EFFECT OF ENERGY SUPPLY ON AMINO ACID UTILIZATION BY GROWING STEERS

by

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D.V.M., University of La Plata, Argentina, 1996
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Effects of energy supply on the efficiency of methionine and leucine utilization in growing steers were evaluated in 3 studies. We hypothesized that increased energy supply would improve efficiency of amino acid utilization. In study 1, treatments were abomasal infusion of 0 or 3 g/d of L-methionine and supplementation with 3 amounts of energy (0, 1.3, and 2.6 Mcal GE/d) in a factorial design. Nitrogen balance was increased ($P<0.05$) by methionine supplementation and increased linearly ($P<0.05$) with energy supply, indicating that efficiency of methionine use was improved by energy supplementation. In study 2, the effects of supplementation with no energy or isocaloric (1.3 Mcal GE/d) supplementation with glucose, fat, acetate, or propionate at 2 levels of L-methionine supplementation (0 or 3 g/d) were evaluated. Supplemental energy increased ($P<0.01$) nitrogen retention, without differences among energy sources. The results indicated that energy supplementation improved the efficiency of methionine utilization, independent of energy source. In study 3, effects of energy supplementation on leucine utilization in growing steers at 2 body weights (150 kg in Exp. 1 and 275 kg in Exp. 2) were evaluated. Treatments were a 3 × 2 factorial with 0, 4, or 8 g/d of L-leucine infused abomasally and 2 amounts of energy (0 and 1.9 Mcal GE/d). In Exp. 1, nitrogen retention linearly increased in response to leucine supplementation when additional energy was supplied. When no energy was supplemented, nitrogen retention was similar for 4 and 8 g/d of leucine. Energy supplementation increased nitrogen retention ($P<0.01$), indicating that energy supplementation improved the efficiency of leucine utilization by modestly increasing nitrogen retention when leucine was limiting and by increasing the ability of steers to respond to the highest amount of supplemental leucine. In Exp. 2, nitrogen retention was not affected by leucine supplementation, indicating that leucine did not limit protein deposition. Energy supply increased nitrogen retention ($P<0.01$) independent of the level of leucine supplemented, demonstrating an increase in capacity for protein deposition when energy was supplemented. Overall, our results indicated that energy affects the efficiency of amino acid utilization, challenging the assumption of a constant efficiency of use.

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May Good bless you all.

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Dedication

To my beloved wife Karina and loving son "Juani". None of this would
be possible without your love and support

To my parents who has always encouraged
me to pursue my dreams

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CHAPTER 1 - LITERATURE REVIEW: INTERACTION BETWEEN PROTEIN AND ENERGY SUPPLY ON PROTEIN UTILIZATION IN GROWING RUMINANTS

INTRODUCTION

Ruminants have the unique capacity to transform relative low quality dietary N into high quality animal proteins (i.e. meat and milk). However, the efficiency of N utilization is usually low, leading to large amounts of N excreted to the environment and high feeding costs. A better match between animals' requirements and dietary supply is needed to maximize the efficiency of N utilization and minimize the environmental issues. Therefore, understanding how growing ruminants respond to variations in nutrient supply as well as interactions among nutrients is critical to estimate animals' requirements, predict their performance, and improve the efficiency of nutrient utilization. The effects of protein and energy supply on protein utilization have been studied during the last 50 years. However, our knowledge on the type of response curves and the efficiencies of protein utilization is relatively poor, because responses have been highly variable depending on the species (i.e. pigs vs. ruminants) and the experimental approach utilized.

Based on information generated with monogastric animals, the relationship between energy and protein supply on protein deposition has been described as protein- and energy-dependent phases of growth (Balch, 1967; Chowdhury and Ørskov, 1997; Titgemeyer, 2003). According to this theory, graphically represented in Figure 1, when protein supply is limiting, protein deposition will increase linearly with increases in protein intake (protein-dependent phase), until a point is reached where energy becomes most limiting, and the animal no longer responds, or, if so, only with a very low efficiency, to additional increases in protein supply. For instance, in Figure 1, increasing protein supply from level "A" to "B", when energy is not limiting (high energy supply), linearly increases protein deposition from level "D" to "E".

Moreover, when the supply of digestible protein is in excess of animals' requirements ("A" at low energy supply), additional protein intake ("B") does not affect protein deposition ("C"), but increases in energy intake will linearly increase N balance (energy-dependent phase), until a point is reached ("D") where protein becomes limiting. Therefore, based on this type of relationship between energy and protein supply, 2 important assumptions are implied:

1) Energy supply is related to the amino acid (AA) requirements, such that specific AA requirements exist only at a given energy level. The higher the energy intake, the greater the potential for growth, resulting in greater AA requirements. In addition, because the relationship between energy intake and rate of protein deposition is linear (Figure 1), AA requirements can be expressed relative to the energy intake.

2) The energy supply does not affect the efficiency of AA utilization. If protein is the limiting nutrient, increases in energy supply does not affect protein accretion. In Figure 1, protein deposition at "A" level of protein supply will result in similar levels of protein deposition ("D") for both intermediate and high levels of energy intake. Moreover, protein deposition is linearly increased by protein supply when protein is limiting growth, independent of the energy level provided. Thus, it is possible to assume a single efficiency of AA utilization for a broad range of energy supply.

These types of relationships and the assumptions derived from them have been the conceptual basis of many simulation models for estimating nutrient requirements for monogastric (NRC, 1998; Sandberg et al., 2005a, b) as well as ruminant animals (ARC, 1980; Ainslie et al., 1993; NRC, 1996). However, the experimental evidence that supports this type of interaction between protein and energy supply are primarily based on experiments using growing pigs, with much less (and more inconsistent) information from growing ruminants.

The objective of this literature review is to examine the currently available evidence to support the protein- and energy-dependent phases of growth in ruminants, comparing it to the evidence observed in growing pigs and discussing the implications of the results and the limitations of the different experimental approaches utilized. Finally, some physiological mechanisms potentially involved in the observed responses are presented, identifying areas in which more research is needed.

PROTEIN- AND ENERGY-DEPENDENT PHASES OF GROWTH IN GROWING PIGS

In nonruminants, the study of the energy- and protein-dependent phases of growth is relatively simple because the level and composition of the protein that reaches the small intestine can be determined through changes in the composition and amount of protein in the diet. Moreover, increased amounts of energy intake do not affect the total amount and composition of the protein reaching the small intestine.

Feeding young growing pigs (7 to 19 kg of body weight, BW) with diets deficient in protein, resulted in a linear increase in protein deposition due to increases in protein intake, independent of the level of energy intake (Campbell and Dunkin, 1983). However, when pigs were fed with levels of protein above their requirements, protein retention increased linearly with increasing amounts of energy intake. Campbell et al. (1983, 1985a), working with growing pigs from 20 to 45 kg BW, observed that when energy was limiting, protein deposition did not change when additional protein was supplied. When energy supply was adequate, however, protein deposition increased linearly by the supplementation of the most limiting AA. In addition, a linear relationship between energy supply and protein deposition (when dietary protein was not limiting) was also demonstrated by those studies using broad ranges of energy supply (Campbell et al., 1983, 1985a). Similar results were observed by Chiba et al. (1991b) working with growing pigs (20 to 50 kg BW), wherein protein deposition was linearly increased by supplementation with the most limiting AA (lysine) when energy supply was not limiting. However, when lysine was limiting protein retention, increasing levels of energy intake did not affect protein deposition. Moreover, a linear relationship between energy supply and protein deposition (when dietary protein was not limiting) was also demonstrated in heavier pigs for a broad range in energy supply (Chiba et al., 1991b). Furthermore, it has been observed that the increase in N retention in growing pigs due to increases in energy intake was similar for fat and carbohydrates supplementation, suggesting that the total energy intake level was more important than the energy sources utilized (Reeds et al., 1981).

Interestingly, all the studies using growing pigs up to 50 kg BW (Campbell and Dunkin, 1983; Campbell et al., 1983; Chiba et al., 1991b) demonstrated that the relationship between energy intake and rate of protein deposition was linear, including when pigs were fed *ad libitum* (maximum energy intake), suggesting that maximal protein retention in growing pigs at the early

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stages of development is beyond the upper limit of appetite. Thus, for those young pigs with high potential for lean tissue deposition, the point "E" of Figure 1, where energy limited the responses to additional protein intake, was not reached. For heavier pigs (50 to 90 kg BW), however, it has been observed that beyond a determined level of energy, when energy supply exceed the requirements for maximum protein deposition, the pigs did not respond to additional increases in energy supply because protein deposition was limited by intrinsic factors (Campbell et al., 1985b). Moreover, the point of maximum protein deposition was reached at lower energy levels in female than entire male pigs (Campbell et al., 1983, 1985b).

