ENHANCING ENERGY EFFICIENCY OF INTENSIFIED COW-CALF PRODUCTION SYSTEMS THROUGH OPTIMAL FEEDING STRATEGIES

A Dissertation

by

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ABSTRACT

A series of experiments was designed to aid in developing optimal solutions for intensive cow-calf production. In the first experiment, we studied potential system limitations regarding inclusion and intake of grain and their effects on risk of digestive upset. Ruminal pH declined rapidly when concentrate diets were fed at high intake levels, but the minimal risk of acidosis observed at high intakes was mitigated through intake restriction. Next, we quantified interactions between dietary energy density and intake on energy digestibility to more accurately predict energy supplies. When high-energy diets were limit-fed to maintenance intake, more complete digestion leads to under estimation of DE intake. In the third experiment, we measured effects of dietary energy density and intake on apparent energy requirements. Divergence between observed and predicted energy retention was observed, suggesting that increasing energy density and restricting intake improved energy metabolism. Finally, in a study involving two experiments, we determined the effects of intake restriction on mass and metabolism of metabolically active organs to determine their role in a cow's ability to adapt under periods of energy deficiency. Dietary energy restriction reduced the mass of metabolically active organs. Overall, limit-feeding high-energy diets to beef cows appears to provide opportunities for increased efficiency of land and feed energy use, with minimal risks to animal health. Previous nutrition models neglect to account for effects of intake restriction on energy metabolism, causing an overestimation of feed requirements for intensively-managed beef cows.

DEDICATION

To Mr. and Mrs. Tony Trubenbach Jr:

Words cannot describe my appreciation for the two of you. You will always be my heroes. You led by example with unblemished integrity, and I hope to one day be worthy of the kind of admiration I have for you.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

INTRODUCTION

Sustainable intensification has been proposed as a necessary element of increasing global protein supplies in the face of decreasing land availability (FAO, 2011). Sustainability of intensified cow-calf systems relies heavily on the control of variable input costs, specifically feed costs associated with cow maintenance. Several reports (Loerch, 1996; Schoonmaker et al., 2003; Sawyer and Wickersham, 2013) have demonstrated potential economic advantages with limit-feeding beef cows, particularly during dry or winter periods, as an alternative to traditional schemes utilizing harvested or stockpiled forages.

Previous work from our laboratory (Trubenbach et al., 2014) has demonstrated that in limit-fed, beef cows, dietary energy utilization may be improved by increasing dietary energy density and restricting intake below conventional intake levels. However, it remains unclear whether this response is attributed to improvements in dietary energy delivery, or if it is the result of reduced energy demands.

Studies in the dairy literature (Moe et al., 1965; Tyrrell and Moe, 1975; Colucci et al., 1982) concluded that energy digestion increases with intake restriction, with the rate of increase becoming greater with more grain inclusion, ultimately suggesting that greater energy density may be the source of observed improvements in energy efficiency. However, several mechanisms for reduced requirements have also been

proposed, including decreased protein turnover, cellular ion transport, and mass and total metabolism of metabolically active organs (McBride and Kelly, 1990). These reports of reduced maintenance requirements have also been supported with evidence of reduced whole-body nutrient balance (Freetly and Nienaber, 1998).

Adding to system complexity, the propensity for subacute ruminal acidosis (SARA) increases with dietary grain inclusion (Johnson et al., 1974; Britton and Stock, 1987; Reinhardt et al., 1993) and may be exacerbated by increased intake (Zinn, 1995), especially during adaptation to energy dense diets (Galyean et al., 1992). Limiting intake may mitigate the risk of acidosis (Preston, 1995), but interactions between grain inclusion level and intake are not well characterized in limit-fed systems.

To develop optimal solutions for intensive cow-calf systems, the following objectives were created: a) evaluate interactions between dietary corn inclusion and intake on ruminal parameters indicative of SARA b) quantify interactions of dietary energy concentration and intake on digestion, c) estimate maintenance requirements (NE_m) as a function of energy density, and d) measure effects of sub-maintenance energy restriction on abdominal and thoracic organ mass and metabolism. The following chapter reviews existing literature associated with our objectives, and outlines potential opportunities and limitations to limit-fed, cow-calf systems.

ACIDOSIS AND TRANSITIONING BETWEEN DIETS

Rapid fermentation causes a surge in acidic fermentation products, increasing the incidence of ruminal acidosis (Owens et al., 1998), which is characterized by the accumulation of organic acids in the rumen without sufficient buffering capacity to

prevent pH depression (Cooper and Klopfenstein, 1996). Intake of fermentable carbohydrate causes a major reduction of fibrolytic bacteria and rapid growth of amylolytic bacteria (Goad et al., 1998; Tajima et al., 2001), which is accompanied by a rapid accumulation of acid and subsequent decrease in ruminal pH. In addition to the risk of acidosis, performance can be compromised by periods of low ruminal pH; ruminal cellulolytic activity is inhibited below pH 6.0 (Mould and Ørskov, 1983), potentially resulting in reduced fiber digestion (Calsamiglia et al., 2002).

In growing cattle, the primary consequences of acidosis include intake depression, diarrhea, reduced fiber fermentation, laminitis, liver abscesses, and inflammation. The same symptoms occur in dairy cows, with the addition of reduced milk fat content. In both instances, the economic significance of SARA manifests in reduced intake and performance. However, for cow-calf producers, maximizing intake of high-energy diets rarely makes economic sense. Differences in system objectives may alter the relative importance of mitigating acid accumulation, as long as animals are not subjected to acute symptoms.

Diagnosis of SARA has been variable. Zebeli and Metzler-Zebeli (2012) indicated that decreased fiber digestion and increased serum acute phase proteins occurred when ruminal pH dropped below 5.8 for at least 360 min/d, which was supported by Beauchemin et al. (2001). Cooper and Klopfenstein (1996) characterized SARA as episodes of ruminal pH between 5.2 and 5.6. Oetzel (2007) asserted that intake depression occurs in dairy cows when pH falls below 5.5, which was supported by Hibbard et al. (1995) and Krause and Oetzel (2006). Owens et al. (1998) added that

duration of pH below 5.6 severely impacted the magnitude of disease, and suggested a threshold 720 min/d for diagnosis of SARA. Gohzo et al. (2005, 2006) determined that SARA can be induced when pH remains below 5.6 for as little as 180 min/d, but confirmed that the extent of the condition is worsened when pH remains below this threshold for longer periods of time.

An abrupt change from a high-forage to a high-concentrate diet can result in acute acidosis or SARA (Goad et al., 1998; Coe et al., 1999). A wealth of literature exists for growing cattle during the adaptation phase (Fulton et al., 1979; Owens et al., 1998; Bevans et al., 2005); however, feeding behaviors are likely very different between growing calves fed *ad libitum* and limit-fed cows, given very small amounts of a high-energy diet.

Absorptive capacity of ruminal acids is a function of size and surface area of ruminal papillae. During periods of low intake or high-forage diets, length and density of papillae decrease by up to 50% (Dirksen et al., 1985). Accordingly, cows rapidly transitioned from pasture to grain-based diets may have compromised ability to absorb fermentative products.

Rate of consumption increases with intake restriction (Cooper et al., 1999), causing accumulation of substrate. Acid production is directly related to starch availability (Oetzel and Nordlund, 1998), which increases as dietary grain inclusion increases. Because intake restriction is a desired element of intensive feeding strategies for beef cows, it is assumed that increased appetite results in rapid consumption of limit-fed rations, potentially resulting in periodic abundance of available starch.

Even after adaptation, animals still experience bouts of acidosis (Nagaraja and Titgemeyer, 2007). Inflammation or parakeratosis of the rumen wall due to low rumen pH for extended periods of time, puts cows at increased risk of SARA. Like the adaptation phase, the propensity for the onset of SARA increases with dietary grain inclusion (Johnson et al., 1974; Britton and Stock, 1987; Reinhardt et al., 1993), with the effects being exacerbated by increased intake (Zinn, 1995; Oetzel and Nordlund, 1998) due to greater starch supply.

For producers using semi-confinement practices, additional consideration of the transition back to pasture is worth considering. Kruse et al. (2010) reported that previous intake restriction had no carryover effects during the first lactation for rumen digesta volume, dry matter intake, or milk yield in dairy heifers. Smith et al. (2017) also reported uneventful transitioning of beef cows back to pasture following a period of intake restriction. However, cattle in these experiments were fed relatively low-energy diets and intake restriction was moderate. Adaptation back to forage diets following a period of feeding high-grain diets at less than 2% of BW is not well documented.

Overall, the risk of SARA appears to become greater with increased intake, largely driven by starch supply, with greater levels of grain potentially exacerbating the problem. While limiting total intake of concentrates may provide an opportunity to decrease the risk of acidosis (Preston, 1995), the interactions between grain inclusion and intake are not well understood. Additionally, to our knowledge, models predicting ruminal pH parameters related to SARA, as a function of grain inclusion or energy

density, are lacking. Therefore, there is a need to identify limitations to dietary grain inclusion and total intake for cow-calf systems employing limit-feeding practices.

ENERGY DELIVERY

In most cases, the largest source of dietary energy loss is in the form of feces (Ferrell and Ojtjen, 2008). Accordingly, the accuracy with which its inverse (TDN and/or DE) is predicted has a substantial effect on forecasts of energy delivery. Published feed composition tables (BCNRM, 2016) contain tabular nutrient values intended to be used with both level 1 and 2 solutions. Level 1 uses a weighted average approach to calculate TDN for a diet, using ingredient values predicted either from previous digestion experiments or from equations used by commercial laboratories, with no regard for intake effects on digestion.

Dairy studies (Tyrrell and Moe, 1974; 1975; Wagner and Loosli, 1967) consistently report greater digestibility with intake restriction, while conclusions from beef literature are less consistent (Murphy et al., 1994; Zinn, 1995; Clark et al., 2007). However, intake restriction in the latter is typically mild, and most responses are observed in growing cattle fed well above maintenance intake levels. Therefore, these data may not be relevant to systems in which intake is restricted to maintenance levels.

Tedeschi et al. (2002) reported that using tabular estimates of TDN causes discrepancy because the values are not discounted for level of intake above maintenance. To account for these effects, the BCNRM (2016) proposes two options for TDN adjustment: 1) the mechanistic level of solution, or 2) equations developed by Tedeschi

et al. (2005) to estimate TDN based on chemical analysis and a predicted discount for concentrate and forage fractions with respect to multiples of intake above maintenance.

Discount equations reported by Tedeschi et al. (2005) were developed using the level 2 solution of the Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004), the same system used to predict TDN in level 2 of BCNRM (2016), which is based on feed carbohydrate fractions and their theoretical rates of digestion and passage. Using chemical composition values from the NRC (2000), TDN was predicted at maintenance (1×), 2×, and 3× levels of DMI in the CNCPS for every ingredient in the feed composition library at that time. Discount equations were estimated by regressing these predicted TDN values on DMI for both concentrate and forages. Results suggested a 5.0% discount in TDN per multiple of maintenance intake for forages and only a 2.3% discount for concentrates.

These discount rates are different from the discount factor in the dairy NRC (2001), which discounts diets high in formulated TDN more severely than those containing lower formulated energy concentrations. The Dairy NRC (2001) concluded, from numerous feeding trials (Moe et al., 1965; Tyrrell and Moe, 1972; Colucci et al., 1982), that the rate of decline in TDN with intake is a function of formulated TDN at maintenance (TDN_{1X}). While energy delivery is not adjusted for intake level in diets with formulated TDN less than 60%, for diets with TDN greater than 60%, the following equation was developed:

TDN percentage unit decline = $0.18 \times TDN_{1X} - 10.3$, ($r^2 = 0.85$)

This equation was converted so that a percentage discount could be applied to DE:

$$\text{Discount} = \{TDN_{1X} - [(0.18 \times TDN_{1X}) - 10.3] \times \text{Intake}\} / TDN_{1X}$$

Greater digestion with restricted intake is often attributed to slower passage of slowly fermented feed constituents (Mertens et al., 1987), along with positive effects from increased rumen pH on fiber digestion (Mould and Ørskov, 1983), and greater starch digestion (Colucci et al.; 1982).

Feeding high-grain diets typically results in reduced rumen pH (Johnson et al., 1974; Britton and Stock, 1987; Reinhardt et al., 1993; Rustomo et al., 2006), with the effects being exacerbated by greater intake (see Chapter II) and grain processing (Yang et al., 2001). When grains are fed at high intakes, rate of fermentation increases more than rate of absorption, due to substrate supply (Oetzel and Nordlund, 1998), causing pH to decline faster and to a greater extent. Rumen cellulolysis is inhibited below pH 6.0-6.1 (Mould and Ørskov, 1983), resulting in reduced fiber digestion (Calsamiglia et al., 2002). Furthermore, as starch availability increases, the negative effects on fiber fermentation appear to be exacerbated in diets containing low-quality forage, including straw (Brown, 1966; Mould and Ørskov, 1983), as Tyrrell and Moe (1975) suggested that the cell wall fractions of the diet have greater reductions in digestion at high intakes than soluble components.

Galyean et al. (1979) observed reductions in starch digestion from 99.6% to 93.8% and 90.4% when intake was increased from 1.00 to 1.67 and 2.00 times maintenance intake, respectively. Russell et al. (1981) reported a tendency for total tract

starch digestion to decrease from 81.4% to 76.4% and 76.0% when intake of corn silage-based diets was increased from 1 to 2 and 3 times maintenance, respectively. Wheeler et al. (1975) tested for interactions between forage:concentrate ratio and intake level on starch digestion. Total starch digestion was not affected by forage:concentrate, but was reduced substantially when intakes were increased from maintenance to lactation levels. However, the authors concluded that because starch represented a larger proportion of DMI in diets with lower forage:concentrate, that reduced starch digestion accounted for more of the total depression in digestible DM as the portion of concentrate increased.

Wheeler et al. (1975) and Colucci et al. (1982) reported kernels of grain in feces from cows fed at lactation levels, while they were minimal or lacking when the same diets were fed at maintenance. The difference in fecal grain kernel presence between intake levels increased with greater inclusion of grain. Greater depressions in digestion of low-forage diets are likely due to additive effects of reduced feed mastication, reduced rumen retention and fermentation time, and increased starch escape through the lower tract due to incomplete physical assimilation (Colucci et al., 1982).

While fecal energy typically represents the majority of dietary energy loss, losses also transpire through production of urine, gas and heat. While the objectives of the current paper do not include quantifying these forms of energy loss, it is important to note that conversion of DE to NE may be affected by diet and intake, in addition to adjustments made in the current model (BCNRM, 2016). For example, Zinn, (1995) and Clark et al. (2007) reported an improvement in apparent DE with intake restriction, but

also that the net energetic value of the diet was not changed, due to greater methane production and reduced conversion of DE:ME.

Previous beef models, including the default option in the current model (BCNRM, 2016), have assumed 82% conversion of DE to ME. However, Hales et al. (2012, 2013, 2014) reported conversions ranging from 89.3 to 95.0% in high-energy diets. They attribute the discrepancy to less methane production than expected. Although these data were observed from growing cattle fed at or near *ad libitum* intakes, they suggest that conversion of energy from grains may be greater than previously thought.

Mills et al. (2001) proposed that the proportion of ingested energy lost as methane increases with intake restriction, which would result in overestimated dietary ME values in feed restricted animals. Furthermore, Vermorel and Bickel (1980) suggested that methane losses are likely greater in mature animals than in young, growing animals. Overall, DE:ME conversion remains poorly understood, especially when diets are limit-fed; however, it should be noted that fermentation is altered considerably when intake is restricted, and that several factors determine overall methane production in these systems.

Efficiency of ME use for maintenance and/or pregnancy could also be affected by a multitude of factors. Increased glucose requirements of the gravid uterus and mammary tissue cause major changes in glucose metabolism in gestating ruminants (Bell and Bauman, 1997), which rely heavily on hepatic gluconeogenesis for glucose supply, even when open. Propionate is the primary exogenous precursor for hepatic gluconeogenesis (Brockman, 1993), which is known to be stressed by substrate supply

during late gestation. Previous studies from our lab have reported greater proportional concentrations of propionate with intake restriction (Trubenbach, 2014; Boardman, 2015), suggesting a potential under-prediction of ME efficiency at low intakes. Propionate production is favored by fermentation of starch by amylolytic bacteria (Elliot, 1980; France and Siddons, 1993); therefore, relative propionate production increases with greater corn inclusion, possibly resulting in even greater under-prediction of ME efficiency with intake restriction as energy density is increased.

Overall, energy delivery appears to be under-predicted by the BCNRM (2016) for limit-fed systems, with fecal energy losses representing much of the difference. Although their interactions have not been well-defined in beef cattle, literature and equations from the dairy NRC (2001) suggests that intake restriction results in greater digestion, with the magnitude of improvement being greater with increasing energy density.

ENERGY REQUIREMENTS

Freetly and Nienaber (1998) reported the effects of intake on maintenance requirements in mature cows. Cows were subjected to one of two treatments: control, fed a fixed amount of chopped brome hay near the estimated maintenance level of intake for 224 d; restricted, fed 65% of control intake for 112 d and subsequently allowed to consume 135% of control intake for 112 d. Treatments were designed so the total amount of feed consumed during the 224-d period was the same between control and restricted cows. Restricted cows were in negative energy balance from d 0 to 84. However, by d 112 cows had returned an RE of 0, suggesting an ability to adapted to

energy restriction, potentially by reducing equilibrium maintenance requirements. Similar reports of reduced maintenance requirements with cows were described by Koong et al., (1985) and Jenkins and Ferrell (1997).

Compensatory gain is an established phenomenon in growing (Lofgreen and Kiesling, 1985; Sainz et al., 1995) and mature cattle (Freetley et al., 1998; Sawyer et al., 2004). A series of experiments with sheep (Koong et al., 1982) and rats (Ferrell and Koong, 1982) demonstrated the effects of intake level on maintenance requirements and feed efficiency. Using similar designs for both species, animals were fed one of three intake levels, designed to achieve low, medium, or high rates of gain. After the feeding period, animals previously fed at high intake levels had 38% (rats) and 74% (sheep) greater fasting heat production and substantially lower feed efficiency than those fed to achieve low rates of gain. Authors also reported that mass of the stomach, small intestine, large intestine, liver, heart, kidneys and spleen all increased with increasing intake, and that the divergence in maintenance requirements was largely driven by energy consumption from these tissues.

Visceral tissues account for disproportionally large amounts of energy expenditure in the ruminant. In cattle, liver and gastrointestinal tissues represent approximately 8-15% of total body weight, yet they account for approximately 40-50% of total body energy consumption (Reynolds et al., 1991). Therefore, relatively small changes in total metabolism of these tissues may represent a large proportion of total energy requirements. Reduced maintenance requirements in intake-restricted cows may

be largely driven by a reduction in mass and overall energy expenditure of metabolically active organs (Sainz and Bentley, 1997; McCurdy et al., 2010).

Camacho et al. (2014) reported the effects of limiting intake on organ mass gestating beef cows. Cows were fed individually either at maintenance or at a restricted rate of 60% of maintenance energy requirements for several combinations of 55-d periods. Efficiency of gain (gain:feed) was greater in restricted cows. Restricted cows had lighter liver and rumen mass compared to control cows. Following realimentation for 55 d, splanchnic tissues, EBW and ultrasonography measurements of backfat and ribeye area were not found to be different between treatments groups, indicating that organ mass corrects rapidly with realimentation. Alternatively, Wood et al. (2013) reported no difference in organ mass, due to intake restriction. In the latter experiment, NE was only restricted to 85% requirements, suggesting that reduced requirements may not manifest in organ catabolism with moderate restriction.

Murray et al. (1977), Winter et al. (1976) and Ledin (1983) reported similar findings that splanchnic tissues, especially the liver and gastrointestinal tract, vary in response intake level. In their review paper, Ferrell and Jenkins (1985) concluded that variation in visceral organ weights may contribute substantially to variation in total animal energy expenditures.

Intake effects on mass specific rate of oxygen consumption in the liver and gastrointestinal tract remains somewhat unclear. General dogma is the reduced tissue energy expenditure in intake-restricted animals results from the overall change in organ mass, rather than a change in energy consumption per g of tissue. Intake restriction

reduced intestinal cellularity, vascularity (Reed et al., 2007; Neville et al., 2008) and ion transport in splanchnic tissues; however, several studies in sheep (McBride and Milligan, 1985; Rompala et al., 1987; 1988), pigs (Nyachoti et al., 2000), rats (Burrin et al., 1988) and lactating cows (McBride and Milligan, 1984) found no difference in mass specific oxygen consumption rate because of intake restriction.

In contrast, Wood et al. (2013) reported lower mass specific hepatic oxygen consumption in pregnant heifers fed 85% of total NE requirements versus those fed 140% of NE requirements. However, no animals were fed at maintenance, making it difficult to draw conclusions

Effects of reducing DMI may be augmented by increasing energy density, which further reduces splanchnic tissue mass and metabolism (Reynolds et al., 1991). Increasing the energy density of a total mixed ration increased energy efficiency and/or efficiency of gain (gain:feed) in lambs (Sainz et al., 1995), heifers (Reynolds et al., 1991), compensating beef cows (Swingle et al., 1979; Sawyer et al., 2004) and dairy cows (Wagner and Loosli, 1967; Tyrrell and Moe, 1975). We recently reported (Trubenbach et al., 2014) reduced heat production and improved energy utilization with increased energy density in limit-fed beef cows, suggesting that part of the enhancement could be attributed to a reduction in maintenance requirements. With sheep, McLeod and Baldwin (2000) reported that cellular hyperplasia in ruminal and intestinal tissues is affected by both diet and intake. However, total oxygen consumption by isolated epithelial cells was unaffected, suggesting that diet and intake alter gut energy

expenditure primarily through changes in tissue mass, rather than mass specific metabolism.

Effects of energy and DM intake on metabolism are often difficult to isolate in a factorial arrangement. By definition, DMI must be altered to achieve isocaloric intakes in diets containing different levels of energy density. Increasing ME intake, however, modifies liver metabolism by increasing metabolic energy load (Reynolds et al., 1991). potentially confounding results on whole-animal energy metabolism.

Overall, it appears that cows may adapt to periods of energy restriction by reducing basal energy requirements, potentially via reduced mass and metabolism of metabolically active organs. Accordingly, dietary energy density and intake may interact to cause overestimated feed requirements, especially in high-grain diets. However, these interactions have not been quantified in beef cows fed near maintenance intake levels, warranting further investigation.

Reviewed literature suggests that rapidly adapting beef cows to high-energy diets may put them at risk for acidosis, but also that intake restriction may effectively mitigate these risks. Evidence also suggests that current models describing dietary energy delivery fail to account for effects of intake restriction, possibly over-estimating feed requirements of intensively-managed beef cows through two primary sources: 1) under-predicted energy digestion, and 2) over-predicted energy requirements.

To develop solutions for intensive cow-calf systems, the following objectives were developed: a) to evaluate interactions between dietary corn inclusion and intake on ruminal parameters indicative of acidosis b) to quantify interactions between dietary

energy concentration and intake on energy digestion, c) to estimate maintenance requirements (NE_m) as a function of dietary energy density, and d) to measure effects of sub-maintenance energy restriction on abdominal and thoracic organ mass and metabolism.

CHAPTER II

LIMITS TO THE SYSTEM: RUMINAL FERMENTATION

INTRODUCTION

Under some production conditions, the total cost per calorie of dietary energy consumed by beef cows may be reduced by decreasing the land requirement and increasing the intensity of cow feeding operations (Sawyer and Wickersham, 2013). However, the level of grain inclusion and total consumption may be constrained by effects of rapid starch fermentation on ruminal health (Paisley, 2003).

Subacute ruminal acidosis (Owens et al., 1998) is characterized by the accumulation of organic acids (VFA and lactate) in the rumen in the absence of sufficient buffering capacity to prevent significant pH depression (Cooper and Klopfenstein, 1996). Propensity for SARA increases with dietary grain inclusion (Johnson et al., 1974; Britton and Stock, 1987; Reinhardt et al., 1993) and may be exacerbated by increased intake (Zinn, 1995), especially during adaptation to more energy dense diets (Galyean et al., 1992). Limiting intake may mitigate the risk of acidosis (Preston, 1995), but interactions between grain inclusion level and intake are not well characterized.

