angus/X	169	306.2	529.77	1.33	8.68	6.63	88.62	15.53	2.08	3.04
Bull												
A	120	Gelbv/Bal	75	514.4	584.08	0.92	10.00	12.11	70.85	13.61	1.76	2.54
B	120	Gelbv/Bal/A	70	382.1	501.84	1.69	13.28	7.99	70.85	13.61	1.76	2.54
C	51	Brv	64	353.7	462.89	1.69	12.46	7.61	89.10	12.25	2.02	3.00
Heifer												
A	121	Gelbv/Bal/A	71	378.6	474.15	1.35	10.86	8.20	70.85	1.41	1.76	2.54
B	50	Braunivieh	57	397.1	437.95	0.73	13.28	19.98	70.85	13.61	1.76	2.54
C	83	Herf X	57	371.0	413.33	0.75	16.68	17.82	60.00	12.25	2.02	3.00
D	116	Hereford	61	376.6	482.77	1.74	11.97	7.42	89.83	12.56	2.04	3.00
E	58	Hereford	65	351.4	436.22	1.28	9.12	7.97	89.83	12.56	2.04	3.00
F	89	Sim/Ang	69	373.5	475.39	1.48	9.75	6.85	87.42	10.68	2.08	3.05

[1]Group name was labeled by letters.

[2]Breed: Herf = Hereford, X = crossbred, Ang = Angus, Brv = Braunvieh, Gelbv = Gebvieh, Bal = Balancer, Sim = Simmental

[3]DOF: Days on feed.

[4]Diet energy and nutrients concentration is determined on a DM basis from dietary formulation and nutrients value in NRC (2000).

Table 2.2 Summary of equations.

Abbreviation		Equation
EBW	Empty body weight; Owens et al., 1995	$EBW(kg) = SBW * 0.917 - 11.39$
EBW	Empty body weight; NRC Beef, 2000	$EBW(kg) = SBW * 0.891$
EE_{diet}	Effective energy yield in diet	$EE_{diet}(MJ/\square gOM) = 1.15ME - 3.84 - 4.67DCP$
EERQ	Effective energy requirement	$EERQ(kJ/g) = MH + 50PR + 56LR + MTHE$
LR	Lipid retention	$Fat\ (g) = (0.037 * EBW + 0.00054 * EBW^2 - 0.610) * 1000$
MTHE	Energy lost as methane	$MTHE(kJ/g) = \ (digsitable\ intake\ in\ gram/180) * 673.4 * 4.184 * 12.2\% * 0.612$
NE_g	NE_g requirement; NRC, 2000	$NE_g(Kcal/kg) = \sqrt[0.9116]{SWG/(13.91 * SBW^{-0.6837})}$
NE_m	NE_m requirement; NRC, 1996, 2000	$NE_m(Kcal/kg) = 77Kcal/E\square W^{0.75}$
PR	Protein retention	$Protein\ (g) = (0.235 * EBW - 0.00013 * EBW^2 - 2.418) * 1000$
Intake prediction equations		
NRC_{ibw}	NRC equation based on iBW	$DMI = 4.54 + 0.0125 * iBW$
NRC_{NEm}	NRC equation based on SBW and NE_m concentration in diet	$DMI = \frac{SBW^{0.75}(0.2435 * NE_{m-diet} - 0.0466 * {NE_{m-diet}}^2 - 0.1128)}{NE_{m-diet}}$
$NRC_{NEm-mon}$	NRC equation based on SBW and NE_m concentration in diet with adjustment	$DMI = 0.96 * \left\{\frac{SBW^{0.75} * [0.2435 * 1.12 * NE_{m-diet} - 0.0466 * (1.12 * NE_{m-diet})^2 - 0.1128]}{1.12 * NE_{m-diet}}\right\}$
NE	NE equation based on NE_m and NE_g requirement	$DMI = (NE_m/NE_{m-diet}) + (NE_g/NE_{g-diet})$ $= [(77Kcal * EBW^{0.75})/NE_{m-diet}] + \left\{\left[\sqrt[0.9116]{S\square G/(13.91 * SBW^{-0.6837})}\right]/NE_{g-diet}\right\}$
$EE_{77KcalEBW}$	EE equation based on 77Kcal maintenance heat per kg $EBW^{0.75}$	$DMI = (EERQ/EE_{diet})$ $= (MH_{77KcalEBW} + 50PR + 56LR + METH)/(1.15ME - 3.84 - 4.67DCP)$
$EE_{77KcalBW}$	EE equation based on 77Kcal maintenance heat per kg $BW^{0.75}$	$DMI = (EERQ/EE_{diet})$ $= (MH_{77KcalBW} + 50PR + 56LR + METH)/(1.15ME - 3.84 - 4.67DCP)$

Table 2.3 Descriptive statistics used to evaluate DMI prediction equations.

Statistic	Abbreviation	Description
Coefficient of determination	r^2	Proportion of variation of measured DMI that can be explained by predicted DMI
Mean absolute error	MAE	$(\sum \lvert M_i - P_i \rvert)/n$, where n = number of paired measured DMI (M_i) and predicted DMI (P_i)
Mean bias	--	Measured DMI minus predicted DMI
Mean proportional bias	--	Slope of simple linear regression of predicted on measured DMI with the intercept forced to 0
0Mean square prediction error	MSPE	$(\sum(M_i - P_i)^2)/n$, where n = number of paired measured (M_i) and predicted (P_i) DMI values
Random variation	--	$(1 - r^2) \times {S_M}^2$, where r^2 = the coefficient of determination for the regression of measured on predicted DMI and ${S_M}^2$ = variance of the measured DMI
Relative prediction error	RPE	MAE as proportion of the mean of measured intake and expressed as a percentage
Root mean square error	RMSE	Square root of error mean square of the linear regression of measured on predicted DMI
Systematic bias (or line bias)	--	$(1 - b)^2 \times {S_P}^2$, where b = the slope of the regression of measured on predicted DMI and ${S_P}^2$ = variance of the predicted DMI

Table 2.4 Ratio of measured DMI to predicted DMI for each equation[1].

Animal group[2]		NRC			NE	EE	
		ibw	NE_m	$NE_{m\text{-}mon}$	77Kcal	77 Kcal EBW	77 Kcal BW
Steer							
A	Ratio[3]	0.90	0.89	0.99	1.01	1.09	1.04
	SD	0.102	0.089	0.099	0.106	0.101	0.095
B	Ratio	1.18	1.14	1.25	1.28	1.25	1.19
	SD	0.140	0.169	0.182	0.164	0.129	0.118
C	Ratio	1.00	0.93	1.03	1.20	1.18	1.12
	SD	0.085	0.095	0.104	0.113	0.105	0.097
D	Ratio	0.84	0.82	0.93	1.02	1.03	0.98
	SD	0.097	0.090	0.101	0.113	0.088	0.083
E	Ratio	1.05	1.01	1.11	1.13	1.06	1.01
	SD	0.089	0.088	0.095	0.083	0.080	0.075
F	Ratio	1.01	0.88	0.98	1.04	1.07	1.02
	SD	0.132	0.103	0.115	0.121	0.108	0.102
G	Ratio	1.04	1.01	1.12	1.23	1.22	1.16
	SD	0.108	0.107	0.117	0.131	0.110	0.102
Bull							
A	Ratio	0.91	0.81	0.88	1.10	1.04	0.98
	SD	0.071	0.059	0.064	0.141	0.106	0.094
B	Ratio	1.42	1.29	1.40	1.27	1.13	1.08
	SD	0.11	0.09	0.09	0.109	0.075	0.069
C	Ratio	1.38	1.36	1.51	1.49	1.52	1.46
	SD	0.202	0.196	0.217	0.186	0.160	0.148
Heifer							
A	Ratio	1.17	1.07	1.16	1.24	1.08	1.03
	SD	0.105	0.095	0.102	0.129	0.093	0.087
B	Ratio	1.40	1.32	1.44	2.18	1.72	1.62
	SD	0.175	0.163	0.177	0.369	0.229	0.209
C	Ratio	1.22	1.25	1.39	2.24	2.12	1.98
	SD	0.222	0.228	0.3247	0.530	0.414	0.379
D	Ratio	1.30	1.27	1.41	1.40	1.41	1.34
	SD	0.139	0.166	0.183	0.268	0.219	0.201
E	Ratio	1.06	1.04	1.16	1.29	1.22	1.16
	SD	0.157	0.157	0.174	0.178	0.14	0.136

[1]Equations are based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon)}$, based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

[2]Group name was labeled as letters.

[3]Ratio and standard deviation (SD) are measured intake to predicted intake ratio and SD of the ratios.

Table 2.5 Statistics from regression of measured DMI on predicted DMI by each of six-prediction equations[1].

		NRC			NE	EE	
Item[2]		ibw	NE_m	$NE_{m\text{-}mon}$	77Kcal	77 Kcal EBW	77 Kcal BW
r^2							
Steer	A[3]	0.49	0.53	0.53	0.58	0.62	0.63
	B	0.24	0.29	0.30	0.56	0.61	0.62
	C	0.28	0.34	0.34	0.55	0.53	0.53
	D	0.26	0.34	0.34	0.46	0.58	0.58
	E	0.48	0.57	0.57	0.69	0.61	0.61
	F	0.01	0.17	0.17	0.42	0.48	0.48
	G	0.14	0.23	0.23	0.43	0.49	0.49
Bull	A	0.23	0.32	0.32	0.38	0.43	0.44
	B	0.33	0.49	0.49	0.61	0.67	0.67
	C	0.30	0.40	0.41	0.50	0.57	0.57
Heifer	A	0.28	0.35	0.35	0.41	0.46	0.49
	B	0.01	0.04	0.04	0.11	0.22	0.22
	C	0.25	0.21	0.21	0.09	0.20	0.21
	D	0.16	0.17	0.17	0.17	0.22	0.23
	E	0.08	0.17	0.17	0.50	0.56	0.56
Mean proportion bias							
Steer	A	1.09	1.12	1.00	0.99	0.91	0.95
	B	0.84	0.88	0.80	0.80	0.80	0.84
	C	0.99	1.08	0.97	0.84	0.86	0.90
	D	1.18	1.22	1.09	0.98	0.97	1.01
	E	0.94	1.00	0.90	0.89	0.95	0.99
	F	0.98	1.12	1.01	0.96	0.93	0.97
	G	0.95	0.99	0.89	0.81	0.82	0.86
Bull	A	1.09	1.25	1.16	0.92	0.97	1.03
	B	0.70	0.77	0.71	0.80	0.89	0.93
	C	0.71	0.73	0.65	0.68	0.63	0.66
Heifer	A	0.85	0.93	0.86	0.81	0.92	0.97

[1]Equations were based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

[2]Definations: r^2 is coefficient of determination of the regression of measured intake on predicted intake; mean proportion bias is slope of simple linear regression of predicted intake on measured intake with the intercept forced to zero.

[3]Group was labeled as letters.

Table 2.6 Accuracy of DMI prediction equations evaluated with steer dataset.

	Accuracy measures[2]				Bias and variation measures[3]		
Intake equation[1]	MAE	RPE	MSPE	RMSE	Mean bias	Systematic bias	Random variation
Group A[4]							
NRC_{ibw}	1.07	11.9	1.61	0.85	49.3	6.7	44.0
NRC_{NEm}	1.16	12.4	1.78	0.82	63.2	0.0	36.8
$NRC_{NEm\text{-}mon}$	0.65	7.8	0.66	0.82	0.4	0.9	98.8
NE_{77Kcal}	0.67	8.3	0.70	0.77	0.2	17.3	82.5
$EE_{77KcalEBW}$	0.80	10.8	0.98	0.73	46.2	|0.7	53.1
$EE_{77KcalBW}$	0.61	7.9	0.64	0.72	19.1	1.4	79.5
Group B							
NRC_{ibw}	1.72	19.5	4.19	1.28	61.3	0.2	38.5
NRC_{NEm}	1.51	15.8	3.41	1.24	43.0	13.4	44.0
$NRC_{NEm\text{-}mon}$	2.14	15.3	6.03	1.23	70.9	4.3	24.8
NE_{77Kcal}	2.25	14.0	6.15	0.98	81.2	3.9	14.5
$EE_{77KcalEBW}$	2.12	19.5	5.37	0.92	83.5	1.1	15.4
$EE_{77KcalBW}$	1.74	20.8	3.88	0.91	77.3	1.7	21.0
Group C							
NRC_{ibw}	0.60	7.0	0.56	0.76	0.2	0.0	99.8
NRC_{NEm}	1.02	11.9	1.38	0.73	42.2	20.5	37.3
$NRC_{NEm\text{-}mon}$	0.68	7.8	0.73	0.72	7.4	22.3	70.3
NE_{77Kcal}	1.45	16.4	2.52	0.60	80.9	5.2	13.9
$EE_{77KcalEBW}$	1.29	14.6	2.07	0.61	77.5	4.8	17.7
$EE_{77KcalBW}$	0.98	11.1	1.29	0.61	63.2	8.7	28.1
Group D							
NRC_{ibw}	1.60	21.0	3.41	0.97	75.0	0	25.0
NRC_{NEm}	1.91	24.6	4.42	0.91	82.7	0.2	17.2
$NRC_{NEm\text{-}mon}$	0.99	13.0	1.43	0.90	47.0	0	53.0
NE_{77Kcal}	0.63	7.8	0.85	0.83	2.0	25.1	72.9
$EE_{77KcalEBW}$	0.57	7.2	0.56	0.73	10.0	2.6	87.3
$EE_{77KcalBW}$	0.48	6.3	0.52	0.73	3.5	3.5	93.1
Group E							
NRC_{ibw}	0.66	7.1	0.76	0.73	26.7	6.3	66.9
NRC_{NEm}	0.62	7.0	0.58	0.66	0.0	27.7	72.3
$NRC_{NEm\text{-}mon}$	0.93	10.4	1.22	0.66	60.5	5.7	33.9
NE_{77Kcal}	1.05	11.8	1.39	0.56	75.1	3.2	21.7
$EE_{77KcalEBW}$	0.63	7.0	0.60	0.63	32.7	4.3	63.0
$EE_{77KcalBW}$	0.50	5.7	0.41	0.62	1.1	7.7	91.2
Group F							
NRC_{ibw}	0.99	11.2	1.45	1.18	0.1	5.9	94.0
NRC_{NEm}	1.25	16.4	2.76	1.08	58.9	0.1	41.0
$NRC_{NEm\text{-}mon}$	0.83	10.1	1.16	1.08	2.8	0.0	97.2
NE_{77Kcal}	0.84	9.0	1.15	0.91	7.9	22.6	69.5
$EE_{77KcalEBW}$	0.85	9.0	1.13	0.86	30.7	6.2	36.1
$EE_{77KcalBW}$	0.72	7.8	0.84	0.86	5.3	9.4	85.2
Group G							
NRC_{ibw}	0.76	8.7	0.88	0.89	10.8	0.9	88.4
NRC_{NEm}	0.75	8.7	0.84	0.85	0.3	15.6	84.1
$NRC_{NEm\text{-}mon}$	1.06	11.8	1.63	0.84	53.0	4.0	43.0
NE_{77Kcal}	1.61	18.3	3.15	0.73	80.3	3.4	16.6
$EE_{77KcalEBW}$	1.53	17.3	2.79	0.69	82.4	0.9	16.7
$EE_{77KcalBW}$	1.21	13.6	1.87	0.69	73.6	1.6	24.8

[1]Equations were based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

[2]Definations: MAE = mean absolute error (kg/d), RPE = relative prediction error (%), MSPE = mean square prediction error (kg^2/d^2), RMSE = root mean square error (kg/d).