In conclusion, the results from experiments using growing pigs support the existence of the protein- and energy-dependent phases of growth as described in Figure 1. In addition, because the levels of energy intake and the rate of protein deposition was linear in most of the experiments (at least in young growing pigs), it is appropriate to express the protein or AA requirements in relation with the energy concentration in the diet (i.e. g AA/Mcal DE). For example, several studies have suggested that in growing pigs the performance was optimized when lysine (the most limiting AA) was 3.0 g/Mcal of DE (Chiba et al., 1991a, b). If AA concentration is not adjusted according to changes in energy concentration of the diet, the rate of protein deposition decreases and the rate of fat deposition increases due to increases in energy intake (Chiba et al., 1991a).

PROTEIN- AND ENERGY-DEPENDENT PHASES OF GROWTH IN GROWING RUMINANTS

Contrary to studies using growing pigs, the experimental information on the relationship between protein and energy supply on the efficiency of protein utilization is limited and highly variable for growing ruminants. This can be partially explained by the difficulties in studying variations in energy and protein supplies independently in ruminants. Because of normal rumen metabolism, it is difficult to alter energy intake without affecting the amount of microbial protein synthesized in the rumen, and consequently the total protein supply to the animal. Moreover, due to the dietary protein degradation and microbial protein synthesis in the rumen, it is relatively difficult to modify the amount and/or composition of protein supply to the animal

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through changes in dietary protein composition. Therefore, the study of the effects of energy and protein supplementation on the efficiency of protein utilization in ruminants requires special experimental models to circumvent the effects of rumen metabolism. The model should be designed so that no nutrients other than protein and energy are limiting protein deposition. The experimental approaches utilized by the studies found in the literature represented different degrees of complexity, resulting in experimental results with different degrees of precision, making it not always possible to compare among them. Therefore, the evidence of protein- and energy- dependent phases of growth in growing ruminants is presented according to the 3 experimental approaches most frequently used.

First experimental approach: Variation in nutrient intake. An approach used to study the interaction between protein and energy supply by ruminants is varying nutrient intake by: 1) fixing feed intake and varying diet composition, 2) feeding the same diets at different levels, or 3) varying the forage to concentrate ratio of the diet. This approach is the simplest, but also is the model that produces the most variable and least conclusive results, leading in some cases to misinterpretations.

Steers fed pelleted diets with 3 levels of protein at or below the estimated maintenance protein requirements and with 3 amounts of digestible energy intake (66, 100, and 133% of estimated maintenance energy requirements) increased daily BW gain with increasing energy intake, even when protein supply was limiting (Elliot et al., 1964). The authors suggested that increased energy supply might produce a protein-sparing effect, reducing the maintenance protein requirements and increasing protein deposition (Elliot et al., 1964). These results indicate that energy intake may increase the efficiency of protein utilization, challenging the assumption of a single efficiency derived from the theory of the protein- and energy-dependent phases of growth. However, in that study the increases in energy intake were achieved by increasing the levels of highly digestible carbohydrate-rich concentrates in the diet, which might also alter the ruminal fermentation and microbial protein synthesis. In addition, the response variable (BW gain) does not measure changes in protein deposition exclusively, and can be highly affected by changes in gut fill.

Working with growing calves grazing native range (limited in crude protein), Scales et al. (1974) observed no increases in daily gain in response to energy (corn grain) supplementation

when dietary crude protein was between 5 to 10%, suggesting that when protein limited growth, calves do not response to increases in energy intakes. On the other hand, when calves received protein supplementation (more than 15% total dietary crude protein), a significant increase in daily gain occurred with energy supplementation. These results seemed to support the existence of protein- and energy-dependent phases of growth, but effects of protein and energy supplementation on forage digestion were not evaluated in that study. It is likely that energy supplementation decreased fiber digestion in the rumen, with that effect being more accentuated at the lower levels of dietary protein (Klevesahl et al., 2003). Thus, energy supplementation at low levels of dietary protein might not necessarily be associated with significant increases in energy supply to the animals.

Andrew and Ørskov (1970), using early-weaned lambs fed with diets containing increasing concentrations of protein at low, medium, or high levels of feeding, observed that when feed intake was limiting N retention, the lambs did not significantly increase protein deposition in response to increasing amounts of protein supplementation, suggesting the existence of an energy-dependent phase of growth. On the other hand, at the highest level of feeding, there was a linear increase in N retention as protein intake increased, until reaching a point where energy (or another factor) became most limiting, at which point no further responses to protein intake were observed. Similarly, growing steers fed concentrate diets containing 3 levels of protein (8, 11, and 14%) and 2 energy concentrations (2.0 and 2.7 Mcal ME/kg) increased N retention in response to protein intake, but not to energy intake (Elsasser et al., 1989). These results might indicate that energy intake did not affect protein utilization when protein intake was limiting. These results, in agreement with previous studies using a similar experimental approach (Balch, 1967), suggested the existence of protein- and energy-dependent phases of growth in growing ruminants as observed in growing pigs. Moreover, the information generated in those early experiments has been the base of some nutrient requirement systems, which assumed that energy supply did not affect the efficiency of AA utilization (ARC, 1980; Ainslie et al., 1993; NRC, 1996).

The most obvious constraint of those experimental approaches based on dietary manipulation is the impossibility of altering the energy supply to the animals without affecting the amount of microbial protein synthesized in the rumen. Moreover, altering total dry matter intake can affect the rate of passage and ruminal degradation of dietary protein, resulting in a

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different amount and composition of AA reaching the small intestine. Therefore, increasing level of feeding to increase energy supply could be associated not only with greater energy supply, but also with a greater total protein supply and an increase in the ratio of metabolizable protein (MP):energy. Thus, it is almost impossible to discern if the animals were increasing growth in response to additional supply of energy or protein. If that is not considered, erroneous interpretations can be made. For example, Chowdhury and Ørskov (1997) re-analyzed the data of Andrew and Ørskov (1970) considering the finding of the complementary study of Ørskov and Fraser (1973). By estimating total N supply in the small intestine for the dietary treatments used by Andrew and Ørskov (1970), it was observed that increasing the feeding level to increase energy intake was also associated with greater amounts of both microbial and dietary N arriving to the small intestine (Ørskov and Fraser, 1973). Those changes in protein supply were not considered in the original paper (Andrew and Ørskov, 1970), which concluded that N balance was increased by increases in protein supply if energy was not limiting, supporting the existence of protein- and energy-dependent phases of growth. However, Chowdhury and Ørskov (1997) demonstrated that those early conclusions were potentially erroneous because the animals were not responding to additional energy supply through changes in N requirements, but rather by direct responses to additional MP supply that resulted from increases in dietary energy supply. Therefore, the earlier conclusions on the existence of protein- and energy-dependent phases in ruminants could not be further supported when considering the effects of rumen metabolism on total protein supply (Chowdhury and Ørskov, 1997).

The effects of protein and energy intake on protein deposition were also evaluated at sub-maintenance levels of energy supply. Fattet et al. (1984) fed growing lambs with 2 amounts of NaOH-treated barley straw (at or below maintenance) without or with fish meal supplementation to increase dietary protein to energy ratio. Using serial slaughter procedures, it was observed that the lambs had positive protein deposition, even at negative energy balance, challenging the assumptions of the energy-dependent phase of growth as described in growing pigs (Figure 1). Thus, it was concluded that, even at negative energy balance, endogenous energy mobilization (adipose tissue) could be used as fuel to sustain protein accretion when no limitations in protein supply exist. Those conclusions were further supported by similar experiments with overfat lambs (Vipond et al., 1989) and using intragastric infusion approaches (see below), but not by other studies (Iason and Mantecon, 1993; Galbraith et al., 1997). In addition, in recent studies

(Pittroff et al., 2006a, b), using diets similar to those used by Fattet et al. (1984), fish meal supplementation did not increase adipose tissue mobilization nor reduce protein mass losses. The genetic potential for lean tissue deposition and wool growth, feeding regime, and analysis methods may partially explain the apparent discrepancies among studies. Moreover, Iason and Mantecon (1993) suggested that adipose tissue mobilization is used to provide energy for protein deposition only when animals are overfat. Therefore, there is not conclusive evidence in the literature to support the theory that increasing MP:energy ratio in energy-deprived animals may consistently induce adipose tissue mobilization to sustain protein accretion.

Overall, the experimental results using this relative simple approach do not provide conclusive evidence to allow acceptance or rejection of the existence of protein- and energy-dependent phases of growth in ruminants.

Second experimental approach: Intragastrically maintained animals. The limitations of research models for independently evaluating the effects of energy and protein supply motivated the development of new research techniques, such as the use of total intragastrically maintained animals. This experimental model is based on the intra-ruminal infusion of volatile fatty acids (VFA), buffers and minerals, and the intra-abomasal infusion of protein, glucose, vitamins, and trace minerals (Ørskov et al., 1979), allowing variation in energy and protein supply independent of each other. Thus, this approach permits a more precise variation in nutrient supply without the confounding effects of digestive transformations by rumen metabolism. However, the evidence of protein- and energy-dependent phases of growth in ruminants generated with the intragastric infusions technique have been variable too.