Limit-feeding a total mixed ration (TMR) to meet requirements of beef cows in a commercial system has been successfully applied (Loerch, 1996). For producers using limit-feeding, the effects of transitioning from the TMR back to pasture warrants consideration. Successful adaptation from limit-feeding strategies to *ad libitum* intake

has been reported (Kruse et al., 2010). These authors reported that previous intake restriction had no carryover effects during the first lactation for ruminal digesta volume, DMI, or milk yield in dairy heifers. Smith et al. (2017) also reported uneventful transitioning of beef cows back to pasture following a period of intake restriction. However, cattle in these experiments were fed relatively low-energy diets and intake restriction compared to *ad libitum* intake was moderate. Adaptation back to forage diets following a period of feeding high-grain diets at less than 2.0% of BW is not well documented.

We hypothesize that a) ruminal pH declines upon introducing high-grain diets, b) the rate and magnitude of decline in ruminal pH upon feeding is related to the level of grain inclusion in the diet, c) that effects of grain inclusion on ruminal pH can be mitigated by limiting substrate availability via intake restriction, and d) that cattle transition back to forage diets rapidly. To obtain information about potential biological limits to feeding highly fermentable diets to beef cows, the objectives of this study were to evaluate interactions between dietary corn inclusion and intake on ruminal parameters indicative of SARA during transition to concentrate diets, after a period of adaptation, and during transition back to a forage diet.

MATERIALS & METHODS

Six crossbred steers (mean BW 449 ± 26 kg) fitted with ruminal cannulae were used in a 6 × 6 Latin square experiment with a 3 × 2 factorial treatment arrangement. Diet energy density was altered (Table 1) by substituting dry rolled corn for wheat straw in a TMR, such that corn inclusion in the diets was 32% (**32C**), 48% (**48C**), or 64%

(**64C**). Energy intake levels were designed to meet 75% of the energy requirements (NRC, 2000) for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal NE_m/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal NE_m/kg $EBW^{0.75}$). Thus, among diets, feeding rates were isocaloric per metabolic weight within intake level. Each steer received 200 mg monensin daily while on treatment diets.

Table 1. Formulated ingredient and nutrient composition of treatment diets

Ingredient[a]	32C[b]	48C	64C
	% DM		
Wheat straw	31.86	15.93	0.00
Corn	31.86	47.79	63.72
Distillers' grain	28.12	28.12	28.12
Urea	1.23	1.23	1.23
Molasses	4.19	4.19	4.19
Mineral	2.74	2.74	2.74
Diet components[c]			
CP, %	16.5	17.5	18.5
TDN, %	69.0	76.5	84.0
ME, Mcal	2.49	2.76	3.03
Net energy (NE_m), Mcal	1.58	1.83	2.07
Net energy (NE_g), Mcal	0.98	1.20	1.42

[a]Steers were fed 200 mg monensin daily, while on treatment diets only.

[b]During the concentrate feeding period, steers were fed diets containing 32 (**32C**), 48 (**48C**) or 64 (**64C**) % cracked corn at either low or high intake levels. Intake levels were designed to meet energy requirements (NRC, 2000) for either a 454-kg, mature, dry, open cow (**L**) or for a 390-kg, primiparous cow at peak lactation (6.09 kg/d) and gaining 0.14 kg/d (**H**).

[c]According to NRC model estimates.

Prior to experiment initiation, steers were housed in common with *ad libitum* access to ryegrass hay. Steers were moved into individual stalls in an enclosed barn 3 days prior to treatment application. For 3 d immediately prior to experiment initiation, steers were fed ryegrass hay *ad libitum* to establish benchmark voluntary intake of the hay.

Treatment diets were applied on d 1-10 of each period. On d 11-14 of each period, steers were fed ryegrass hay at 90% (88.4 g/kg $EBW^{0.75}$) of observed voluntary intake established before experiment initiation.

Steers were fed daily at 0600 h. Throughout experimental periods, feed refusals (if present) were collected daily. Steers were given *ad libitum* access to fresh water throughout the experiment.

On d 1 and 2 of each treatment application period, immediate ruminal responses to treatment diets were characterized. A suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) was used to collect a total of 50 mL of fluid in equal portions from anterior, midline and posterior locations of the rumen prior to feeding (0 h) and at 2, 4, 6, 9 and 12 h after feeding. A portable pH meter with a combined electrode (VWR SympHony, Radnor, PA) was used to measure pH of ruminal fluid at the time of sampling. Sub-samples of ruminal fluid (8 mL) were combined with 2 mL of 25% *m*-phosphoric acid and then frozen at -20°C for subsequent determination of VFA concentrations. Samples of ruminal fluid were thawed and centrifuged at 20,000 × *g* for 20 min, and VFA concentrations were measured using gas chromatography as described by Vanzant and Cochran (1994).

On d 10, adapted ruminal fermentation was characterized by collecting ruminal fluid prior to feeding (0 h) and at 2, 4, 6, 9 and 12 h after feeding. Collection, pH measurement and preservation procedures were identical to those described for d 1 and 2.

Ruminal responses to re-feeding a forage diet were characterized on d 11-14 by collecting ruminal fluid prior to (0 h), and 4 h after feeding each day. Ruminal fluid collection procedures and pH measurements were identical to those described above.

Time at which pH declined below and returned above threshold pH values of 5.6 or 6.0 was calculated using linear interpolation between time points with observed pH values that bracketed the target value. The difference between predicted time points (transition below and above the threshold) represented duration of time below the threshold value. Area above the curve and under the threshold (**AUT**; pH × min) was estimated by trapezoidal summation between the time pH declined below and returned above threshold values.

Data were analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included intake, steer, period, hour and day. Diet was included as a regression variable. For single responses related to ruminal fluid pH measured on d 1, 2 and 10 (including minimum pH, duration and AUT for pH < 5.6 and 6.0), model terms included intake and diet × intake, with random effects of steer and period.

Ruminal fluid pH and VFA responses measured within d 1, 2 and 10 were analyzed as repeated measures. Model effects included diet, intake, diet × intake, hour, diet × hour, intake × hour and diet × intake × hour, with hour as the repeated variable,

steer within period as the subject, and steer and period used as random effects. Regression parameters were determined for diet effects or interactions including the diet term by removing from the model all remaining terms which included diet. Data from VFA analysis is not shown for d 1 and 2.

Preprandial (immediately prior to feeding) and postprandial (4 h after feeding) ruminal fluid pH responses on days 11 through 14 were analyzed as repeated measures. To determine the rate of adaptation back to forage diets, model terms included diet, intake, diet × intake, day, diet × day, intake × day and diet × intake × day, with day as the repeated variable, steer within period as the subject, and steer and period used as random effects. Data from VFA analysis is not shown for d 11-14.

Second order diet effects were initially included in the models; however, effects were not significant ($P \geq 0.10$) for any responses, and were therefore removed from subsequent models.

RESULTS AND DISCUSSION

Objectives of this experiment were to analyze the effects of dietary corn inclusion and intake level on ruminal fermentation and pH parameters. These observations may be used to refine recommendations for limit-feeding systems both during and after adaptation to high-energy diets, and when returning cattle to a forage diet following a period of limit-feeding a TMR.

Ruminal pH responses during adaptation

No diet × intake × hour interactions were observed ($P \geq 0.24$) for ruminal pH during d 1 or d 2 of feeding concentrate diets. On d 1, an intake × hour interaction was detected ($P < 0.01$; Figure 1). Prior to feeding, pH was similar ($P = 0.14$) between steers fed H or L levels of intake. Immediately following feeding, pH declined across all treatments; the decline was greater ($P < 0.01$) in steers fed H vs. those fed L, such that the magnitude of difference between intake levels increased over time. The rapid decline in ruminal pH upon abrupt transition from a forage to a concentrate diet was expected (Galyean et al., 1992; Brown et al., 2006), as fermentation substrate was provided via fermentable carbohydrate. Greater DMI in steers fed H further increased fermentable carbohydrate supply, which is directly related to acid production, leading to negative impacts on subsequent ruminal pH (Oetzel and Nordlund, 1998).

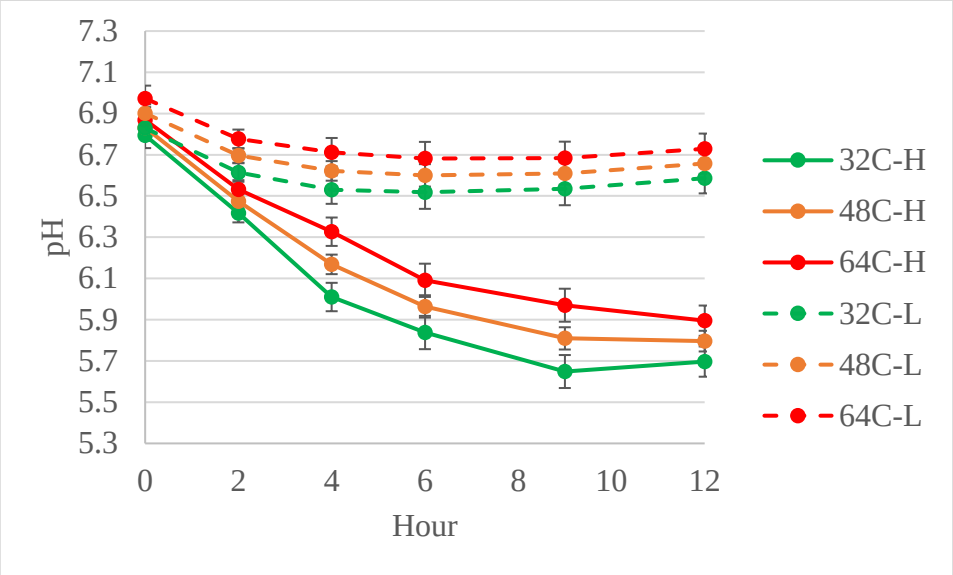

Figure 1. Ruminal pH in the first 24 hours of feeding concentrate diets. Diets contained 32 (**32C**), 48 (**48C**) or 64 (**64C**) % cracked corn at intake levels designed to meet 75 % of NE_m requirements (NRC, 2000) for a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100 % of requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg/d) gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$). Intake × hour interaction was observed ($P < 0.01$), but diet × intake × hour, diet × intake and diet × hour interactions were not significant ($P \geq 0.12$). Main effects of diet, intake and hour ($P < 0.01$) were observed. Regression coefficient for the diet effect = 0.0041 ± 0.0011.

Diet × intake and diet × hour interactions were not significant ($P \geq 0.12$) for pH on d 1. Ruminal pH increased with increased corn inclusion ($P < 0.01$). Grain inclusion moderated the decline in pH following feeding on d 1. Risk of SARA during the adaptation phase is typically perceived to increase with greater energy density (Nagaraja and Lechtenberg, 2007), which is inconsistent with our d 1 responses. Rumen microbial populations adapt rapidly to changes in host diet (Fernando et al., 2010). However, it is possible that fermentation capacity of the microbial population was inhibited for grain particles due to a lack of adaptation at the time of grain introduction. Because diets were isocaloric within intake levels, and because DDG inclusion was held constant across diets, total intake of DDG, and therefore fermentable fiber, increased with decreasing grain inclusion. If microbial populations were more adapted to fiber fermentation following a period of consuming forage diets, then total supply of fermentable substrate and relative fermentation capacity may have been reduced with grain inclusion within an intake level.

Minimum ruminal pH (Tables 2 and 3) was lower in steers fed H vs. those fed L ($P < 0.01$). A diet × intake interaction was observed ($P = 0.03$), but was largely the result of an overall positive effect of corn inclusion on pH, rather than a difference between intake levels.

Table 2. Regression coefficients for ruminal pH parameters in the first 24 hours of steers consuming concentrate diets as a function of dietary intake and corn inclusion

		Estimate[2]		*P*-value		Main effects[3]	
Item	Intake[1]	β_0	β_1	β_0	β_1	Intake	D×I
Minimum pH	H	5.41 ± 0.15	0.007 ± 0.003	< 0.01	0.03	< 0.01	0.03
	L	6.31 ± 0.15	0.005 ± 0.003	< 0.01	0.11		
Duration, pH<6.0, min/d	H	1287 ± 249	-11.2 ± 5.01	< 0.01	0.04	< 0.01	0.10
	L	0.00 ± 249	0.00 ± 5.01	1.00	1.00		
Duration, pH<5.6, min/d	H	204 ± 106	-2.76 ± 2.13	0.07	0.21	0.18	0.45
	L	0.00 ± 106	0.00 ± 2.13	1.00	1.00		
Area, pH<6.0, min×pH/d	H	242 ± 83.4	-1.72 ± 1.66	0.01	0.31	0.03	0.59
	L	0.00 ± 83.4	0.00 ± 1.66	1.00	1.00		
Area, pH<5.6, min×pH/d	H	19.2 ± 15.7	-0.23 ± 0.32	0.23	0.47	0.48	0.76
	L	0.00 ± 15.7	0.00 ± 0.32	1.00	1.00		

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Parameter estimates, β_0 = intercept; β_1 = dietary corn inclusion (%)

[3]*P*-values for main effects of intake level and the intake × corn inclusion interaction

Table 3. Effects of dietary corn inclusion and intake level on ruminal parameters in the first 24 hours of steers consuming concentrate diets

	High intake			Low intake			
Item	32C	48C	64C	32C	48C	64C	SEM
Minimum pH	5.63	5.75	5.86	6.47	6.56	6.64	0.073
Duration, pH<6.0, min/d	928	749	570	0.00	0.00	0.00	104.9
Duration, pH<5.6, min/d	116	71	27	0.00	0.00	0.00	44.0
Area, pH<6.0, min×pH/d	187	160	132	0.00	0.00	0.00	36.4
Area, pH<5.6, min×pH/d	11	7	4	0.00	0.00	0.00	6.5

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Diet energy density was altered by substituting dry rolled corn for wheat straw in a total mixed ration, such that corn inclusion in the diets was 32% (**32C**), 48% (**48C**), or 64% (**64C**).

Fifteen out of 18 observations of steers fed H resulted in pH < 6.0, and 5 out of 18 resulted in pH < 5.6; pH in steers fed L did not go below either threshold on d 1. A tendency for a diet × intake interaction was observed ($P = 0.10$) for duration with pH < 6.0. In steers fed H, the duration below pH 6.0 decreased ($P = 0.04$) with increasing corn inclusion, but pH did not fall below the threshold in steers fed at L, obviating any relationship to corn inclusion ($P = 1.00$). Minimal time below the pH 5.6 threshold resulted in no effects of intake or diet × intake ($P \leq 0.18$) for duration with pH < 5.6. Diet × intake interactions were not significant ($P \geq 0.59$) for AUT for pH < 6.0 or 5.6. Area under pH 6.0 was greater ($P = 0.03$) in steers fed H vs. those fed L, but AUT for pH < 5.6 was not affected by intake ($P \geq 0.23$).

Similar ruminal parameters have been reported in the literature (Gozho et al., 2005; Khafipour et al., 2009). Ruminal cellulolytic activity is inhibited below pH 6.0 (Mould and Ørskov, 1983), resulting in reduced fiber digestion (Calsamiglia et al., 2002). Ruminal pH of 5.6 is a common threshold for the diagnosis of SARA (Cooper and Klopfenstein, 1996).

Owens et al. (1998) suggested a minimum of 720 min/d < pH 5.6 for diagnosis of SARA. None of the steers in the current study approached this metric during d 1. Alternatively, Gohzo et al. (2005, 2006) suggested that SARA can be induced when pH remains below 5.6 for as little as 180 min/d, but that the impact of the condition worsens when pH remains below this threshold for longer periods of time. While three steers (one from 32C-H; two from 48C-H) experienced pH < 5.6 for > 180 min on d 1, the

maximum amount of time pH remained below the threshold in a single steer was 555 min, suggesting that the upset was relatively mild.

Intake restriction affectively mitigated perceived risks from the abrupt introduction of concentrate diets. Ruminal pH declined rapidly in steers fed H; however, the perturbation was not sufficiently severe to induce symptoms of acidosis and with apparently minimal risk of SARA on d 1. Grain inclusion had an opposite effect from our predictions by having slightly beneficial effects on ruminal parameters during the transition.

No interactions among intake, diet, or hour ($P \geq 0.28$) were observed for ruminal pH on d 2 (Figure 2). Unlike d 1, on d 2 preprandial ruminal pH was lower ($P < 0.01$) in steers fed H (6.18 ± 0.043) than in those fed L (6.98 ± 0.043), and remained lower ($P < 0.01$) through h 12. Overall, pH declined ($P < 0.01$) following feeding on d 2, and pH was lowest at h 4 for both levels of intake (5.72 ± 0.053 and 6.58 ± 0.053 for H and L, respectively). Diet did not affect pH on d 2 ($P = 0.11$).

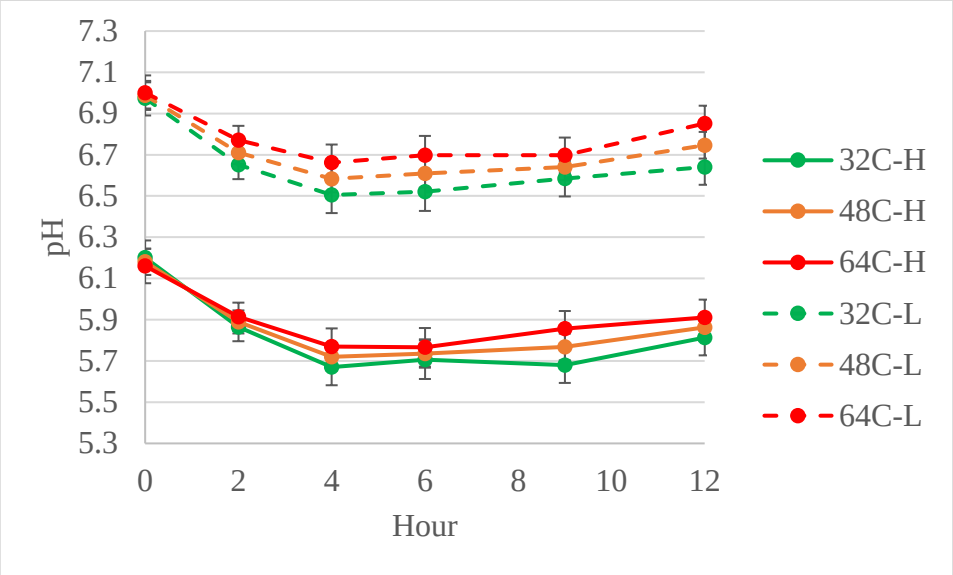

Figure 2. Ruminal pH on d 2 of feeding concentrate diets. Diets contained 32 (**32C**), 48 (**48C**) or 64 (**64C**) % cracked corn at intake levels designed to meet 75 % of NE_m requirements (NRC, 2000) for a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100 % of requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg/d) gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$). No interactions between diet, intake or hour were observed ($P \geq 0.28$). Main effects of intake and hour were observed ($P < 0.01$), but the diet effect was not significant ($P = 0.11$).

Table 4. Regression coefficients for ruminal pH parameters on day 2 of steers consuming concentrate diets as a function of dietary intake and corn inclusion

		Estimate[2]		*P*-value		Main effects[3]	
Item	Intake[1]	β_0	β_1	β_0	β_1	Intake	D×I
Minimum pH	H	5.53 ± 0.17	0.00 ± 0.003	< 0.01	0.37	< 0.01	0.24
	L	6.31 ± 0.17	0.00 ± 0.003	< 0.01	0.15		
Duration, pH<6.0, min/d	H	1186 ± 288	-5.76 ± 5.71	< 0.01	0.32	< 0.01	0.61
	L	0.00 ± 288	0.00 ± 5.71	1.00	1.00		
Duration, pH<5.6, min/d	H	185 ± 154	-0.45 ± 154	0.24	0.88	0.50	0.99
	L	0.00 ± 3.03	0.00 ± 3.03	1.00	1.00		
Area, pH<6.0, min×pH/d	H	202 ± 112	0.33 ± 2.20	0.08	0.88	0.22	0.99
	L	0.00 ± 112	0.00 ± 2.20	1.00	1.00		
Area, pH<5.6, min×pH/d	H	22.6 ± 19.2	-0.09 ± 0.38	0.25	0.81	0.51	0.97
	L	0.00 ± 19.2	0.00 ± 0.38	1.00	1.00		

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Parameter estimates, β_0 = intercept; β_1 = dietary corn inclusion (%)

[3]*P*-values for main effects of intake level and the intake × corn inclusion interaction

Table 5. Effects of dietary corn inclusion and intake level on ruminal parameters on day 2 of steers consuming concentrate diets

	High intake			Low intake			
Item	32C	48C	64C	32C	48C	64C	SEM
Minimum pH	5.62	5.67	5.72	6.46	6.54	6.62	0.082
Duration, pH<6.0, min/d	1002	909	817	0.00	0.00	0.00	126.6
Duration, pH<5.6, min/d	171	164	156	0.00	0.00	0.00	70.8
Area, pH<6.0, min×pH/d	213	218	223	0.00	0.00	0.00	51.0
Area, pH<5.6, min×pH/d	19	18	16	0.00	0.00	0.00	8.9

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Diet energy density was altered by substituting dry rolled corn for wheat straw in a total mixed ration, such that corn inclusion in the diets was 32% (**32C**), 48% (**48C**), or 64% (**64C**).

No diet × intake interactions were observed (P > 0.24) for ruminal pH parameters on d 2 of feeding concentrate diets (Tables 4 and 5). Minimum pH was lower (P < 0.01) in steers fed H vs. steers fed L, but was not affected by corn inclusion (P ≥ 0.15).

Seventeen out of 18 observations of steers fed H experienced pH < 6.0, and 7 out of 18 experienced pH < 5.6 on d 2; pH in steers fed L did not decline below either threshold. Duration with pH < 6.0 was greater (*P* < 0.01) in steers fed H vs. those fed L. However, due to negligible time below the thresholds, effects of intake and diet × intake were not significant for duration of pH < 5.6 (*P* = 0.50) or AUT for pH < 5.6 and 6.0 (*P* ≥ 0.22).

The most severe effects of intake level on ruminal pH during adaptation to concentrate diets were observed on d 2, when pH remained below 6.0 for 909 ± 88 min in steers fed H, possibly due to more rapid or complete fermentation and thus acid production by a more adapted microbial population. One steer from 32C-H experienced pH < 5.6 for 738 min, which is slightly greater than the 12-h threshold proposed by Owens et al. (1998). While pH certainly declined sufficiently to levels capable of reducing fiber digestion in steers fed L, symptoms of acidosis were not observed. However, ruminal pH in steers fed L did not fall below 6.4 during this period (regardless of corn inclusion level), suggesting that the amount of fermentable substrate was likely insufficient to result in excess acid accumulation (Oetzel and Nordlund, 1998; Owens et al., 1998), as it is not anticipated that rate of fermentation was reduced by limiting intake (Galyean et al., 1979).

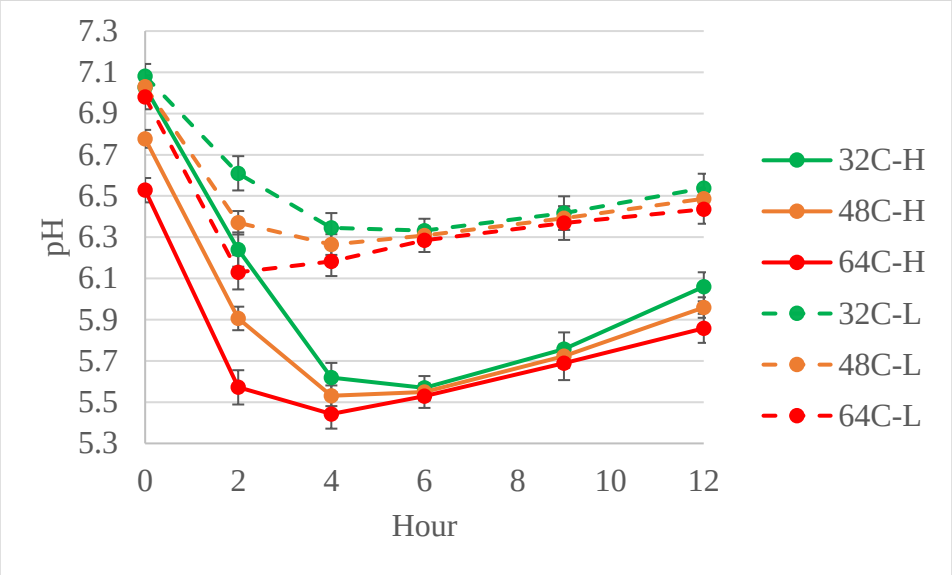

Figure 3. Ruminal pH on d 10 of feeding concentrate diets. Diets contained 32 (**32C**), 48 (**48C**) or 64 (**64C**) % cracked corn at intake levels designed to meet 75 % of NE_m requirements (NRC, 2000) for a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100 % of requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg/d) gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$). Diet × intake × hour, diet × hour and intake × hour interactions were observed ($P \leq 0.01$), but diet × intake interaction was not significant ($P = 0.22$). Main effects of diet, intake and hour were significant ($P \leq 0.01$). See Table 6 for regression parameters.