[3]Definations: bias and variations were decomposed from MSPE into three components; mean bias = $(\bar{M} - \bar{P})^2$, attributed to difference between means of measured ($\bar{M}$) and predicted ($\bar{P}$) intake; systematic bias = ${S_P}^2 * (1 - b)^2$, a product of variance of predicted intake (${S_P}^2$) and the square of the deviation from one of the slope (b) of the regression of measured on predicted intake; random variation = ${S_M}^2 * (1 - r^2)$, a product of variance of measured intake (${S_M}^2$) and the deviation from one of the coefficient of determination (r^2) of the regression of measured on predicted intake.

[4]Steer group was labeled as letters.

Table 2.7 Accuracy of DMI prediction equations evaluated with bull dataset.

Intake equation[1]	Accuracy Measure[2]				Bias and variation measures[3]		
	MAE	RPE	MSPE	RMSE	Mean bias	Systematic bias	Random variation
Group A[4]							
NRC_{ibw}	1.09	11.3	1.56	0.79	60.6	0.1	39.3
NRC_{NEm}	2.55	26.1	7.03	0.74	92.1	0.3	7.6
$NRC_{NEm\text{-}mon}$	1.69	17.4	3.34	0.74	83.6	0.3	16.1
NE_{77Kcal}	1.04	10.4	1.75	0.71	33.8	38.0	28.3
$EE_{77KcalEBW}$	0.74	7.4	0.93	0.68	8.3	43.5	48.2
$EE_{77KcalBW}$	0.80	8.1	0.95	0.67	8.9	44.5	46.7
Group B							
NRC_{ibw}	3.96	29.4	16.81	1.03	93.4	0.4	6.2
NRC_{NEm}	2.97	22.0	9.62	0.90	91.6	0.2	8.2
$NRC_{NEm\text{-}mon}$	3.77	28.1	15.03	0.90	94.5	0.2	8.2
NE_{77Kcal}	2.73	20.1	8.20	0.78	90.6	2.2	7.3
$EE_{77KcalEBW}$	1.46	11.0	2.69	0.72	77.2	3.5	19.1
$EE_{77KcalBW}$	1.07	8.1	1.53	0.72	59.7	7.3	33.0
Group C							
NRC_{ibw}	3.57	27.6	14.88	1.51	84.3	0.7	15.0
NRC_{NEm}	3.37	26.5	12.99	1.40	85.3	0.0	14.7
$NRC_{NEm\text{-}mon}$	4.25	33.4	19.87	1.40	90.3	0.1	9.6
NE_{77Kcal}	4.01	31.9	17.63	1.28	90.9	0.1	9.1
$EE_{77KcalEBW}$	4.52	35.9	21.90	1.19	93.5	0.2	6.3
$EE_{77KcalBW}$	4.21	33.4	19.09	1.18	92.6	0.2	7.2

[1]Equations were based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

[2]Defination: MAE = mean absolute error (kg/d), RPE = relative prediction error (%), MSPE = mean square prediction error (kg^2/d^2), RMSE = root mean square error (kg/d).

[3]Defination: bias and variations were decomposed from MSPE to three components; mean bias = $(\bar{M} - \bar{P})^2$, attributed to difference between of means of measured ($\bar{M}$) and predicted ($\bar{P}$) intake; systematic bias = ${S_P}^2 * (1 - b)^2$, a product of variance of predicted intake (${S_P}^2$) and the square of the deviation from one of the slope (b) of the regression of measured on predicted intake; random variation = ${S_M}^2 * (1 - r^2)$, a product of variance of measured intake (${S_M}^2$) and the deviation from one of the coefficient of determination (r^2) of the regression of measured on predicted intake.

[4]Bull group was labeled as letters.

Table 2.8 Accuracy of DMI prediction equations evaluated with heifer dataset.

	Accuracy Measure[2]				Bias and variation measures[3]		
Intake equation[1]	MAE	RPE	MSPE	RMSE	Mean bias	Systematic bias	Random variation
Group A[4]							
NRC_{ibw}	1.65	14.6	3.50	0.99	71.9	0.8	27.4
NRC_{NEm}	0.97	8.7	1.38	0.94	35.9	0.8	63.3
$NRC_{NEm\text{-}mon}$	1.56	13.9	3.12	0.94	72.1	0.0	27.9
NE_{77Kcal}	2.04	18.5	5.07	0.90	82.2	2.2	15.7
$EE_{77KcalEBW}$	0.95	8.6	1.38	0.84	45.6	4.2	50.2
$EE_{77KcalBW}$	0.74	6.8	0.85	0.83	12.1	7.8	80.2

[1]Equations were based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

[2]Definations: MAE = mean absolute error (kg/d), RPE = relative prediction error (%), MSPE = mean square prediction error (kg^2/d^2), RMSE = root mean square error (kg/d).

[3]Definations: bias and variations were decomposed from MSPE to three components; mean bias = $(\overline{M} - \overline{P})^2$, attributed to difference between of means of measured ($\overline{M}$) and predicted ($\overline{P}$) intake; systematic bias = ${S_P}^2 * (1 - b)^2$, a product of variance of predicted intake (${S_P}^2$) and the square of the deviation from one of the slope (b) of the regression of measured on predicted intake; random variation = ${S_M}^2 * (1 - r^2)$, a product of variance of measured intake (${S_M}^2$) and the deviation from one of the coefficient of determination (r^2) of the regression of measured on predicted intake.

[4]Heifer group was labeled as letters.

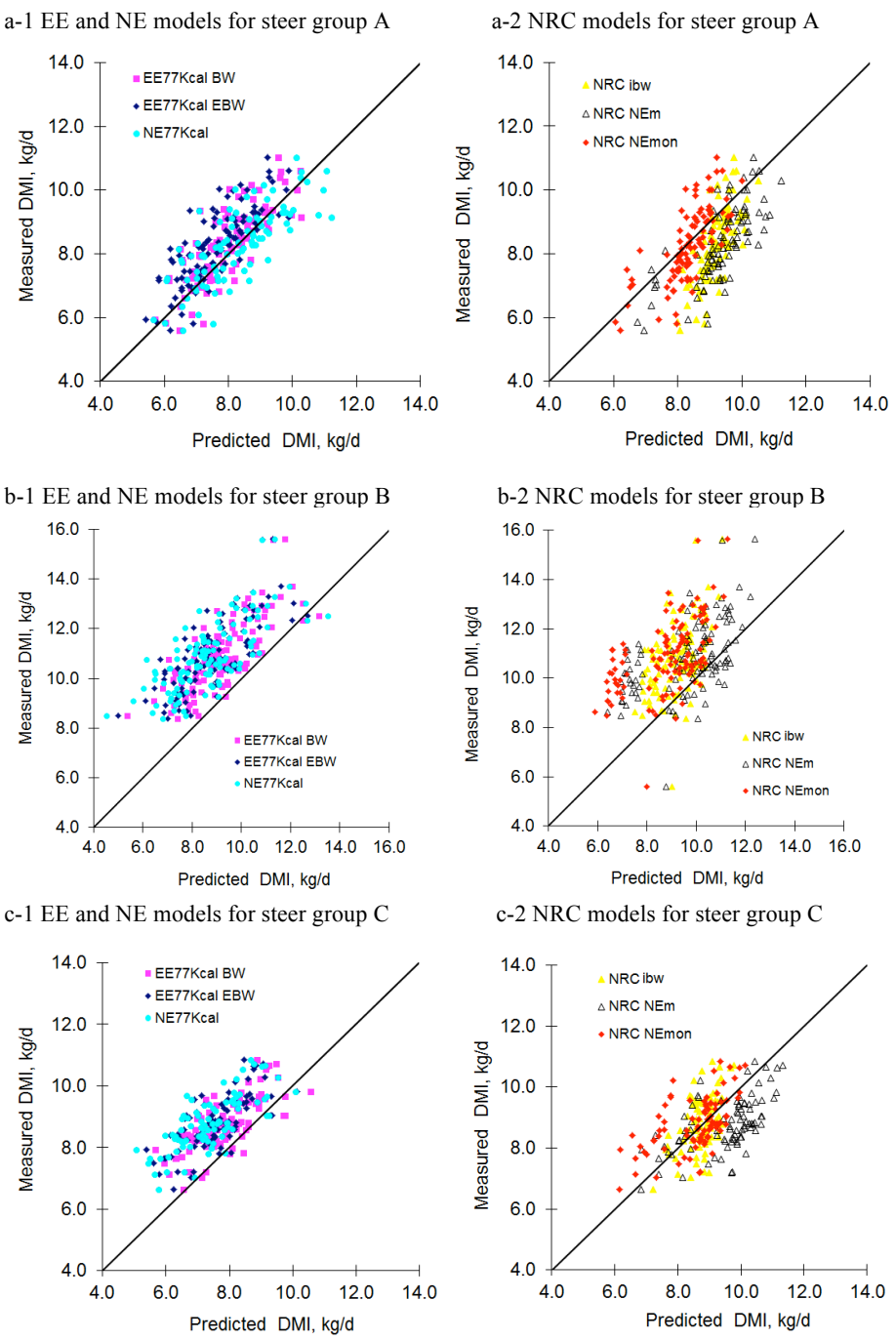

Figure 2.1 Relationship between measured DMI and predicted DMI for three steer groups[1].

[1]Equations are based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

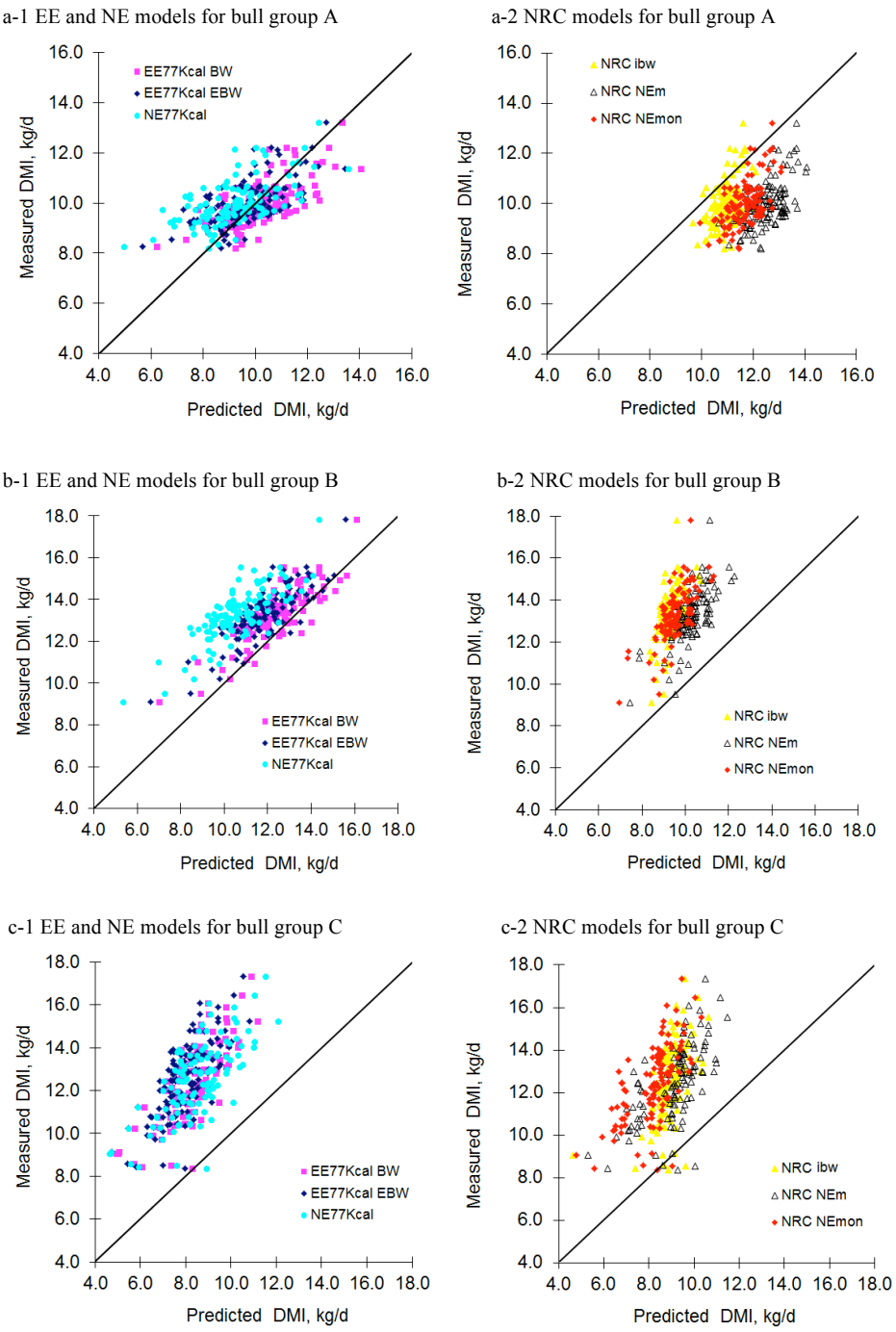

Figure 2.2 Relationship between measured DMI and predicted DMI for three bull groups[1].