Lindberg and Jacobsson (1990) observed in wether sheep that increasing the level of casein infusion, when the sheep received a high level of energy supply, linearly increased N retention. However, when energy infusion was limited, N retention reached a plateau when increasing levels of protein were infused. These results suggest the existence of protein- and energy-dependent phases of growth, but the efficiency of N utilization was not constant across different levels of N intake. In contrast to those results, other studies with lambs (Chowdhury et al., 1997) and steers (Chowdhury et al., 1990) demonstrated that N retention increased due to increases in N supply, even when sub-maintenance levels of energy were supplied. Those results supported the earlier studies (see above) with fish meal supplementation (Fattet et al., 1984;

Vipond et al., 1989), suggesting that endogenous fuel (fat mobilization) can be used to maintain protein deposition in situations when energy (but not protein) supply is limiting. These results challenge the assumption that when energy is limiting there should be no responses to additional protein supply. Moreover, Ørskov et al. (1999) observed that steers wholly nourished by intragastric infusion with a broad range of energy supply had linearly increased N retention in response to casein infusion, but there was little effect of energy supply when it was more than that needed to reduce β-hydroxybutyrate concentrations to basal levels. In addition, infusion of glucose or its precursors seemed to be more important in reducing urinary N excretion than total energy supply, suggesting that dietary energy supply affected protein utilization when a deficit of glucose existed (Ørskov et al., 1999). Similarly, fasting N excretion in sheep was reduced by both glucose and fat supplementation, although the effect of glucose was of greater magnitude (Asplund et al., 1985). Those results suggest that energy supply may affect the efficiency of protein utilization due to a sparing effect on AA by glucogenic energy sources.

Although most of the results from intragastric infusion experiments seemed to suggest that the existence of protein- and energy-dependent phases of growth are unlikely, some of the earlier conclusions can not be confirmed with experiments involving normally fed animals. Under conditions of total intragastric infusion, the metabolism of the animals may differ from that of normally fed animals because they usually present a non-functional rumen, as well as a regressed thin-walled intestine (Ørskov et al., 1979), resulting in different overall metabolism and reduced growth rate. Moreover, Pittroff et al. (2006a) suggested that direct comparisons between the results obtained with intragastrically maintained and normally fed animals may be misleading due to differences in synchronization of nutrient supply, stage of maturity, and planned vs. actually achieved variations in protein and energy supply to the animals.

Third experimental approach: Pre-ruminant calves. Another experimental approach to study the existence of protein- and energy-dependent phases of growth has been the use of preruminant calves. As the rumen is not yet functional, it is possible to alter both protein and energy intakes through changes in their concentration in milk replacers or altering the feeding levels, avoiding the issues generated by the normal rumen metabolism.

A quantitative analysis of more than 290 individual N balance results from 10 studies using milk-fed lambs indicated that, when lambs received a protein deficient diet, N balance

increased linearly as protein intake increased, with an estimated efficiency of absorbed protein utilization of 72% (Black and Griffiths, 1975). In addition, N balance was also increased in response to increases in energy intake, but only when dietary protein was above the lambs' requirements, indicating that energy intake increased the potential for protein deposition, and consequently the protein requirements. However, this increase in N balance in response to additional energy intake was of less magnitude when the lambs were heavier (Black and Griffiths, 1975), suggesting that as animals approach maturity less energy is partitioned to protein synthesis and more to adipose tissue accumulation. Overall, the analysis of that data set supports the existence of protein- and energy-dependent phases of growth in growing lambs, suggesting that efficiency of protein utilization was not affected by energy supply, although dependent of the stage of maturity of the lambs.

Conversely, other studies using preruminant cattle and the serial slaughter approach did not support these observations with milk-fed lambs. Some of the results with preruminant calves are summarized in Figure 2. To facilitate comparisons among studies, results were standardized to a metabolic BW basis ($BW^{0.75}$) as recommended by Black and Griffiths (1975) for preruminants.

Donnelly and Hutton (1976) compared 6 levels of protein intake at 2 levels of energy supply in Friesian bull calves (40 kg BW). As protein intake increased, protein deposition linearly increased (Figure 2A), indicating that protein supply was limiting protein accretion. Increased energy supply was also associated with increases in protein deposition, even when protein intake was limiting (Figure 2A). These results did not support the hypothesis of protein- and energy-dependent phases of growth. The greater protein retention in response to increases in energy supply at similar levels of protein intake indicated that the efficiency of protein utilization was increased by energy supply. In that study (Donnelly and Hutton, 1976), the marginal efficiency of utilization of digestible protein intake was approximately 45%, being similar for both levels of energy intake. In addition, calves receiving higher energy supply reached their maximum protein deposition capacity when the digestible protein intake was about 18 g/kg $BW^{0.75}$ daily (Figure 2A). Fat deposition was inversely related with protein deposition, indicating that when dietary protein is insufficient, the extra energy consumed is diverted to fat accretion.

Gerrits et al. (1996) also evaluated the rates of protein and fat deposition in preruminant calves with a range of BW from 80 to 240 kg and fed with 12 dietary treatments, combining 6 amounts of protein intake at 2 levels of energy supply. Independent of the BW of the calves, increased energy intakes enhanced fat and protein deposition, even at the lowest levels of protein intake (Figure 2B). Moreover, calves weighing between 160 to 240 kg BW and receiving the high energy supply reached a maximum protein deposition capacity at approximately 11 g of digestible protein/kg BW$^{0.75}$ daily (Figure 2B) as previously observed by Donnelly and Hutton (1976). However, maximum protein deposition was reached at a lower level of digestible protein intake (11 vs. 18 g of digestible protein/kg BW$^{0.75}$ daily) compared with that previous study (Donnelly and Hutton, 1976). The marginal efficiency of digestible protein utilization was between 40% to less than 30%, but was not affected by energy intake (Gerrits et al., 1996).

Similar results were observed in a recent study (Bartlett et al., 2006) with preruminant calves (46 kg of BW) fed increasing amounts of protein at 2 feeding levels. At low levels of energy supply, protein deposition was linearly increased as protein supply increased until it reached a point where energy become most limiting, such that calves did not respond to additional increases in protein intake (Figure 2C). At higher levels of energy intake, the potential for protein deposition was higher, resulting in linear increases in protein retention in response to all the amounts of protein intake evaluated. The efficiency of digestible protein utilization increased from 52 to 60% when calves received higher feeding levels, indicating that increased energy supply improved dietary protein utilization. However, in that study the higher energy intake was partially confounded with higher total protein supply and when requirements of maintenance were subtracted, the estimated efficiency of digestible protein use for protein gain was similar (75%) for both feeding levels. Conversely to the findings of previous studies (Donnelly and Hutton, 1976; Gerrits et al., 1996), maximum protein capacity was not reached (Figure 2C), possibly because of the lower protein intake levels evaluated and the differences in the genotype of the animals utilized (U.S. Holsteins vs. European Friesians).

Direct comparisons among studies are difficult because of the difference in levels of protein and energy evaluated, in BW, ages, and genotypes of the experimental animals, in the baseline used for analyzing changes in body composition, and in the protein source of the milk replacers (dried skim milk- vs. whey protein-based). It also was recently demonstrated that feeding frequency can affect the efficiency of protein utilization in preruminant calves (van den

Borne et al., 2006). In spite of this, several general conclusions can be made from the studies that used preruminant calves as experimental model. First, energy intake seems to improve the efficiency of protein utilization, even at low levels of protein intake, challenging the assumption of the current models of a single efficiency for different levels of energy intake (Ainslie et al., 1993; NRC, 1996). Thus, it is evident that the type of curves obtained with preruminant calves and summarized in Figure 2 are different than those assumed by theory of the protein- and energy-phases of growth (Figure 1). Second, alterations in dietary protein to energy ratio affect energy utilization and composition of gain. At similar energy intake, the lower the protein supply, the higher the fat deposited (Donnelly and Hutton, 1976; Diaz et al., 2001; Blome et al., 2003; Bartlett et al., 2006). Moreover, increasing levels of protein supply were associated with increases in fat deposition, even when protein intake was limiting (Donnelly and Hutton, 1976; Gerrits et al., 1996), indicating that nutrients were not used preferentially for protein accretion during the protein-dependent phase as it is usually observed in growing pigs. However, these changes in composition of gain due to alterations in dietary protein to energy ratio are not adequately modeled by the current NRC (2001) system for dairy calves (Diaz et al., 2001; Bartlett et al., 2006). Third, the efficiencies of digestible protein utilization observed in most of the studies, although variable, were lower than those usually found with monogastric animals, and also lower than the fixed value (66%) assumed by the current NRC (2001) model for preruminant calves. Fourth, although the use of preruminant calves seems to be a suitable experimental approach, it has not been well documented if ruminant animals have the same type of response to energy and protein supply. For instance, energy metabolism changes from the extensive use of glucose and long-chain fatty acids to a VFA-based metabolism. Moreover, the development of a functional rumen may also imply a higher demand for absorbed AA (Reynolds, 2002), and this may affect the efficiency of AA utilization. However, it was suggested that the metabolism of absorbed AA should be similar in preruminants and ruminants animals at similar BW in terms of their response to protein and energy supply (Black and Griffiths, 1975; Gerrits et al., 1997a). More experimental information using ruminant animals are needed to validate those conclusions.