Table 6. Regression coefficients for hourly ruminal pH on day 10 of steers consuming concentrate diets as a function of dietary intake and corn inclusion

Hour	Intake[1]	Estimate[2]	SEM[3]	*P*-value
0	H	-0.0156	0.0025	< 0.01
	L	-0.0032	0.0025	0.21
2	H	-0.0209	0.0038	< 0.01
	L	-0.0150	0.0038	0.00
4	H	-0.0055	0.0032	0.08
	L	-0.0051	0.0032	0.11
6	H	-0.0013	0.0024	0.60
	L	-0.0015	0.0024	0.54
9	H	-0.0021	0.0037	0.56
	L	-0.0015	0.0037	0.68
12	H	-0.0063	0.0031	0.04
	L	-0.0032	0.0031	0.31

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Parameter estimates = dietary corn inclusion (%)

[3]Standard error of the mean

Table 7. Regression coefficients for ruminal pH parameters on day 10 of steers consuming concentrate diets as a function of dietary intake and corn inclusion

		Estimate[2]		*P*-value		Main effects[3]	
Item	Intake[1]	β_0	β_1	β_0	β_1	Intake	D×I
Minimum pH	H	5.61 ± 0.16	-0.003 ± 0.003	< 0.01	0.31	< 0.01	0.16
	L	6.46 ± 0.16	-0.005 ± 0.003	< 0.01	0.11		
Duration, pH<6.0, min/d	H	203 ± 113	9.13 ± 2.25	0.09	< 0.01	0.22	< 0.01
	L	-8.19 ± 113	0.65 ± 2.25	0.94	0.77		
Duration, pH<5.6, min/d	H	-38.8 ± 113	5.57 ± 2.26	0.74	0.02	0.94	0.07
	L	0.00 ± 113	0.00 ± 2.26	1.00	1.00		
Area, pH<6.0, min×pH/d	H	19.9 ± 65.6	4.03 ± 1.31	0.76	0.01	0.95	0.02
	L	-2.31 ± 65.6	0.10 ± 1.31	0.97	0.94		
Area, pH<5.6, min×pH/d	H	-34.5 ± 28.7	1.45 ± 0.57	0.24	0.02	0.50	0.06
	L	0.00 ± 28.7	0.00 ± 0.57	1.00	1.00		

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Parameter estimates, β_0 = intercept; β_1 = dietary corn inclusion (%)

[3]*P*-values for main effects of intake level and the intake × corn inclusion interaction

Table 8. Effects of dietary corn inclusion and intake level on ruminal parameters on day 10 of steers consuming concentrate diets

	High intake[1]			Low intake			
Item	32C[2]	48C	64C	32C	48C	64C	SEM
Minimum pH	5.51	5.45	5.40	6.29	6.20	6.11	0.067
Duration, pH<6.0, min/d	495	641	788	12.7	23.1	33.6	48.7
Duration, pH<5.6, min/d	139	228	318	0.00	0.00	0.00	48.6
Area, pH<6.0, min×pH/d	149	213	278	0.84	2.42	3.99	28.5
Area, pH<5.6, min×pH/d	12	35	58	0.00	0.00	0.00	12.8

[1]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$).

[2]Diet energy density was altered by substituting dry rolled corn for wheat straw in a total mixed ration, such that corn inclusion in the diets was 32% (**32C**), 48% (**48C**), or 64% (**64C**).

Ruminal responses after adaptation

After 10 d of feeding concentrate diets, diet × intake × hour, diet × hour and intake × hour and interactions were observed ($P < 0.01$) for ruminal pH (Figure 3). Ruminal pH declined in all treatments immediately after feeding ($P < 0.01$); however, the magnitude of decline was greater in steers fed H than in those fed L. Furthermore, the decline in pH was exacerbated by increasing corn inclusion (Table 6), with the effect being more pronounced for H than for L. Overall, ruminal pH decreased with increasing corn inclusion ($P < 0.01$), but the magnitude of difference in the diet effect between H and L was greatest at h 2 and decreased throughout the remainder of d 10.

Minimum ruminal pH on d 10 (Tables 7 and 8) was lower ($P < 0.01$) in steers fed H than in those fed at the L intake level, but the diet × intake interaction was not significant ($P = 0.16$). All observations of steers fed at the H intake level experienced pH below both 6.0 and 5.6 on d 10; however, only 3 out of 18 steers fed at L experienced pH below 6.0, with none falling below 5.6. A diet × intake interaction was observed ($P < 0.01$) for the duration of ruminal pH < 6.0. Duration increased with greater corn inclusion within H ($P < 0.01$), but there was no relationship to corn inclusion when steers were fed at L ($P = 0.77$). A corresponding tendency for a diet × intake interaction was observed ($P = 0.07$) for duration of ruminal pH < 5.6; duration below pH 5.6 was not related to corn inclusion level within L ($P = 1.00$), but duration below pH 5.6 increased with increasing corn inclusion within H ($P = 0.01$).

A diet × intake interaction was observed ($P = 0.02$) for AUT for pH < 6.0. Area increased with greater corn inclusion within H ($P = 0.01$), but was not affected by corn

inclusion in the diet ($P = 0.94$) within L. A tendency for a similar diet × intake interaction was observed ($P = 0.06$) for AUT for pH < 5.6. Area increased with increasing corn inclusion for H ($P = 0.02$), but had no effect on AUT for L ($P = 1.00$).

Restricting intake on d 10 moderated the decline in pH upon feeding, similar to responses observed on d 1 and 2. Zinn (1995) also observed greater ruminal pH and lower total VFA concentration when DMI was restricted, in steers, from 2.4 to 1.6% BW of high-energy diets. However, Montgomery et al., (2004) applied a comparable degree of restriction (2.4 to 1.6% BW), and did not observe an effect on pH. In the Montgomery et al. (2004) experiment, steers consuming 2.4% their BW in DM were actually fed *ad libitum*, potentially resulting in a greater number of meals and slower rate of consumption, resulting in a more stable rumen environment and thus outcomes more comparable to the limit fed scenario (Montgomery et al., 2003). Owens et al. (1998) also noted that cattle consuming small meals were at lower risk for SARA than those eating large meals.

Increasing forage content of a diet is thought to reduce the relative risk for acidosis by reducing starch availability, increasing saliva production, and decreasing the rate and size of meal consumption (Owens et al., 1998). Our measures of ruminal pH on d 10 are consistent with these assertions, as the large decline in pH following feeding at H was somewhat alleviated by increasing forage in the diet.

Khafipour et al. (2009) subjected steers to a grain-based SARA challenge. In their experiment, control steers had a mean pH of 6.17, while challenged steers had a mean pH of 5.97. The control treatment in that study had a pH response similar to H in

the current experiment, but much lower than L. Challenged steers in the Khafipour et al. (2009) study spent a similar amount of time with ruminal pH below 5.6 or 6.0 (279 and 678 min/d, respectively) as steers fed 48C-H (276 and 639 min/d) and 64C-H (294 and 789 min/d) in the current study. However, in both 48C-H and 64C-H, AUT for pH < 5.6 (39 and 53 min × pH/d, respectively) was lower than in those challenged steers (102 min × pH/d) in the previous study (Khafipour et al., 2009. Duration and AUT for pH < 5.6 and 6.0 for steers fed the control diet in Khafipour et al. (2009) were similar to steers fed 32C-H in the present study: no steers fed L in the present study had lower pH than the previous studies' control diets.

In another challenge experiment, Gozho et al. (2006) observed a systemic inflammatory response when duration below 5.6 was greater than 174 min/d, which is less than we observed for 48C-H and 64C-H. They also stated that the severity of SARA in their experiment was milder than in studies by Krajcarski-Hunt et al. (2002), who reported duration and AUT for pH < 5.6 of 594 ± 189 min/d and 228 ± 89 min × pH/d, respectively. In a practical setting, however, cattle limit-fed in a group setting may be at a greater risk for SARA than these data suggest, due to variance in individual rate and degree of intake (Cooper et al., 1999) among pen mates. Episodes of pH remaining below pH 5.6 for 180 min or more were detected (1 from 32C-H, 4 from 48C-H, and 4

Table 9. Effects of dietary intake and corn inclusion level on ruminal VFA concentrations[1] on day 10 of steers consuming concentrate diets[2]

		Diet corn inclusion[4]				P-value						
Item	Intake[3]	32C	48C	64C	SEM	Diet	Intake	Hour	D×I	D×H	I×H	D×I×H
Acetate	H	48.5	46.1	43.7	1.681	0.13	0.06	0.20	< 0.01	< 0.01	< 0.01	< 0.01
	L	42.9	42.7	42.5								
Propionate	H	25.3	28.8	32.4	2.317	< 0.01	0.77	0.42	< 0.01	< 0.01	0.01	0.04
	L	20.6	22.7	24.7								
Isobutyrate	H	0.86	0.83	0.80	0.058	0.52	0.91	0.52	< 0.01	0.01	0.19	0.03
	L	0.94	0.94	0.94								
Butyrate	H	11.6	12.0	12.3	0.956	0.97	0.45	0.45	< 0.01	< 0.01	0.01	0.11
	L	8.08	7.72	7.35								
Isovalerate	H	1.53	1.90	2.27	0.233	< 0.01	0.34	0.48	0.03	0.03	0.30	0.09
	L	1.18	1.71	2.24								
Valerate	H	1.09	1.64	2.18	0.165	< 0.01	0.37	0.01	0.01	0.01	0.78	0.62
	L	0.62	0.69	0.75								
Acetate:	H	1.99	1.73	1.46	0.112	< 0.01	0.92	0.38	< 0.01	< 0.01	0.23	0.23
propionate	L	2.16	2.00	1.84								
Total VFA	H	88.8	91.2	93.6	3.051	0.10	0.09	0.91	< 0.01	< 0.01	< 0.01	< 0.01
	L	74.3	76.4	78.4								

[1]mM concentration

[2]Values reported as least squares means and standard error of the mean.

[3]Energy intake levels were designed to meet 75% of the energy requirements for either a 454-kg, mature, dry, open cow (L; 57.7 kcal/kg EBW0.75) or 100% of the requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg milk/d) and gaining 0.14 kg/d (H; 184 kcal/kg EBW0.75).

[4]Diet energy density was altered by substituting dry rolled corn for wheat straw in a total mixed ration, such that corn inclusion in the diets was 32% (32C), 48% (48C), or 64% (64C).

from 64C-H), but this duration never exceeded 720 min, suggesting that the severity of insult was relatively low (Owens et al., 1998).

In the current experiment, steers fed L were at minimal risk for SARA, by most commonly accepted measures (Owens et al., 1998; Krause and Oetzel, 2006; Khafipour et al., 2009). While symptoms of acidosis were not observed in steers fed at the H intake level, the risk for SARA after 10 d of adaptation appeared to increase with increasing dietary energy density, due to greater duration and AUT for pH < 5.6, with the risk being augmented by increasing corn inclusion.

Diet × intake × h interactions were observed ($P \leq 0.09$) for ruminal acetate, propionate, isobutyrate, isovalerate and total VFA concentrations (Table 9). For molar concentration of acetate, the interaction was driven primarily by a large change in the effect of corn inclusion within H. Prior to feeding, diet had minimal effects on acetate, but between h 4-12 within H, acetate concentration decreased over time; the rate of change was more negative as corn inclusion increased.

The acetate:propionate ratio declined with increasing corn inclusion, but the magnitude of effect was dependent upon intake level and time. The slope across corn inclusion within L was consistent between h 0-6 and approached zero thereafter. Within H, the diet effect became more negative from h 2 to 12.

The diet × intake × h effect for total VFA concentration on d 10 was primarily driven by rapid changes immediately following feeding. At h 2, total VFA concentration increased with increasing corn inclusion, but the size of difference was greater in steers fed H vs. those fed L. However, diet had minimal effects on total VFA concentrations

between h 4-12. Propionate and butyrate concentrations followed similar pattern as total VFA. The remaining VFA concentrations were numerically insignificant, but followed patterns similar to the acetate:propionate ratio.

Ruminal pH responses upon returning to a forage diet

A diet × intake × day interaction was observed ($P < 0.01$) for preprandial ruminal pH between d 11 and 14 (Figure 4). The interaction was primarily driven by immediate re-ranking of treatments between d 1 and 2 of returning to a forage diet. Prior to initial forage feeding on d 11, pH was similar ($P = 0.31$) between steers fed H and L, and decreased with increasing corn inclusion within both H and L intake levels ($P \leq 0.07$). However, 24 h after the return to a forage diet (i.e., preprandial sample on d 12), pH tended to be lower ($P = 0.10$) in steers previously fed L vs. those fed H, and continued to be slightly lower ($P \leq 0.03$) through d 14. Additionally, prior corn inclusion level had no effect ($P > 0.10$) on ruminal pH from d 12 through 14. By d 14 all steers had a pH similar to ($P \geq 0.10$) mean pH at h 0 prior to the start of feeding treatment diets (6.92 ± 0.10), effectively returning to original baseline.

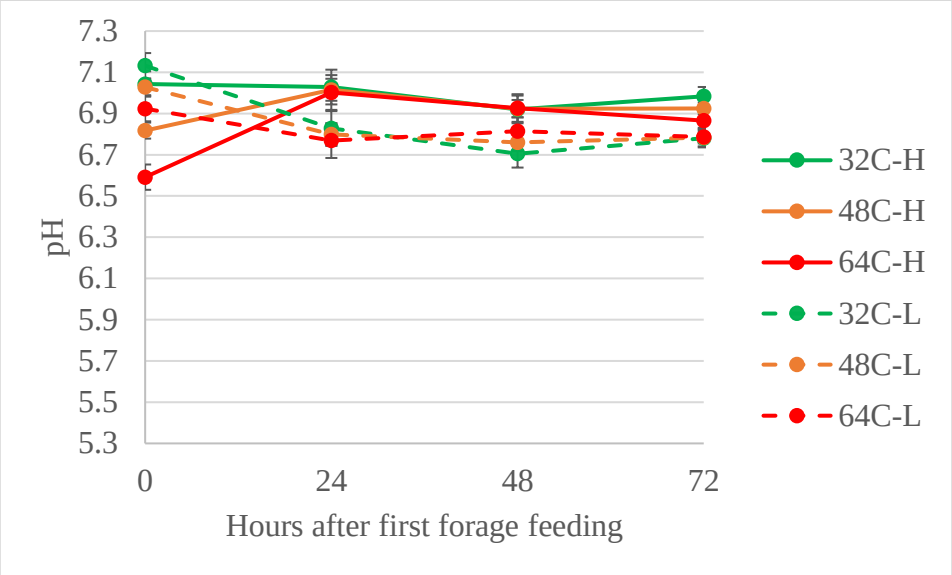

Figure 4. Preprandial ruminal pH of steers consuming a forage diet after a 10-d period of feeding concentrate diets. Concentrated diets contained 32 (**32C**), 48 (**48C**) or 64 (**64C**) % cracked corn at intake levels designed to meet 75 % of NE_m requirements (NRC, 2000) for a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100 % of requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg/d) gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$). Diet × day interaction was observed ($P < 0.01$), and along with a tendency for a diet × intake interaction ($P = 0.06$), but diet × intake × day and intake × day interactions were not significant ($P \geq 0.23$). Main effects of diet, intake and day were all observed ($P < 0.01$). Regression coefficients for the diet × day interactions were: d 11 = -0.0103 ± 0.0021; d 12 = -0.0011 ± 0.0020; d 13 = 0.0018 ± 0.0016; d 14 = -0.0018 ± 0.0012. Regression coefficients for the diet × intake interactions were: H = -0.0058 ± 0.0011; L = -0.0014 ± 0.0011.

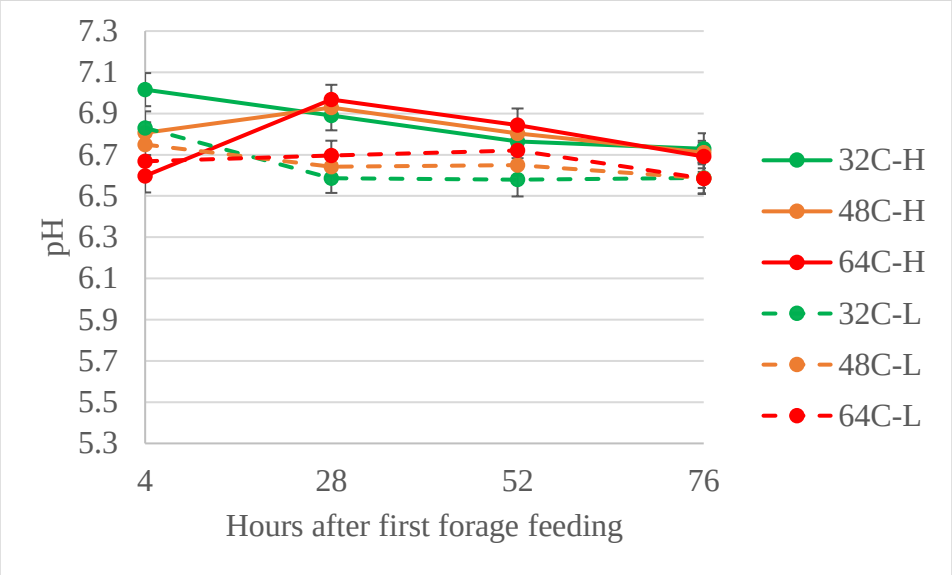

Figure 5. Postprandial ruminal pH of steers consuming a forage diet after a 10-d period of feeding concentrate diets. Concentrate diets contained 32 (**32C**), 48 (**48C**) or 64 (**64C**) % cracked corn at intake levels designed to meet 75 % of NE_m requirements (NRC, 2000) for a 454-kg, mature, dry, open cow (**L**; 57.7 kcal/kg $EBW^{0.75}$**)** or 100 % of requirements for a 390-kg, primiparous cow at peak lactation (6.09 kg/d) gaining 0.14 kg/d (**H**; 184 kcal/kg $EBW^{0.75}$). A diet × day interaction was observed ($P < 0.01$), but diet × intake × day, diet × intake and intake × day interactions were not significant ($P \geq 0.31$). Main effects of intake ($P = 0.04$) and day ($P < 0.01$) were observed, but the effect of diet was not significant ($P = 0.59$). Regression coefficients for the diet × day interactions were: d 11 = -0.0091 ± 0.0028; d 12 = 0.0032 ± 0.0021; d 13 = 0.0034 ± 0.0026; d 14 = -0.0006 ± 0.0013.

A diet × intake × day interaction was observed ($P < 0.01$) for postprandial ruminal pH between d 11 and 14 (Figure 5). Much like preprandial pH, the interaction was primarily driven by immediate changes between d 0 and 1 of returning to a forage diet. Immediately after the first forage feeding, ruminal pH decreased with increased prior corn inclusion ($P < 0.01$), but was similar ($P = 0.11$) between prior intake levels. By d 12, ruminal pH was greater ($P < 0.01$) in steers previously fed H vs. those fed L, with no effect of prior corn inclusion ($P > 0.10$). On d 14, steers previously fed all treatments had pH similar to ($P > 0.05$) mean pH at h 4 prior to the start of feeding treatment diets (6.51 ± 0.04), except for those previously fed 32C-H, which were only slightly greater (6.75 ± 0.07).

After returning to a forage diet ruminal pH, measured prior to feeding, rapidly returned to levels similar to those measured prior to the beginning of the experiment. Although pH, measured 4 h post-feeding, remained greater in all treatments 4 d after returning to a forage diet, the difference was biologically insignificant, suggesting that rumen fermentation can rapidly return to baseline following a period of feeding concentrate diets. While pH during this adaptation stage has not been previously reported, results from Kruse et al. (2010) and Smith et al. (2017) suggested no carryover effects on intake capacity or potential subsequent performance from being previously limit-fed.

CONCLUSIONS

Ruminal pH declines rapidly when concentrate diets are introduced at high intake levels, but the minimal risk of SARA observed at high intakes appears to be mitigated

through intake restriction. After a period of adaptation to concentrate diets, increasing intake increases the rate and extent of decline in ruminal pH after feeding, with effect of corn inclusion augmenting the perturbation. Similar to the adaptation period, restricting intake to our L level appears to mitigate the risk of SARA. Upon returning to a forage diet, cattle appear to transition rapidly with no apparent risks relative to ruminal fermentation.

CHAPTER III

PREDICTING DIETARY ENERGY SUPPLY

INTRODUCTION

Several reports (Schoonmaker et al., 2003; Sawyer and Wickersham, 2013; Trubenbach et al., 2014) have demonstrated potential economic advantages with limit-feeding beef cows, particularly during dry or winter periods, as an alternative to traditional schemes utilizing harvested or stockpiled forages. In addition, limit-feeding a mixed ration offers potential savings through manipulation of maintenance requirements (Freetly and Nienaber, 1998) and increased energy efficiency through inclusion of feed additives (Boardman, 2015).

We have recently observed (Trubenbach et al., 2014; Boardman, 2015) greater energy digestion in cows fed at 80% of maintenance energy requirements compared to those fed at maintenance, and the improvement in energy utilization may be amplified by increasing energy concentration. Others have also reported an inverse relationship between intake and digestion in dairy cattle (Wagner and Loosli, 1967; Colucci et al., 1982; Llamas-Lamas and Combs, 1991). The Dairy Cattle NRC (2001) uses intake, measured in multiples of maintenance, and formulated TDN concentration to estimate a discount factor for DE at a respective intake, with greater formulated TDN concentrations resulting in larger discounts. The current empirical model for predicting energy delivery in beef cattle (BCNRM, 2016) assumes a constant formulated DE concentration for a respective diet. Furthermore, the current mechanistic beef model

(BCNRM, 2016) predicts only slight reductions in energy digestion with greater intakes, with the degree of change being greater in low-energy diets than in high-energy diets.

Studies in beef cattle examining the effects of energy concentration and intake on digestion have primarily drawn conclusions from animals fed well above maintenance levels of intake, and the interactions have not been characterized at sub-maintenance intake levels. Because evidence suggests that intake restriction, particularly with high-energy diets, may result in under-estimation of energy delivery, coupled with potentially over-estimated maintenance requirements, there exists a need to more accurately obtain optimal solutions for precision feeding of limit-fed beef cows. The objectives of the current experiment were 1) to quantify interactions between dietary energy concentration and intake on digestion, and 2) to develop an equation for precise estimation of apparent DE concentration using intake and formulated energy concentration, across a range of diet energy concentrations and intakes realistic to a drylot cow-calf system.

MATERIALS & METHODS

Twenty-five crossbred (3⁄4 Angus × 1⁄4 Nellore) and angus cows were used in a 25 × 6 incomplete Latin square to analyze the effects of dietary energy concentration and intake on nutrient digestion and apparent deviation from expected energy intake. Cows were randomly assigned to seven pens of three or four cows per pen. Cows were individually fed a total mixed ration at approximately 0730 h daily using the Calan gate system.