[1]Equations are based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

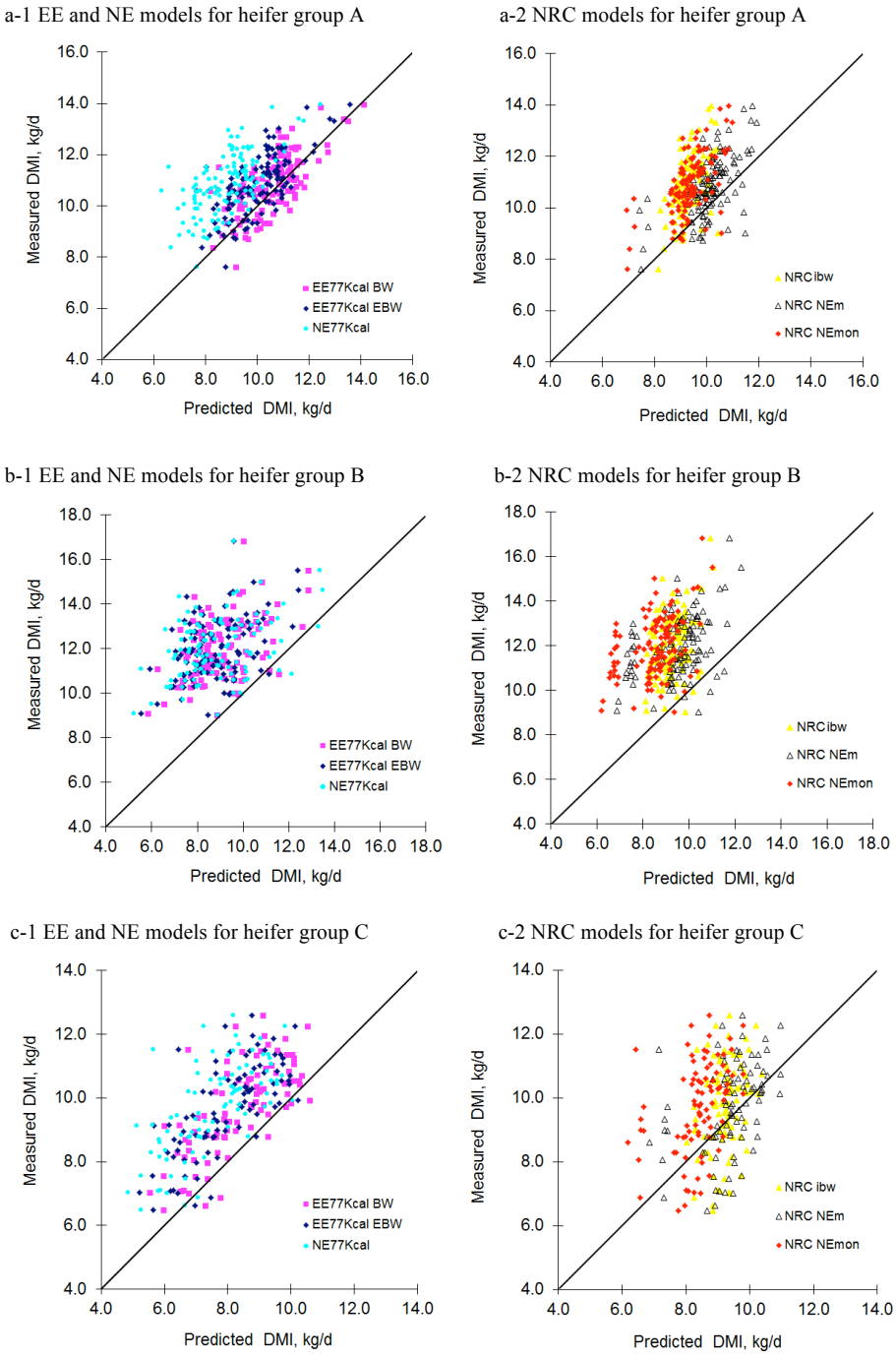

Figure 2.3 Relationship between measured DMI and predicted DMI for heifer groups[1].

[1]Equations are based on ibw (NRC_{ibw}), based on shrunk body weight and dietary NE_m concentration without (NRC_{NEm}) and with adjustment ($NRC_{NEm\text{-}mon}$), based on NE_m and NE_g requirement (NE), based on EE with 77Kcal per kg of $EBW^{0.75}$ maintenance heat ($EE_{77KcalEBW}$) and 77Kcal per kg of $BW^{0.75}$ maintenance heat ($EE_{77KcalBW}$).

CHAPTER 3

RUMEN DEGRADABLE NITROGEN SUPPLY AFFECTS MICROBIAL EFFICIENCY IN CONTINUOUS CULTURE AND FEED EFFICIENCY IN HEIFERS

ABSTRACT

Objectives of these studies were: a) to determine if ammonia-nitrogen (NH_3-N) level affected ruminal microbial efficiency and to evaluate accuracy of a prediction model for determining NH_3-N and degradable protein required by rumen bacteria. Diets consisting of corn, SoyPLUS (West Central®, Ralston, IA), bloodmeal and urea were formulated to provide adequate RDP peptide (RDPep) and inadequate to adequate levels of NH_3-N in *in vitro* and *in vivo* experiments. In Exp.1, four diets varying in ruminal degradable nitrogen (RDN) levels were fed to continuous culture fermenters. The RDN levels, calculated as predicted RDN supplied relative to RDN required by rumen microbes expressed as percentage of crude protein, were -1.03%, -0.62%, -0.08% and 0.39%. These RDN levels were achieved by differing urea inclusion in diets (0, 0.21, 0.46, and 0.73% urea as DM basis). As urea increased in diet digestibility of OM increased linearly ($P = 0.01$), grams of bacterial nitrogen outflowing from rumen and microbial efficiency (MOEFF) increased in a cubic response ($P < 0.01$) with the highest measurement being for the -0.08% RDN diet. Increasing RDN level increased pH (quadratic, $P = 0.02$) but units of difference

among treatments were small and may lack biological significance. Ammonia concentration increased linearly ($P < 0.01$) when RDN increased. There was a cubic response ($P < 0.05$) for total VFA, acetate and propionate concentrations. No difference was measured for either molar percentage of acetate or propionate, or acetate to propionate ratio. Bacterial N production and MOEFF were maximized when RDPep and RDN requirement and supply was balanced (-0.08% RDN diet). In Exp.2, similar diet were fed and urea inclusion was 0%, 0.12%, 0.22%, and 0.34% (DM basis) to formulate four diets with RDN balance levels of -0.92%, -0.69%, -0.49% and -0.26%, respectively. Sixty crossbred Angus heifers were grouped into 12 pens randomly and each diet treatment was assigned to three pen replications. There was no significant difference on ADG and DMI among treatments. The gain to feed ratio (G: F) responded quadratically ($P < 0.01$) to RDN level with the greatest G: F occurring in calves fed -0.69% RDN diet. Maximal MOEFF was achieved when RDN requirement was met but not exceeded. Greatest feed efficiency for the -0.69% RDN diet could be due to NH_3-N not supplied via diet being compensated by recycled nitrogen. Once NH_3-N requirements are met, excessive nitrogen metabolism requires energy, incresing intake without improving gain. The model to predict RDN required optimized growth performance. We concluded ruminant diets can be formulated to meet microbes requirement for RDPep and RDN.

INTRODUCTION

Microbial efficiency (MOEFF) is a function of dilution rate (Meng et al., 1999), with achieving maximum efficiency dependent upon adequate fermentation substrates (carbohydrate and rumen degradable protein) supply. Russell et al. (1983, 1992) suggested that bacteria capable of degrading non-structural carbohydrate (NSC) required both ammonia (NH_3) and peptides for maximum protein synthesis. Bryant (1973) found that structural carbohydrate (SC) degrading bacteria utilized ammonia as their nitrogen source. Fu et al. (2000, 2001) determined typical finishing diets (high corn content) could provide a greater level of RDP peptide (RDPep) than required for maximal microbial efficiency. Brooks et al. (2012) showed MOEFF was greatest when RDPep supply did not exceed predicted microbial requirement of RDPep. However, even when the RDPep requirement was met, ruminal degradable nitrogen (RDN) or NH_3-N could be limiting and negatively influence MOEFF. Inadequate RDN decreased organic matter digestion and microbial nitrogen flow (Brooks et al., 2012). Therefore, to maximize MOEFF, requirements for NH_3-N and RDPep must be met.

Requirements for RDPep and RDN are projectable if fermentable substrate (starch, fiber and protein) amount is known. Brooks et al. (2009, 2012) found variation of degradation rates for starch, NDF, and protein was similar among different feedstuffs when expressed as the potentially digestible fraction. Therefore, it is feasible to project rumen microbes RDPep and RDN requirements when available

nutrients are estimated using rumen degradation rates and an average dilution rate for the type of diet fed.

We hypothesized MOEFF, organic matter digestion, and, subsequently, animal growth would respond to increasing supply of RDN until NH_3-N requirement was met. This research was conducted to measure the influence upon fermentation characteristics and growth performance when levels of RDN supplied were less than, equal to or greater than estimated requirement and to support the validity and the accuracy of a prediction model estimating RDPep and RDN requirement by rumen microbes.

MATERIALS AND METHODS

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee.

Treatment Diet Design

Rumen degradable protein and peptide requirement was calculated and met during diet formulation. Rumen degradable protein and peptide requirement and different RDN supply levels were determined by balancing available rumen degradable protein (peptide) and non-protein nitrogen to available rumen degradable carbohydrate. Rumen degradable carbohydrate and protein mass was calculated using the degradation rate (h^{-1}; k_d) of each nutrient (CP, NDF, NSC), along with an estimated passage rate (k_p) of 0.06 h^{-1}. Nutrient mass degraded at each hour was calculated as (Brooks et al., 2012):

$$g\ degraded = grams\ of\ nutrient\ available \times k_d$$

Nutrient mass available at each hour was calculated as:

$$g\ available\ at\ h_n$$
$$= grams\ of\ nutrient\ remaining\ at\ h_{n-1} - g\ degraded$$
$$- \left(g\ nutrient\ available\ at\ h_{n-1} \times k_p\right)$$

Degradation rates were 2.92% and 4.79% h^{-1} for NDF and NSC, respectively, and 2.2% h^{-1} for corn and bloodmeal protein, 2.8% h^{-1} for Soyplus (West Central, Ralston, IA) protein, and 3.8% h^{-1} for soybean meal protein (Brooks, 2009; Brooks et al., 2012).

Dietary treatments were composed of different amounts of urea to vary RDN supply. Available RDN supply was calculated as urea N plus predicted RDPep supply minus predicted RDPep requirement. RDN balance was calculated as RDN supply relative to estimated RDN required by microbes and presented as percentage of CP (Table 3.3). RDN balance ranged from negative (deficient) to positive (excess). Diet composition fed to continuous culture fermenters is listed in Table 3.1. Additional urea was added to diets allowing overall urea levels to be 0%, 0.21%, 0.46% and 0.73% as DM and resulted in calculated RDN balance levels of -1.03, -0.62, -0.08 and 0.39% CP (Table 3.3).

Continuous Culture Fermentation Experiment

The continuous culture experiment was conducted as a completely randomized design with RDN balance level as treatment. As formulated in Table 3.1, two RDN deficient diets (diet 1 and diet 2), one RDN balanced diet (diet 3) and one RDN excess diet (diet 4) were fed. Each RDN diet was allotted to five fermenters randomly. Rumen fluid was collected from two ruminally fistulated multiparous lactating Holstein cows which were provided free access to a lactation diet (24.5% corn silage, 12.6% alfalfa hay, 15.3% alfalfa haylage with 47.7% concentrate; 17.0% CP, 24% ADF and 41% NDF), and these cows were housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research and Teaching Farm. Rumen fluid was kept in a vacuum flask and transported from farm to lab (estimated travel time 15 minutes) immediately strained through four layers of cheesecloth and mixed with buffer solution at a 1:4 ratio (rumen fluid: solution). Twenty single-flow continuous culture fermenter polycarbonate vessels (Nalgene, Rochester, NY) were inoculated with rumen fluid and buffer solution mixture and maintained as described by Meng et al. (1999). The rumen fluid and buffer mixture was added to each fermenter up to the effluent outflow port (approximately 1460 ml). Buffer solution (Slyter, 1990) contained 3.72 g sodium carbonate, 4.82 g potassium carbonate, 1.11g disodium hydrogen phosphate, 1.35 g dipotassium hydrogen phosphate, 282 mg sodium chloride, 342 mg potassium chloride, 77 mg magnesium chloride hexhydrate, 32 mg calcium chloride, and 250 mg L-Cysteine-HCl per liter and was infused continuously into fermenters by peristaltic pump (Masterflex model 7520-10, Cole-

Parmer Instrument Co., Chicago, IL) at dilution rate of 6% ± 0.2% h^{-1}. Fermenters were flushed with CO_2 gas continuously, stirred with magnetic stir plates and immersed in a 39 °C water bath (model 730, Fisher Scientific, Pittsburgh, PA).

Forty-five grams of diet was fed to each fermenter daily and split into two equal meals at a12-h interval. Incubation time was an 8-d period, with 5 d of adaptation and samples were collected from d 6 to d 8. On each sampling day, 5 ml of fermenter fluid was collected from each fermenter before feeding (0 hour sample) and 4 hours after feeding (4 hours sample), and pH was measured at the same time fermenter fluid was sampled. Fermenter fluid samples were placed in 50 ml centrifuge tubes and frozen immediately (-20 ˚C). Samples were composited by hour for each fermenter. Fermenter effluent for each sampling day was collected into plastic graduated cylinders (Fisher Scientific, Hampton, Rockingham, NH) immersed in ice-cooled water. Fermenter effluent volume was recorded daily, mixed thoroughly, and 1 L subsample was taken and stored at 4 ˚C. Fermenter effluent samples were pooled for each fermenter and stored at 4 ˚C until further analyses. On the last sampling day, fermenter contents including undigested feed pellet were blended (Model 34BL22, Waring, New Hartford, CT) for three 20-second pulses to release particle associated bacteria and then strained through two layers of cheesecloth. Fermenter content samples were stored at 4 ˚C for bacteria isolation by differential centrifugation processing described by Meng et al. (1999).

Laboratory Analyses

Fermenter fluid samples were thawed, vortexed and 2 ml subsample was placed into a centrifuge tube. Subsamples were centrifuged at 10,000 × g for 10 min at 4 ˚C to clarify supernatant for ammonia and VFA analysis. Ammonia concentration was determined with phenol-hypochlorite assay (Broderick and Kang, 1980) using a DU-50 spectrophotometer (Beckman, Pal Alto, CA) and VFA concentration was analyzed with gas chromatography method (Salanitro and Muirhead, 1975) using a gas chromatograph (Model3400, Varian, Walnut Greek, CA).

A 500 ml subsample of fermenter effluent was lyophilized at 10 ˚C (Genesis 25XL, SP Industries, Warminster, PA). Isolated bacteria were lyophilized under the same conditions. Lyophilized effluent and fermenter content samples were kept at room temperature to balance moisture, then weighed and ground using a motar and pestle. Effluent residue and isolated bacteria from fermenter content were analyzed for DM and OM according to the AOAC methods (AOAC method 934.01, AOAC method 942.05) and nitrogen content by combustion analysis (N content, LECO FP-428 Leco Co., St. Joseph, MI; AOAC 990.03). Purine content was determined as described by Zinn and Owens (1986). Effluent bacterial nitrogen was calculated under the assumption that purine to N ratio was consistent in both effluent residue and isolated bacteria. MOEFF was calculated as grams of bacterial N produced per kilogram of OM truly digested.