POSSIBLE MECHANISMS INVOLVED IN THE NUTRITIONAL EFFECTS ON EFFICIENCY OF PROTEIN USE

The information on the physiological mechanism potentially involved in the responses in protein deposition due to changes in the nutritional status (primary protein and energy supply) is very limited. Several speculations have been proposed, but no conclusive evidence exists. Changes in the circulating hormone concentrations, and consequently in the magnitude of protein synthesis and degradation (protein turnover), can be affected by nutrition and may explain the effects on amount and efficiency of protein deposition. Although circulating hormone concentrations and protein turnover are closely related, they are discussed separately because only a few studies determined both of them simultaneously.

Changes in hormone concentrations. Determination of circulating levels of some key hormones is of interest in growing ruminants because they can, at least partially, modulate the nutritional effects on protein turnover, which finally will determine the net gain or loss of protein in the body (Davis et al., 2003; Lobley, 2003).

The growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis plays an important role in nutrient partitioning and protein metabolism (Breier et al., 1988). Produced primarily by the liver, but also by muscle, IGF-I is a mediator of GH actions on muscle. Once IGF-I is bound to cell surface-receptors, it initiates a cascade of signaling that results in stimulation in gene transcription and enhanced protein synthesis (Czech, 1989). Thus, although the biological significance of circulating levels of GH and IGF-I is not well understood yet (LeRoith et al., 2001), they were measured in some studies evaluating the protein- and energy-dependent phases of growth in growing cattle as a means to explain the changes (if any) in the efficiency of AA utilization by assuming that changes in serum hormone concentrations are directly associated with potential effects on protein turnover and AA oxidation. For example, in growing steers, long-term (14-d) injections of GH decreased AA oxidation, increased whole-body protein synthesis, without affecting protein degradation, resulting in increased N retention and efficiency of AA utilization (Eisemann et al., 1989), actions apparently mediated by IGF-I. The growth promoting effects of IGF-I, and its positive relationship with protein intake and protein deposition have also been demonstrated in several studies with growing calves (Gerrits et al.,

1998; Smith et al., 2002; Bartlett et al., 2006), lambs (Davenport et al., 1995), and steers (Maloney et al., 1998; Lobley et al., 2000). Although increased levels of feeding were often associated with greater serum IGF-I concentrations (Elsasser et al., 1989; Smith et al., 2002; Brown et al., 2005, Bartlett et al., 2006), the circulating levels of IGF-I and GH were not always closely related. It was observed in growing steers that GH concentrations were higher, but IGF-I was lower, at low levels of feeding (below maintenance) compared with higher levels of feeding, indicating that the GH/IGF-I axis is uncoupled in steers with a low level of nutrition (Dawson et al., 1998). This interaction between level of feeding and concentrations of circulating GH and IGF-I may partially explain the apparent inconsistency among studies. Although it is unknown if dietary energy or protein intake is more important for regulating a functional GH/IGF-I axis (Dawson et al., 1998), studies with growing cattle suggest that circulating levels of IGF-I are more strongly influenced by protein than energy intake (Elsasser et al., 1989; Gerrits et al., 1998; Bartlett et al., 2006).

Insulin also plays an important role in mediating the nutritional effects on muscle protein deposition, which affects the efficiency of AA utilization in growing animals (Davis et al., 2003). Positive relationships between plasma insulin concentrations and protein turnover were observed in growing heifers (Smith et al., 1992), but insulin stimulated protein synthesis more than protein degradation, resulting in a net increase in protein retention (Rooyackers and Nair, 1997). In growing calves, plasma insulin concentration was positively correlated ($r = 0.37$) with whole-body protein gain (Bartlett et al., 2006). Moreover, the low plasma levels of insulin observed at low levels of nutrition have been associated with decreases in the ability of GH to stimulate liver IGF-I secretion (Dawson et al., 1998). Thus, significant interactions among hormones in regulating protein and lipid metabolism in growing animals make the interpretation of the biological significance of a single hormone difficult.

Circulating levels of leptin have also been determined to be reflective of the energy status of animals. As adipocytes are the primary site of leptin production in cattle (Ji et al., 1998), it was hypothesized that serum concentrations of leptin are positively correlated with greater adiposity in ruminants (Geary et al., 2003). That relationship was also demonstrated in growing lambs in negative energy balance, where leptin concentration decreases as animals lose weight and fat tissue (Pittroff et al., 2006a). Fish meal supplementation did not accelerate fat mobilization or affect leptin level (Pittroff et al., 2006a, b). In addition, leptin was more strongly

related with energy intake than with fat mass in lambs in positive energy balance (Pittroff et al., 2006a).

Overall, these results on hormone concentrations indicate that they are actively involved in modulating the nutritional effects on protein metabolism. Care has to be exerted, however, when considering circulating levels of hormones to explain changes in protein and lipid metabolism because of the interactions among hormones, their interactions with other factors, and the local effects of other growth regulators. In addition, in all experiments there was a very high degree of variability in circulating hormone concentrations among animals, limiting the ability to detect statistically significant differences when the number of experimental units was small.

Changes in protein turnover. Protein turnover is the continuous process of protein synthesis and degradation, and the difference between these 2 opposing processes results in net protein gain or loss. This important physiological process, however, is associated with energy costs (primarily for protein synthesis), as well as with the oxidation of AA that reduces the overall efficiency of protein utilization (Lobley, 2003). Because the magnitude of protein turnover is very significant [i.e., only 6% of protein synthesized was retained in finishing steers (Lobley et al., 2000)], slight changes in protein synthesis or degradation may lead to important changes in protein retention. Therefore, evaluating changes in the magnitude of protein turnover may be useful to explain the effects of protein and energy supply on the efficiency of protein deposition (Reeds et al., 1981; Lobley, 2003).

In ruminants, the magnitude of protein turnover can be influenced by nutrient supply (Lobley et al., 1987). Growing steers fed increasing levels of the same grass-barley diet (from 0.8 to 2.6 times maintenance energy requirements) linearly increased daily BW gain and plasma AA flux, and reduced whole-body AA oxidation (Dawson et al., 1998). Similarly, there was a decline in the rates (activity per unit of metabolic BW) of both protein synthesis and degradation as steers' weight (maturity) increased (Lobley et al., 2000). Moreover, this decline was reversed as the level of nutrition increased (Lobley et al., 2000), supporting the hypothesis that nutritional status significantly affects the magnitude of protein turnover. It is not well understood, however, if increases in dietary energy or protein supply are more important for modulating those changes in protein turnover. Increases in metabolizable protein supply (Raggio et al., 2004) and most

limiting AA supply (Wessels et al., 1997), as well as in total metabolizable energy intake (Lobley et al., 1987; Smith et al., 1992) seemed to increase protein turnover, although the increases in protein synthesis were greater than the increase in protein degradation, resulting in increased protein deposition.

These nutritional effects on protein turnover have been modeled to explain responses in the efficiency of AA utilization. For example, models of results with preruminant calves (Gerrits et al., 1996) assumed a fixed fractional degradation rate (FDR) of 2% of total protein mass per day, an AA oxidation rate dependent on plasma AA concentrations, and a fractional synthesis rate (FSR) dependent on the AA and acetyl-CoA concentrations (Gerrits et al., 1997a, b). Although acetyl-CoA is not directly related with muscle protein synthesis, it represents a simple way to model the observed positive effect of energy intake on the efficiency of protein utilization (Gerrits et al., 1996), because it is assumed that increasing energy intake increases acetyl-CoA concentrations resulting in greater FSR at similar FDR and lower oxidation rates (due to reduced plasma AA concentration). In addition, AA imbalances increase the plasma concentrations, and consequently the oxidation rate, of those AA provided in excess (Gerrits et al., 1997a). Roux (2005) also proposed several equations describing protein turnover to estimate the efficiency and energy cost of protein deposition in growing ruminants. If appropriately validated, this type of quantitative approach, which considers efficiency of protein retention as a function of potential protein mass at maturity, protein synthesis activity according to the proportion of active nuclei in tissues, and level of intake (Roux, 2005, 2006), could potentially be applied to quantitatively evaluate the effects of nutrition and other factors on protein metabolism at the tissue level. However, due to the limited information for specific AA, most of the recently proposed models (Gerrits et al., 1997a; Roux, 2006), as well as current nutrient requirements systems (Ainslie et al., 1993; NRC, 1996) assume a similar efficiency of utilization for most of the essential AA. Thus, more experimental information is needed to enhance and validate these types of models.

SUMMARY AND CONCLUSIONS

Studying the effects of protein and energy supply in growing ruminants is difficult due to the normal rumen metabolism, and experiments have yielded variable results.