Treatments were arranged as 5 × 5 factorial with five levels of dietary energy concentration being the first factor. Diets (Table 10) were constructed by substituting dry

Table 10. Formulated ingredient and nutrient composition of treatment diets

NE_m, Mcal/kg	1.09	1.34	1.58	1.83	2.07
Ingredient			% DM		
Wheat straw	63.72	47.79	31.86	15.93	0.00
Cracked corn	0.00	15.93	31.86	47.79	63.72
Dried distiller's grain	28.12	28.12	28.12	28.12	28.12
Urea	1.23	1.23	1.23	1.23	1.23
Molasses	4.19	4.19	4.19	4.19	4.19
Mineral	2.74	2.74	2.74	2.74	2.74
Diet components[a]					
CP, %	14.5	15.5	16.5	17.5	18.5
TDN, %	54.9	61.4	68.9	76.3	83.8
ME, Mcal/kg	1.95	2.22	2.49	2.76	3.03
DE[b], Mcal/kg	2.42	2.70	3.03	3.36	3.69
Chemical composition[c]					
CP, %	11.4	12.0	12.7	13.0	13.2
OM, %	90.5	91.3	92.7	93.3	95.8
ADF, %	46.8	36.0	26.6	16.9	7.2
Acid detergent insoluble ash, %	4.3	3.3	2.3	1.6	0.5
GE, Mcal/kg	4.46	4.43	4.46	4.46	4.52

[a]According to NRC model estimates

[b]DE (Mcal/kg) calculated as: (4.4 × TDN%) / 100%

[c]Chemical analysis

Table 11. Formulated NE_m intake[a] of treatment diets

	Diet				
NE_m Mcal/kg	1.09	1.34	1.58	1.83	2.07
Intake level					
1	53.9	53.9	53.9	53.9	53.9
2	70.0	78.2	86.4	86.4	86.4
3	86.2	102.6	118.8	118.8	118.8
4	102.3	126.9	151.3	151.3	151.3
5	118.5	151.3	183.8	183.8	183.8

[a]kcal/kg $EBW^{0.75}$

rolled corn for wheat straw in a total mixed ration; such that formulated NE_m concentrations (Mcal/kg) in the diets were: **1.09**, **1.34**, **1.58**, **1.83**, and **2.07**. For the

second factor, each diet was fed at one of five levels of intake. The lowest level of NE_m intake (53.9 kcal NE_m/kg $EBW^{0.75}$; Table 11) was designed to meet 70% of NE_m requirements (NRC, 2000) for a 454-kg mature, dry, open cow (4.7 Mcal), which presumably represents the least energetic requirement in a breeding herd managed in a drylot system. The highest level of intake (183.8 kcal NE_m/kg $EBW^{0.75}$) was estimated to meet requirements for a 390-kg primiparous cow at peak lactation (6.09 kg/d) gaining 0.14 kg/d (14.4 Mcal), characterizing the greatest requirement in the same management system.

According to NRC (2000) estimates, predicted maximum DMI of 1.09 and 1.34 Mcal NE_m/kg was less than the intake levels required to achieve an energy intake of 183.8 kcal/kg $EBW^{0.75}$. Therefore, to minimize the likelihood of excessive orts, maximum intake levels were limited to forecasted DMI for 1.09 and 1.34 Mcal NE_m/kg, such that energy intakes were 118.5 and 151.3 kcal NE_m/kg $EBW^{0.75}$, respectively. Three intermediate intake levels were evenly spaced between minimum and maximum intake levels for each of the five diets. Cows were given *ad libitum* access to fresh water throughout the experiment.

Experimental periods were 14 d in length, including 10 d for adaptation to treatments, followed by 4 d of fecal collection for measurement of digestion. Representative feed ingredient samples were obtained and composited within period following feeding on d 10 through 13. On d 11 through 14, fecal samples obtained per rectum were collected 3 times daily and immediately frozen at -20°C for subsequent

analysis. Samples were collected every 8 h, with the sampling time advanced by 2 h each d, such that samples were represented in 2-h intervals post feeding across 24-h.

Samples of feces and ingredients were dried at 55°C in a forced-air oven for 96 h, allowed to air-equilibrate, and weighed to determine partial DM. All dried samples were ground with a Wiley mill to pass a 1-mm screen. Samples were dried at 105°C for DM determination. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450°C. Acid detergent fiber analysis was performed using an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY), and acid detergent insoluble ash (ADIA) was determined as the remaining DM upon combustion of ADF DM residue in a muffle furnace at 450°C. Gross energy (Mcal/kg DM) of ingredient and fecal samples was determined by direct calorimetry using a Parr 6300 Calorimeter (Parr Instrument Company, Moline, IL).

Nutrient digestibility was calculated using the following formula: [1 – (fecal output of nutrient/intake of nutrient)] × 100. Fecal production was calculated by dividing dietary ADIA intake by fecal ADIA concentration. Apparent DE concentrations were calculated by dividing apparent DE intake (gross energy intake – fecal energy) by DMI. Formulated DE concentration was calculated for each diet by the equation:

DE, Mcal/kg = (TDN% × 4.4) / 100%

A second forecast of DE intake (forage-adjusted) was estimated using *in vitro* estimates of DM digestibility of wheat straw samples, which was measured as DM disappearance during a 48-h ruminal incubation period. Digestion of straw *in vitro* was assumed to be synonymous with TDN (%). Using *in vitro* estimates for wheat straw

TDN and NRC (2000) TDN values for remaining ingredients, a forage-adjusted, weighted average TDN value was calculated for each diet. Forage-adjusted DE (Mcal/kg) was calculated from TDN using the equation listed previously. Forage-adjusted DE intake was calculated by multiplying forage-adjusted DE concentration (Mcal/kg) by DMI. Deviation from forecasted DE was calculated as ($Observed_{DE}$ – $Forecasted_{DE}$) / $Predicted_{DE}$ × %, such that positive values represent under-predicted DE and negative values represent over-predicted DE.

Data were analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included animal and experimental period, which were both random terms in the model, while diet NE_m concentration (Mcal/kg) and NE_m intake (kcal/kg $EBW^{0.75}$) were used as continuous variables. Main effects included NE_m concentration, NE_m intake, their interaction, and a second order term for each. If second order terms were not different than zero ($P > 0.05$), they were removed from the model. An ESTIMATE statement was used to estimate empirical responses at measured intake levels.

RESULTS

A diet × intake interaction was observed ($P < 0.01$) for DM digestion (Table 12). Digestion increased slightly with greater intake of the diet containing 1.09 Mcal NE_m/kg, and essentially no change in the 1.34 Mcal NE_m/kg diet; however, in each of the remaining diets, digestion decreased with increasing intake, and the degree of decrease became greater as energy concentration increased. Rate of decline was 0.17% per additional kcal/kg $EBW^{0.75}$ in the highest-energy diet (2.07 Mcal NE_m/kg). Digestion

increased as energy density increased ($P < 0.01$), but the rate of change was greater when the diets were fed at the lowest intake levels (37.1% per Mcal NE_m/kg) versus highest intake levels (11.9% per Mcal NE_m/kg) intake.

Table 12. Effects of dietary energy density and intake on DM digestion[a]

Effect	NE_m[b]	Intake[c]	N × I	N^2	I^2
Estimate	48.9209	0.28	-0.2178	-	-
Standard error	1.1929	0.0289	0.0171		
Pr > |t|	> 0.01	> 0.01	> 0.01	-	-
	Diet NE_m, Mcal/kg				
Intake level[d]	1.09	1.34	1.58	1.83	2.07
1	55.7 ± 1.10	64.8 ± 1.20	73.9 ± 1.35	83.3 ± 1.56	92.1 ± 1.78
2	56.4 ± 1.27	64.6 ± 1.16	71.8 ± 1.17	79.5 ± 1.30	86.6 ± 1.51
3	57.1 ± 1.60	64.3 ± 1.31	69.8 ± 1.16	75.6 ± 1.21	81.1 ± 1.42
4	57.8 ± 2.00	64.1 ± 1.59	67.7 ± 1.33	71.7 ± 1.32	75.7 ± 1.55
5	58.5 ± 2.44	63.8 ± 1.94	65.7 ± 1.61	67.9 ± 1.59	70.2 ± 1.86

[a]Percent
[b]N = NE_m concentration, Mcal/kg
[c]I = NE_m intake, kcal/kg $EBW^{0.75}$
[d]Intake levels specified for each treatment in Table 11

A similar diet × intake interaction was observed ($P < 0.01$) for OM digestion (Table 13). Digestion of OM increased by 0.15 and 0.01% per kcal/kg $EBW^{0.75}$ increase in intake of the diets containing the lowest energy concentrations, 1.09 and 1.34 Mcal NE_m/kg, respectively, but decreased with increasing intake of the remaining diets. Like DM digestion, the rate of decline in OM digestion with increasing intake of higher-energy diets was amplified by greater energy density. Rate of decline was 0.05% in the diet containing 1.58 Mcal NE_m/kg, and 0.18% per kcal/kg $EBW^{0.75}$ in the diet containing 2.07 Mcal NE_m/kg. Digestion of OM increased ($P < 0.01$), with energy density, with the

extent of increase being greater at the low intake level (36.5% per Mcal NE_m/kg) than at the high intake level (2.9% per Mcal NE_m/kg).

Table 13. Effects of dietary energy density and intake on OM digestion[a]

Effect	NE_m[b]	Intake[c]	N × I	N^2	I^2
Estimate	50.309	0.3524	-0.2569	-	-
Standard Error	1.1458	0.02789	0.01651	-	-
Pr > |t|	> 0.01	> 0.01	> 0.01	-	-
	Diet NE_m, Mcal/kg				
Intake level[d]	1.09	1.34	1.58	1.83	2.07
1	58.7 ± 1.05	67.7 ± 1.14	76.6 ± 1.29	85.9 ± 1.49	94.5 ± 1.70
2	61.1 ± 1.21	68.0 ± 1.10	74.9 ± 1.11	82.0 ± 1.24	88.6 ± 1.44
3	63.5 ± 1.52	68.3 ± 1.24	73.1 ± 1.10	78.1 ± 1.14	82.7 ± 1.35
4	65.9 ± 1.91	68.6 ± 1.51	71.4 ± 1.26	74.2 ± 1.25	76.8 ± 1.49
5	68.2 ± 2.34	68.9 ± 1.86	69.6 ± 1.54	70.3 ± 1.52	71.0 ± 1.79

[a]Percent
[b]N = NE_m concentration, Mcal/kg
[c]I = NE_m intake, kcal/kg $EBW^{0.75}$
[d]Intake levels specified for each treatment in Table 11

For ADF digestion (Table 14), a diet × intake interaction was observed ($P < 0.01$). A second order effect for diet energy density was also detected ($P = 0.04$). Digestion of ADF increased with increasing intake of diets containing 1.09 and 1.34 Mcal NE_m/kg, with the average rate of increase being greater in the lowest-energy diet (1.09 Mcal/kg; 0.12% per kcal/kg $EBW^{0.75}$) than that containing 1.34 Mcal/kg (0.02% per kcal/kg $EBW^{0.75}$). For diets containing greater energy values, ADF digestion decreased with increased intake, with the rate of decrease being faster with greater energy concentration. Rate of decrease in the diet containing 1.58 Mcal NE_m/kg was small at 0.07% per kcal/kg $EBW^{0.75}$, but was 0.26% per kcal/kg $EBW^{0.75}$ in the diet

containing 2.07 Mcal NE_m/kg. At the lowest NE_m intake level, a maximum value (55.7%) for ADF digestion was observed within the range of diets, with digestion declining with energy concentration greater than 1.58 Mcal NE_m/kg and below 1.34 Mcal NE_m/kg. At the highest NE_m intake level, ADF digestion declined with energy concentration, with the rate of decline becoming more severe with greater energy concentration.

Table 14. Effects of dietary energy density and intake on ADF digestion[a]

Effect	NE_m[b]	Intake[c]	N × I	N^2	I^2
Estimate	57.857	0.5375	-0.3847	-12.8057	-
Standard Error	11.5944	0.1482	0.08518	6.3351	-
Pr > |t|	> 0.01	> 0.01	0.01	0.04	-
	Diet NE_m, Mcal/kg				
Intake level[d]	1.09	1.34	1.58	1.83	2.07
1	54.2 ± 2.96	55.7 ± 3.14	55.7 ± 2.97	53.9 ± 2.95	50.9 ± 3.99
2	56.1 ± 2.46	56.3 ± 2.51	53.4 ± 2.52	48.4 ± 2.53	42.6 ± 3.17
3	58.0 ± 3.30	56.9 ± 2.54	51.1 ± 2.37	42.9 ± 2.40	34.2 ± 2.75
4	59.9 ± 4.81	57.5 ± 3.20	48.8 ± 2.58	37.4 ± 2.61	25.8 ± 2.92
5	61.9 ± 6.55	58.0 ± 4.20	46.5 ± 3.06	31.9 ± 3.09	17.5 ± 3.59

[a]Percent
[b]N = NE_m concentration, Mcal/kg
[c]I = NE_m intake, kcal/kg $EBW^{0.75}$
[d]Intake levels specified for each treatment in Table 11

A diet × intake interaction was observed ($P < 0.01$) for apparent DE concentration (Table 15). In the diets containing 1.09 and 1.34 Mcal NE_m/kg, apparent DE increased with increasing intake at rates of 7.28 kcal NE_m/kg $EBW^{0.75}$ and 0.51 kcal NE_m/kg $EBW^{0.75}$, respectively. In the higher-energy diets, apparent DE decreased with increasing intake, with the rate of decline being augmented by increasing energy

concentration. Apparent DE decreased at a rate of 2.69 kcal/kcal NE_m/kg $EBW^{0.75}$ with increasing intake of the diet containing 1.58 Mcal NE_m/kg, while this rate of decline was 9.00 kcal/kcal NE_m/kg $EBW^{0.75}$ in the most energy-dense diet (2.07 Mcal NE_m/kg). At lower intakes, apparent DE increased with increasing formulated energy concentration. When intake was 53.9 kcal/kg $EBW^{0.75}$, apparent DE increased with increasing energy concentration at a rate of 59.5% per Mcal NE_m/kg. However, the rate of increase in DE was reduced as intake became greater. At the greatest NE_m intake level, apparent DE declined with greater formulated energy concentration.

Table 15. Effects of dietary energy density and intake on apparent DE concentration[a]

Effect	NE_m[b]	Intake[c]	N × I	N^2	I^2
Estimate	2.2659	0.01743	-0.01274	-	-
Standard Error	0.0586	0.001408	0.000833	-	-
Pr > |t|	> 0.01	> 0.01	.01	-	-
	Diet NE_m, Mcal/kg				
Intake level[d]	1.09	1.34	1.58	1.83	2.07
1	2.66 ± 0.06	3.05 ± 0.06	3.43 ± 0.07	3.84 ± 0.08	4.21 ± 0.09
2	2.78 ± 0.06	3.06 ± 0.06	3.35 ± 0.06	3.64 ± 0.07	3.91 ± 0.08
3	2.89 ± 0.08	3.08 ± 0.07	3.26 ± 0.06	3.45 ± 0.06	3.62 ± 0.07
4	3.01 ± 0.10	3.09 ± 0.08	3.17 ± 0.07	3.25 ± 0.07	3.33 ± 0.08
5	3.13 ± 0.12	3.10 ± 0.10	3.08 ± 0.08	3.06 ± 0.08	3.04 ± 0.09

[a]Mcal/kg
[b]N = NE_m concentration, Mcal/kg
[c]I = NE_m intake, kcal/kg $EBW^{0.75}$
[d]Intake levels specified for each treatment in Table 11

A diet × intake interaction was observed ($P < 0.01$) for percent deviation from formulated DE intake (Table 16). For the diet containing 1.09 Mcal NE_m/kg DE was underpredicted, with degree of under-prediction becoming larger (0.10% per kcal/kg

$EBW^{0.75}$) with increased intake. Apparent DE was also under-predicted in diets containing 1.34 and 1.58 Mcal NE_m/kg, but the magnitude of difference declined with increasing intake of these diets. At low intakes, apparent DE was under predicted for the remaining diets. However, the underestimation became smaller with increasing intake, until becoming negative at the highest intake levels, indicating the model overpredicted DE at high intakes. The deviation declined at a rate of 0.24% per kcal/kg $EBW^{0.75}$ for the diet highest in energy concentration. Under-prediction of DE was greatest for the 1.09 Mcal NE_m/kg diet, and declined with increasing energy concentration ($P < 0.01$). Rate of decline in underprediction of DE was slower at the lowest intake level (3.57% per Mcal NE_m/kg) than at the greatest intake level (42.2% per Mcal NE_m/kg).

Table 16. Effects of dietary energy density and intake on percent deviation from formulated DE intake[a]

Effect	NE_m[b]	Intake[c]	N × I	N^2	I^2
Estimate	12.3192	0.3715	-0.2951	-	-
Standard Error	2.043	0.04705	0.02781	-	-
Pr > |t|	> 0.01	> 0.01	0.01	-	-
	Diet NE_m, Mcal/kg				
Intake level[d]	1.09	1.34	1.58	1.83	2.07
1	16.1 ± 2.08	15.2 ± 2.26	14.3 ± 2.52	13.4 ± 2.85	12.6 ± 3.20
2	17.8 ± 2.38	14.5 ± 2.27	11.2 ± 2.30	7.9 ± 2.50	4.7 ± 2.79
3	19.4 ± 2.89	13.8 ± 2.51	8.1 ± 2.31	2.3 ± 2.36	-3.1 ± 2.63
4	21.0 ± 3.52	13.0 ± 2.92	5.0 ± 2.54	-3.3 ± 2.49	-10.9 ± 2.77
5	22.6 ± 4.21	12.3 ± 3.45	1.9 ± 2.94	-8.9 ± 2.84	-18.8 ± 3.17

[a]Ingredient DE concentration (Mcal/kg) was calculated as: (4.4 × TDN%) / 100%. Diet DE concentration was calculated as a weighted average of ingredient concentrations. Concentration of DE in all ingredients was calculated using NRC (2000) values for TDN.
[b]N = NE_m concentration, Mcal/kg
[c]I = NE_m intake, kcal/kg $EBW^{0.75}$
[d]Intake levels specified for each treatment in Table 11

A diet × intake interaction ($P < 0.01$) was observed for percent deviation from predicted DE values when the TDN value of wheat straw was adjusted based on *in vitro* analysis (Table 17). A second order effect for diet energy density was different from zero ($P < 0.01$). At low levels of energy concentration, DE was slightly over predicted from forage-adjusted forecasts, with the magnitude of difference increasing at greater intakes. However, at low intakes of higher-energy diets, DE was under-predicted, with the magnitude of under-prediction declining with increasing intake. Apparent DE was over-predicted when all diets were fed at high intakes, but the effect of intake magnified with increasing energy concentration. In the diet containing 1.09 Mcal NE_m/kg the rate of change was less than 0.01% per kcal/kg $EBW^{0.75}$, becoming 0.19% per kcal/kg

Table 17. Effects of dietary energy density and intake on percent deviation from formulated DE intake after adjustment for forage *in vitro* DM digestibility[a]

Effect	NE_m[b]	Intake[c]	N × I	N^2	I^2
Estimate	-15.3349	0.2037	-0.1907	12.6779	-
Standard Error	10.1019	0.1241	0.0713	5.3633	-
Pr > |t|	0.13	0.10	0.01	0.01	-

	Diet NE_m, Mcal/kg				
Intake level[d]	1.09	1.34	1.58	1.83	2.07
1	-1.9 ± 3.28	-0.6 ± 3.51	2.2 ± 3.46	6.7 ± 3.44	12.3 ± 4.05
2	-2.0 ± 3.00	-2.3 ± 3.15	-1.0 ± 3.21	1.9 ± 3.19	6.0 ± 3.48
3	-2.1 ± 3.54	-4.0 ± 3.18	-4.2 ± 3.13	-2.9 ± 3.11	-0.2 ± 3.20
4	-2.3 ± 4.61	-5.6 ± 3.58	-7.4 ± 3.24	-7.7 ± 3.21	-6.5 ± 3.29
5	-2.4 ± 5.94	-7.3 ± 4.26	-10.6 ± 3.52	-12.5 ± 3.49	-12.8 ± 3.71

[a]Forage dry matter digestibility was measured in vitro, which was used to represent adjusted TDN. Ingredient DE concentration (Mcal/kg) was calculated as: (4.4 × TDN%) / 100%. Diet DE concentration was calculated as a weighted average of ingredient concentrations. Concentration of DE in all ingredients except wheat straw was calculated using NRC (2000) values for TDN.

[b]N = NE_m concentration, Mcal/kg

[c]I = NE_m intake, kcal/kg $EBW^{0.75}$

[d]Intake levels specified for each treatment in Table 11

$EBW^{0.75}$ in the diet greatest in energy concentration. When the diets lowest in energy concentration were fed at the lowest intake level, DE was slightly over-predicted; when NE_m increased to 1.58 Mcal/kg, DE was underpredicted, and the magnitude of under-prediction increased, with the rate of increase becoming greater as energy concentration increased. At the greatest intake level, deviation became greater with increasing energy concentration, with the rate of decline being moderated as energy concentration increased.

DISCUSSION

Experiment objectives were 1) to quantify interactions between dietary energy concentration and intake on digestion and 2) to generate an equation for precise estimation of DE intake across a range of diet energy concentrations and intakes realistic in a drylot cow-calf system. Accordingly, empirical responses were not necessarily of primary interest; more importantly, the response surface parameterized by treatment application is useful for applicable interpretation.

The accuracy with which TDN and/or DE are predicted has a substantial effect on forecasts of energy delivery. The published feed composition table (BCNRM, 2016) contains tabular nutrient values intended to be used with both level 1 and 2 solutions. The level 1 solution uses a weighted average approach to calculating diet TDN, using ingredient values predicted either from previous digestion experiments or from equations used by commercial laboratories. Using tabular estimates of TDN causes discrepancy because the values are not discounted for level of intake above maintenance (Tedeschi et al., 2002). The BCNRM (2016) proposes two options for TDN adjustment: 1) use the

mechanistic level of solution, or 2) use equations developed by Tedeschi et al. (2005) to estimate TDN based on chemical analysis and a predicted discount for concentrate and forage fractions with respect to multiples of intake above maintenance.

Discount equations reported by Tedeschi et al. (2005) were developed using the level 2 solution of the Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004), the same system used to predict TDN in the level 2 solution of BCNRM (2016), which is based on feed carbohydrate fractions and their theoretical rates of digestion and passage. Using chemical composition values from the NRC (2000), TDN was predicted at maintenance (1×), 2×, and 3× levels of DMI in the CNCPS for every ingredient in the feed composition library at that time. Discount equations were estimated by regressing these predicted TDN values on DMI for both concentrate and forages. Results suggested a 5.0% discount in TDN per multiple of maintenance intake for forages and only a 2.3% discount for concentrates.

These discount rates are distinctly different from the discount factor in the dairy NRC (2001), which discounts diets high in formulated TDN more severely than those containing lower formulated energy concentration. The Dairy NRC (2001) concluded from numerous feeding trials (Moe et al., 1965; Wagner and Loosli, 1967; Tyrrell and Moe, 1972) that the rate of decline in TDN with intake is a function of formulated TDN at maintenance (TDN_{1X}): TDN percentage unit decline = $0.18 \times TDN_{1X} - 10.3$, ($r^2$ = 0.85), with the rate of decline being greater in high-TDN diets. This equation was converted so that a percentage discount could be applied to DE:

$$\text{Discount} = \{TDN_{1X} - [(0.18 \times TDN_{1X}) - 10.3] \times \text{Intake}\} / TDN_{1X}$$

Diets with TDN less than 60% are not discounted for intake level. If maintenance intake equals 77 kcal/kg $EBW^{0.75}$, DE would be projected by the dairy NRC (2001) to decline at rates of 0.0, 1.2, 3.1, 4.5 and 5.7% per multiple of maintenance for diets containing 1.09, 1.34, 1.58, 1.83 and 2.07 Mcal NE_m/kg, respectively. When expressed as a percentage of forecasted DE, apparent DE increased at rates of 23.1 and 1.5% per multiple of maintenance for diets containing 1.09 and 1.34 Mcal NE_m/kg, and declined at rates of 6.9, 13.9 and 19.1% for diets containing 1.58, 1.83 and 2.07 Mcal NE_m/kg, respectively.

Zinn (1995) observed increases in apparent DE of 2.5 and 6.2% when intake of diets containing 74% dry rolled or steam flaked corn, respectively, was reduced from 100.6 to 69.8 g DM/kg $EBW^{0.75}$, although ME intake was not affected by treatment, due to greater proportional methane production in low-intake steers.