RDN diet samples were ground through a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 2-mm screen, and determined DM,

OM, and CP (AOAC method 934.01; AOAC method 942.05; AOAC 990.03; LECO FP 428 Leco Co., St. Joseph, MI). True digestibility of OM and protein were calculated from the amount of effluent residue OM/CP after correction for microbial contribution and the amount of OM/CP fed to fermenters.

Animal Growth Experiment

Sixty crossbred Angus heifers (353.9 kg, SD was 43.7 kg) were offered *ad libitum* access to water and a transition diet (55% whole corn, 35% receiving and 10% hay) for 14 d before experiment started. Two consecutive day weights were taken at the beginning of experiment to calculate initial body weight (IBW). Animals were assigned by weight to 1 of 12 pens. During 115 days on feed (Sep 28, 2009 to Jan 21, 2010), body weights were taken on d 36, d 64 and d 92 to determine interval growth rate and on d 114 and d 115 to calculate final body weight (FBW).

Diet was formulated as described previously in continuous culture section. Diets contained whole corn and pelleted supplement (Table 3.2). Urea levels were 0%, 0.12%, 0.22% and 0.34% (DM basis) which resulted in RDN balance levels (Table 3.3) of -0.92%, -0.69%, -0.49% and -0.26% CP, respectively. One of four diets was randomly assigned to 3 of 12 pens. Pen intake was recorded daily and diet samples were collected weekly. Animal diet samples were ground through a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 2-mm screen and then analyzed for DM, OM and CP (AOAC method 934.01; AOAC method 942.05; AOAC 990.03; LECO FP 428 Leco Co., St. Joseph, MI).

Individual ADG was obtained by calculation from on and off test weight and days on feed. Gain to feed ratio was calculated by using pen average ADG divided by pen average DMI.

Statistical Analysis

All statistical analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC). *In vitro* continuous culture fermentation characteristics were analyzed by ANOVA using PROC GLM. Data were analyzed as a completely randomized design using 4 treatments with fermenter as the experimental unit. Growth performance was analyzed as a completely randomized design, with pen as the experimental unit, by ANOVA using PROC GLM. For both *in vitro* and *in vivo* experiments CONTRAST statements were used to test for linear, quadratic and cubic effects. Statistical significance was determined using $P \leq 0.05$-probability level.

RESULTS

Fermentation Characteristics

Adding urea to continuous culture diets showed a quadratic ($P \leq 0.02$) response on fermenter pH at feeding and 4 hours after feeding (Table 3.5). Ammonia concentration at feeding increased quadratically ($P < 0.01$) to increasing RDN level. Ammonia concentration responded linearly at 4 hours after feeding ($P < 0.01$, Table 3.5).

Total VFA, acetate and propionate concentrations had a cubic response ($P \leq$ 0.04, Table 3.5) to increasing RDN level, with the greater concentration occurring for diets adequate in RDN (-0.08% and 0.39% RDN diets). However, neither the molar percentage of acetate and propionate, nor the acetate to propionate ratio showed significant differences between treatments indicating fermentation pattern wasn't affected by RDN level. The butyrate, isovalerate and valerate concentration increased linearly ($P \leq 0.01$) as RDN increased.

True OM digestibility increased linearly ($P = 0.03$, Table 3.6) as RDN increased. OM digestibility for diets adequate in RDN was significantly greater ($P =$ 0.01) than RDN deficient diets. Grams of bacterial N outflowing from the rumen had a cubic response ($P < 0.01$) with -0.08% RDN diet being the greatest. A cubic response was also displayed for MOEFF ($P < 0.01$). There were no significant differences in RUP among treatments. The measured RDP for each diet was greater and the measured MOEFF was lower than predicted (Table 3.7).

Growth Experiment

DMI, ADG and gain data are presented in Table 3.8. Only ADG for d 0 to d 36 showed a quadratic response ($P \leq 0.02$) to increasing RDN. DMI, ADG of other periods and ADG over the feeding period did not differ. However, the gain to feed ratio, as a measure of feed efficiency, had a quadratic response ($P = 0.01$) to increasing RDN. Figure 3.1 presents a graph displaying feed to gain ratio response to RDN level. The lowest feed cost per unit gain occurred for diet B and diet C (RDN balance was -0.69% and -0.49% respectively).

DISCUSSION

Diet Formulation and Rumen Degradable Nitrogen Balance

Diets were formulated to provide adequate RDPep and inadequate to adequate RDN for microbial growth. Estimation of RDPep and RDN requirement for microbes required knowledge of substrate fermented in the rumen. In both continuous culture and animal growth experiment diets used the same supplement formulation and inclusion rate. Fermentation substrate fractions such as NSC and SC, were similar in both diets. In order to achieve maximal microbial protein synthesis, two thirds of nitrogen source for non-structural carbohydrate (NSC) fermenting bacteria must come from peptides or amino acids; structural carbohydrate (SC) degrading bacteria are capable of using only ammonia (Bryant, 1973; Russell et al., 1992). Estimation of RDPep and RDN requirement for all diets are listed in Table 3.3.

When intake was estimated and diet formulation was known, rumen degradable carbohydrate mass was calculated using degradation rate (h^{-1}; k_d), along with an estimated passage rate (k_p) of 0.06 h^{-1} as described previously. Nitrogen mass required for NSC and SC fermentation was calculated base on fermentable NSC and SC mass and MOEFF where MOEFF followed a quadratic function (Meng et al., 1999): $MOEFF_{starch} = 7.1 + 341.6\times D - 965.3\times D^2$, and $MOEFF_{NFC} = 1.7 + 368.7\times D - 586.9\times D^2$. Two thirds of nitrogen for fermentable NSC should come from peptides (Russell et al., 1992). RDPep requirement was met in typical finishing diets (Fu et al., 2000, 2001). RDPep supply and requirement in experiment diets (Table 3.3) agreed with this conclusion. After RDPep requirement was met, surplus

RDPep may exist and was hydrolyzed to produce ammonia. RDN supply was calculated as RDPep supply minus RDPep requirement plus urea in diet. Nitrogen requirement for fermentable SC and one third of NSC was added up to RDN requirement. Subsequently, RDN balance (difference between RDN supply and RDN requirement) presenting as percentage of CP (Table 3.3) was formulated to be negative (deficient) to positive (excess).

Microbial Fermentation and Efficiency

Adhesion ability of *F. succinogenes* to cellulose decreased when pH was reduced below 6.0 or changed to above 7.5. Adhesion of *R. albus* was impacted little by pH between 5.5 and 8.0 but decreased remarkably when pH was below 5.0 (Morris, 1988; Roger et al., 1990). Statistical difference on pH at feeding and 4 hours after feeding found in this experiment was less than 0.2 pH units among treatments and was outside the range that negatively influence fibrolytic activity.

The increases in ammonia concentration among diets were attributed to urea being rapidly degraded to ammonia. The elevation of ammonia concentration 4 hours after feeding for diet with excess RDN (diet 4, Table 3.5) was believed to have occurred because after ammonia-nitrogen requirement was met it would accumulate in rumen fluid. However, MOEFF was not found to peak in diet with RDN excess. Ammonia concentration would not enhance bacteria utilization of ammonia once ammonia started to accumulate (Satter and Roffler, 1975). Brooks et al. (2012) also found further increasing RDPep showed little improvement in fermentation once RDN was balanced. The ammonia concentration (1.87 to 2.73 mg/dl) in this study

appeared limiting if compared with other experiments. There is a wide disparity of ammonia required for maximum rumen microbial growth (Hume et al., 1970; Satter and Slyter, 1974; Mehrez and Øskov, 1977; Slyter et al., 1979; Kang-Meznarich and Broderick, 1980; Erdman et al., 1986). Satter and Slyter (1974) concluded 5 mg/dl was enough to maximize rumen bacteria growth rate. Moreover, in Slyter's research, 2 mg/dl was proposed as a sufficient ammonia level to maximal rumen function.

Total VFA concentration was greatest when RDN was adequate. Bacterial N production and true OM digestibility agreed with this tendency. No fermentation pattern shifting occurred among treatments. The greatest OM digestibility was expected to result in greater total VFA concentration, which occurred.

Griswold et al. (2003) observed urea infusion in both low RDP (8% as dietary DM) and high RDP (11% as dietary DM) diets increased OM digestibility. Additionally, high RDP diet without urea improved SC and NSC digestibility compared with low RDP diets without urea (Griswold et al., 2003). Conversely, no response in SC digestibility was measured when ruminally available amino acids were increased (Griswold et al., 1996). Rihani et al. (1993) reported OM digestion was not improved by adding ruminal ammonia in high-fiber diets. Russell et al. (1992) suggested SC fermenting bacteria used ammonia-nitrogen as their sole nitrogen source. However, some hemicellulose fermenting bacteria (e.g. *Butyrivibrio fibrisolvens*) have the ability to compete with NSC fermenting bacteria for peptide and amino acids as a nitrogen source when ammonia-nitrogen was limiting (Cotta and Hespell, 1986). Therefore, to maximize bacteria growth RDPep and RDN must both

meet microbial requirements. All treatment diets in these experiments would have provided adequate RDPep for NSC fermenting bacteria (Table 3.4, 4.68% supplied in diets vs. approximately 2.85% required), but RDN balance was insufficient, adequate or excessive. The greater total VFA concentration, greater bacterial nitrogen production, increased OM digestibility and the greatest MOEFF occurred when adequate RDN was fed.

Animal Growth Performance

RDN level in diets did not affect ADG and DMI, but a numerically lower intake and greater ADG allowed an improved gain to feed ratio. There are few studies using rumen microbial requirement for RDPep and RDN to formulate a diet. Research investigating non-protein nitrogen (urea) rarely had available RDN estimates or RDN balance calculated. In two growth trials Milton et al. (1997) showed a quadratic response to urea for gain to feed ratio with the ratio being greatest in dry-rolled corn diet-fed steers with 0.5% urea (treatments were 0, 0.5, 1.0 and 1.5% urea as DM basis). Chizzotti et al. (2008) observed a quadratic effect on DMI (% of BW) by steers as urea level increased from 0 to 1.95%. In another study cattle were fed steam-flaked barley based finishing diets (Zinn et al., 2003) with increasing urea level (0, 0.4, 0.8, and 1.2% as DM), and there was a trend to maximize ADG (quadratic, $P = 0.13$) at 0.8% urea. Compared to current study urea level (0, 0.12, 0.22 and 0.34% as DM basis) to referenced publications, we used relatively lower and a more narrow range of urea. A similar quadratic response on gain to feed ratio was observed when urea level increased in treatment diets. Nevertheless, our goal was to test growth response

to different RDN levels when RDPep was met while test an estimation model for RDPep and RDN balance accuracy. All four treatment diets in animal growth experiment were adequate in RDPep but deficient in RDN balance. Results suggested feed efficiency could be maximized when calculated RDN supply was lower than predicted RDN requirement. Recycled nitrogen has ability to compensate for a diet ammonia nitrogen shortage. With adequate RDN supplied to rumen, microbes were capable to grow to their optimized capacity, as documented by the *in vitro* experiment. Brake et al. (2010) observed the percentage of recycled nitrogen used for anabolism tended to be greater for low protein (corn based diet with 10.2% CP) diet than high protein diet (DDGS diet with 14.5% CP). Nitrogen recycling was critical for rumen bacteria if protein degradation in rumen was limited.

When RDN was balanced, ADG was poor in RDPep deficient diets and the greatest feed to gain ratio was achieved by calves fed a RDPep adequate diet (Brooks et al., 2012). Insuring RDN requirements are met but not excessive when RDP peptide is not limiting could be an effective strategy to maximize microbial protein yield, microbial efficiency, and feed efficiency.

CONCLUSION

RDPep and RDN are required by rumen microbes to promote microbial growth. Maximal MOEFF and, consequently, greater feed efficiency could be achieved when RDN is balanced in the diet and RDPep is not limiting. With understanding of NSC, SC, and CP degradation rates, and rumen dilution rate, RDPep and RDN requirements can be estimated and diets formulated to prevent deficiencies. We conclude maximizing feed efficiency requires RDN requirement to be balanced to meet but not exceed microbial requirement.

Table 3.1 Diet composition fed in continuous culture experiment.

	Treatment[1]			
Item	Diet 1	Diet 2	Diet 3	Diet 4
Ingredient (inclusion rate, % DM)				
Corn	85	84.9	84.75	84.6
Urea[2]	0.0	0.1	0.2	0.4
Supplement pellet	15	15	15	15
Supplement Pellet Composition (inclusion rate, % DM in supplement)				
Corn	1.89	1.22	0.66	0.00
Bloodmeal	25.65	25.62	25.60	25.57
SoyPLUS[3]	55.83	55.77	55.72	55.66
Urea	0.00	0.77	1.42	2.18
Dyna-K[4]	1.42	1.42	1.42	1.42
Limestone	10.51	10.49	10.48	10.47
NaCL	1.42	1.42	1.42	1.42
Vitamin Premix[5]	1.42	1.42	1.42	1.42
TM Premix[6]	1.42	1.42	1.42	1.42
Rumensin 80[7]	0.22	0.22	0.22	0.22
Oil	0.22	0.22	0.22	0.22

[1]Treatments consisted of four RDN balance levels: RDN balance deficient diet: diet 1 = -1.03%, diet 2 = -0.62%, balanced RDN diet: diet 3 = -0.01%, and RDN balance excess diet: diet 4 = 0.39%.

[2]Added additional urea into diets besides of urea in supplement pellet; the overall urea level for each diet was 0, 0.21, 0.46, and 0.73% as DM basis, respectively.

[3]SoyPLUS: product of West Central® (Ralston, IA), contained (DM basis) 49.84% CP, 60% RUP 60%, and 40% RDP (%CP).

[4]Dyna-K® (Plymonthm, MN) contained (as-fed basis) 50% K, 46.4% Cl.

[5]Contained (as-fed basis) 4,000,000 IU of vitamin A, 800,000 IU of vitamin D and 1,250 IU of vitamin E per kilogram.

[6]Contained (as-fed basis) 10% Fe, 10% Mn, 10% Zn, 2% Cu, 1,500 ppm Se, 1,000 ppm I, and 500 mg/kg Co.

[7]Contained (as-fed basis) Monensin, USP, 80 g per pound (176 g per kilogram).

Table 3.2 Diet composition fed to crossbred Angus heifers.