Experimental approaches that allow more controlled experimental conditions (intragastric infusions and preruminant calves) suggest that increasing energy supply affects the efficiency of AA utilization. That indicates that the current assumption of a single efficiency of AA use is not appropriate for growing ruminants, and that the models need to consider the level of energy supply in order to more precisely estimate AA requirements. The underlying mechanisms involved in the improvement of AA utilization are not totally understood, but they may involve changes in hormone profiles and in the magnitude of protein synthesis and degradation. Several aspects remain to be elucidated, however. The effect of the energy source supplemented (i.e., glucogenic vs. lipogenic) may differentially affect the efficiency of AA use. Moreover, the effects of energy supply could be different for the different AA. Thus, if the efficiency of use differs among AA, the first limiting AA should be evaluated in each specific experimental condition. The interaction between the effects of energy supply and other factors that affect efficiency of AA utilization, such as weight/age (maturity), genotype, use of growth promoters, environmental and infectious conditions, etc., remain to be determined. In addition, evaluating changes at tissue and cellular levels rather than whole-body responses using modern technologies (i.e., mRNA abundance, gene expression, etc.) may identify the tissues more responsive to nutritional effects and the physiological mechanisms involved in that response (i.e., expression of specific receptors, abundance and activity of AA catabolic enzymes, etc). Therefore, more research is needed evaluating the effects of energy supply on protein utilization in growing ruminants to build, enhance, and validate more complex models considering variable efficiencies of AA utilization.

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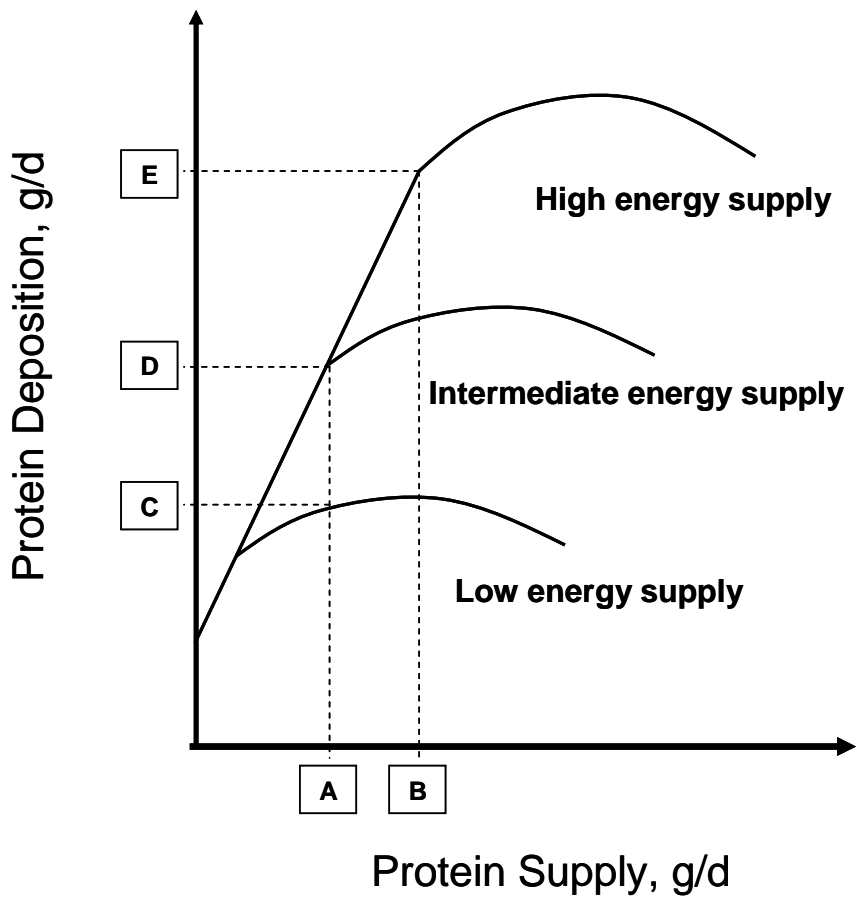
Adapted from Balch, 1967 and Titgemeyer, 2003

Figure 1-1. Representation of protein- and energy-dependent phases of growth

Protein Deposition, g/kg BW$^{0.75}$ daily

A

High energy intake
(0.29 Mcal ME/kg BW$^{0.75}$ daily)

Low energy intake
(0.23 Mcal ME/kg BW$^{0.75}$ daily)

B

High energy intake
(0.24 Mcal ME/kg BW$^{0.75}$ daily)

Low energy intake
(0.18 Mcal ME/kg BW$^{0.75}$ daily)

C

High energy intake
(0.25 Mcal ME/kg BW$^{0.75}$ daily)

Low energy intake
(0.17 Mcal ME/kg BW$^{0.75}$ daily)

Digestible Protein Intake, g/kg BW$^{0.75}$ daily
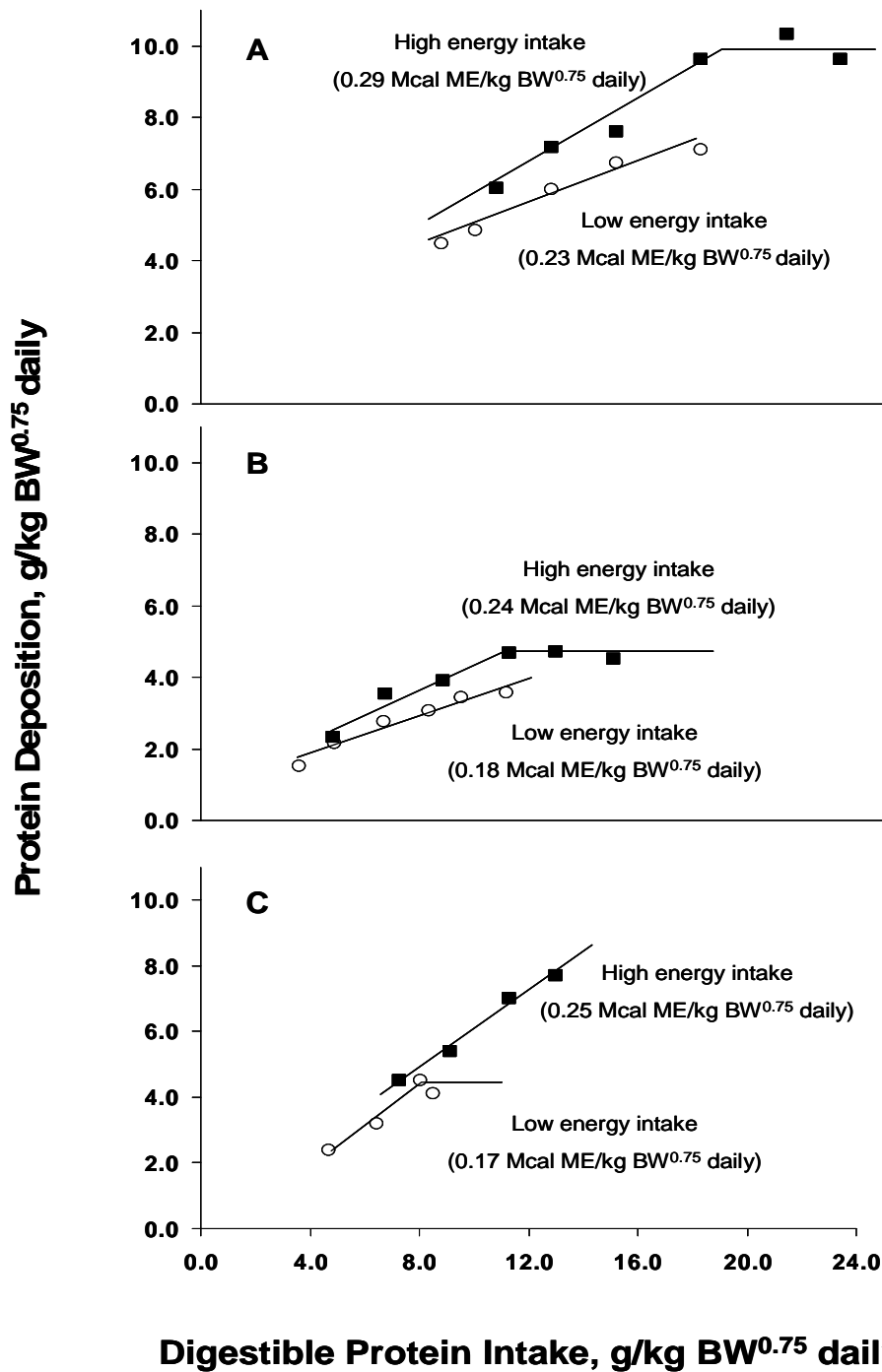
Figure 1-2. Protein deposition in preruminant calves fed increasing amounts of digestible protein intake at two levels of energy intake. Panel A = Adapted from Donnelly and Hutton (1976) using Friesian calves of 40 kg BW. Panel B = Adapted from Gerrits et al. (1996) using Holstein Friesian × Dutch Friesian calves of 160 to 240 kg BW. Panel C = Adapted from Bartlett et al. (2006) using Holstein calves of 46 kg BW.