Although the absolute rates of change in apparent DE were more profound than expected in the current experiment, the perception that the rate of decline in DE with intake becomes greater with increasing energy concentration is in good agreement with empirical evidence presented by the dairy NRC (2001).

A greater decline than anticipated in digestion of the high-energy diets could have been caused by the effects of increasing inclusion and total intake of non-structural carbohydrate on fiber digestion. Feeding high-grain diets typically results in reduced rumen pH (Johnson et al., 1974; Britton and Stock, 1987; Rustomo et al., 2006), with the effects being exacerbated by greater intake (see Chapter II) and grain processing (Yang et al., 2001).

When grains are fed at high intakes, rate of fermentation increases more than rate of absorption, due to increased substrate availability, causing pH to decline faster and to a greater extent (see Chapter II). Rumen cellulolysis is inhibited below pH 6.0-6.1 (Mould and Ørskov, 1983), a threshold below which pH has been observed in matching diets fed at equal intakes (see Chapter II). Furthermore, as starch availability increases, the negative effects on fiber fermentation appear to be exacerbated in diets containing low-quality forage, including straw (Brown, 1966; Mould and Ørskov, 1983). Tyrrell and Moe (1975) suggested that the cell wall fractions of the diet have greater reductions in digestion at high intakes than soluble components. This supports large reductions in ADF digestion in high-energy diets fed in the current study. However, total reductions in DE cannot be accounted for by fiber digestion alone, especially in diets containing little to no straw.

Although starch digestion was not measured in the current experiment, it is probable that a major component of the observed reduction in DE concentration can be attributed to losses from fecal starch. Galyean et al. (1979) observed starch digestion decreased from 99.6% to 93.8% and 90.4% when intake was increased from 1.00 to 1.67 and 2.00 times maintenance intake, respectively. Intake of NE_m was similar (87.5, 146.1, and 182.4 kcal/kg $EBW^{0.75}$, respectively) to mid- and high-intake groups observed in the current experiment.

Russell et al. (1981) also reported a tendency for total tract starch digestion to decrease from 81.4 to 76.4 and 76.0% when intake of corn silage-based diets was increased from 1 to 2 and 3 times maintenance, respectively. Wheeler et al. (1975) tested

for interactions between forage:concentrate ratio and intake level on starch digestion. Total starch digestion was not affected by forage:concentrate, but was reduced substantially when intakes were increased from maintenance to lactation levels. However, the authors concluded that because starch represented a larger proportion of DMI in diets with lower forage:concentrate, that reduced starch digestion accounted for more of the total depression in digestible DM as the portion of concentrate increased, which is likely consistent with the current experiment.

Wheeler et al. (1975) and Colucci et al. (1982) reported that kernels of grain were present in feces from cows fed at lactation levels of intake, while they were minimal or lacking when the same diets were fed at maintenance intake levels. These authors also reported that the difference in fecal grain kernel presence between intake levels increased with inclusion of grains in the diets. Larger depressions in digestion of low-forage diets are likely due to additive effects of reduced feed mastication, reduced rumen retention and fermentation time, and increased starch escape through the lower tract due to incomplete physical assimilation (Colucci et al., 1982).

Apparent DE concentration of the diet containing the most wheat straw was in good agreement with forage-adjusted forecasts, but deviated significantly from original NRC (2000) estimates. Results suggest an under-under prediction for wheat straw digestion, which is not surprising, as variance in digestibility can be quite high in wheat straw (Acock et al., 1978) and other forages (Oba and Allen, 1999). The accuracy of forage-adjusted estimates demonstrates the importance of *in vitro* tests in amending

estimates of energy intake when information about actual ingredients is lacking, especially when forages are fed.

CONCLUSIONS

Interactions between formulated energy concentration and level of intake appear to affect energy digestion in limit-fed cattle, such that intake restriction causes improvement in digestion in high-grain diets, with the degree of improvement becoming greater with increased grain inclusion. The current level 2 model for predicting energy delivery in beef cattle (BCNRM, 2016) attempts to correct for reduced digestion at intakes above maintenance; however, this model assumes greater increases in theoretical rate of passage for forage ingredients, leading to greater estimated reductions in digestion for those fractions. Empirical data suggests that large energy losses occur from undigested fiber and starch when diets high in minimally processed or unprocessed grains are fed at high intakes. Alternatively, when high-energy diets are limit-fed to maintenance or sub-maintenance levels of intake, more complete digestion of grains and fiber components leads under estimation of DE intake. We advise that users should not extrapolate energy delivery beyond the range of intake levels measured in the current experiment.

CHAPTER IV

EFFECTS OF DIETARY ENERGY DENSITY AND INTAKE ON ENERGY REQUIREMENTS

INTRODUCTION

Limit-feeding a total mixed ration to beef cows offers potential opportunities for energy and cost savings (Schoonmaker et al., 2003; Sawyer and Wickersham, 2013; Trubenbach et al., 2014). We recently observed (Trubenbach et al., 2014) reduced heat production and improved energy utilization with increased dietary energy density in limit-fed beef cows, suggesting that part of the enhancement could be attributed to a reduction in maintenance requirements. Others have reported similar improvements in energy efficiency with increased dietary energy density (Swingle et al., 1979; Sawyer et al., 2004; Trubenbach et al., 2014). Feed restriction has been shown to reduce maintenance requirements (Jenkins and Ferrell, 1997; Freetly and Nienaber, 1998). These effects of reducing DMI may be augmented by increasing dietary energy density by further reducing splanchnic tissue mass and metabolism (Reynolds et al., 1991; McLeod and Baldwin, 2000), but the effects on maintenance requirements have not been quantified across a range of applicable diets in gestating beef cows. We hypothesize that increasing dietary energy density and restricting intake reduce total heat production and maintenance requirements in gestating beef cows. The experimental objectives were: 1) estimate maintenance requirements (NE_m) as a function of dietary energy density 2)

evaluate effects of altering energy intake and dietary energy density of maternal diet on postnatal calf performance.

MATERIALS & METHODS

The experimental protocol was approved by the Agricultural Animal Care and Use Committee of Texas A&M Agrilife Research.

Fifty-six pregnant, crossbred ($^{3}/_{4}$ Angus × $^{1}/_{4}$ Nellore) and angus cows (393 ± 34.20 kg) were used in an experiment designed to examine effects of dietary energy concentration and intake on energy utilization. Cows were stratified by day of gestation and BW (collected approximately one month prior to the experiment) and randomly assigned into 14 pens of four head each. Adjacent pens (blocks) contained one cow from each treatment. Treatments were arranged as 4 × 2 factorial with four levels of dietary energy concentration and two levels of intake. Diets (Table 18) were constructed by substituting dry rolled corn for wheat straw in a total mixed ration; such that corn concentration in the diets were approximately: **16%** (1.335 Mcal NE_m/kg), **32%** (1.580 Mcal NE_m/kg), **48%** (1.825 Mcal NE_m/kg), and **64%** (2.070 Mcal NE_m/kg). For the second factor, each diet was fed at restricted (**R**) or maintenance (**M**) levels of intake. Intake requirements of each diet (NE_m equivalents) were estimated per NRC (2000) model using inputs for a dry, 454-kg cow, 225-d in gestation. Intake of M was designed to meet predicted intake requirements for each diet, while R was estimated to meet only 75% of predicted intake requirements. Cows were fed individually at approximately 0730 h daily using a Calan gate system.

Table 18. Formulated ingredient and nutrient composition of treatment diets

	Diet			
Ingredient[1]	16C	32C	48C	64C
Wheat straw	47.79	31.86	15.93	0.00
Cracked corn	15.93	31.86	47.79	63.72
Dried distiller's grain	28.12	28.12	28.12	28.12
Urea	1.23	1.23	1.23	1.23
Molasses	4.19	4.19	4.19	4.19
Mineral	2.74	2.74	2.74	2.74
Diet components[2]	DM basis			
CP, %	15.5	16.5	17.5	18.5
TDN, %	61.0	69.0	76.5	84.0
ME, Mcal/kg	2.22	2.49	2.76	3.03
NE_m, Mcal/kg	1.34	1.58	1.83	2.07
NE_g, Mcal/kg	0.76	0.98	1.20	1.42
Formulated treatment intake	kcal/kg $EBW^{0.75}$			
Restricted	74.9	76.0	76.8	77.5
Maintenance	99.8	101.4	102.4	103.4

[1]Dry matter basis

[2]According to NRC model estimates; see Chapter III for complete analysis

Total requirements were estimated by summing NE_m equivalent requirements for maintenance and pregnancy:

$$NE_{ma} = 0.077 \times EBW^{0.75}$$

$$EBW = SBW \times 0.891$$

$$SBW = BW \times 0.96$$

$$NE_{preg} = [CBW \times (0.4504 - 0.0000766t) \times e^{(0.03233-0.0000275t)t}] / 1000 * k_m$$

$$k_m = NE_m/ME$$

Where:

NE_{ma} = NE requirement for maintenance, Mcal

EBW = empty body weight, kg

SBW = shrunk body weight, kg

BW = body weight, kg, 454 kg

NE_{preg} = NE_m equivalent requirement for pregnancy, Mcal

CBW = calf birth weight, 33 kg

t = days in gestation, 225 d

NE_m = NE_m concentration, Mcal/kg

ME = ME concentration, Mcal/kg

Cow BW was collected every other week, and rib fat thickness was measured via ultrasonography every 28 d. After termination of the trial, all cows were placed on a common pasture in anticipation of calving and allowed to graze while being fed a protein supplement, such that all were managed as a single group following the experimental period. Birthdate, calf BW, and calf sex were recorded within 24 h of parturition. At weaning (227 d after the end of the experimental period), cow BW and body condition score (**BCS**), and calf age and BW were collected. Pregnancy determination was made at pre-weaning, 20 d prior to weaning.

This study was conducted alongside a digestion trial (see Chapter III), which consisted of a group of cohort females that were housed in the same barn during the same period. Diets in both experiments were constructed from the same ingredients, and intake levels in the current experiment were within the range of intakes measured in the digestion experiment. Results were extrapolated from the accompanying experiment to estimate DE concentration in the current experiment. A model developed in our

laboratory (see Chaper III) used to estimate DE concentration as a function of formulated NE_m concentration and intake:

$$DE = 2.2659 \times NE_m + 0.01743 \times Intake - 0.01274 \times NE_m \times Intake$$

Where:

DE = digestible energy concentration, Mcal/kg

NE_m = formulated NE_m concentration, Mcal/kg

Intake = formulated NE_m intake, kcal/kg $EBW^{0.75}$

For the purposes of estimating DE concentration, initial BW was used to calculate EBW using the equations listed above. Metabolizable energy concentration was estimated for each diet by multiplying DE by 0.82 (NRC, 2000).

A calculated measure of BCS was estimated every 28 d using a regression equation (rBCS) developed from observations of fat thickness corresponding to observed BCS (Herd and Sprott, 1998):

$$rBCS = -1.2927x^2 + 6.0916x + 2.2114$$

Where:

x = Rib fat thickness (cm) determined by ultrasound

Equations published in NRC (2000) were used to calculate empty body energy. Body energy (BE) was calculated as:

$$BE\ (Mcal) = 9.4 \times TF + 5.7 \times TP$$

Where:

TF = total fat, kg

TP = total protein, kg

Body components were calculated as:

$$TF = AF \times EBW$$

$$TP = AP \times EBW$$

Where:

AF = proportion of empty body fat

AP = proportion of empty body protein

Body composition was estimated using the following equations:

$$AF = 3.768 \times rBCS$$

$$AP = 20.09 - 0.668 \times rBCS$$

$$MW = BW - GU$$

Where:

MW = maternal body weight, kg

GU = gravid uterine weight, kg

Gravid uterine weight was estimated each time BW was collected to adjust MW using an equation reported by Ferrell et al. (1976):

$$GU = CBW \times 19.32e^{0.02t - 0.0000143t\text{^}2} / 1000$$

RE and HE were calculated for each period as:

$$RE_{total}, Mcal = (BE_f + UE_f) - (BE_i + UE_i)$$

$$HE_{total}, Mcal = MEI_{total} - RE_{total}$$

Where:

BE_f = final body energy, Mcal

BE_i = initial body energy, Mcal

UE_f = final gravid uterine energy, Mcal

UE_i = initial gravid uterine energy, Mcal

MEI_{total} = total metabolizable energy intake, Mcal.

Gravid uterine energy was estimated (BCNRM, 2016) at each period to estimate energy retention in the gravid uterus:

$$UE = CBW \times 1.811e^{0.03233t - 0.0000275t\ ^{\wedge}2} / 1000$$

Daily RE, HE and MEI were calculated for each period by dividing RE_{total}, HE_{total} and MEI_{total} by d within the period. Results for RE, HE and MEI are reported in kcal/kg $EBW^{0.75}$. Average $EBW^{0.75}$ was calculated as $[(\text{initial EBW} + \text{final EBW}) / 2]^{0.75}$.

Maintenance level of intake for metabolizable energy (ME_m) was calculated for both H and L using a linear regression of the means of RE on MEI. The linear functions representing each diet were solved for RE = zero; the solution of which represented ME_m for the respective diet.

Fasting heat production was estimated using the linear regression of the means of log (HE) on MEI. The linear functions representing each diet were solved for MEI = zero; the solution of which represented the estimate of fasting heat production (FHP) for each respective diet.

Data were analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included diet, intake, block, day, period and cow. Changes in maternal BW and backfat thickness and estimates of total RE were analyzed using the repeated measures technique. Model terms included diet, intake, day, diet × intake, diet

× day, intake × day and diet × intake × day. Block served as the random effect. Day was used as the repeated variable, with unstructured covariance and cow used as the subject. The repeated measures technique was also used for measures of daily RE and heat production, along with difference between observed and predicted RE, with model terms including diet, intake, period, diet × intake, diet × period, intake × period and diet × intake × period, a random block effect, cow used as the subject, and an unstructured variance. For measures of DM and energy intake, birthweight, postnatal cow and calf performance and total difference between observed and predicted RE, model terms included diet, intake and diet × intake, with a random block effect.

Requirements were predicted retrospectively, using measured calf birth weight, and average BW and d in gestation within each period as predictor variables in the equations listed above. Estimated RE was calculated by subtracting these retrospective estimates from observed NE_m intake. The difference between observed and predicted RE was then estimated for each period. Total difference in observed and predicted RE was calculated by adding the differences from each period.

RESULTS

A diet × intake interaction was observed ($P = 0.03$) for DMI (Table 19), with DMI declining as corn inclusion increased at both levels of intake, but at different rates. At the R level of intake, DMI declined ($P < 0.01$) with corn inclusion, while at the M intake level intake declined at a greater rate, although this rate of changed declined as corn inclusion increased ($P \leq 0.05$).

Table 19. Dry matter and energy intake of cows fed diets differing in corn inclusion at or below maintenance intake levels

		Diet[1]					*P*-value		
Item		16C	32C	48C	64C	SEM	Diet	Intake	D×I
DMI, kg	R intake[2]	3.84	3.36	2.82	2.59	0.088	< 0.01	< 0.01	0.03
	M intake[23]	5.06	4.41	3.67	3.45				
DMI, g/kgEBW$^{0.75}$	R intake[23]	48.0	40.5	34.9	31.1	0.328	< 0.01	< 0.01	< 0.01
	M intake[23]	63.6	54.1	47.5	42.0				
DE, Mcal/kg	R intake[2]	3.05	3.41	3.76	4.12	0.003	< 0.01	< 0.01	< 0.01
	M intake[2]	3.06	3.35	3.63	3.91				
DE intake, kcal/kgEBW$^{0.75}$	R intake[23]	146	138	132	128	1.038	< 0.01	< 0.01	< 0.01
	M intake[23]	195	181	172	164				
ME, Mcal/kg	R intake[2]	2.50	2.79	3.09	3.37	0.002	< 0.01	< 0.01	< 0.01
	M intake[2]	2.51	2.75	2.98	3.21				
ME intake, kcal/kg EBW$^{0.75}$	R intake[23]	120	113	108	105	0.851	< 0.01	< 0.01	< 0.01
	M intake[23]	160	149	142	135				
NEm, Mcal/kg	R intake[23]	1.61	1.86	2.10	2.34	0.002	< 0.01	< 0.01	< 0.01
	M intake[23]	1.62	1.82	2.01	2.20				
NEm intake, kcal/kg EBW$^{0.75}$	R intake[2]	77.2	75.3	73.5	72.6	0.555	< 0.01	< 0.01	< 0.01
	M intake[2]	103	98.4	95.7	92.5				

[1]Cracked corn inclusion of each diet was 16% (**16C**), 32% (**32C**), 48% (**48C**) and 64% (**64C**) on a DM basis. Designed to deliver maintenance (103.1 kcal NE_m/kg EBW$^{0.75}$; **M**) or 75% of maintenance (77.3 kcal NE_m/kg EBW$^{0.75}$; **R**) intake levels. Values reported are least squares means with standard error of the means.

[2]Linear effect ($P \leq 0.05$) within intake level

[3]Quadratic effect ($P \leq 0.05$) within intake level

When expressed relative to $EBW^{0.75}$, an interaction was observed ($P < 0.01$) with rate of decline across corn inclusion differing between intake levels. Quadratic effects of corn inclusion were observed ($P \leq 0.05$) at both levels of intake, with the rate of decline decreasing with corn inclusion, but more severely for M.

Diet × intake interactions were observed ($P < 0.01$) for all measures of dietary energy concentration. For both DE and ME, linear effects of corn inclusion were observed ($P \leq 0.05$) at both levels of intake, with the rate and extent of increase in energy concentration across corn inclusion being greater for R. Quadratic effects of corn inclusion were observed at both levels of intake for NE_m concentration ($P < 0.05$). While the overall rate and extent of increase in energy concentration across corn inclusion was greater for R, the rate of increase across corn inclusion slowed with greater corn inclusion at both intake levels. Diet × intake interactions were observed ($P < 0.01$) for all measures of dietary energy intake. By design, energy intake was greater for M ($P < 0.01$), but for DE and ME intake, energy intake declined with corn inclusion at decreasing rates ($P \leq 0.05$) as corn inclusion increased, with the overall magnitude and rate of decline being greater for M. Intake of NE_m declined linearly ($P \leq 0.05$) with increasing corn inclusion, but the rate of decline was greater for M.

Diet × intake × day ($P = 0.03$), diet × day ($P < 0.01$) and intake × day ($P < 0.01$) interactions were all observed for change in maternal BW (Figure 6), but the diet × intake interaction was not significant ($P = 0.18$). The three-way interaction was driven by a linear effect of diet within the R intake level ($P = 0.02$) at d 28, where loss in maternal BW decreased with corn inclusion. No other tests for linear, quadratic or cubic

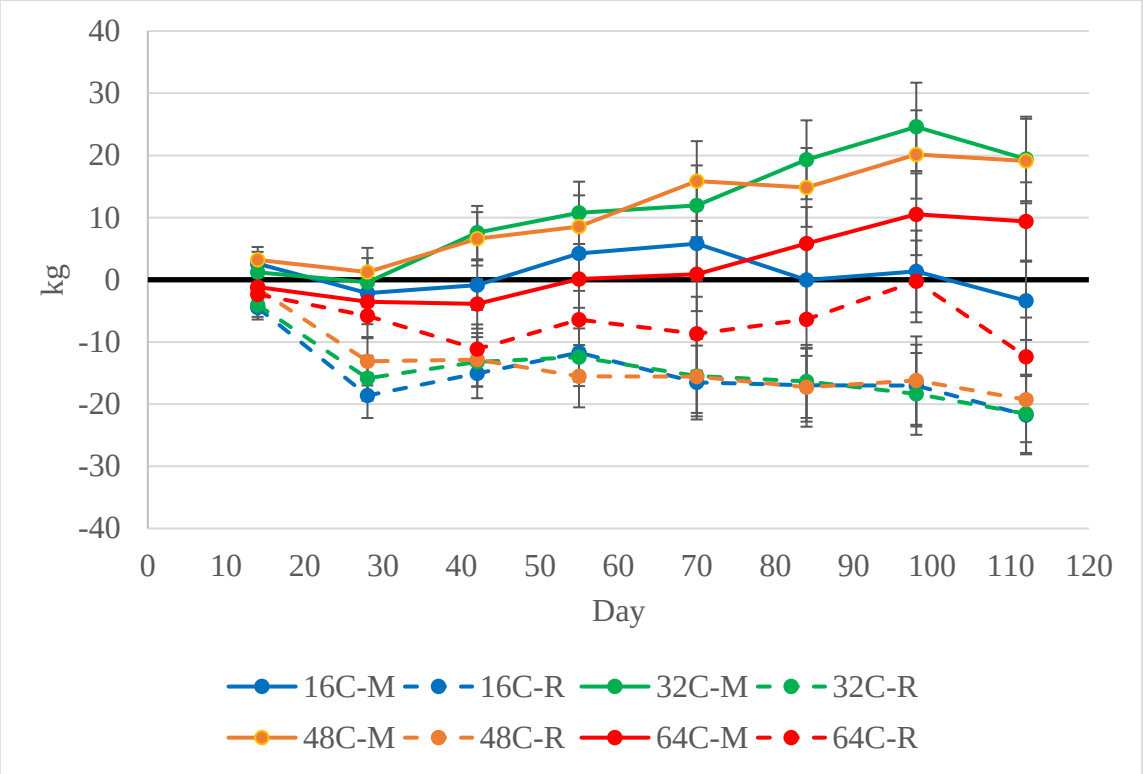

Figure 6. Change in maternal BW in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. Diet × intake × day ($P = 0.03$), diet × day ($P < 0.01$) and intake × day ($P < 0.01$) interactions were all observed, but the diet × intake interaction was not significant ($P = 0.18$). The main effects of intake ($P < 0.01$) and day ($P < 0.01$) were significant, but the diet effect was not ($P = 0.52$). Linear effect of diet within the R intake level was significant ($P = 0.02$) at d 28. No other tests for linear, quadratic or cubic effects of diet within R or M intake levels were significant ($P \geq 0.07$).

effects of diet within R or M intake levels were significant ($P \geq 0.07$). Additionally, change in maternal BW at d 98 in cows fed 64C-R was not different from zero ($P = 0.96$), and at least tended ($P \leq 0.10$) to be different from cows fed any other diet at the R intake level. Change in maternal BW across diets was positive ($P \leq 0.01$) or not different ($P \geq 0.14$) from zero in cows fed M and less than zero ($P < 0.01$) in cows fed R. The difference between intake levels became greater over time. Across intake levels, there were no linear, quadratic or cubic effects of corn inclusion observed ($P \geq 0.17$); however, by d 112 change in BW was lower ($P \leq 0.07$) in cows fed 16C than in any other diet.

No interactions were observed ($P \geq 0.11$) for change in backfat thickness (Figure 7). Loss in backfat was greater in cows fed R ($P = 0.01$) than M, with the losses becoming greater over time ($P < 0.01$), in both intake levels. Change in thickness was not different from zero ($P \geq 0.17$) through d 84 in cows fed at the M intake level, but was slightly negative (-0.049 ± 0.012 cm; $P < 0.01$) by d 112. Cows fed at the R intake level did not lose backfat in the first 28 d ($P = 0.75$); however, by d 55, change in thickness was negative (-0.023 ± 0.011 cm; $P = 0.03$), declining further, through d 112 (-0.088 ± 0.011 cm). Diet did not affect change in backfat thickness ($P = 0.28$).