	Treatment[1]			
Item	Diet A	Diet B	Diet C	Diet D
Ingredient (inclusion rate, % as-fed)				
Corn	85	85	85	85
Supplement pellet[2]	15	15	15	15
Supplement Pellet Composition (inclusion rate, as-fed % in supplement)				
Corn	2.0	1.3	0.7	0.0
Bloodmeal	25.5	25.5	25.5	25.5
SoyPLUS[3]	55.5	55.5	55.5	55.5
Urea[4]	0.0	0.7	1.3	2.0
Dyna-K[5]	1.3	1.3	1.3	1.3
Limestone	9.6	9.6	9.6	9.6
NaCL	1.3	1.3	1.3	1.3
Vitamin Premix[6]	1.3	1.3	1.3	1.3
TM Premix[7]	1.3	1.3	1.3	1.3
Rumensin 80[8]	0.2	0.2	0.2	0.2
Oil	2.0	2.0	2.0	2.0

[1]Treatments consisted of four RDN balance deficiency diets: diet A through D, diet A = -0.92%, diet B = -0.69%, diet C = -0.49%, and diet D = -0.26%.

[2]Supplement pellet were using the same formulation as continuous culture experiment.

[3]SoyPLUS: product of West Central® (Ralston, IA), contained (DM basis) 49.84% CP, RUP 60% (%CP), RDP 40% (%CP).

[4]Urea level in each diet was: 0, 0.12, 0.22, and 0.34% as DM basis, respectively.

[5]Dyna-K® (Plymonthm, MN) contained (as-fed basis) 50% K, 46.4% Cl.

[6]Contained (as-fed basis) 4,000,000 IU of vitamin A, 800,000 IU of vitamin D and 1,250 IU of vitamin E/kg.

[7]Contained (as-fed basis) 10% Fe, 10% Mn, 10% Zn, 2% Cu, 1,500 ppm Se, 1,000 ppm I, and 500mg/kg Co.

[8]Contained (as-fed basis) Monensin, USP, 80 g per pound (176 g per kilogram).

Table 3.3 Estimation of substrate fractions, RDPep requirement, RDN requirement, and RDN balance for diets used in continuous culture experiment and animal growth study.

	Treatment[1]							
	Continuous culture				Crossbred Angus heifers			
Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet A	Diet B	Diet C	Diet D
Estimated substrate fraction[2], %								
CP	17.2	17.8	18.5	19.3	17.7	18.0	18.3	18.7
NSC	66.3	65.9	65.3	64.7	66.0	65.7	65.4	65.2
SC	16.4	16.3	16.2	16.0	16.4	16.3	16.2	16.2
Calculation and estimation[3],								
DM, %	86.24	86.22	86.24	86.20	86.08	86.09	86.11	86.12
Urea, % as DM	0.00	0.21	0.46	0.73	0.00	0.12	0.22	0.34
CP, % as DM	14.18	14.76	15.45	16.20	14.54	14.87	15.15	15.49
RDP_{total}[4], % of DM	4.68	5.21	5.84	6.52	4.78	5.08	5.34	5.64
RDPep[5],% of DM	4.68	4.67	4.66	4.65	4.78	4.77	4.77	4.77
RDPep required[6], % of DM	2.85	2.85	2.84	2.83	2.83	2.83	2.83	2.82
RDN required[7], %	2.85	2.98	3.14	3.30	2.87	2.94	3.01	3.08
RDN balance[8], g	-0.16	-0.10	-0.01	0.06	-0.14	-0.11	-0.08	-0.04
RDN balance, CP/DM%[9]	-1.03	-0.62	-0.08	0.39	-0.92	-0.69	-0.49	-0.26

[1]Treatments consisted of four RDN balance levels for continuous culture experiment: RDN balance levels were -1.03, -0.62, -0.08 and 0.39% respectively; and four RDN balance level deficiency diets (diet A to diet D) for animal growth experiment: RDN balance levels were -0.92, -0.69, -0.49 and -0.26% respectively.
[2]Substrate fraction included crude protein, non-structural carbohydrate, and structural carbohydrate.
[3]Calculation and estimation was based on nutrient tabular values from NRC, dilution rate and rumen degradation rate.
[4]RDP_{total}: calculated RDP including urea as crude protein source; % of DM.
[5]RDPep: calculated RDP without including urea; % of DM.
[6]RDPep required: calculated value, based on previous research two thirds of nitrogen source must come from peptide for non-structural carbohydrate fermentation and peptide efficiency was assumed to be 80% (Russell, 1992).
[7]RDN required: value is percentage of CP (converting N to CP by multiplying6.25) as DM basis; ammonia-N requirement includes nitrogen for fermenting protein, structural carbohydrate and non-structural carbohydrate.
[8]RDN balance in gram: available ruminal degradable nitrogen minus ruminal degradable nitrogen required when 100 g diet was fed.
[9]RDN balance in CP/DM%: RDN balance level presented as CP as DM basis (converting RDN to CP by multiplying 6.25); negative = RDN balance deficient, zero = RDN balanced, positive = RDN balance excess

Table 3.4 Diets nutrient composition fed to continuous culture fermenters and to crossbred Angus heifers.

	Treatment[1]							
	Continuous culture				Crossbred Angus heifers			
Item[2]	Diet 1	Diet 2	Diet 3	Diet 4	Diet A	Diet B	Diet C	Diet D
DM, %	87.21	86.62	87.28	88.24	84.29	84.36	84.45	84.32
OM, %	95.28	95.60	94.80	95.02	96.08	96.16	95.40	95.21
CP, %	14.41	14.59	15.44	18.10	14.25	14.35	15.25	16.67

[1]Treatments consisted of four RDN balance levels (diet 1 to diet 4) for continuous culture experiment: RDN balance levels were -1.03, -0.62, -0.08 and 0.39% respectively; and four RDN balance level deficiency diets (diet A to diet D) for animal growth experiment: RDN balance levels were -0.92, -0.69, -0.49 and -0.26% respectively.

[2]Nutrients were determined by using AOAC method 934.01, AOAC method 942.05 and LECO nitrogen analyzer (Model FP 428 Leco Co., St. Joseph, MI).

Table 3.5 Effect of increasing ruminal degradable nitrogen concentration on pH, ammonia and VFA concentration in continuous culture[1].

Item	Treatment[2]				*SEM*	*P*[3]		
	Diet 1	Diet 2	Diet 3	Diet 4		Lin	Quad	Cub
pH								
0 hr[4]	6.56[b]	6.66[a]	6.62[ab]	6.56[b]	0.02	0.58	<0.01	0.34
4 hr	6.59[ab]	6.68[a]	6.62[ab]	6.54[b]	0.03	0.18	0.02	0.32
NH_3, mg/dl								
0 hr	0.97[b]	0.94[b]	0.93[b]	1.24[a]	0.06	<0.01	<0.01	0.26
4 hr	1.87[b]	2.01[b]	2.18[b]	2.73[a]	0.13	<0.01	0.13	0.55
Total VFA-4h, m*M*	89.30[ab]	77.93[b]	97.48[a]	95.34[a]	4.01	0.05	0.27	<0.01
Ace/Pro	1.97	2.14	1.83	2.02	0.14	0.80	0.97	0.14
VFA-4h, mM								
Acetate	53.76[a]	46.52[b]	55.14[a]	55.44[a]	2.40	0.22	0.13	0.04
Propionate	27.50[ab]	22.05[b]	31.98[a]	27.78[ab]	2.63	0.37	0.81	0.02
Isobutyrate	0.58	0.60	0.65	0.63	0.04	0.30	0.61	0.65
Butyrate	6.11[c]	7.01[bc]	7.74[ab]	9.05[a]	0.52	<0.01	0.70	0.74
Isovalerate	1.17[b]	1.35[ab]	1.43[ab]	1.62[a]	0.11	0.01	0.96	0.69
Valerate	0.77[c]	1.00[bc]	1.20[ab]	1.45[a]	0.11	<0.01	0.95	0.88
VFA-4h, molar %								
Acetate	60.2	59.8	56.7	58.2	1.71	0.25	0.59	0.35
Propionate	30.7	28.2	32.6	30.0	1.92	0.92	0.79	0.10
Isobutyrate	0.7	0.8	0.7	0.7	0.05	0.70	023	0.15
Butyrate	6.9[c]	8.9[ab]	7.9[bc]	9.5[a]	0.50	<0.01	0.62	0.02
Isovalerate	1.3[b]	1.7[a]	1.5[ab]	1.7[a]	0.11	0.12	0.43	0.04
Valerate	0.9[b]	1.3[a]	1.2[ab]	1.5[a]	0.13	<0.01	0.57	0.17

[1]Means with no superscripts ([abc]) in common within the same row are statistically significant.
[2]Treatments consisted of four RDN balance levels; RDN balance deficient diet; diet 1 = -1.03% and diet 2 = -0.62%, balanced RDN diet: diet 3 = -0.08%, and RDN balance excess diet: diet 4 = 0.39%.
[3]Significant linear, quadratic or cubic response to RDN treatment diets were confirmed when *P* - value being less than 0.05.
[4]Sample at feeding (0 hr) and 4 hours after feeding (4 hr) were tested for pH, ammonia nitrogen and VFA; only 4 hours after feeding VFA were reported in table.

Table 3.6 Effect of increasing ruminal degradable nitrogen concentration on true digestibility of OM, microbial efficiency and RUP[1].

Item	Treatment[2]				*SEM*	*P*[3]		
	Diet 1	Diet 2	Diet 3	Diet 4		Lin	Quad	Cub
Intake OM, g/d	43.50	43.43	43.37	44.09	--	--	--	--
OM digested, g/d	17.64^{b}	17.54^{b}	18.01^{b}	20.10^{a}	0.64	0.01	0.11	0.73
Bacterial N, g/d	0.38^{ab}	0.34^{b}	0.42^{a}	0.41^{ab}	0.01	0.02	0.43	<0.01
True OM dig, %	40.56^{b}	40.38^{b}	41.53^{ab}	45.58^{a}	1.47	0.03	0.17	0.82
MOEFF[4]	21.47^{ab}	19.69^{b}	23.34^{a}	20.20^{b}	0.74	0.97	0.37	<0.01
RUP, % of CP	50.95	54.67	51.95	49.38	1.85	0.38	0.11	0.44

[1]Means with no superscripts (^{abc}) in common within the same row are statistically significant.
[2]Treatments consisted of four RDN balance levels; RDN balance deficient diet; diet 1 = -1.03% and diet 2 = -0.62%, balanced RDN diet: diet 3 = -0.08%, and RDN balance excess diet: diet 4 = 0.39%.
[3]Significant linear, quadratic or cubic response to RDN treatment diets were confirmed when *P* -value being less than 0.05.
[4]Microbial efficiency was calculated as grams of microbial nitrogen produced per kilogram organic matter truly digested.

Table 3.7 Comparison between measured microbial efficiency and protein digestibility by continuous culture fermenters and model prediction.

	Treatment[1]			
Item	Diet 1	Diet 2	Diet 3	Diet 4
Measured				
MOEFF	21.47	19.69	23.34	20.20
RUP, % of CP	50.95	54.67	51.95	49.38
RDP, % of CP	49.05	45.33	48.05	50.62
Predicted				
MOEFF	25.1	25.2	25.3	25.4
RDP, % of CP	32.99	35.29	37.80	40.26

[1]Treatments consisted of four RDN balance levels; RDN balance deficient diet; diet 1 = -1.03% and diet 2 = -0.62%, balanced RDN diet: diet 3 = -0.08%, and RDN balance excess diet: diet 4 = 0.39%.

Table 3.8 Effect of increasing ruminal degradable nitrogen concentration on dry matter intake, average daily gain and feed efficiency of heifers[1].

Item	Treatment[2]				*SEM*	*P*[3]		
	Diet A	Diet B	Diet C	Diet D		Lin	Quad	Cub
IBW, kg	356.76	354.15	354.48	350.09	11.58	0.71	0.94	0.88
FBW, kg	488.64	496.91	494.36	489.52	15.57	1.00	0.68	0.90
DMI, kg/d	8.26	8.14	7.96	8.30	0.21	0.93	0.29	0.53
ADG, kg/d								
0-36 day	0.55^{b}	0.83^{a}	0.79^{a}	0.61^{b}	0.09	0.71	0.02	0.66
0-64 day	1.16	1.12	1.13	1.07	0.07	0.46	0.81	0.72
0-92 day	1.13	1.19	1.78	1.15	0.06	0.80	0.50	0.87
0-115 day	1.16	1.25	1.23	1.22	0.04	0.41	0.29	0.49
G:F	0.140^{b}	0.154^{a}	0.154^{a}	0.147^{ab}	0.003	0.17	0.01	0.69

[1]Means with no superscripts (^{abc}) in common within the same row are statistically significant.
[2]Treatments consisted of four RDN balance deficiency diets: diet A through D, diet A = -0.92%, diet B = -0.69%, diet C = -0.49%, and diet D = -0.26%.
[3]Significant linear, quadratic or cubic response to RDN treatment diets was confirmed when *P* - value being less than 0.05.

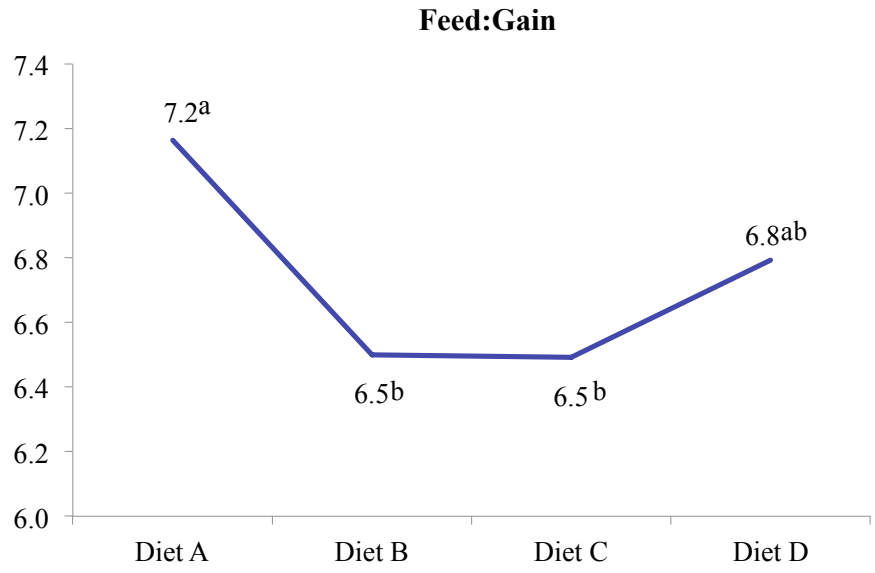

Figure 3.1 Feed to gain ratio of heifers fed diets increasing in ruminally degradable nitrogen.
[1]Means with no superscripts ([abc]) in common are statistically significant.
[2] X-axis were four RDN balance deficiency diets: diet A through D, diet A = -0.92%, diet B = -0.69%, diet C = -0.49%, and diet D = -0.26%.
[3]Significant quadratic response to RDN treatment diets were confirmed with *P* -value being less than 0.05.