■ = High energy intake, ○ = Low energy intake

CHAPTER 2 - EFFECTS OF ENERGY LEVEL ON METHIONINE UTILIZATION BY GROWING STEERS[1]

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ABSTRACT: We evaluated the effect of energy supplementation on methionine (**Met**) utilization in growing steers. Six ruminally cannulated Holstein steers (228 ± 8 kg BW) were used in a 6 × 6 Latin square and fed 2.8 kg DM/d of a diet based on soybean hulls. Treatments were abomasal infusion of two amounts of Met (0 or 3 g/d) and supplementation with three amounts of energy (0, 1.3, and 2.6 Mcal GE/d) in a 2 × 3 factorial design. The 1.3 Mcal/d treatment was supplied through ruminal infusion of 90 g/d acetate, 90 g/d propionate, and 30 g/d butyrate and abomasal infusion of 30 g/d glucose and 30 g/d fat. The 2.6 Mcal/d treatment supplied twice these amounts. All steers received basal infusions of 400 g/d of acetate into the rumen and a mixture (125 g/d) containing all essential AA except Met into the abomasum. No interactions between Met and energy level were observed. Nitrogen balance was increased ($P <$ 0.05) by Met supplementation from 23.6 to 27.8 g/d, indicating that Met limited protein deposition. Nitrogen retention increased linearly ($P <$ 0.05) with energy supply from 23.6 to 27.7 g/d. Increased energy supply also linearly reduced ($P <$ 0.05) urinary N excretion from 44.6 to 39.7 g/d and reduced plasma urea concentrations from 2.8 to 2.1 mM. Total tract apparent OM and NDF digestibilities were reduced linearly ($P <$ 0.05) by energy supplementation, from 78.2 and 78.7% to 74.3 and 74.5%, respectively. Whole-body protein synthesis and degradation were numerically increased by energy supplementation. Energy supplementation linearly increased ($P <$ 0.05) serum IGF-I from 694 to 818 ng/mL and quadratically increased ($P <$ 0.05) serum insulin concentrations (0.38, 0.47, and 0.42 ng/mL for 0, 1.3, and 2.6 Mcal/d, respectively). In growing steers, N retention was improved by energy supplementation, even when Met limited protein deposition, suggesting that energy supplementation affects the efficiency of AA utilization.

Key words: Methionine, Energy, Growth, Utilization

INTRODUCTION

Energy supply can limit protein deposition when dietary protein supply is not limiting. Campbell et al. (1985) found in pigs that when energy was limiting, protein deposition did not change in response to protein supply. When energy was adequate, protein deposition in pigs increased linearly with Lys supply, but when Lys limited protein retention, increased energy did

not affect protein deposition (Chiba et al., 1991). The relationship between energy and protein supplies on protein deposition in pigs has been described as protein- and energy-dependent phases of growth (Titgemeyer, 2003). With this model, the efficiency of AA use is not affected by energy intake, and AA requirements may be expressed relative to energy intake.

Protein- and energy-dependent phases of growth have been assumed for growing cattle by most nutrient requirements systems (Ainslie et al., 1993). Because of the difficulty in varying energy intake without affecting ruminal microbial protein production, the relationship between AA and energy supplies on protein deposition is poorly understood in ruminants. Lindberg and Jacobsson (1990), using sheep intragastrically infused with increasing protein at three rates of energy supplementation, found a linear relationship between N infusion and N retention when energy supply was high. At lower energy supplementation rates, N retention increased initially then plateaued as N supply increased, indicating protein- and energy-dependent phases of growth. In preruminant calves, increasing energy intakes improved protein retention at both high and low protein intakes (Gerrits et al., 1996), suggesting that energy intake affects efficiency of AA use, challenging the assumption of a constant efficiency of AA use across different energy levels.

Our objective was to determine effects of energy supply on Met utilization for whole-body protein retention in growing steers. We hypothesized that increased energy supply would improve efficiency of Met use.

MATERIALS AND METHODS

Animals and Treatments

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee. Six ruminally cannulated Holstein steers (228 ± 8 kg initial BW) were allocated in a 6×6 balanced Latin square design. All data from 1 observation were lost due to problems not related to treatment, and data for whole-body protein turnover were eliminated from 1 additional observation because it was an outlier (z-score [(value – mean)/(3 SD)] was 5.7; Morris, 1999).

The steers were housed in individual metabolism crates with continuous lighting and controlled temperature (22°C). The animals had continuous access to fresh water and were limit-

fed (2.82 kg/d of DM) a diet based on soybean hulls (Table 1) at 12-h intervals. The basal diet provided a low protein:energy ratio, small amounts of ruminally undegradable protein, and enough ruminally available N to maximize ruminal microbial growth (Campbell et al., 1997). Feed restriction was designed to maintain a small supply of AA to create a limitation in Met, such that a clear response to its supplementation could be achieved. A mixture of 20 g/d L-lysine·HCl, 10 g/d L-threonine, 10 g/d L-histidine·HCl·H$_2$O, 10 g/d L-phenylalanine, 5 g/d L-tryptophan, 20 g/d L-leucine, 10 g/d L-isoleucine, 10 g/d L-valine, 10 g/d L-arginine, and 20 g/d glycine was continuously infused abomasally to supply all essential AA in excess of the animals' requirements to prevent limitations in protein synthesis by AA other than Met, thereby allowing protein deposition until limited by energy or Met supply. The profile of AA infused was based on the supplies and requirements of AA estimated for growing Holstein steers fed with a diet similar to that used in our study (Greenwood and Titgemeyer, 2000). Amino acid solutions for each steer were prepared by dissolving L-Leu, L-Ile, and L-Val in 1 kg of water containing 60 g of 6 *M* HCl. Once these AA were dissolved, the remaining AA were added to the mixture, and, after addition of glucose and L-Met (see below), water was added to bring the total weight of the daily infusate to 4 kg. All steers received 10 mg/d pyridoxine·HCl, 10 mg/d folic acid, and 100 μg/d cyanocobalamin mixed with the abomasal infusate, to avoid deficiencies in those vitamins (Lambert et al., 2004), and 400 g/d of acetate into the rumen as an energy source.

Treatments were abomasal infusion of two amounts (0 or 3 g/d) of L-Met and supplementation with three amounts of energy (0, 1.3, and 2.6 Mcal GE/d, Table 2) in a 2 × 3 factorial. Methionine was the AA selected for study because it is the first-limiting AA for cattle when metabolizable protein is supplied primarily by ruminal bacteria (Richardson and Hatfield, 1978; Greenwood and Titgemeyer, 2000). The amounts of Met were selected to be in the range of linear response (0 to 6 g/d) for our experimental model (Campbell et al., 1997; Lambert et al., 2002). Continuous infusion of VFA into the rumen, continuous infusion of glucose into the abomasum, and pulse doses of lipid into the abomasum at 12-h intervals (Table 2) allowed increases in energy supply to the animal without increasing ruminal microbial protein synthesis. The VFA were mixed with water such that the total weight of the ruminal infusate was 4 kg/d. A peristaltic pump and polyvinylchloride tubing (2.4 mm i.d.) were used to infuse solutions into the rumen and abomasum. Abomasal lines were placed through the reticulo-omasal orifice and into

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the abomasum, and were retained by a rubber flange (8 cm diameter) attached at the end of the line.

Sample Collection and Analyses

Each experimental period consisted of 2 d for adaptation and 4 d for sample collection. It has been demonstrated, in our experimental model (Schroeder et al., 2006), and by others (Hovell et al., 1983; Moloney et al., 1998), that ruminants adapt within the 2-d time frame to changes in nutrient supply when ruminal adaptation is not required. Feed samples and refusals (if any) were collected from d 2 through 5 of each period, composited by period, and stored (-20ºC) for later analysis. Total urinary (into buckets containing 1.3 L of 1.38 *M* HCl to keep pH below 3) and fecal outputs were collected daily, with samples of urine and feces (1% and 10%, respectively) saved, composited by period within animal, and stored at -20ºC. Before analysis, samples were thawed at room temperature and homogenized. Feed, feed refusals, and fecal samples were partly dried at 55ºC for 36 h, air-equilibrated for 36 h, and ground with a Willey mill to pass a 1-mm screen. Partly dried diet and fecal samples were analyzed for DM (105ºC for 24 h), ash (450ºC for 8 h), and NDF with an ANKOM-Fiber Analyzer 200 (ANKOM-Technology, Fairport, NY) using Na_2SO_3 and heat-stable amylase. Total N was determined on diet, wet fecal samples, and urinary samples with a LECO FP 2000 N Analyzer (LECO, St. Joseph, MI). Urine samples were analyzed colorimetrically for NH_3 (Broderick and Kang, 1980) and urea concentrations (Marsh et al., 1965).