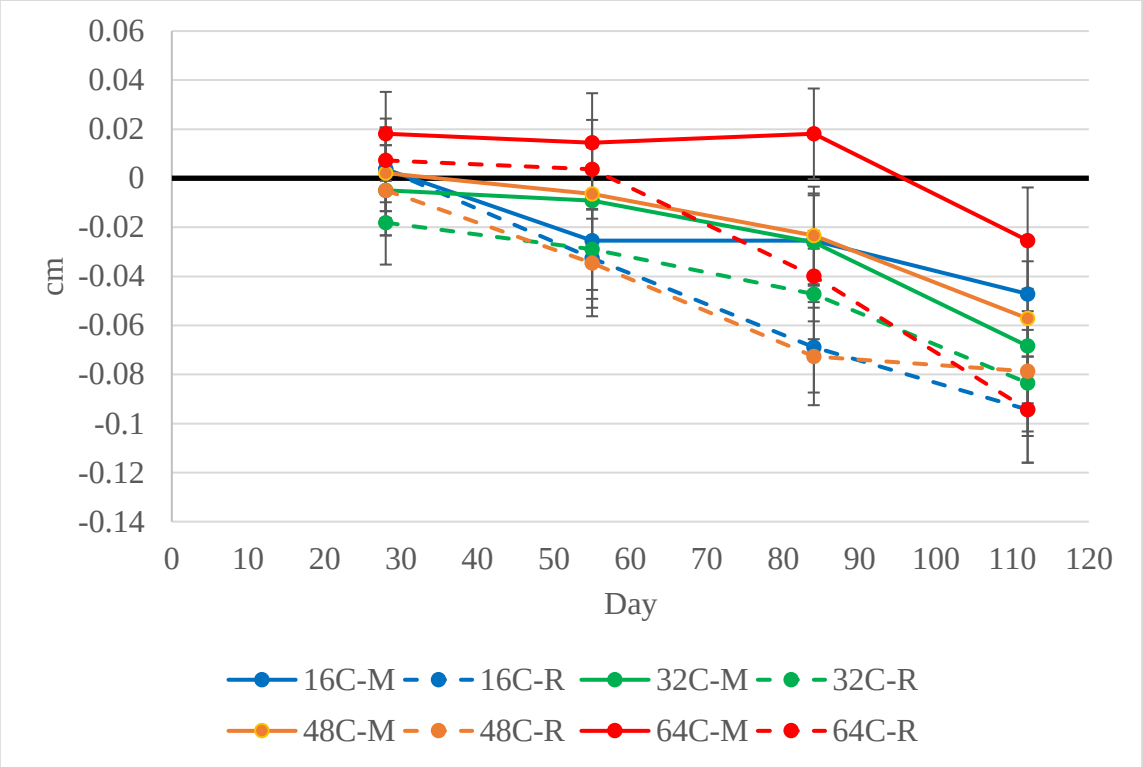

Figure 7. Change in backfat in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. No interactions were observed ($P \geq 0.11$). Main effects of intake ($P = 0.01$) and day ($P < 0.01$) were significant, but the diet effect was not ($P = 0.28$).

Diet × intake × day ($P = 0.77$), diet × day ($P = 0.44$) and diet × intake ($P = 0.49$) interactions were not significant for total RE (Figure 8). However, an intake × day interaction was observed ($P < 0.01$), with RE being greater ($P < 0.01$) in cows fed at M vs. those fed R, and the difference becoming greater over time. At d 28 RE was not

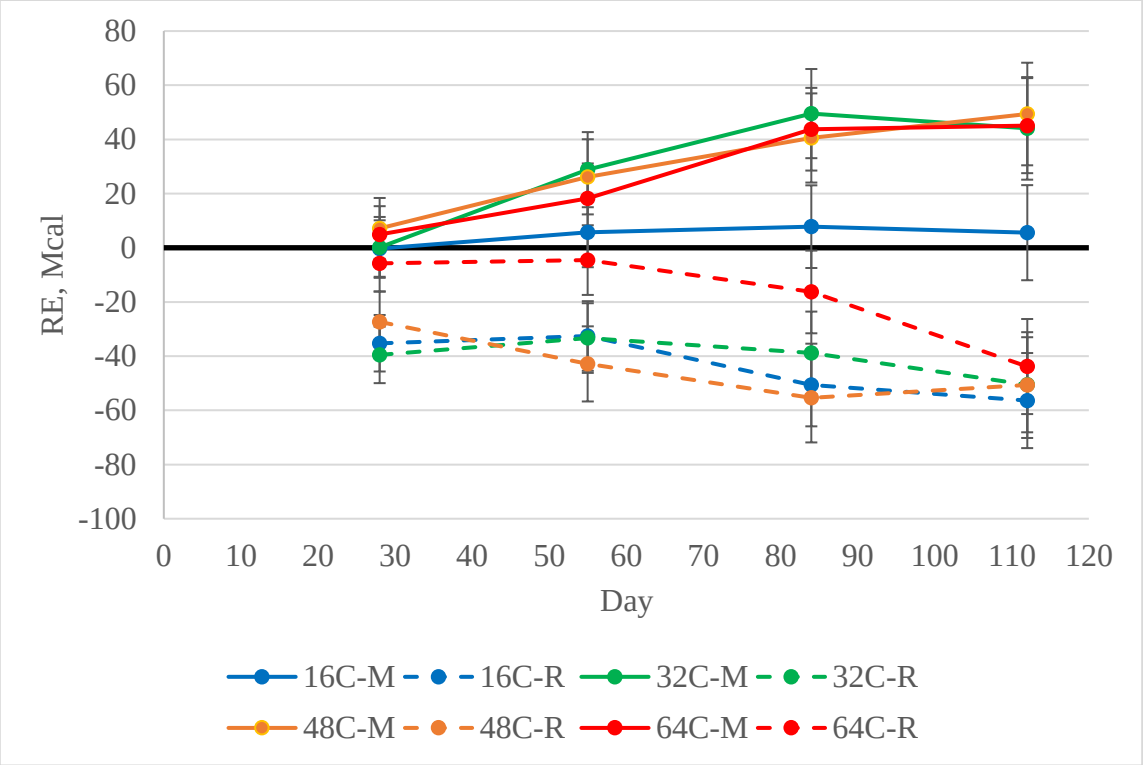

Figure 8. Total energy retention in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. The intake × day interaction was significant ($P < 0.01$), but diet × intake × day ($P = 0.77$), diet × day ($P = 0.44$) and diet × intake ($P = 0.49$) interactions were not. The main effect of intake was significant ($P < 0.01$), but the effects of diet ($P = 0.20$) and day ($P = 0.11$) were not.

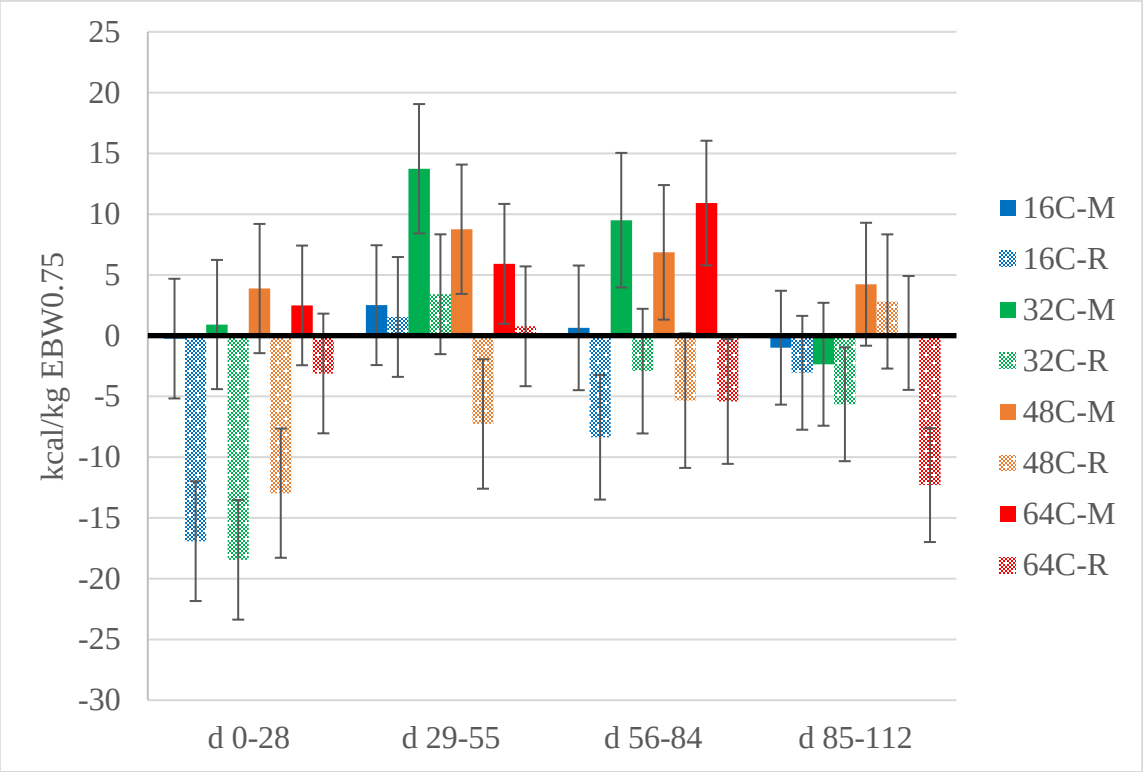

Figure 9. Daily energy retention in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. No interactions were observed ($P \geq 0.18$). Main effects of intake ($P < 0.01$) and period ($P < 0.01$) were significant, but the diet effect was not ($P = 0.33$).

different from zero ($P = 0.62$) in cows fed M, but by d 55, they had achieved positive RE (19.7 ± 7.21 Mcal; $P < 0.01$), which continued to increase through d 112 (36.0 ± 9.52 Mcal). Cows fed at the R intake level lost body energy during the first 28 d (-27.0 ± 5.97 Mcal; $P < 0.01$), and continued to lose energy through d 112 (-50.4 ± 9.43 Mcal).

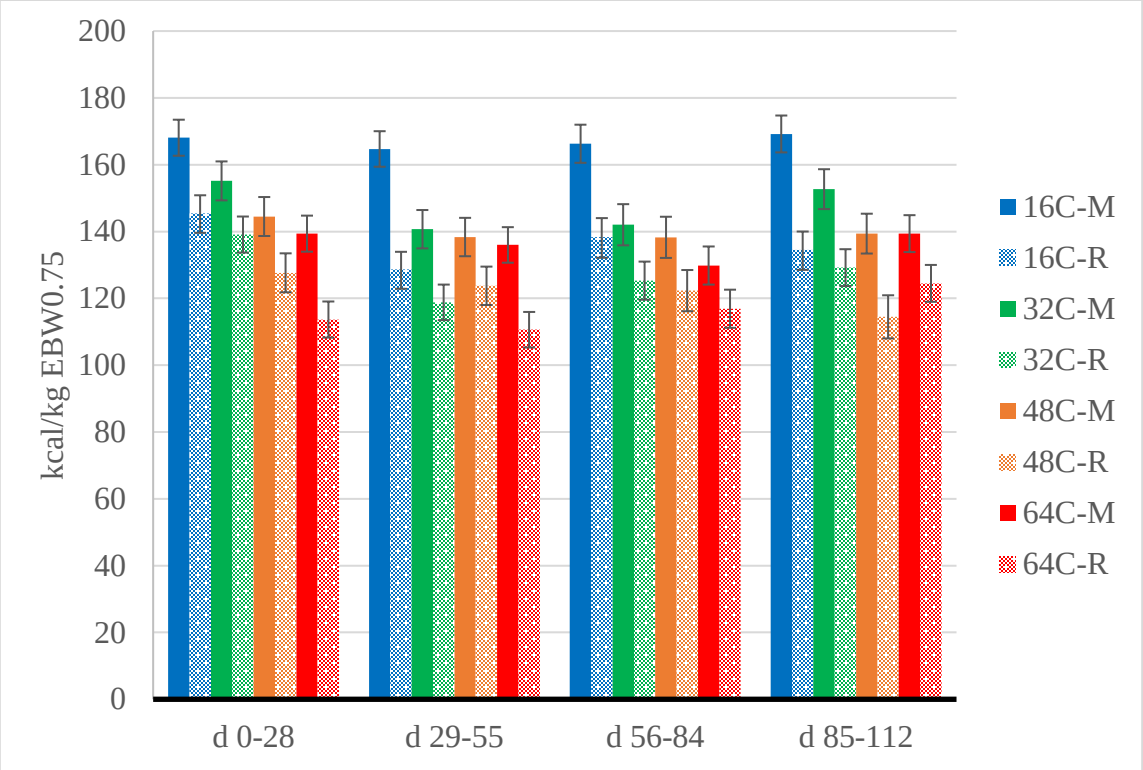

Figure 10. Daily heat production in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. No interactions were observed ($P \geq 0.19$). Main effects of diet ($P < 0.01$), intake ($P < 0.01$) and period ($P < 0.01$) were all significant.

No interactions were observed ($P \geq 0.18$) for daily RE (Figure 9). Retention was greater in cows fed at M than in those fed the R intake level ($P < 0.01$). Retention was also affected by period, increasing from period 1 (d 0-28; M = 1.76 ± 2.62 kcal/kg $EBW^{0.75}$/d; R = -12.9 ± 2.57 kcal/kg $EBW^{0.75}$/d) to period 2 (d 29-55; M = 7.73 ± 2.62

kcal/kg $EBW^{0.75}$/d; R = -0.39 ± 2.57 kcal/kg $EBW^{0.75}$/d), and then declining in period 4 (d 84-112; M = 0.27 ± 2.49 kcal/kg $EBW^{0.75}$/d; R = -4.55 ± 2.51 kcal/kg $EBW^{0.75}$/d).

No interactions were observed ($P \geq 0.19$) for daily heat production (Figure 10). Heat production declined with increasing corn inclusion ($P < 0.01$) and intake restriction ($P < 0.01$). A period effect was also observed ($P = 0.01$), with heat production decreasing from period 1 (d 0-28) to period 2 (d 29-55), and then increasing to period 4 (d 85-112).

No interactions were observed ($P \geq 0.26$) for daily difference in observed and predicted RE (Figure 11). The difference was greater in cows fed R vs. cows fed at the M intake level. The difference also increased linearly ($P = 0.01$) with corn inclusion and increased over time ($P < 0.01$). The diet × intake interaction was not significant ($P = 0.36$) for total difference between observed and predicted RE (Figure 12), but the difference increased linearly ($P = 0.01$) with corn inclusion and increased ($P < 0.01$) with intake restriction.

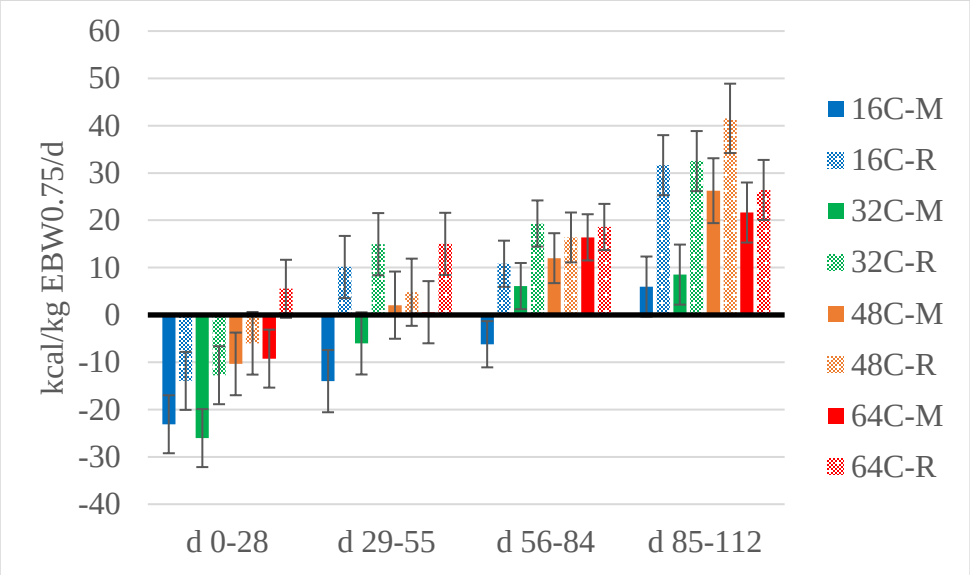

Figure 11. Daily difference between observed and predicted energy retention in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. No interactions were observed ($P \geq 0.20$). Main effects of diet ($P = 0.01$), intake ($P < 0.01$) and day ($P < 0.01$) were all significant.

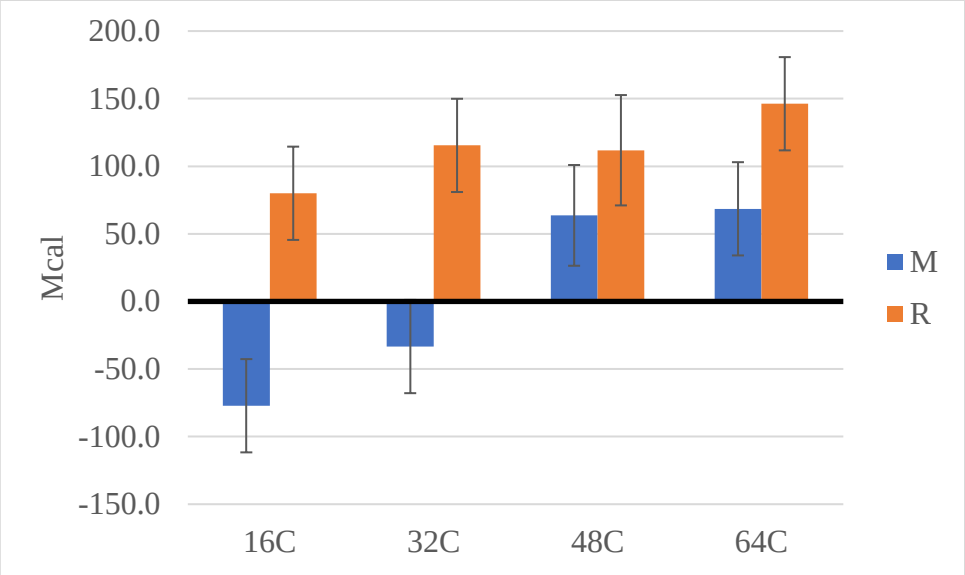

Figure 12. Total difference in observed and predicted energy retention in cows fed concentrate diets at or below maintenance intake levels. Diets contained 16 (**16C**), 32 (**32C**), 48 (**48C**) or 64% (**64C**) cracked corn at maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or restricted to 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. The diet × intake interaction was not significant ($P = 0.36$). Both diet ($P = 0.01$) and intake ($P < 0.01$) effects were significant.

No interactions ($P \geq 0.28$) were observed for birthweight or calf age, calf BW, cow BW, cow BCS, days in gestation, or pregnancy rate at weaning (Table 20). Neither diet ($P \geq 0.08$) nor intake ($P \geq 0.13$) affected birthweight or calf age, calf BW, cow BW BCS, or days in pregnancy at weaning. Pregnancy rate was lower ($P = 0.02$) in cows fed R vs. those fed at the M intake level.

Table 20. Effects of maternal dietary energy density and intake on birthweight and postnatal cow and calf performance

		Diet[1]					*P*-value		
Item		16C	32C	48C	64C	SEM	Diet	Intake	D×I
Birth weight, kg	R intake	36.0	34.3	33.6	32.7	2.823	0.87	0.84	0.44
	M intake	31.4	36.0	36.3	34.3				
Weaning[2]									
Cow BW, kg	R intake	399	412	377	419	14.40	0.08	0.12	0.79
	M intake	419	417	401	423				
Cow BCS	R intake	3.86	3.79	3.60	4.00	0.201	0.27	0.35	0.52
	M intake	4.00	3.64	4.00	4.07				
Calf age, d	R intake	219	223	218	220	5.174	0.95	0.73	0.76
	M intake	222	217	218	219				
Calf BW, kg	R intake	170	179	160	170	12.49	0.48	0.87	0.28
	M intake	189	161	169	165				
Days in gestation[3]	R intake	114	104	122	107	15.63	0.80	0.13	0.77
	M intake	102	101	100	102				
Pregnancy rate[3], %	R intake	28.5	71.5	20.2	42.8	19.41	0.60	0.02	0.47
	M intake	85.7	71.5	66.7	71.5				

[1]Cracked corn inclusion of each diet was 16% (**16C**), 32% (**32C**), 48% (**48C**) and 64% (**64C**) on a DM basis. Designed to deliver maintenance (103.1 kcal NE_m/kg $EBW^{0.75}$; **M**) or 75% of maintenance (77.3 kcal NE_m/kg $EBW^{0.75}$; **R**) intake levels. Values reported are least squares means with standard error of the means.

[2]Weaning occurred 227 d after the end of the experimental period.

[3]Pregnancy determination was made 20 d prior to weaning.

DISCUSSION

Our objective was to quantify the effects of energy density (corn inclusion) and intake on estimates of maintenance requirements. We have previously used methods described by Garrett (1987) to estimate FHP by regressing log (heat production) on ME intake. However, because the intercepts of these regressions are highly sensitive to slight changes in LSM estimates, our results produced spurious estimates that are not useful for interpretation. Regardless, results from the current experiment show discrepancies in energy balance, similar to those we have recently observed (Trubenbach et al., 2014). This divergence could result from reduced requirements, increased feed energy utilization, or a combination thereof.

Fecal losses represent the most significant source of dietary energy losses in beef cattle, and are accounted for in the net energy system by converting GE to DE. We attempted to quantify interactions between formulated dietary energy density and intake on fecal losses by measuring apparent DE concentration of these diets in a group of cohort cows that were fed the same diets at similar intake levels.

Our estimates of energy delivery assumed an 82% DE:ME conversion rate (NRC, 2000), which has been previously disputed (BCNRM, 2016). Hales et al. (2012, 2013, 2014) reported greater conversions rates (89.3 to 95.0%) in growing cattle fed high-energy diets, and have attributed the differences to reduced methane production. While increasing DE utilization with greater corn inclusion could explain some of the widening difference between observed and predicted RE in the current experiment, the rate would have to reach 98% to completely explain the diversion. Mills et al. (2001)

proposed that the proportion of ingested energy lost as methane increases with intake restriction, and Vermorel and Bickel (1980) suggested that methane losses are likely greater in mature animals than in young, growing animals, making an extremely high conversion rate unlikely in this experiment.

Efficiency of ME use for maintenance and/or pregnancy could represent some of the observed divergences in energy balance in the current experiment. Increased glucose requirements of the gravid uterus and mammary tissue cause major changes in glucose metabolism in gestating ruminants (Bell and Bauman, 1997), which rely heavily on hepatic gluconeogenesis for glucose supply, even when they are not pregnant. Propionate is the primary exogenous precursor for hepatic gluconeogenesis (Brockman, 1993), which is known to be stressed by substrate supply during late gestation. Previous studies from our lab have reported greater proportional concentrations of propionate with intake restriction (Trubenbach et al., 2014; Boardman, 2015), suggesting a potential under-prediction of ME efficiency at low intakes. Because propionate production is favored by fermentation of starches by amylolytic bacteria (Elliot, 1980; France and Siddons, 1993), total propionate production increases with greater corn inclusion (see Chapter II), possibly resulting in even larger under-prediction of ME efficiency with intake restriction when dietary energy density is increased.

Reduced nutrient requirements could also explain part of the discrepancy between observed and predicted RE. Freetly and Neinaber (1998) reported that cows fed below requirements returned to a maintenance state (RE = 0) after 112 d of a constant intake level. Recent observations in our laboratory (Trubenbach et al., 2014) have also

suggested that requirements may be negatively related to dietary energy concentration. Compensatory gain is well-documented in growing animals (Sainz et al., 1995), which is attributed to mobilization and reduced energy expenditure of metabolically costly organs (Sainz and Bentley, 1997; McCurdy et al., 2010). Similar reductions in maternal organ mass were reported by Camacho et al. (2014), who also reported greater efficiency of nutrient use after a period of intake restriction.

Because energy requirements for pregnancy are calculated in Mcal of ME and converted to NE_m equivalents by multiplying by k_m, and because k_m increases with increasing dietary energy density, pregnancy requirements (NE_m equivalents) increase with dietary energy density. We accounted for this in our experimental design by estimating total NE_m equivalent requirements for each respective diet. To determine if the large separation between observed and predicted RE was caused by over-predicting requirements for cows fed high-energy diets, we estimated RE again, using a common k_m across diets, normalizing pregnancy requirements.

Although the magnitude of separation across diets decreased slightly, the under-prediction of RE continued to be greater in cows fed R vs. those fed M, and was positively related to corn inclusion when k_m was normalized. At the beginning of the experiment total predicted NE_m equivalent requirements for cows fed the 64C were less than 1% greater than cows fed 16C, and less than 7% greater by termination, which represents only a small amount of the observed deviation from predicted RE.