CHAPTER 4

INFLUENCE OF RUMINAL DEGRADABLE NITROGEN SUPPLY IN CORN-BASED FINISHING DIETS WITH AND WITHOUT FORAGE ON GROWTH PERFORMANCE, RFI AND CARCASS CHARACTERISTICS OF BEEF CATTLE

ABSTRACT

Two growth experiments were conducted at University of Missouri Teaching and Research Farm from December 2010 to June 2011 to test growth performance and feed efficiency response to diet formulations containing deficient or adequate ruminal degradable nitrogen (RDN) and balanced or unbalanced for post-ruminal arginine levels (most limiting amino acid). In Exp. 1, 80 crossbred steers were blocked by initial body weight into two groups (light and heavy) and then randomly assigned to pens (5 per pen) within group. Diets were whole corn and pelleted protein supplement, and designed to provide inadequate to excessive RDN supply and adequate or deficient in absorbable arginine. An additional diet was fed containing 10% (as-fed basis) grass hay with RDN and post-ruminal arginine supplied to meet requirement (BALHay). This diet was included to test roughage inclusion response. There was no significant difference on ADG during 142 days on feed, but DMI and feed to gain ratio were greater due to roughage inclusion ($P < 0.01$). In the second growth experiment, three post-ruminal

arginine supply (deficient, adequate and exceeding) levels were fed to 118 crossbred steers. No significance was found on ADG during 168 days on feed. DMI and feed to gain ratio were greatest on balanced (adequate) arginine diet ($P \leq 0.03$). This result was unexpected. However, ADG was greater ($P \leq 0.08$) and feed to gain ratio was lower during 0-28 days and 0-87 days for calves fed arginine adequate diet. Animals consumed more energy than predicted by effective energy model, which was possibly caused by diet energy density overestimation. Alternatively, three diets may not have been arginine deficient. No further feed efficiency improvement could be made if post-ruminal amino acid requirements were met. Feed efficiency may be improved by formulating diets to supply adequate ruminal degradable nitrogen and absorbable amino acids.

INTRODUCTION

Typical finishing diets contain 82 to 90 % grain with 6 to 10% forage. Recently our laboratory suggested that removing forage from finishing diets to improve feed efficiency. Early in 1968, Wise et al. reported feeding all-concentrated diets to beef cattle would become one feeding system due to grain price. Grain level fed to animals could be as high as 100% by using whole corn. This kind of high grain diet did not cause rumen disorder or other secondary health problems. Synchronizing ruminal degradable protein with fermentable carbohydrate in high grain diets is key to a healthy rumen environment and maximizes microbial efficiency. Rumen microbial protein supplies 40% to 90 % of amino acid requirement (Firkins, 1996) and protein is typically considered the most expensive feedstuff. However, microbial protein may not supply adequate absorbable amino acids for rapidly growing animals (Merchen and Titgemeyer, 1992). Single amino acid deficiencies in absorbable protein would limit use of other amino acids (Cole and Lunen, 1994). To maximize microbial protein yield and prevent post-ruminal amino acid deficiency requires microbial growth requirement and post-ruminal amino acid flow estimation. Nitrogen source and requirement for rumen bacteria has previously been discussed (Russell et al., 1992; Fu et al., 2001; Brooks, 2010; Brooks et al., 2011, 2012). Research studying amino acid requirement of ruminants started on sheep with methionine concluded the most limiting amino acid followed by lysine (Nimrick et al., 1970; Owens et al., 1973; Reis et al., 1973). Richardson and Hatfield (1978) fed growing Holstein steers semi-purified diets, infused amino acids or amino acids combinations, and found methionine, lysine and threonine were the first three limiting amino acids in growing

steers. Lysine was found the first limiting for calves less than 3 months age who were fed a corn and corn gluten meal diet; but when using a corn and soybean meal diet methionine was first limiting and lysine was second (Abe et al., 1997, 1998). Limiting amino acids for calves and growing cattle were different due to multiple reasons: inadequate rumen function, different growth rate, diet ingredient differences and so on. However, it is well agreed diet amino acid profile has impact on the first limiting amino acid. Greenwood and Titgemeyer (2000) reported in steers fed a soyhull based diet (73% soyhulls, 19% alfalfa and intraruminal infusion of acetate as energy supplement), methionine was first limiting amino acid, histidine second and at least one of the branched-chain AA was limiting as well. Instead of lysine and methionine, Ludden and Kerley (1997) reported arginine was projected as the first limiting amino acid in post-ruminal flow studies feeding cannulated Holstein steers a basal diet (corn 56.1%, soyhulls 18%, cottonseed hulls 15%, corn gluten meal 5.6% and animal fat 4.25%) at different energy levels. In another growth experiment, arginine was the first limiting for crossbred steers fed the same basal diet top-dressed with one of following: rumen-stable lysine, rumen-stable lysine and methionine combination, or blood meal (Ludden and Kerley, 1998).

We hypothesized diets formulated by an empirical model to contain adequate (or balanced) ruminal degradable nitrogen (RDN) and post-ruminal arginine would enhance microbial protein yield from rumen and improve feed efficiency compared with inadequate RDN and post-ruminal arginine supply. Conventional feedlot diet (containing

10% roughage) with adequate RDN and post-ruminal arginine was also tested to compare growth response to no forage diets.

MATERIALS AND METHODS

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee.

Experimental Design and Animal Management

One hundred and ninety-eight crossbred steers were transported to University of Missouri Beef Research and Teaching Farm from December 4 to December 14, 2010. Animals were vaccinated and dewormed and fed receiving diet *ad libitum* for 5 to 14 days depending on arrival date to allow adaption to a high concentration diet and GrowSafe® feeding system (GrowSafe® System Ltd., Airdrie, AB, Canada). Electronic ID tags (Allflex US INC., Dallas-Fort Worth Airport, TX) were put on the left ear to track intake and associated eating behaviors using the GrowSafe® Feed Intake System. Animals with depression or eating disorder issues were isolated in a hospital pen, observed and treated during receiving period. Before experiment started, animal body weight was taken on 2 consecutive days (Dec 20 and 21) to calculate initial BW (IBW). First day weight was used to sort and group animals. Animals were sorted in ascending body weight order, in which the 80 heavier animals were used for Exp. 1, and the remaining 118 lighter animals were used for Exp. 2.

Experiment 1

Experiment 1 was a completely randomized block design with body weight blocked as heavy or light. Eighty steers were ordered from greatest to smallest body weight, and heaviest 40 were randomly placed into five pens, similarly lightest 40 were randomly placed into another five pens. Each pen contained 8 steers and 2 feeding bunks that allowed only a single animal access to each feed bunk. One of five treatment diets were randomly assigned to 8 steers in heavy pen and 8 steers in light pen. Experimental unit was individual animal with 16 replications for each treatment diet, 8 steers per block. Four of five diets consisted of whole kernel corn and protein supplement, pelleted and consisting of blood meal, SoyPLUS (West Central, Ralston, IA), wheat middlings, urea and vitamin and minerals. Diets provided balanced ruminal degradable nitrogen (RDN) and post-ruminal arginine (Arg) supply based on requirement (diet BALANCE), balanced RDN but deficient post-ruminal arginine supply (diet AA-), balanced post-ruminal arginine but deficient RDN supply (RDN-), and deficient RDN and post-ruminal arginine supply (diet NEG). A fifth diet treatment was added that contained 10% (as-fed basis) grass hay and balanced for both RDN and post-ruminal arginine (BALHay) to compare growth response of a traditional feedlot diet to a balanced no-roughage diet. Table 4.1 displays ingredient composition of five treatment diets and Table 4.2 presents calculated nutrient values, RDN and post-ruminal arginine balance estimations. All ingredients except for corn and hay were mixed and pelleted into supplements. Supplement, whole corn and hay were mixed as a total mixed ration using a truck

mounted ribbon mixer. Animals were fed once daily at approximately 0800, with feed and water provided *ad libitum* during the 142 days feeding period.

Experiment 2

Experiment 2 was a completely randomized design with 118 steers randomly assigned to one of three pens. Average initial body weight was similar across pen (306.9 kg, 306.9 kg and 308.9 kg). Three diet treatments were assigned to three pens randomly. Diets containing whole corn and pelleted protein supplement were formulated to provide increasing post-ruminal arginine supply (post-ruminal arginine supply lower than requirement: LowArg, n=39; post-ruminal arginine supply balanced with requirement: BalanceArg, n=39; post-ruminal arginine supply greater than requirement: HighArg, n=40). Table 4.3 and Table 4.4 present ingredient and chemical composition, and estimated RDN and dietary post-ruminal arginine supply. The treatment BalanceArg was the same formula as BALANCE diet in Exp. 1. Diet mixing procedure and feeding operation were the same as described in Exp. 1.

Data Collection

Daily individual animal feed intake for both studies were measured by GrowSafe® Feed Intake System (GrowSafe® System Ltd., Airdrie, AB, Canada). Two consecutive day live body weights were taken at the beginning and end of study (IBW and FBW) to calculate average daily gain ($ADG = (FBW - IBW)/Days\ on\ feed$, *kg/day*). Body weights were taken approximately every 28 days during study. Feed conversion ratio (FCR) was calculated as DMI divided by ADG. In addition, residual feed intake (RFI) was computed based on measured feed intake minus expected intake, which was

predicted by regressing ADG and metabolic middle weight ($MMWT = [(IBW + FBW)/2]^{0.75}$) against measured DMI using the regression procedure of SAS (PROC REG). The model fitted was:

$$Y_i = \beta_0 + \beta_1 * ADG + \beta_2 MMWT;$$

where Y_i = expected feed intake of animal I; β_0 = the regression intercept; β_1 = partial regression coefficient for ADG; and β_2 = partial regression coefficient for MMWT.

Diet samples for both studies were collected weekly and tested for initial dry matter (55˚C). Dried samples were ground through a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 2-mm screen for determination of DM, OM and CP (AOAC 934.01; AOAC 942.05; AOAC 990.03; LECO FP 428, Leco Co., St. Joseph, MI). At the end of two experiments, steers were fasted 24 hours before harvest. Carcass data measured by the plant is listed in Table 4.7 and 4.10.

For Exp. 1, fecal samples were taken from each animal before feeding for two consecutive days. Samples were put in 55˚C oven for 4 days minimum until dry. Sample from two consecutive days for each individual animal was combined at the same proportion and ground through a mill to pass through a 2- mm screen. Fecal DM, OM and CP were analyzed using the same AOAC methods as diet samples. Acid insoluble ash (AIA) of diet and fecal samples were analyzed using a 2N HCL procedure (Van Keulen and Young, 1977). DM digestibility was calculated as:

$dig = [(diet\ AIA\%)/(fecal\ AIA\%)] * 100.$

Statistical Analysis

All statistical analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC). Animal performance, apparent DM digestibility and carcass characteristic data from Exp. 1 were analyzed using PROC MIXED procedure of SAS with weight block as random factor and diet treatment as fixed factor. Experimental unit was animal. Least square means of DMI, ADG, FCR and carcass traits were compared using LSD, and significant treatment effect were reported at $P \leq 0.05$.

Animal performance and carcass characteristic data from Exp. 2 were statistically analyzed using PROC MIXED of SAS with diet treatment as fixed factor. Individual steer was experimental unit. Mean comparison of DMI, ADG, FCR and carcass traits were made as described for experiment 1 using LSD, and significant treatment effect were reported at $P \leq 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Table 4.5 shows steer growth performance for study 1 during 142 day feeding period. There was no significant difference on 0-142 days ADG due to treatments. Dry matter intake and feed conversion ratio was greater for cattle fed BALHay diet. There was also no period difference among treatment for ADG. Greater intake by cattle fed BALHay diet caused poor feed conversion ratio. Oljen et al. (1971) fed either an all concentrate corn-based diet or a pelleted alfalfa-based diet to 24 weanling calves and

found ADG and feed to gain ratio were: 1.27 kg, 5.71 for concentrate diet and 1.05 kg, 10.06 for alfalfa diet respectively. Low forage digestibility was the reason for greater intake and poorer feed efficiency. In this experiment, apparent DM digestibility was lower in BALHay diet ($P = 0.06$). Increasing hay level in whole corn-based diet decreased DM digestibility from 78.8% to 71.9% when hay level was increased from 4% to 24% (Paterson et al., 1985). Increasing forage inclusion levels in high concentrate diets or increasing forage to concentrate ratios decreased DM digestibility in small ruminants (Cantalapiedra-Hijar et al., 2009), dairy cows (Yang et al., 2001) and beef steers (Fieser and Vanzant, 2004). Digestion site and retention time in the gastrointestinal tract explained digestion variations for diets with different forage to concentrate ratios (Cole et al., 1976; Paterson et al., 1985).

Comparing arginine deficient (diet AA- and NEG) to adequate diet (diet BALANCE and RDN-) did not result in growth performance response. Table 4.6 shows diet nutrient analysis in Exp. 1, and RDN/post-ruminal arginine balance estimated using measured body weight and gain in estimation model. Using NRC table values underestimated diet CP content. Measured DMI was greater than projected in estimation model; likely caused by animals on trial being heavier than was proposed in estimation model (365 kg on trial vs. 250 kg proposed). The consumed EE to required EE ratio was greater than 1 because BW of animals on trial and growth rate (Table 4.6) differed from proposed variables used in the estimation (Table 4.2), diet effective energy density could have been overestimated, and effective energy required by animal could have been underestimated due to cold environmental conditions. Both growth studies were

conducted when local weather had been through the coldest winter in 29 years (unpublished data, Guinan et al., University of Missouri Extension and State Climatologist). Intake would be increased since more energy was needed for maintenance requirement (NRC, 1987).

Consuming more energy than required also caused post-ruminal arginine supply to be increased. We hypothesized efficiency would not be maximized until post-ruminal arginine requirement was met, however, with measured variables in model, post-ruminal arginine deficient diets (diet AA- and NEG) were not deficient, and could be a reason for lack of response on DMI or ADG. Also, we hypothesized efficiency would be better when RDN and post-ruminal arginine were balanced, however, growth data showed no response on ADG and FCR comparing adequate (diet BALANCE and AA-) with deficient RDN diets (diet RDN- and NEG).

Residual feed intake (RFI), computed as difference between measured intake and expected intake predicted from regression on gain and metabolic middle weight (Koch et al., 1963), was greater (less efficient) in calves fed BALHay than other treatments. Davis (2009) fed 87 spring-born crossbred Angus steers diets varying in post-ruminal amino acid supply and found RFI was not influenced by diet ($P > 0.05$). Looking at the four no roughage diet treatment groups, DMI was similar but calves fed inadequate RDN and post-ruminal arginine diet (diet NEG) had lower RFI (more efficient). Numerical difference on DMI for calves fed NEG diet (9.27 kg for AA- group vs. 8.77 kg for NEG group; $P > 0.05$) was not great enough to cause significance in RFI (0.19 for AA- group vs. -0.53 for NEG group; $P < 0.01$).