On the last day of each period, whole-body protein turnover was measured by the continuous infusion of labeled phenylalanine (L-$^2H_5$-Phe, Cambridge Isotope Laboratories, Andover, MA). Labeled phenylalanine replaced an equal amount (molar basis) of the phenylalanine contained in the basal AA mixture, and the labeled mixture was continuously infused (83.3 mg/h) into the abomasum for 10 h. Blood samples were collected 2 h before (background correction) and 10 h after infusion (Lobley et al., 2000; Löest et al., 2002), and enrichment of L-$^2H_5$-Phe in plasma was determined through gas chromatography-mass spectrometry (Calder and Smith, 1988) by monitoring ions of weight 336 and 341. Phenylalanine irreversible loss rate (mmol/h) was estimated as:

[(enrichment of L-$^2H_5$-Phe infused/ enrichment of $^2H_5$-Phe in plasma) – 1] × infusion rate of L-$^2H_5$-Phe.

This result was converted to whole-body protein flux (**WBPF**) by dividing by 0.035 (body protein concentration of Phe; Lobley et al., 2000). Whole-body protein synthesis (**WBPS**) and degradation (**WBPD**) were calculated (Wessels et al., 1997) from the relationships:

WBPF = WBPD + (N absorbed × 6.25) = WBPS + (urinary N excretion × 6.25). Thus, WBPS was calculated as:

$$\text{WBPS} = \text{WBPF} - (\text{urinary N excretion} \times 6.25),$$

and WBPD was calculated as:

$$\text{WBPD} = \text{WBPF} - (\text{N absorbed} \times 6.25).$$

On d 6 of each period, 10 h after the morning feeding, jugular blood was collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ). Blood collected in tubes containing sodium heparin was placed immediately on ice and then centrifuged for 20 min at $1,000 \times g$ to obtain plasma that was frozen (-20ºC) for later analysis. Plasma was analyzed for glucose, urea, and AA as described by McCuistion et al. (2004). Subsamples of plasma (2 mL) were deproteinized (0.5 mL of 6 M HClO$_4$) and centrifuged ($13,800 \times g$), and the supernatant was neutralized (0.26 mL of 6 M KOH) for β-hydroxybutyrate analysis (Kientsch-Engel and Siess, 1985). Blood samples were also collected in tubes without anticoagulant, left for 30 min at room temperature, and centrifuged for 20 min at $1,000 \times g$, and the serum was stored (-20ºC) for later analysis of IGF-I and insulin. Concentrations of IGF-I were determined by using an active IGF-I coated-tube immunoradiometric assay kit (intra-assay CV of 8.7% and sensitivity of 5.0 ng/mL; DSL-5600, Diagnostic Systems Laboratories, Webster, TX), and concentrations of insulin were determined with a RIA kit (intra-assay CV of 9.9% and sensitivity of 0.006 ng/mL; DSL-1600, Diagnostic Systems Laboratories).

To characterize the ruminal environment, ruminal fluid was collected after the last period. Ruminal samples were collected from the dorsal, ventral, and caudal areas of the rumen just before, and 4 and 8 h after feeding, and squeezed through four layers of cheesecloth. The pH of the ruminal fluid was measured immediately (Orion portable pH meter 230A, Orion Research Inc., Boston, MA) and an 8-mL aliquot was preserved with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen at -20ºC. Samples were subsequently centrifuged at $15,000 \times g$ at 4ºC for 30 min and analyzed for NH$_3$, as described for urine samples, and for VFA by gas chromatography as described by Vanzant and Cochran (1994).

Statistical Analyses

Statistical analyses were performed by using the MIXED procedure of SAS System for Windows 8.1 (SAS Inst. Inc., Cary, NC). To allow determination of possible carry-over effects, the experimental design was a balanced Latin square. The first analysis included the effects of Met, energy, Met × energy, period, and carry-over effect (Morris, 1999), with steer included as a random variable. Seven levels of carry-over effect were included in the model (6 treatments plus a pre-treatment effect for period 1). Carry-over effect was not significant for any variable studied, and it was subsequently excluded from the model (Morris, 1999). Linear and quadratic effects of energy supplementation rate, and their interactions with Met, were tested by single degree of freedom orthogonal contrasts. Treatment means were determined by using the LSMEANS option.

RESULTS AND DISCUSSION

Average ruminal NH_3 concentrations (6.2 ± 2.9 mM) were sufficient to maximize microbial growth and rumen digestion (Satter and Slyter, 1974). Total VFA concentrations and pH in the rumen were 78 ± 23 mM and 6.4 ± 0.4, respectively.

Nitrogen balance and diet apparent total tract digestibilities are shown in Table 3; the interaction between Met and energy was not significant for any of these variables. Apparent digestibilities of OM and NDF were linearly reduced by energy supplementation, which could be due to the VFA infusion into the rumen affecting ruminal conditions for fiber digestion. These negative effects of energy supplementation on OM digestion slightly reduced basal energy supply by 0.18 and 0.43 Mcal/d of DE. Thus, the planned differences in the amount of energy supplied among treatments was reduced from 1.3 and 2.6 Mcal/d of GE to 1.1 and 2.2 Mcal/d of GE, respectively. It is also possible that the supply of Met from the diet would be decreased by the energy supplements, but reduction in Met supply would strengthen our conclusions regarding responses to energy and Met supplies.

As expected, infusion of 3 g/d Met increased N retention (23.6 vs. 27.8 g/d), indicating that this AA limited protein deposition. Previous studies with the same experimental model demonstrated a linear response in N retention when Met was infused up to 6 g/d (Campbell et al., 1997) or 10 g/d (Lambert et al., 2002). If it is assumed that the empty BW of Holstein steers

contains 3.15% N (Fortin et al., 1980), the extra 4.2 g/d N retained would represent an increase of 133 g/d in ADG. Assuming that retained N is directly converted in protein deposition (N retention × 6.25) and protein for the whole empty body contains 2.0 g of Met/100 g protein (Ainslie et al., 1993), the calculated efficiency of supplemental Met utilization was 18% (0.53 g of Met deposited/3 g of Met infused). This efficiency was not significantly affected by energy supplementation.

Energy supplementation linearly increased N retention at both amounts of Met infusion (Table 3). This improvement in N retention was related to a decrease ($P < 0.01$) in urinary N excretion without changes in fecal N output, and it would represent about 68 and 149 g/d added gain for the 1.3 and 2.6 Mcal of GE/d treatments, respectively. The increase in N retention in response to energy supplementation at similar (or perhaps lower) levels of Met supply, which were less than the steers' requirements, demonstrates that there was an increase in the efficiency of Met utilization as energy supply increased. If the basal absorbable Met supply was 1.0 g/kg DM intake, as was measured for our diet by Campbell et al. (1997), the basal dietary Met supply was 2.8 g/d. The estimated efficiencies of utilization of dietary Met when steers did not receive Met supplementation were 96, 106, and 112% for the 0, 1.3, and 2.6 Mcal of GE/d treatments, respectively. Although the absolute values may be overestimated by overestimations of N retention (Gerrits et al., 1996), the relative changes suggest that the overall efficiency of Met utilization increased with the increase in energy supply. These results indicate that the assumption of a single efficiency of AA utilization is unlikely to be appropriate for growing cattle across a broad range of AA supplementation rates. Comparing energy supplementation rates similar to those used in our study, Gerrits et al. (1996) also observed increased protein deposition in preruminant calves (80 to 240 kg BW) when energy supply was increased, even at low protein intakes, supporting the idea that energy supply may affect the efficiency of AA utilization. Ørskov et al. (1999) observed that steers wholly nourished by intragastric infusion had linearly increased N retention in response to casein infusion, but there was little effect of energy supply when it was more than that needed to reduce β-hydroxybutyrate concentrations to basal levels. Moreover, infusion of glucose or its precursors seemed to be more important in reducing N excretion than total energy supply, suggesting that dietary energy supply affected protein utilization when a deficit of glucose existed (Ørskov et al., 1999). In our study, although energy supplementation slightly reduced β-hydroxybutyrate concentrations in plasma (Table 5),

it seems unlikely that our steers were grossly deficient in glucose; plasma glucose concentrations averaged 4.8 mM and were not different among treatments (Table 5). In the current study, we infused a mix of different energy sources (Table 2) with the goal of determining if broad effects of energy supplementation were present. Further research will be needed to evaluate effects of specific energy sources.

Of note, the efficiency of Met utilization was greater for the basal than for supplemental Met (96 to 112% for basal supplies vs. 18% for supplemental Met), indicating that the efficiency decreased as Met supply increased. Gradual decreases in the efficiency of utilization of sulfur-containing AA as their supply increased were reported in studies with growing rats, and the relationship between AA supply and the efficiency of AA utilization followed a sigmoidal response curve (Heger and Frydrych, 1989). In our study, as energy supplementation increased, it might be expected that the requirement for Met increased and that, in turn, the supply of Met as a percentage of its requirement decreased. Under these conditions, the improvement in the efficiency of Met utilization in response to energy supplementation might be a result of the position of our steers on a sigmoidal response of Met utilization to Met supply, with energy supplementation shifting the steers to a portion of the curve with a steeper slope. Data of Campbell et al. (1997), however, demonstrated a constant efficiency of Met utilization across amounts of Met supplementation that corresponded to the amounts used in our study. It is possible that the efficiency of Met utilization is not constant across all levels of Met supply, but rather is greater when supplies of Met are less than the amounts absorbed by our control steers.