Deviations from predicted RE increased over time, which is confounded with increased requirements of the gravid uterus. Because energy requirements for pregnancy are calculated using an exponential function, minor errors in estimated day of gestation result in large discrepancies in estimated requirements. Cows from this experiment were shipped to another location to calve one d after the end of the experiment. It is possible that shipping stress induced pre-term labor in some cows, as the average birthdate across treatments was 7 d after shipping, resulting in over predicted days in gestation and requirements. However, because age at weaning was not affected by treatment, this potential error in predicting requirements for pregnancy would be the same across treatments, meaning that the observed effects of dietary energy density and intake on deviations from predicted RE would remain valid.

Observed energy intake declined with corn inclusion at both intake levels, yet no diet effects were observed in change in backfat or energy retention, and increased corn inclusion provided an advantage in BW loss in the first 28 d or treatment application. These results are directionally supportive of the reported deviation from expected RE.

The reduced pregnancy rate in cows fed at the R intake level is not surprising, as pre-partum intake level and cow BCS at calving have been shown to have significant effects on post-partum reproductive function (Selk et al., 1988; Houghton et al., 1990). Although our results do support the conjecture that energy efficiency is related to dietary energy density and intake, it appears that cows should not be fed less than 75% of their estimated requirements, due to negative effects on reproductive efficiency.

CONCLUSIONS

Overall, we conclude that both intake restriction and increasing dietary energy density reduce total heat production in gestating beef cows. Although we cannot definitively conclude that these treatments effect maintenance requirements, our data demonstrated a divergence between observed and prediction for RE, which could result from either enhanced dietary energy supply or reduced requirements. The divergence was directly related to dietary energy density and augmented by intake restriction, suggesting that large improvements in overall dietary energy utilization can be realized by limit-feeding high-energy diets to gestating beef cows. However, because reproductive efficiency was compromised by intake restriction, we do not recommend feeding gestating beef cows at or below 75% of total requirements.

CHAPTER V

EFFECTS OF INTAKE RESTRICTION ON THORACIC AND ABDOMINAL ORGAN MASS AND METABOLISM

INTRODUCTION

Limit-feeding beef cows may provide opportunities for feed savings and overall increased profitability and efficiency of feed energy utilization. Previous studies have reported reduced maintenance requirements in limit-fed cattle (Jenkins and Ferrell, 1997; Freetly and Nienaber, 1998), suggesting that it may be possible to restrict intake of total mixed rations beyond forecasted maintenance requirements without excessive tissue loss, particularly during the dry period. Several mechanisms for reduced requirements have been proposed, including decreased protein turnover, cellular ion transport, and mass and total metabolism of metabolically active organs (McBride and Kelly, 1990). Previous work from our lab (Trubenbach et al., 2014) suggests that the reduction in requirements may be a function of dietary energy density, as effects of DM and energy intake may have confounding effects on blood flow and subsequent energetic requirements associated with digestion and absorption, which was proposed by Reynolds et al. (1991). Effects of intake on organ mass and metabolism have historically been measured in growing animals or mature animals fed near maintenance levels of intake, but to our knowledge, have not been measured on mass-specific tissue oxygen consumption in pregnant females subjected to this degree of energy restriction. We hypothesize that intake restriction beyond maintenance levels of intake results in further

declines in abdominal and thoracic organ mass and metabolism, and that these effects are larger when dietary energy density is increased. The experimental objectives were to measure the effects of sub-maintenance energy restriction on abdominal and thoracic organ mass and metabolism, and to determine if these effects are similar between forage and concentrate diets.

MATERIALS & METHODS

The experimental protocol was approved by the Agricultural Animal Care and Use Committee of Texas A&M Agrilife Research.

Experiment 1

Eight cows, pregnant with their first calf, were used in an experiment designed to analyze the effects of intake of a concentrate diet on thoracic and abdominal organ mass and fill. Cows were pregnant (via embryo transfer) with four pairs of identical female twins as part of an accompanying experiment designed to determine the effects of maternal energy intake on fetal development and postnatal growth performance (cite JL here). One cow from each pair of pregnancies was randomly assigned to one of two treatments, so that each pair of pregnancies was represented in each treatment. Between d 158 and 270 of gestation, cows were fed a total mixed ration (Table 21) either at maintenance (**M**) or restricted to 70% of maintenance (**R**) energy intake levels using a Calan gate system (American Calan, Northwood, NH).

Table 21. Formulated ingredient and nutrient composition of concentrate diet

Ingredient	% As fed
Wheat straw	34.52
Cracked corn	29.46
Dried distiller's grain	27.46
Urea	1.10
Molasses	5.00
Mineral	2.46
Diet components[a]	DM basis[b]
CP, %	16.30
ME, Mcal/kg	2.45
NE_m, Mcal/kg	1.54
Chemical composition	
CP, %	12.7
OM, %	92.7
ADF, %	26.6

[a]According to NRC model estimates

[b]Dry matter contents = 83.42%

Cow BW was measured immediately prior to starting the experiment. Maintenance requirements (NE_m, Mcal/d) were estimated for each cow using equations from the NRC (2000):

$$NE_m = 0.077 \times EBW^{0.75}$$

$$EBW = BSW \times 0.891$$

$$SBW = BW \times 0.96$$

Where:

EBW = empty body weight, kg

SBW = shrunk body weight, kg

BW = body weight, kg

Intake was increased bi-weekly to account for increased requirements for pregnancy. Pregnancy requirements were estimated using NE_m equivalents (NRC, 2000):

$$NE_m = k_m \times CBW \times (0.4504 - 0.000766) \times e^{(0.03233 - 0.0000275t) \times t}$$

$k_m = 0.6368$

CBW = 32

Where:

$k_m = NE_m / ME$

CBW = calf birth weight, kg

t = days in gestation

Total requirements (maintenance + pregnancy) were estimated for each cow, with total requirements multiplied by 0.7 for cows fed R. Cows were weighed bi-weekly, with ribeye area (REA) and back fat (BF) measured via ultrasonography every 28 d.

Cows were weighed immediately prior to necropsy. At the time of necropsy, cows were euthanized using 100 mL phenytoin/pentobarbital (Beuthanasia-D, Merck Animal Health, Madison, NJ). Thoracic and abdominal organs were dissected, removed of excessive adipose tissue, and weighed fresh. Segments of the gastrointestinal tract were tied off with string at each junction: gastroesophageal sphincter, pyloric sphincter and ileocecal valve. Contents of the tract were removed and weighed, and the empty weights of each segment and lengths of the small intestine and colon were recorded. The gastrocnemius was dissected from the right, hind leg, and its weight recorded.

Empty BW was calculated as BW less gastrointestinal contents and gravid uterus. Maternal BW was calculated as BW less gravid uterine weight (GU, kg), which was estimated using the following equation (NRC, 2000):

$$GU = CBW \times 19.32 \times e^{0.02 \times t - 0.0000143 \times t^{\wedge}2} / 1000$$

Calf birth weight was estimated by re-writing the equation above, using gravid uterine weight measured at necropsy on d 270 to solve for CBW:

$$CBW = e^{-0.02 \times t + 0.0000143 \times t^{\wedge}2 + \ln(1000 \times GU)} / 19.32$$

Calf birth weight was then substituted into the prior equation to estimate GU for each respective d.

Data were analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included treatment, day and cow. Changes in BW, maternal BW, backfat thickness and REA were analyzed using the repeated measures technique. Model terms included treatment, day and the treatment × day interaction. Day was used as the repeated variable, with unstructured covariance and cow ID used as the subject. For responses collected at necropsy, model terms included treatment and initial cow BW, which served as a covariate.

Experiment 2

Ten cows, pregnant with their first calf, were used in an experiment designed to analyze the effects of intake of a forage diet on thoracic and abdominal organ mass and metabolism. Cows with common days in gestation (estimated via rectal palpation 41 d prior to the start of the experiment) were stratified by weight and randomly assigned to one of two treatments. Between d 146 and 244 of gestation, cows were fed a hay diet

(Table 22) either at maintenance (**M**) or restricted to 70% of maintenance (**R**) energy intake levels using a Calan gate system (American Calan, Northwood, NH). Energy requirements for maintenance and pregnancy were estimated using the same methods described above, with the exception that k_m was lower (0.5917) than in the previous experiment due to lower dietary energy density. Requirements were estimated for each cow, with total requirements (maintenance + pregnancy) multiplied by 0.7 for cows fed R. Body weight, when used to estimate requirements, was calculated as the mean of cow BW measured on three consecutive d prior to starting the experiment. After the start of the experiment, cows were weighed bi-weekly, with ribeye area (REA) and back fat (BF) measured via ultrasonography every 28 d.

Table 22. Formulated ingredient and nutrient composition of forage diet

Ingredient	% DM
Alfalfa hay	78.13
Wheat straw	21.87
Diet components[a]	DM basis[b]
CP, %	16.49
ME, Mcal/kg	2.07
NE_m, Mcal/kg	1.22
Chemical composition	
CP, %	16.49
OM, %	91.47
ADF, %	40.05

[a]According to NRC model estimates

[b]Dry matter contents =86.82%

Collection procedures at necropsy were identical to those described in experiment 1, with the addition of liver and jejunal tissue samples being collected for determination of mitochondrial respiration. Immediately following euthanasia and removal of the gravid uterus, was removal of the liver. After collecting liver weight, a small section (10 g) was removed from the right lobe. This section was minced and subsamples were immediately stored in ice-cold BIOPS. The jejunum was identified by measuring 11 m distal to the pyloric sphincter. At this reference point, a cross sectional section was removed and immediately rinsed in phosphate-buffered saline (PBS). Following rinsing in PBS, the sample was minced and stored in ice-cold BIOPS.

High-resolution respirometry

Tissue samples (liver: cubes, 4.5 ± 3.45 mg; jejunum: single piece, 19.4 ± 3.49 mg) were removed from BIOPS and added to each respirometer chamber of the Oxygraph-2k (O2k; Oroboros, Innsbruck, Austria), containing 2 mL of MiR06 (MiR05 + 5μL 280 U/mL catalase) and maintained at 37°C, and allowed to incubate for 10 min. Throughout the entire substrate-uncoupler-inhibitor titration (SUIT) protocol, hyperoxic O_2 concentrations (200 - 500 μM O_2) were maintained by titration of H_2O_2 (100 mM) to prevent O_2 limitation.

Oxygen flux and respiratory states for liver and jejunal tissue were determined using two different protocols, modified from previous validated protocols in equine and bovine skeletal muscle (Li et al., 2016; White et al., unpublished data). The protocol for liver tissues included: 1) pyruvate (5 mM) and malate (2 mM) to support electron flow through complex I (CI) of the ETS (LEAK respiration, L); 2) adenosine diphosphate

(ADP; 2.5 mM) to stimulate respiration (OXPHOS, P_{CI}); 3) cytochrome *c* (cyt *c*; 10 μM) to assess outer mitochondrial membrane integrity (samples with responses to cyt *c* greater than 15% were excluded from the dataset); 4) glutamate (10 mM) as an additional CI substrate; 5) succinate (10 mM) to support convergent electron flow through complex II (CII) of the ETS (P_{CI+II}); 6) uncoupler carbonyl cyanide m-chloro phenyl hydrazone (CCCP; 0.5 μM steps) to assess maximum ETS capacity (E_{CI+II}); 7) Rotenone(0.5 μM), a CI inhibitor, which allowed assessment of ETS with only CII support (E_{CII}); 8) Antimycin A (2.5 μM), an inhibitor of complex III, to measure residual oxygen flux independent of the ETS. The protocol for jejunal tissue mas modified slightly by removing the addition of glutamate from step 4 and including it with the addition of pyruvate and malate during step 1, because addition of glutamate did not increase O_2 consumption beyond that of pyruvate and malate with ADP when optimizing the protocol (data not shown).

Data were analyzed using MIXED procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Class variables included treatment, day and cow. Changes in BW, maternal BW, backfat thickness and REA were analyzed using the repeated measures technique. Model terms included treatment, day and the treatment × day interaction. Day was used as the repeated variable, with unstructured covariance and cow ID used as the subject. For responses collected at necropsy, along with measures of mitochondrial respiration, model terms included treatment and initial cow BW, a continuous variable which served as a covariate.

RESULTS

Experiment 1

The treatment × day interaction was not significant ($P = 0.28$) for change in BW (Figure 13). Change in BW was greater ($P < 0.01$) in cows fed at maintenance vs. those fed R throughout the experiment. By gestational d 172, cows fed R had lost a small amount of BW (14.3 ± 5.91 kg; $P = 0.02$), but cows fed M had little change ($P = 0.29$). From d 172 through 265, cow BW increased ($P < 0.01$) in both treatments. Change in BW was positive ($P \leq 0.01$) from d 186 (15.5 ± 5.91 kg) through 265 (61.2 ±5.91 kg) for cows fed M, and cows fed R returned to a positive total change ($P < 0.01$) by d 265 (23.4 ± 5.91 kg). The treatment × day interaction was not significant ($P = 0.18$) for change in maternal BW (Figure 14). Change in maternal BW was greater ($P < 0.01$) in cows fed H than in those fed R from gestational d 172 (M = 3.07 ± 5.71 kg vs. R = -17.6 ± 5.71 kg) through 265 (M = 15.4 ± 5.71 kg vs. R = -24.5 ± 5.71 kg). The day effect was not significant ($P = 0.99$) for change in maternal BW.

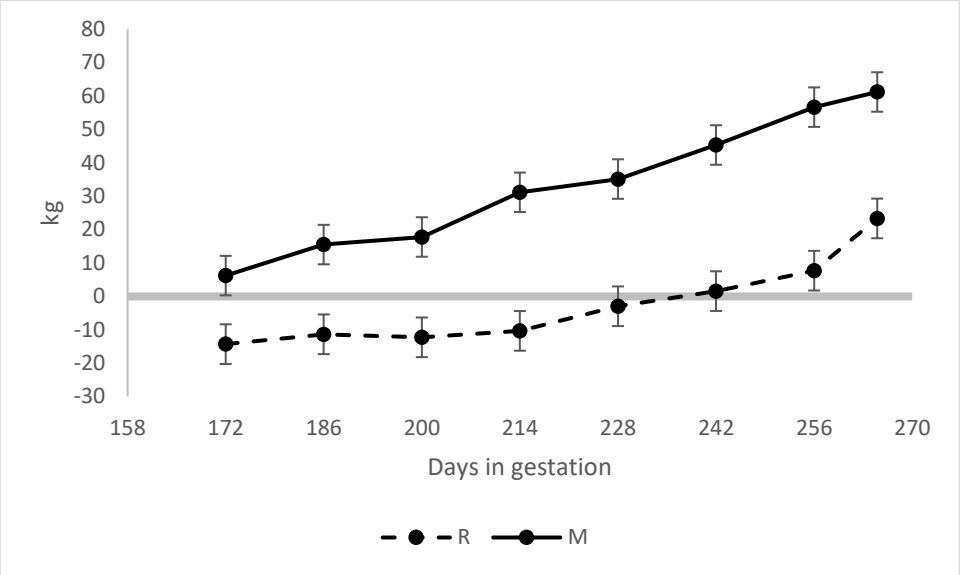

Figure 13. Change in BW in cows fed a concentrate diet at or below maintenance intake levels. Cows were fed a total mixed ration at (**M**) or restricted to 70% (**R**) total NE_m intake levels from d 158-270 of gestation. The treatment × day interaction was not significant ($P = 0.28$), but effects of treatment and day were both significant ($P < 0.01$).

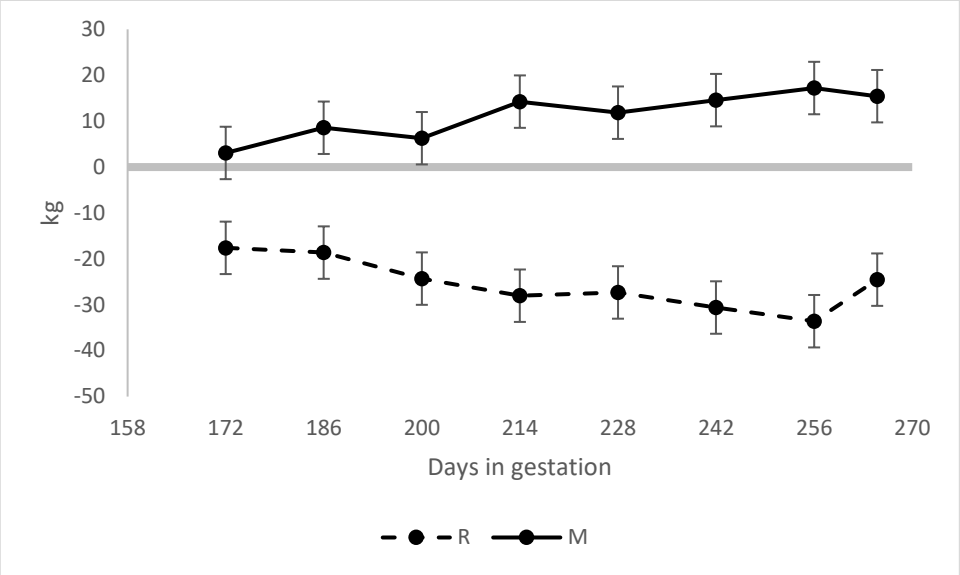

Figure 14. Change in maternal BW in cows fed a concentrate diet at or below maintenance intake levels. Cows were fed a total mixed ration at (**M**) or restricted to 70% (**R**) total NE_m intake levels from d 158-270 of gestation. The treatment × day (P = 0.18) interaction and effect of day (P > 0.99) were not significant. The treatment effect was significant (P < 0.01).

A treatment × day interaction was observed (P < 0.01) for change in backfat (Figure 15). Loss in backfat became greater over time in both treatments (P < 0.01), with the extent being greater in restricted cows vs. those fed at maintenance. Change in backfat was similar between treatments through d 214 (P > 0.81), but tended to be more negative (P = 0.06) in cows fed R (-0.15 ± 0.029 cm) than in those fed M (-0.06 ± 0.030 cm) by d 242, with the difference widening by d 270 (R = -0.29 ± 0.051 vs. M = -0.12 ± 0.050 cm). A treatment × day interaction was observed (P = 0.05) for change in REA (Figure 16). Changes were minimal in both treatments through d 214, remaining greater

($P = 0.03$) than or not different from zero ($P \geq 0.12$), but were negative ($P < 0.01$) in cows fed R (-5.80 ± 1.79 cm^2) by d 242, while cows fed M remained near zero (1.66 ± 1.82 cm^2). By d 270, cows fed M had no change ($P = 0.52$) in REA (-1.51 ± 2.35 cm^2), while REA in cows fed R had declined further (-10.6 ± 2.41 cm^2).

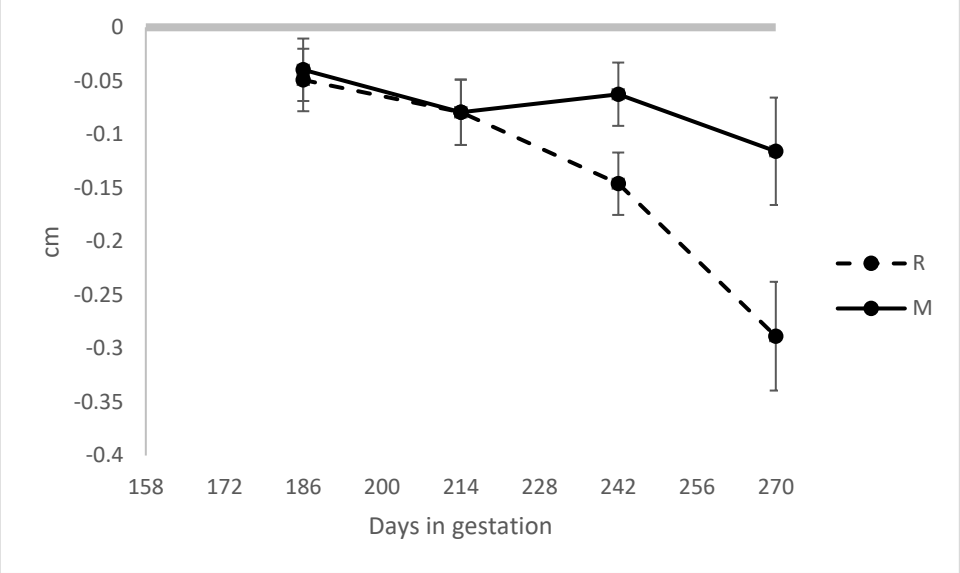

Figure 15. Change in backfat thickness in cows fed a concentrate diet at or below maintenance intake levels. Cows were fed a total mixed ration at (**M**) or restricted to 70% (**R**) total NE_m intake levels from d 158-270 of gestation. A treatment × day interaction was observed ($P = 0.01$). The effect of day was significant ($P < 0.01$), but the treatment effect was not significant ($P = 0.13$).

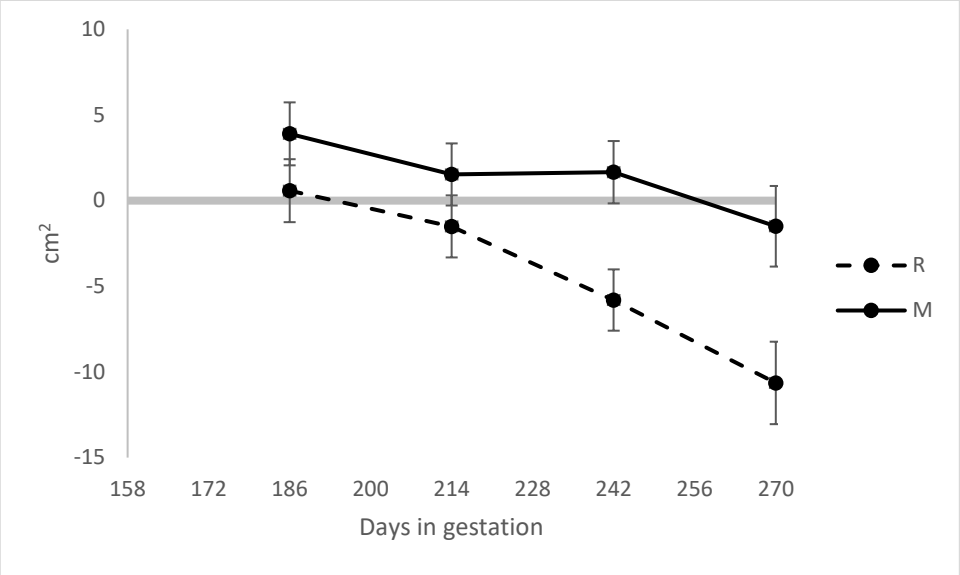

Figure 16 Change in ribeye area in cows fed a concentrate diet at or below maintenance intake levels. Cows were fed a total mixed ration at (**M**) or restricted to 70% (**R**) total NE_m intake levels from d 158-270 of gestation. A treatment × day interaction was observed ($P = 0.05$). Both treatment ($P = 0.01$) and day ($P < 0.01$) effects were significant.

On the d of harvest cow BW and empty BW (Table 23) were greater ($P \leq 0.04$) in cows fed M vs. those fed R. Intestinal lengths were not affected by treatment ($P \geq 0.18$). Contents of the stomach complex and total gastrointestinal tract were greater ($P = 0.04$) in cows fed M than in those fed R, but contents of the small intestine, cecum and colon were not affected ($P > 0.31$) by intake.

Table 23. Body weights, intestinal lengths, and gastrointestinal contents of cows fed a concentrate diet at or below total maintenance requirements from d 158-270 of gestation

	Treatment[1]			
Item	R	M	SEM	*P*-value
Body weight, kg	473	507	8.884	0.04
Empty body weight, kg	359	393	6.633	0.02
Small intestine length, m	31.7	34.1	2.062	0.45
Large intestine length, m	6.46	7.51	0.473	0.18
Gastrointestinal contents				
Stomach complex, kg	41.8	51.8	2.551	0.04
Small intestine, kg	3.49	4.03	0.343	0.31
Cecum, kg	1.54	1.38	0.199	0.59
Colon, kg	1.98	1.31	0.537	0.42
Total tract, kg	48.8	58.6	2.516	0.04

[1]Values reported are least squares means with standard error of the means. R = cows restricted to 70% total NE_m requirements; M = cows fed 100% total NE_m requirements.