No difference due to diet was found on carcass characteristics (Table 4.7). Marbling score showed a slight increasing in calves fed BALANCE diet. Oljen et al. (1971) reported steers fed on forage were graded low choice while steers fed on concentrate were average choice. Additionally cost per unit gain would be greater in forage fed steers. All experimental animals were harvested at the same time, which most likely jeopardized growth performance for BALANCE treatment due to greater lipid accumulation in later finishing phase. This conclusion is supported by interim weight data (Table 4.5 and Figure 4.1).

Experiment 2

The growth performance of Exp. 2 is shown in Table 4.8. No difference in ADG occurred among diet. Animals fed HighArg had lower DMI than other two diets (P = 0.03). Feed conversion ratio was significantly greater (poor feed efficiency) in BalanceArg diet ($P < 0.01$), which was opposite of what was expected. However, significant responses were found on ADG for day 0-28 (P = 0.02) and day 0-87 (P = 0.08) with greatest ADG and better feed efficiency (FCR: 4.03 and 5.6 in Table 4.8) occurring in calves fed BalanceArg diet. As hypothesized, feed efficiency would be maximized when post-ruminal arginine requirement was met. There is a quadratic relationship between empty body weight and body fat and protein in male British beef breeds: $Protein\ (kg) = 0.235 * EBW - 0.00013 * EBW^2 - 2.418$; and $Fat\ (kg) = 0.037 * EBW + 0.00054 * EBW^2 - 0.610$ (NRC, 2000). Equations indicated protein gain rate decreased and fat gain rate increased along with increasing body weight. Energy requirement for protein gain and lipid gain differs since heat of combustion of protein and

lipid are 23.8 and 39.6 kJ/g, respectively (Emmans, 1994). Therefore, during late phase of finishing more energy is needed and post-ruminal amino acids required for animal growth is decreased. High level of bypass amino acids in the diet (ME = 3.10 Mcal/kg, CP = 20.8%) depressed feed efficiency during finishing phase (Davis, 2009). Dividing days on feed into two or three phases and formulating multiple diets to match the change of animal growth requirement on energy and protein is beneficial to feed efficiency.

Table 4.9 shows nutrients analyses of diets, and RDN/post-ruminal arginine balance estimation based on measured DMI and gain for Exp. 2. The LowArg diet was designed to be post-ruminal arginine deficient (Table 4.4) but due to higher intake than expected it was not (Table 4.9). The EE consumed to EE required ratio was greater than one and post-ruminal arginine supply was over 100% of requirement if measured DMI and animal weight were used in model estimation. As noted in previous discussion, there is hardly any improvement when post-ruminal arginine requirement is met. Exp. 2 was carried out in outdoor pens at the same time as Exp. 1; severe cold weather possibly caused energy requirement to increase and differ from expected intake. IBW and FBW of animal on trial were 306 kg and 530 kg, and actual days on feed (168 days) were a wider range than proposed weights and feeding days (Table 4.4). Difference between measured gain and proposed gain potential contributed to changing energy requirement and subsequently, changed post-ruminal arginine supply.

Carcass data showed no difference among diets for hot carcass weight, fat thickness, kidney, pelvic and heart fat percentage, preliminary yield grade, retail product percentage, marbling scores and yield grade. Ribeye tended to increase ($P = 0.1$) in

calves fed LowArg diet. As noted above, LowArg diet was not actually “deficient” and therefore did not cause negative influence on carcass. We presumed that protein and energy supply was greater than animal requirement for all treatments.

CONCLUSION

Diets balanced for ruminal degradable peptide and nitrogen requirements could maximize animal feed efficiency. Roughage inclusion (10% as fed basis) in balanced diet negatively influenced feed efficiency but had no effect on carcass characteristics. No further improvement on feed efficiency was made once absorbable amino acid requirements were met when ruminal degradable nitrogen was not limiting. The implication was that phase feeding by shifting diet formulation to match changing on protein requirement (or absorbable amino acids) would promote feed efficiency and decrease feed cost.

Table 4.1 Composition of diets fed to steers in experiment 1.

	Treatment[1]				
	BALANCE	AA-	RDN-	NEG	BALHay
	RDN+	*RDN+*	*RDN-*	*RDN-*	*RDN+*
Ingredient (% as fed)	*AA+*	*AA-*	*AA+*	*AA-*	*AA+*
Grass hay	--	--	--	--	10
Whole Shell Corn	78.31	84.78	81.58	85.38	70.50
Blood Meal	4.00	4.00	4.00	4.00	4.00
SoyPLUS[2]	9.00	2.00	6.00	2.00	7.00
Wheat Middling	6.00	6.00	6.00	6.00	6.00
Urea	0.27	0.60	--	--	0.18
Oil	0.40	0.40	0.40	0.40	0.40
Dyna-K[3]	0.20	0.40	0.20	0.40	0.20
NaCl	0.20	0.20	0.20	0.20	0.20
Limestone	1.40	1.40	1.40	1.40	1.30
TM Premix[4]	0.10	0.10	0.10	0.10	0.10
Vitamin Premix[5]	0.10	0.10	0.10	0.10	0.10
Rumensin 90[6]	0.02	0.02	0.02	0.02	0.02

[1]Treatments consisted of five balanced or unbalanced requirements of RDN and post-ruminal arginine (AA) diets: RDN and AA both are balanced: BALANCE; RDN is balanced but AA is deficiency: AA-; RDN is deficiency with balanced AA: RDN-; RDN and AA are both deficiency: NEG; RDN and AA both balanced with 10% roughage in diet: BALHay.
[2]SoyPLUS: product of West Central® (Ralston, IA), contained (DM basis) 49.84% CP, RUP 60% (%CP), RDP 40% (%CP).
[3]Dyna-K® (Plymonthm, MN) contained (as-fed basis) 50% K, 46.4% Cl.
[4]Contained (as-fed basis) 10% Fe, 10% Mn, 10% Zn, 2% Cu, 1,500 ppm Se, 1,000 ppm I, and 500mg/kg Co.
[5]Contained (as-fed basis) 4,000,000 IU of vitamin A, 800,000 IU of vitamin D and 1,250 IU of vitamin E per kilogram.
[6]Contained (as-fed basis) Monensin USP, 90 g per pound or 198 g per kilogram.

Table 4.2 Nutrient estimation of diets in experiment 1, and RDN/post-ruminal arginine estimation based on theoretically proposed variables.

	Treatment[1]				
Item	BALANCE *RDN+ AA +*	AA- *RDN+ AA-*	RDN- *RDN- AA+*	NEG *RDN- AA-*	BALHay *RDN+ AA+*
Calculated Values[2]					
DM, %	88.56	88.55	88.50	88.48	88.73
CP, % of DM	16.50	14.55	12.69	12.68	15.86
NE_m, Mcal/kg	2.06	2.06	2.07	2.07	1.92
NE_g, Mcal/kg	1.41	1.41	1.42	1.42	1.28
ME, Mcal/kg	3.02	3.01	3.03	3.03	2.86
EE, MJ/kg OM	10.07	10.09	10.21	10.26	9.34
Model estimation					
Proposed IBW, kg	250	250	250	250	250
Proposed FBW, kg	410	410	410	410	410
Days on Feed, day	80	80	80	80	100
Gain potential, kg/day	2.0	2.0	2.0	2.0	1.6
Estimated DMI[3], kg/day	7.0	7.0	7.0	7.0	7.0
DMI[4], % of MBW,	2.1	2.1	2.1	2.1	2. 1
RDP[5], % of DM	6.14	6.14	4.97	4.94	6.42
RDPep[6], % of DM	5.36	4.41	4.97	4.94	5.89
RDPep required[7]	3.25	3.45	3.36	3.43	2.91
RDN balance[8], % of CP	-0.43	-0.68	-1.45	-2.00	0.09
AA to required ratio[9], %	103	85	98	86	102

[1]Treatments consisted of five balanced or unbalanced requirements of RDN and post-ruminal arginine (AA) diets: RDN and AA both are balanced: BALANCE; RDN is balanced but AA is deficiency: AA-; RDN is deficiency with balanced AA: RDN-; RDN and AA are both deficiency: NEG; RDN and AA both balanced with 10% roughage in diet: BALHay.

[2]Nutrients were calculated from NRC table values; effective energy density in diet was estimated as $\mathrm{EE_{diet}(MJ/kgOM)} = 1.15\mathrm{ME} - 3.84 - 4.67\mathrm{DCP}$.

[3]DMI was predicted by EE density in diet and EE requirement of proposed weight and gain.

[4]DMI was expressed as percentage of middle body weight (proposed weight).

[5]RDP: calculated RDP including urea if applicable; presented as percentage of DM.

[6]RDPep: calculated RDP true protein (no urea); value is presented as percentage of DM.

[7]RDPep required: calculated value based on previous research that at least two thirds of nitrogen source must come from peptide for non-structural carbohydrate fermentation and peptide efficiency was assumed 80% (Russell, 1992).

[8]RDN balance: ruminal degradable nitrogen available minus ruminal degradable nitrogen required by rumen microbes, presented as percentage of CP.

[9]Post-rumial arginine supply to requirement ratio, value was percentage of required.

Table 4.3 Composition of diets fed to steers in experiment 2.

	Treatment[1]		
	LowArg	BalanceArg	HighArg
Whole Shell Corn	81.88	78.315	73.55
Blood Meal	4.00	4.00	6.00
SoyPLUS[2]	--	9.00	12.00
DDGs	5.00	--	--
Wheat Middling	6.00	6.00	6.00
Urea	0.27	0.27	--
Oil	0.40	0.40	0.53
Dyna-K[3]	0.20	0.20	0.20
NaCl	0.20	0.20	0.20
Limestone	1.40	1.40	1.30
TM Premix[4]	0.10	0.10	0.10
Vitamin Premix[5]	0.10	0.10	0.10
Rumensin 90[6]	0.015	0.015	0.02

[1]Treatments consisted of three growing steer diets with increasing post-ruminal arginine levels: arginine is deficiency compared with required: LowArg; arginine is balanced: BalanceArg; arginine supplied is over the required: HighArg.
[2]SoyPLUS: product of West Central® (Ralston, IA), contained (DM basis) 49.84% CP, RUP 60% (%CP), RDP 40% (%CP).
[3]Dyna-K® (Plymonthm, MN) contained (as-fed basis) 50% K, 46.4% Cl.
[4]Contained (as-fed basis) 10% Fe, 10% Mn, 10% Zn, 2% Cu, 1,500 ppm Se, 1,000 ppm I, and 500mg/kg Co.
[5]Contained (as-fed basis) 4,000,000 IU of vitamin A, 800,000 IU of vitamin D and 1,250 IU of vitamin E per kilogram.
[6]Contained (as-fed basis) Monensin USP, 90 g per pound or 198 g per kilogram.

Table 4.4 Nutrient estimation of diets in experiment 2, and RDN/post-ruminal arginine estimation based on theoretically proposed variables.

	Treatment[1]		
	LowArg	BalanceArg	HighArg
Calculated Values[2]			
DM, %	88.63	88.56	88.57
CP, % of DM	15.47	16.50	18.69
NE_m, Mcal/kg	2.07	2.06	2.06
NE_g, Mcal/kg	1.42	1.41	1.40
ME, Mcal/kg	2.98	3.02	3.02
EE, MJ/kg OM	10.14	10.07	9.98
Model estimation			
Proposed IBW, kg	250	250	250
Proposed FBW, kg	410	410	410
Days on Feed, day	80	80	80
Gain potential, kg/day	2.0	2.0	2.0
Estimated DMI[3], kg/day	7.0	7.0	7.0
DMI[4], % of MBW,	2.1	2.1	2.1
RDP[5], % of DM	6.60	6.14	6.00
RDPep[6], % of DM	4.18	5.36	6.00
RDPep required[7]	3.53	3.25	3.12
RDN balance[8], % of CP	-0.37	-0.43	-0.19
AA to required ratio[9], %	84	103	129

[1]Treatments consisted of three growing steer diets with increasing post-ruminal arginine levels: arginine is deficiency compared with required: LowArg; arginine is balanced: BalanceArg; arginine supplied is over the required: HighArg.
[2]Nutrients were calculated from NRC table values; effective energy density in diet was estimated as $\mathrm{EE_{diet}(MJ/kgOM)} = 1.15\mathrm{ME} - 3.84 - 4.67\mathrm{DCP}$.
[3]DMI was predicted by EE density in diet and EE requirement of proposed weight and gain.
[4]DMI was expressed as percentage of middle body weight (proposed weight).
[5]RDP: calculated RDP including urea if applicable; presented as percentage of DM.
[6]RDPep: calculated RDP true protein (no urea); value is presented as percentage of DM.
[7]RDPep required: calculated value based on previous research that at least two thirds of nitrogen source must come from peptide for non-structural carbohydrate fermentation and peptide efficiency was assumed 80% (Russell, 1992).
[8]RDN balance: ruminal degradable nitrogen available minus ruminal degradable nitrogen required by rumen microbes, presented as percentage of CP.
[9]Post-rumial arginine supply to requirement ratio, value was percentage of requirement.

Table 4.5 Growth performance responses to diets with balanced and unbalanced RDN and post-ruminal arginine in experiment 1.

	Treatment[1]					*SEM*	*P*
Growth Traits[2]	BALANCE *RDN+ AA+*	AA- *RDN+ AA-*	RDN- *RDN- AA+*	NEG *RDN- AA-*	BALHay *RDN+ AA+*		
IBW, kg	366.11	364.18	364.63	365.71	366.11	4.70	0.99
FBW, kg	595.95	588.71	587.76	587.24	584.89	12.31	0.97
DMI, kg	8.9^{b}	9.27^{b}	8.57^{b}	8.77^{b}	10.10^{a}	0.40	< 0.01
DM dig, %	70.29^{a}	70.38^{a}	67.87^{ab}	72.55^{a}	57.54^{b}	5.70	0.06
ADG, kg							
0-29 day	2.53	2.40	2.09	2.44	2.39	0.18	0.49
0-56 day	2.05	1.99	2.03	2.02	1.91	0.09	0.82
0-86 day	1.63	1.70	1.70	1.76	1.56	0.07	0.33
0-112 day	1.56	1.59	1.57	1.61	1.42	0.09	0.42
0-142 day	1.61	1.58	1.57	1.56	1.54	0.07	0.97
FCR							
0-29 day	3.43	4.01	3.88	3.53	4.07	0.28	0.31
0-56 day	4.54^{bc}	4.99^{ab}	4.26^{c}	4.46^{c}	5.37^{a}	0.19	< 0.01
0-86 day	5.35^{b}	5.46^{b}	5.01^{b}	5.00^{b}	6.40^{a}	0.19	< 0.01
0-112 day	5.76^{b}	5.90^{b}	5.51^{b}	5.55^{b}	6.90^{a}	0.22	< 0.01
0-142 day	5.63^{b}	5.94^{b}	5.50^{b}	5.57^{b}	6.49^{a}	0.18	< 0.01
RFI	-0.16^{bc}	0.19^{b}	-0.49^{c}	-0.53^{c}	0.95^{a}	0.20	< 0.01

[1]Treatments consisted of five balanced or unbalanced requirements of RDN and post-ruminal arginine (AA) diets: RDN and AA both are balanced: BALANCE; RDN is balanced but AA is deficiency: AA-; RDN is deficiency with balanced AA: RDN-; RDN and AA are both deficiency: NEG; RDN and AA both balanced with 10% roughage in diet: BALHay.

[2]IBW = initial body weight, FBW = final body weight, DMI = dry matter intake, DM dig = dry matter digestibility, ADG = average daily gain, FCR = feed conversion ratio: FCR = DMI/ADG, RFI = residual feed intake.

Table 4.6 Nutrients analyses of diets, and RDN/post-ruminal arginine estimation from actual intake and growth data in experiment 1.

	Treatment[1]				
	BALANCE	AA-	RDN-	NEG	BALHay
	RDN+	*RDN+*	*RDN-*	*RDN-*	*RDN+*
Item	*AA+*	*AA-*	*AA+*	*AA-*	*AA+*
Analysis Values					
DM, %	86.07	83.04	86.20	86.13	85.54
OM, %	95.80	95.97	95.71	95.88	94.91
CP, %	16.63	15.19	15.72	13.59	16.57
Estimations					
IBW, kg	366.11	364.18	364.63	365.71	366.11
FBW, kg	595.95	588.71	587.76	587.24	584.89
Days on Feed	142	142	142	142	142
ADG, kg/day	1.61	1.58	1.57	1.56	1.54
DMI, kg/day	8.9	9.27	8.57	8.77	10.10
DMI[2], % of MBW,	1.85	1.95	1.80	1.84	2.12
EE to required ratio	1.03	1.09	1.03	1.06	1.10
AA to required ratio[4], %	143	115	134	120	136

[1]Treatments consisted of five balanced or unbalanced requirements of RDN and post-ruminal arginine (AA) diets: RDN and AA both are balanced: BALANCE; RDN is balanced but AA is deficiency: AA-; RDN is deficiency with balanced AA: RDN-; RDN and AA are both deficiency: NEG; RDN and AA both balanced with 10% roughage in diet: BALHay.

[2]DMI was expressed as percentage of actual middle body weight.

[3]Ratio between effective energy consumed and effective energy required based on actual weight and gain.

[4]Post-rumial arginine supply to requirement ratio, value was percentage of required.

Table 4.7 Carcass characters responses to diets with balanced and unbalanced RDN and post-ruminal arginine level in experiment 1.

	Treatment[1]					*SEM*	*P*
Traits[2]	BALANCE *RDN+ AA+*	AA- *RDN+ AA-*	RDN- *RDN- AA+*	NEG *RDN- AA-*	BALHay *RDN+ AA+*		
Hot Cwt, kg	374.39	376.76	374.77	375.48	373.58	7.76	0.99
REA, in^2	12.89	13.51	13.08	13.41	12.91	0.41	0.67
FAT, in	0.61	0.53	0.51	0.51	0.55	0.06	0.70
KPH, %	1.57	1.63	1.59	1.63	1.58	0.05	0.82
PYG	3.53	3.34	3.29	3.21	3.38	0.14	0.50
RP, %	62.00	63.46	63.18	63.59	62.62	0.96	0.72
Marbling	5.95	5.82	5.09	5.34	5.24	0.30	0.12
YG	3.08	2.82	2.69	2.94	3.00	0.22	0.66

[1]Treatments consisted of five balanced or unbalanced requirements of RDN and post-ruminal arginine (AA) diets: RDN and AA both are balanced: BALANCE; RDN is balanced but AA is deficiency: AA-; RDN is deficiency with balanced AA: RDN-; RDN and AA are both deficiency: NEG; RDN and AA both balanced with 10% roughage in diet: BALHay.

[2]Hot Cwt = carcass weight; REA = ribeye area; FAT = fat thickness; KPH = kidney, pelvic and heart fat as percentage of hot carcass weight; PYG = preliminary yield grade; RP = percentage of retail product; Marbling = marbling score, a 9 score scale with a subunits ranging from 00 to 99 was used; YG = yield grade, expressed as numerical scores of 1, 2, 3, 4 and 5.

Table 4.8 Growth performance responses to diets with balanced and unbalanced RDN and post-ruminal arginine in experiment 2.

	Treatment[1]				
Growth Traits[2]	LowArg	BalanceArg	HighArg	*SEM*	*P*
IBW, kg	306.91	306.86	308.92	3.41	0.88
FBW, kg	536.68	525.47	537.03	6.07	0.31
DMI, kg	8.85[a]	8.95[a]	8.46[b]	0.14	0.03
ADG, kg					
0-28 day	1.94[b]	2.30[a]	2.12[ab]	0.09	0.02
0-87 day	1.53[b]	1.65[a]	1.54[ab]	0.04	0.08
0-112 day	1.50	1.59	1.54	0.04	0.21
0-142 day	1.47	1.42	1.48	0.03	0.43
0-168 day	1.37	1.30	1.36	0.03	0.24
FCR					
0-28 day	5.29	4.03	6.68	1.57	0.48
0-87 day	6.00	5.61	5.78	0.21	0.42
0-112 day	5.89	5.73	5.73	0.14	0.64
0-142 day	6.08[ab]	6.34[a]	5.89[b]	0.13	0.05
0-168 day	6.53[b]	6.93[a]	6.33[b]	0.13	< 0.01
RFI	0.05[a]	0.31[a]	-0.35[b]	0.11	< 0.01

[1]Treatments consisted of three growing steer diets with increasing post-ruminal arginine levels: arginine is deficiency compared with required: LowArg; arginine is balanced: BalanceArg; arginine supplied is over the required: HighArg.

[2]IBW = initial body weight, FBW = final body weight, DMI = dry matter intake, ADG = average daily gain, FCR = feed conversion ratio: FCR = DMI/ADG, RFI = residual feed intake.

Table 4.9 Nutrients analyses of diets, and RDN/post-ruminal arginine estimation from actual intake and growth data in experiment 2.

	Treatment[1]		
	LowArg	BalanceArg	HighArg
Analysis Values			
DM, %	85.83	86.07	86.30
OM, %	96.00	95.80	95.46
CP, %	15.44	16.63	19.53
Model estimation			
IBW, kg	306.91	306.86	308.92
FBW, kg	536.68	525.47	537.03
Days on Feed	168	168	168
ADG, kg/day	1.37	1.30	1.36
DMI, kg/day	8.85	8.95	8.46
DMI[2], % of MBW,	2.10	2.15	2.00
EE to required ratio	1.23	1.27	1.16
AA to required ratio[4], %	120	157	168

[1]Treatments consisted of three growing steer diets with increasing post-ruminal arginine levels: arginine is deficiency compared with required: LowArg; arginine is balanced: BalanceArg; arginine supplied is over the required: HighArg.
[2]DMI was expressed as percentage of actual middle body weight.
[3]Ratio between effective energy consumed and effective energy required based on actual weight and gain.
[4]Post-rumial arginine supply to requirement ratio, value was percentage of required.

Table 4.10 Carcass characters responses to diets with balanced and unbalanced RDN and post-ruminal arginine level in experiment 2.

	Treatment[1]			*SEM*	*P*
Traits[2]	LowArg	BalanceArg	HighArg		
Hot Cwt, kg	325.43	321.21	323.98	3.80	0.72
REA, in^2	12.35	11.98	11.78	0.20	0.10
FAT, in	0.47	0.47	0.48	0.03	0.94
KPH, %	1.73	1.70	1.67	0.03	0.17
PYG	3.17	3.19	3.20	0.07	0.94
RP, %	64.17	63.78	63.38	0.43	0.41
Marbling	4.93	4.89	4.93	0.18	0.98
YG	2.65	2.67	2.70	0.09	0.92

[1]Treatments consisted of three growing steer diets with increasing post-ruminal arginine levels: arginine is deficiency compared with required: LowArg; arginine is balanced: BalanceArg; arginine supplied is over the required: HighArg.

[2]Hot Cwt = carcass weight; REA = ribeye area; FAT = fat thickness; KPH = kidney, pelvic and heart fat as percentage of hot carcass weight; PYG = preliminary yield grade; RP = percentage of retail product; Marbling = marbling score, a 9 score scale with a subunits ranging from 00 to 99 was used; YG = yield grade, expressed as numerical scores of 1, 2, 3, 4 and 5.

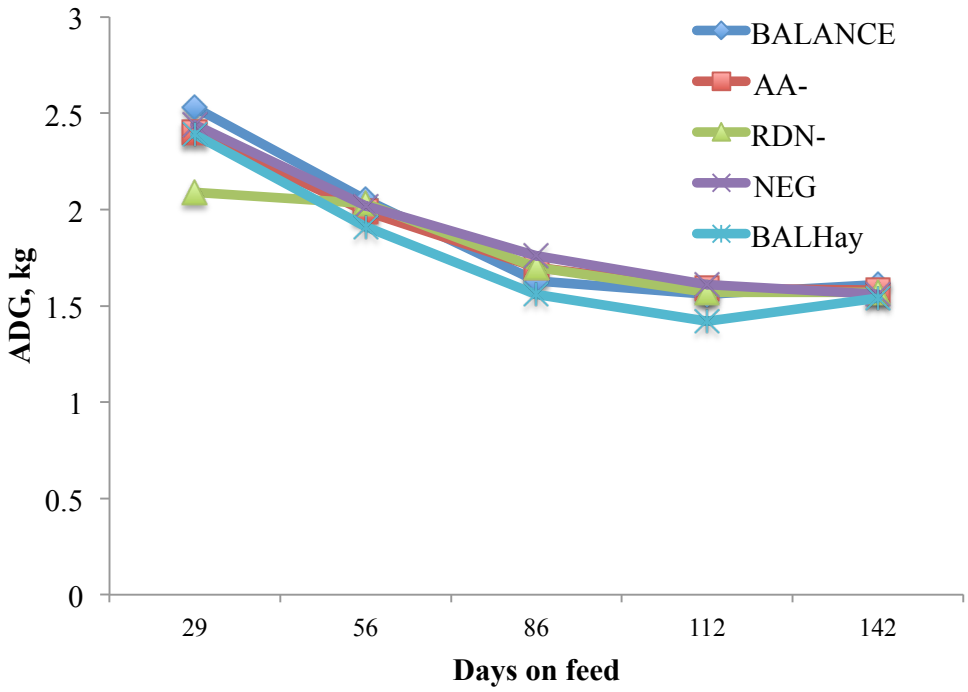

Figure 4.1 Change on growth rate for experiment 1.

[1]Treatments consisted of five balanced or unbalanced requirements of RDN and post-ruminal arginine (AA) diets: RDN and AA both are balanced: BALANCE; RDN is balanced but AA is deficiency: AA-; RDN is deficiency with balanced AA: RDN-; RDN and AA are both deficiency: NEG; RDN and AA both balanced with 10% roughage in diet: BALHay.

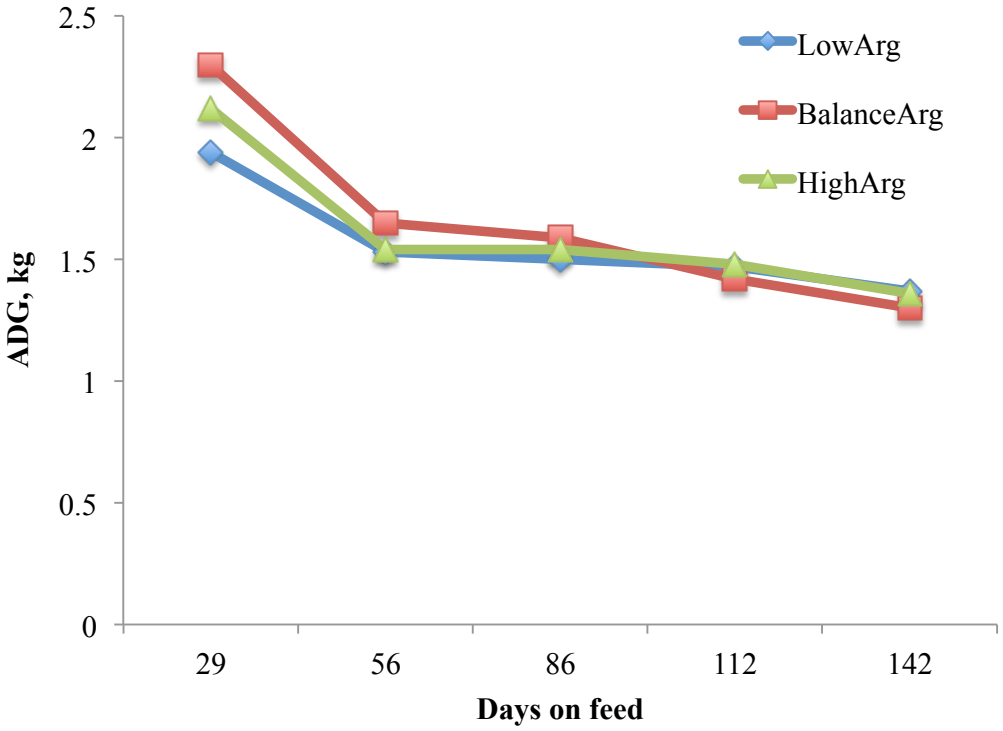

Figure 4.2 Change on growth rate for experiment 2.

[1]Treatments consisted of three growing steer diets with increasing post-ruminal arginine levels: arginine is deficiency compared with required: LowArg; arginine is balanced: BalanceArg; arginine supplied is over the required: HighArg.

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VITA

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Yi went to China Agricultural University in 2001 for undergraduate study in Department of Animal Science and Technology. She graduated in 2005 with a Bachelor degree in Animal Science. After spending one year working as a volunteer in agricultural technology extension in Yunnan province in China, she went back to China Agricultural University for her further study in September 2006. She conducted her research in national key nutritional laboratory under the direction of Dr. Qingxiang Meng, and she was awarded her M.S. in Animal Nutrition and Feed Science in June 2008. Yi started her doctoral program at University of Missouri in August of 2008 under the direction of Dr. Monty S. Kerley. Yi has been a member of the American Society of Animal Science, American Dairy Science Association and Chinese Association of Animal Science and Veterinary Medicine science since 2003. Upon completion of her doctoral degree, she will work as a postdoctoral fellow in Novus International at St Charles, Missouri under the direction of Dr. Geoffrey Zanton and Dr. Mercedes Vazquez-Anon.