Treatment did not affect mass of the pancreas, heart lungs, gastrocnemius, spleen, cecum, colon, gravid uterus or fetus (Table 24; $P \geq 0.14$). Cows fed R had smaller liver ($P = 0.05$) and kidney ($P = 0.02$) mass and tended to have reduced small intestinal mass ($P = 0.08$) than those fed M, but mass of the uteroplacenta tended to be greater ($P = 0.10$) in restricted cows vs. cows fed at maintenance. However, when expressed relative to EBW, these tissues were unaffected by treatment ($P \geq 0.13$). Absolute mass of the stomach complex was not affected by treatment ($P = 0.24$); however, when expressed relative to EBW, stomach complex mass of cows fed R was less than that of cows fed M ($P = 0.05$).

Table 24. Organ and fetal mass of cows fed a concentrate diet at or below total maintenance requirements from d 158-270 of gestation

	Treatment[1]			
Item	R	M	SEM	*P*-value
Liver, kg	3.98	4.53	0.152	0.05
g/kg EBW	11.6	11.9	0.724	0.76
Pancreas, g	276	305	18.63	0.32
g/kg EBW	0.88	0.99	0.063	0.28
Heart, kg	1.76	1.75	0.122	0.94
g/kg EBW	4.79	4.95	0.298	0.72
Lungs, kg	2.78	2.74	0.274	0.92
g/kg EBW	9.95	8.00	1.179	0.28
Gastrocnemius, g	768	852	34.36	0.14
g/kg EBW	2.07	1.80	0.226	0.43
Spleen, kg	0.86	1.08	0.141	0.34
g/kg EBW	2.76	2.95	0.513	0.80
Kidneys, kg	0.73	0.86	0.024	0.02
g/kg EBW	2.14	2.34	0.102	0.20
Stomach complex, kg	18.6	20.5	1.057	0.24
g/kg EBW	38.3	46.2	2.365	0.05
Small intestine, kg	4.80	5.61	0.258	0.08
g/kg EBW	13.5	14.8	0.901	0.33
Cecum, kg	0.39	0.57	0.073	0.14
g/kg EBW	1.98	2.09	0.418	0.86
Colon, kg	4.19	4.48	0.174	0.31
g/kg EBW	10.7	10.5	1.082	0.89
Gravid uterus, kg	65.4	55.5	4.731	0.20
g/kg EBW	122	108	8.224	0.26
Uteroplacenta, kg	18.4	15.4	1.047	0.10
g/kg EBW	27.3	22.1	2.201	0.13
Fetus, kg	39.6	35.3	2.376	0.26
g/kg EBW	74.6	67.4	5.235	0.34

[1]Values reported are least squares means with standard error of the means. R = cows restricted to 70% total NE_m requirements; M = cows fed 100% total NE_m requirements.

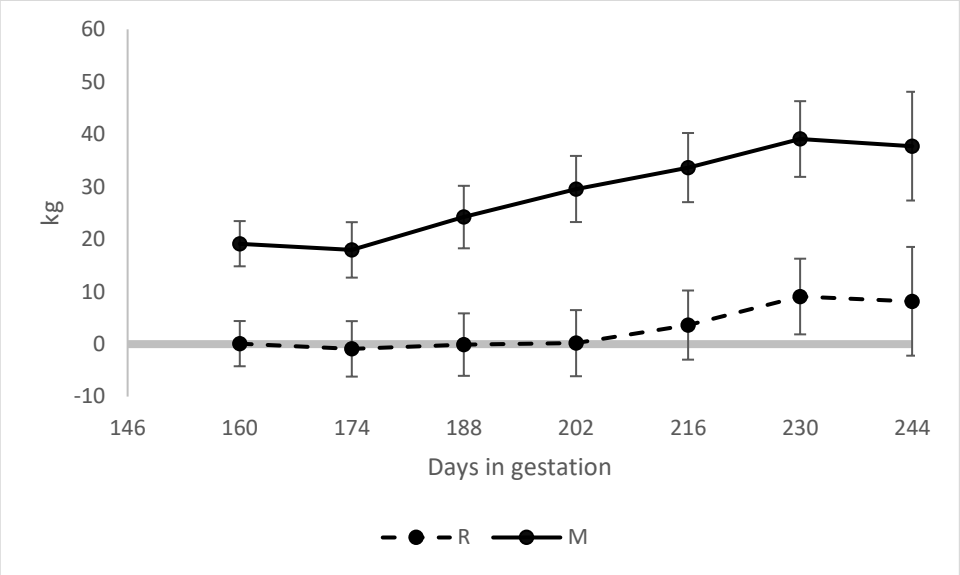

Figure 17. Change in BW in cows fed a forage diet at or below maintenance intake levels. Cows were fed a hay diet at (**M**) or restricted to 70% (**R**) total NE_m intake levels from approximately d 146-244 of gestation. A treatment × day interaction was observed ($P = 0.01$). Both treatment ($P < 0.01$) and day ($P < 0.01$) effects were significant.

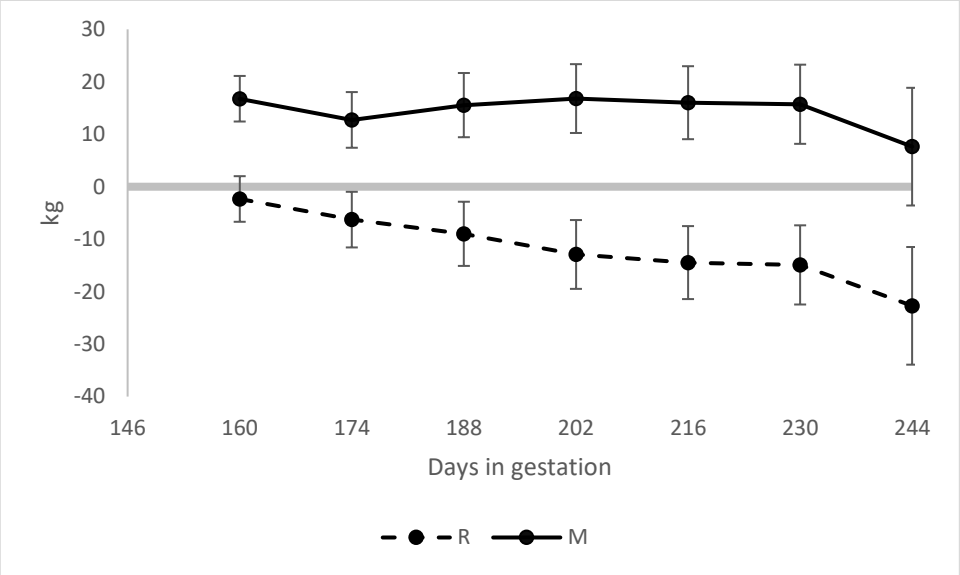

Figure 18. Change in maternal BW in cows fed a forage diet at or below maintenance intake levels. Cows were fed a hay diet at (**M**) or restricted to 70% (**R**) total NE_m intake levels from approximately d 146-244 of gestation. A treatment × day interaction was observed ($P < 0.01$). The effect of treatment was significant ($P = 0.02$), and a tendency ($P = 0.06$) was detected for the day effect.

Experiment 2

A treatment × day interaction was observed ($P = 0.01$) for change in BW (Figure 17), with the change being greater ($P < 0.01$) in cows fed M vs. those fed R, and the difference increasing over time. Body weight in cows fed R did not change ($P > 0.24$) throughout the experimental period; however, by gestational d 160, cows fed M had positive ($P < 0.01$) BW gain (19.1 ± 4.31 kg), with the gain increasing through d 244 ($P < 0.01$). A treatment × day interaction was also observed for change in maternal BW (Figure 18; $P < 0.01$). Maternal BW in cows fed R declined over time, with the change tending to be less than zero ($P \leq 0.08$) between gestational d 202 (-12.9 ± 6.56 kg) and 244 (-22.7 ± 11.2 kg), while the change in cows fed M was greater than zero ($P \leq 0.08$) between d 160 (16.8 ± 4.34 kg) and 230 (15.7 ± 7.54 kg), only returning to zero ($P = 0.51$) by d 244.

No treatment × day interaction was observed ($P = 0.56$) for change in backfat (Figure 19). Change in backfat declined ($P < 0.01$) over time; although losses were not significant in cows fed M ($P \geq 0.13$), backfat was negative in cows fed R from gestational d 230 (-0.22 ± 0.050 cm) through 244 (-0.32 ± 0.070 cm). No treatment × time interaction ($P = 0.08$) nor treatment effect ($P = 0.76$) was observed for change in REA (Figure 20). Ribeye area in both treatments declined over time, with the change falling below zero by gestational d 202 (6.20 ± 2.21 cm^2; $P = 0.02$) and declining through d 244 (-10.3 ± 2.50 cm^2).

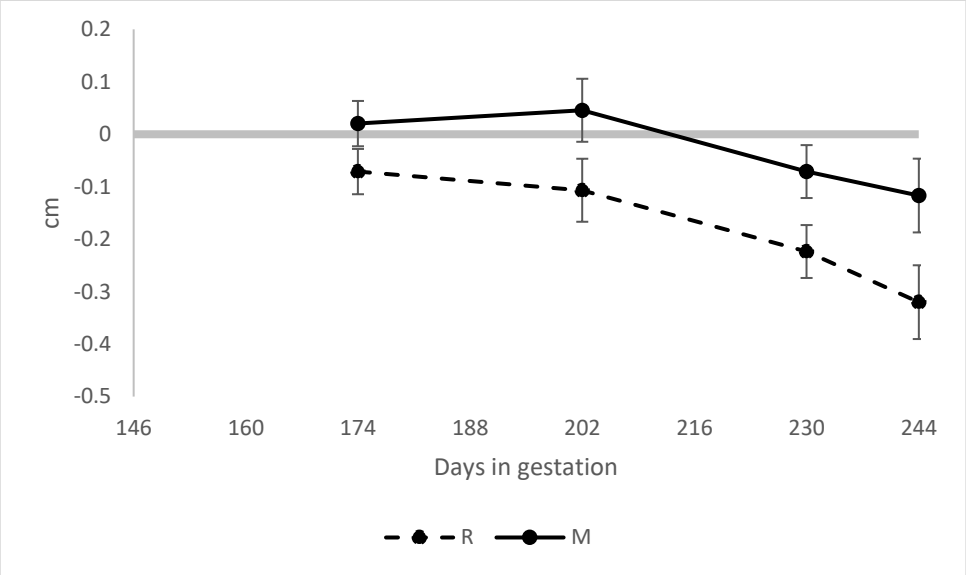

Figure 19. Change in backfat in cows fed a forage diet at or below maintenance intake levels. Cows were fed a hay diet at (**M**) or restricted to 70% (**R**) total NE_m intake levels from approximately d 146-244 of gestation. No treatment × day interaction was observed ($P = 0.53$). Both treatment ($P = 0.05$) and day ($P < 0.01$) effects were observed.

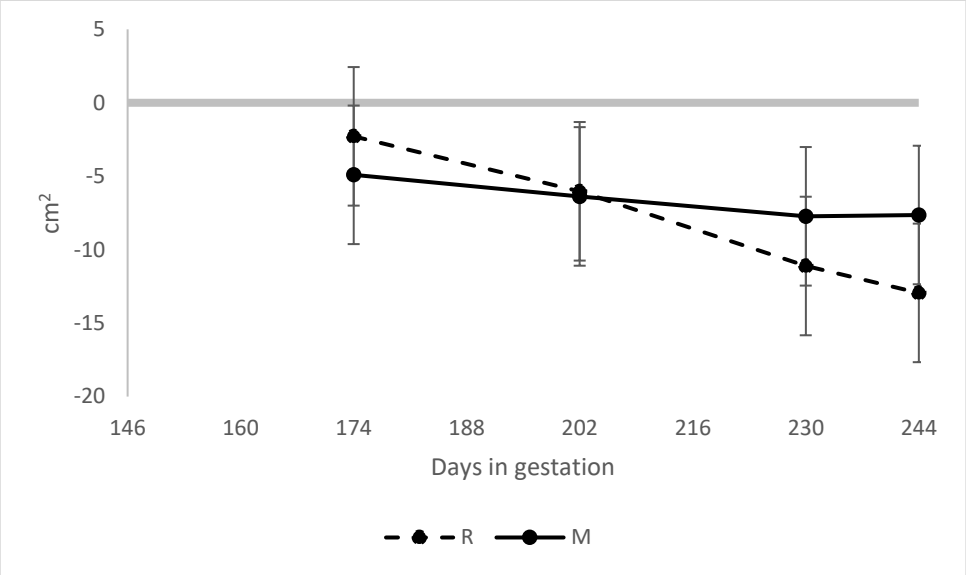

Figure 20. Change in ribeye area in cows fed a forage diet at or below maintenance intake levels. Cows were fed a hay diet at (**M**) or restricted to 70% (**R**) total NE_m intake levels from approximately d 146-244 of gestation. No treatment × day interaction nor diet effect ($P > 0.05$) was observed. A day effect was observed ($P = 0.01$).

On the d of harvest maternal BW was lower (Table 25; $P = 0.04$) and BW tended to be lower ($P = 0.08$) in cows fed R vs. cows fed M, but treatment did not affect intestinal length ($P \geq 0.24$) or contents of the stomach complex, small intestine, cecum, colon or total gastrointestinal tract ($P > 0.22$).

Table 25. Body weights, intestinal lengths, and gastrointestinal contents of cows fed a forage diet at or below total maintenance requirements from approximately d 146-244 of gestation

	Treatment[1]			
Item	70	100	SEM	*P*-value
Body weight, kg	461	484	16.40	0.08
Empty body weight, kg	349	381	14.70	0.04
Small intestine length, m	40.9	44.7	2.115	0.24
Large intestine length, m	6.04	6.07	0.562	0.96
Gastrointestinal contents				
Stomach complex, kg	59.0	65.7	3.498	0.22
Small intestine, kg	6.31	5.95	0.865	0.77
Cecum, kg	2.44	2.26	0.290	0.68
Colon, kg	2.64	2.31	0.466	0.63
Total tract, kg	70.4	76.3	3.325	0.26

[1]Values reported are least squares means with standard error of the means. R = cows restricted to 70% total NE_m requirements; M = cows fed 100% total NE_m requirements.

Mass of the liver lungs, gastrocnemius, spleen, cecum, colon, gravid uterus, uteroplacental and fetus were not affected by treatment (Table 26; $P \geq 0.13$). Mass of the kidneys was lower ($P = 0.05$), and mass of the pancreas, heart and small intestine tended to be lower ($P \leq 0.08$) in cows fed R vs. those fed M. However, when expressed relative to EBW, these measures were not different between treatments ($P \geq 0.20$). Both absolute

Table 26. Organ and fetal mass of cows fed a forage diet at or below total maintenance requirements from approximately d 146-244 of gestation

	Treatment[1]			
Item	70	100	SEM	*P*-value
Liver, kg	3.99	4.36	0.167	0.16
g/kg EBW	11.6	11.9	0.724	0.76
Pancreas, g	304	361	18.69	0.07
g/kg EBW	0.88	0.99	0.063	0.28
Heart, kg	1.65	1.80	0.054	0.08
g/kg EBW	4.79	4.95	0.298	0.72
Lungs, kg	3.45	2.94	0.434	0.43
g/kg EBW	9.95	8.00	1.179	0.28
Gastrocnemius, g	711	668	91.40	0.75
g/kg EBW	2.07	1.80	0.226	0.43
Spleen,g kg	0.94	1.08	0.187	0.62
g/kg EBW	2.76	2.95	0.513	0.80
Kidneys, kg	0.74	0.86	0.036	0.05
g/kg EBW	2.14	2.34	0.102	0.20
Stomach complex, kg	13.1	17.0	0.596	< 0.01
g/kg EBW	38.3	46.2	2.365	0.05
Small intestine, kg	4.64	5.41	0.238	0.06
g/kg EBW	13.5	14.8	0.901	0.33
Cecum, kg	0.70	0.75	0.133	0.81
g/kg EBW	1.98	2.09	0.418	0.86
Colon, kg	3.72	3.89	0.373	0.76
g/kg EBW	10.7	10.5	1.082	0.89
Gravid uterus, kg	42.2	41.1	2.016	0.71
g/kg EBW	122	108	8.224	0.26
Uteroplacenta, kg	9.46	8.42	0.680	0.30
g/kg EBW	27.3	22.1	2.201	0.13
Fetus, kg	25.8	25.6	1.332	0.93
g/kg EBW	74.6	67.4	5.235	0.34

[1]Values reported are least squares means with standard error of the means. R = cows restricted to 70% total NE_m requirements; M = cows fed 100% total NE_m requirements.

Table 27. Hepatic mitochondrial oxygen flux[1] of cows fed a forage diet at or below total maintenance requirements from approximately d 146-244 of gestation

Item[3]	R[2]	M	SEM	P-value
Leak	2.57	1.66	1.980	0.76
P_{CI}	15.1	14.0	1.560	0.63
P_{CI+II}	31.8	35.1	3.722	0.54
E	51.8	55.9	6.514	0.67
E_{CII}	37.7	37.6	4.335	1.00

[1]pmol/(s×mg)

[2]Values reported are least squares means with standard error of the means. R = cows restricted to 70% total NE_m requirements; M = cows fed 100% total NE_m requirements.

[3]P_{CI} = maximum ADP-simulated respiration with complex I substrates; P_{CI+CII} = maximum ADP-simulated respiration with complex I and II substrates; E = maximum electron transport activity; E_{CII} = non-coupled electron transport capacity

Table 28. Jejunal mitochondrial oxygen flux[1] of cows fed a forage diet at or below total maintenance requirements from approximately d 146-244 of gestation

Item	R^2	M	SEM	P-value
Leak	1.07	0.90	0.297	0.69
P_{CI}	1.80	1.57	0.230	0.50
P_{CI+II}	5.64	6.91	0.749	0.26
E	6.87	8.20	0.866	0.31
E_{CII}	4.41	5.17	0.743	0.49
% change, cyt *c*	18.9	22.0	3.374	0.51

[1]pmol/(s×mg)

[2]Values reported are least squares means with standard error of the means. R = cows restricted to 70% total NE_m requirements; M = cows fed 100% total NE_m requirements.

[3]P_{CI} = maximum ADP-simulated respiration with complex I substrates; P_{CI+CII} = maximum ADP-simulated respiration with complex II substrates; E = maximum electron transport activity; E_{CII} = non-coupled electron transport capacity

[4]Percent change in oxygen flux with addition of cyt *c*

($P < 0.01$) and relative ($P = 0.05$) stomach complex mass were reduced in cows fed R vs. cows fed M. Hepatic mitochondrial respiration (Leak, P_{CI} and $P_{CI + CII}$) and electron transport capacity (E; Table 27) were not affected by treatment ($P \geq 0.54$). Likewise, jejunal mitochondrial respiration and electron transport capacity (Table 28) were not different between treatments ($P \geq 0.26$). Percent change in oxygen flux with the addition of cyt *c* was also similar in both treatments ($P = 0.51$).

DISCUSSION

Visceral tissues account for disproportionally large amounts of heat production in the ruminant. In cattle, liver and gastrointestinal tissues represent approximately 8-15% of total body weight, yet they account for approximately 40-50% of total body energy consumption (Reynolds et al., 1991). Therefore, relatively small changes in their metabolism may represent a large proportion of total energy requirements.

Effects of intake on blood flow and total oxygen consumption across splanchnic tissues are well documented (Burrin et al., 1990; Reynolds et al., 1991). However, effects on mass specific rate of oxygen consumption in the liver and gastrointestinal tract appears remain somewhat unclear. The general dogma is that whole organ oxygen consumption is positively correlated with intake, and that the response manifests in the overall change in organ mass, rather than a change in consumption per unit of tissue mass.

While intake restriction has been shown to effect intestinal cellularity, vascularity (Reed et al., 2007; Neville et al., 2008) and ion transport in splanchnic tissues, several studies in sheep (McBride and Milligan, 1985; Rompala et al., 1987; 1988), pigs

(Nyachoti et al., 2000), rats (Burrin et al., 1988) and lactating cows (McBride and Milligan, 1984) have found no difference in mass specific oxygen consumption rate because of intake restriction. Wood et al. (2013) reported lower mass specific hepatic oxygen consumption in pregnant heifers fed 85% of total NE requirements vs. those fed at 140% of requirements, suggesting that the reduction may be an important regulator in nutrient partitioning in energy-restricted cattle. However, this conjecture is confounded, due to the lack of a control. While a treatment difference was detected, it remains unclear whether the response was the result of energy restriction in cows fed below maintenance or was actually a response induced by cows being fed well over maintenance. Our data, although not a direct measure of oxygen consumption, agrees with most historical literature, indicating no effect of intake on mass-specific oxygen consumption.

Decreased absolute weights of several thoracic and splanchnic organs, including heart, kidneys, liver and gastrointestinal tract are commonly a result of intake restriction (Rompala et al., 1988; Wang et al., 2009; Meyer et al., 2010), while changes in relative organ mass (g/kg BW or g/kg EBW), while more indicative of targeted attrition, are more sparsely reported. In cows fed TMR and forage diets, respectively, we observed 9 and 22% reductions in absolute stomach complex mass, with 17% reductions in relative mass in both treatments, which were somewhat greater than other reports.

Meyer et al (2010) reported an 11% reduction in actual stomach complex mass in cows fed 68.1% energy requirement vs. those fed at maintenance, with no difference detected in relative mass. Wood et al. (2013) reported only a 6% reduction in absolute

rumen mass, with no difference in relative mass, between cows fed 85 and 140% NE requirements. Burrin et el. (1990) reported a 37% in in relative stomach mass of lambs fed to maintain BW vs. those fed *ad libitum*; however, the magnitude of difference in intake was much larger in the lambs (maintenance vs. 2.6 times maintenance) by the final day of harvest. The rate of organ attrition reported by Burrin et al. (1990) is interesting, indicating that short-term energy restriction may allow for rapid declines equilibrium maintenance requirements, which could potentially provide opportunities for increased feed efficiency if applied to intensive cow-calf systems, although this hypothesis has not been tested.

Differences in BW, backfat and REA change between treatments were not surprising, as lower energy intake should reasonably result in reduced body energy; however, the minimal differences through approximately d 200 of gestation suggest that the degree of restriction may have been sustainable during early-mid gestation. This timing matches well with entry into the third trimester of pregnancy, when maternal glucose metabolism is known to adapt in support of fetal development (Bell and Bauman, 1997). The small magnitude reduction in backfat and minimal or lack of change in REA suggest that cows fed M were Large changes in BW immediately following treatment application are likely attributed to rapid changes in fill. In experiment 1, cows fed R experience a rapid decline in BW immediately following the start of the experiment.

These results are similar in direction and magnitude to those reported recently in our lab (see Chapter IV) and are consistent with the reduction in gastrointestinal contents

measured at necropsies. Cows fed R in experiment 2 did not experience the same decline in gastrointestinal contents or BW immediately following treatment application. This could be the result of increased ruminal retention time, which has been observed in similar studies in our lab (Trubenbach, 2014). Cows fed R in experiment 2 continue to lose weight, while cows fed R in experiment 1 did not, which could suggest that cows fed the TMR adapted to intake restriction by reducing maintenance requirements more rapidly than cows fed the forage diet.

CONCLUSIONS

In conclusion, dietary energy restriction reduced the mass of metabolically active organs, regardless of which diet was fed. Additionally, our data is consistent with historical literature, suggesting that mass specific oxygen consumption rate is not affected by intake level, and that reductions in total organ oxygen consumption manifest completely in reduced organ mass. Cows fed a TMR appeared to achieve weight stasis following a period of immediate losses, suggesting a potential performance advantage over cows fed a forage diet at intakes below maintenance. This is consistent with other data collected in our lab; however, the mechanism by which these efficiencies are attained remain unclear. Additional data regarding how the degree of intake restriction affects the rate and magnitude of decline in metabolically active organs is warranted.

CHAPTER VI

OVERALL CONCLUSIONS

Overall, limit-feeding high-energy diets to beef cows appears to be a reasonable solution for increasing efficiency of feed energy use, with minimal risks to ruminal health, especially when intake is controlled. Previous nutrition models neglect to account for effects of intake restriction on energy metabolism, causing in overestimation of feed requirements for intensively-managed beef cows.

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