In our study, WBPS and WBPD increased numerically with Met and energy supplementation (Table 4). The increase for WBPS was numerically greater than for WBPD (Table 4), however, resulting in an increase ($P < 0.05$) in N retention in response to Met and energy supplementation (Table 3). These results are in agreement with previous studies in which increased total intake (Lobley et al., 2000) or supply of the most limiting AA (Salter et al., 1990; Wessels et al., 1997) resulted in increases in both WBPS and WBPD. Rates of WBPF in our study were greater than those observed in previous studies with growing steers (Wessels et al., 1997; Lobley et al., 2000). This difference may be explained by the fact that we infused the labeled phenylalanine abomasally rather than intravenously and, thus, our values include first-pass metabolism by splanchnic tissues, which accounts for a substantial amount of WBPF (32 to 45%; Reynolds, 2002; Lobley, 2003). Furthermore, our estimates of WBPS (Table 4) were close

to the values (2.5 to 2.7 kg/d) reported by Lobley (2003) for steers at the height of productive performance. The amount of protein retained as percentage of the total protein synthesized (Table 4) was not significantly affected by the treatments, and was near the value (6%) observed for steers by Lobley et al. (2000).

Hormones are likely to be major regulators of protein turnover. Insulin plays an important role in mediating the nutritional effects on muscle protein deposition, which affects the efficiency of AA utilization in growing animals (Davis et al., 2003). Positive relationships between plasma insulin concentrations and WBPS and WBPD have been observed, but insulin increases WBPS more than WBPD, resulting in a net increase in protein retention (Rooyackers and Nair, 1997). In agreement with those results, energy supplementation increased serum insulin concentrations (quadratic, $P < 0.05$; Table 5), as well as WBPS, WBPD, and protein retention (Table 4). In response to different sources of energy, Schroeder et al. (2006) observed that protein retention was increased similarly by the sources of energy tested, but that serum insulin concentrations were quite different among sources. This suggests that insulin may be not the most critical regulation of protein deposition in our model. The growth promoting effects of IGF-I, and its positive relationship with protein intake and N retention, have also been demonstrated in growing lambs (Davenport et al., 1995). In our study, the serum concentrations of IGF-I were increased linearly by energy supplementation, but it was not affected by Met supplementation (Table 5). In contrast with our results, it has been observed in preruminant calves (Gerrits et al., 1998) and growing steers (Schroeder et al., 2006) that IGF-I increased with increasing protein supply but not with increasing energy intake, even when energy supplementation resulted in greater protein retention. Therefore, it is difficult to determine if the changes in hormone concentrations mediated the nutritional effects, or reflect changes in the metabolic status due to the alterations in energy and protein supply.

Plasma AA concentrations are presented in Table 5. Supplementation of Met was associated with a significant increase in plasma Met, although the small magnitude of increase indicates that Met supply did not exceed Met requirements. Plasma concentrations of valine, leucine, isoleucine, phenylalanine, lysine, tryptophan, serine, proline, asparagine, and ornithine were decreased by Met supplementation. These changes in plasma AA concentrations may indicate that supplementation with the AA that limited protein synthesis (Met) increased uptake and utilization of other AA, thereby decreasing their concentrations in the plasma. Previous

studies also observed reductions in plasma AA concentrations when supplementation with the most limiting AA was provided to growing steers (Campbell et al., 1997; Wessels et al., 1997). Energy supplementation linearly (leucine) or quadratically (lysine, aspartate, asparagine, and ornithine) decreased plasma concentrations of some AA, indicating that energy supplementation also increased AA uptake for protein synthesis. Both glutamate and glutamine plasma concentrations were linearly increased by energy supplementation. In as much as these 2 non-essential AA represent an important energy source for the gastrointestinal tract (Reynolds, 2002; Lobley, 2003), their increase in plasma concentrations may indicate that there was a reduction in the oxidation of these AA as a result of the increased availability of alternative energy sources. This hypothesis is supported by the linear decrease in plasma urea concentrations by the increase in amount of energy supplemented (Table 5). It is also possible that a decrease in the N that passed through the urea cycle could influence concentrations of glutamate and glutamine, which are important carriers for interorgan transport of N. Plasma serine concentrations were increased by energy supplementation, likely due to reductions in transsulfuration of Met as energy supplementation increased Met utilization for protein deposition.

IMPLICATIONS

Energy supplementation increased the efficiency of methionine utilization, indicating that the assumption of a single efficiency for methionine utilization is not likely to be appropriate for growing cattle. Thus, modeling of amino acid requirements in growing cattle may require consideration of the amount of dietary energy supplied.

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Table 2-1. Composition of diet fed to steers

Ingredient	% of DM
Pelleted soybean hulls	82.9
Wheat straw	7.6
Cane molasses	4.1
Dicalcium phosphate	1.9
Sodium bicarbonate	1.2
Calcium carbonate	1.0
Urea	0.42
Magnesium oxide	0.41
Trace mineralized salt [a]	0.20
Vitamin premix[b]	0.22
Sulfur	0.10
Bovatec-150[c]	0.01
Nutrient	
OM	90.3
N	2.19
NDF	50.9

[a] Composition (g/kg, minimum guarantee): NaCl (960 to 990); Mn (>2.4); Fe (>2.4); Mg (>0.5); Cu (>0.32); Zn (>0.32); I (>0.07); and Co (>0.04).

[b] Provided 4,410 IU of vitamin A; 2,205 IU of vitamin D; and 45 IU of vitamin E per kg diet DM.

[c] Provided 35 mg of lasalocid per kg diet DM.

Table 2-2. Energy sources infused into the rumen (acetate, propionate, and butyrate) or abomasum (glucose and lipid)

Energy source	GE, Mcal/d		
	0	1.3	2.6
	-------- g/d (Mcal/d of GE)--------		
Acetate	0	90 (0.31)	180 (0.62)
Propionate	0	90 (0.45)	180 (0.90)
Butyrate	0	30 (0.18)	60 (0.36)
Glucose	0	30 (0.11)	60 (0.22)
Lipid [a]	0	30 (0.28)	60 (0.56)

[a] Contained 20% stearic acid, 50% oleic acid, and 30% corn oil with a composition of 4.2% C16:0, 21.3% C18:0, 54.7% C18:1, 19.1% C18:2, and 0.7% C18:3, analyzed as described by Sukhija and Palmquist (1988).

Table 2-3. Effects of energy and methionine supplementation on nitrogen balance and apparent diet digestion in growing steers

	No methionine			3 g/d L-methionine			
	Gross Energy, Mcal/d						
Item	0	1.3	2.6	0	1.3	2.6	SEM[a]
No. of observations	6	6	5	6	6	6	
N, g/d							
Infused	19.0	19.0	19.0	19.3	19.3	19.3	-
Dietary intake	62.2	62.1	62.2	62.2	62.2	62.2	0.1
Total intake[b]	81.2	81.1	81.2	81.4	81.4	81.4	0.1
Fecal excretion	13.1	14.0	14.5	12.9	14.1	13.7	1.1
Urinary [b,c]	46.5	43.2	41.6	42.8	39.8	37.8	1.1
Urea [b,c]	32.9	30.4	29.9	30.7	26.4	24.4	1.7
Ammonia	1.6	1.7	1.9	1.0	1.3	1.1	0.4
Retained[b, c]	21.6	24.0	25.3	25.7	27.6	30.0	1.3
Diet apparent digestibility, %							
OM[b,c]	78.1	75.3	72.3	78.4	77.1	76.3	1.5
NDF[c]	79.0	76.5	72.1	78.5	76.9	76.9	1.6

[a] For n = 6.

[b] Effect of methionine ($P < 0.05$).

[c] Linear effect of energy supplementation ($P < 0.05$).

Table 2-4. Effects of energy and methionine supplementation on whole-body protein turnover in growing steers

| Item | No methionine | | | 3 g/d L-methionine | | | SEM[a] |
| | Gross Energy, Mcal/d | | | | | | |
	0	1.3	2.6	0	1.3	2.6	
No. of observations	6	6	4	6	6	6	
Protein, kg/d							
Flux	2.61	2.69	2.99	2.74	2.99	3.17	0.38
Synthesis	2.32	2.42	2.73	2.47	2.74	2.93	0.38
Degradation	2.19	2.27	2.58	2.31	2.57	2.75	0.38
Retention, as % of synthesis[b]	5.6	6.2	5.5	6.2	6.2	6.5	1.0

[a] For n = 6.

[b] (N retention × 6.25)/(protein synthesis) × 100.

Table 2-5. Effects of energy and methionine supplementation on serum hormone and plasma metabolite concentrations in growing steers

Item	No methionine Gross Energy, Mcal/d 0	1.3	2.6	3 g/d L-methionine Gross Energy, Mcal/d 0	1.3	2.6	SEM[a]
No. of observations	6	6	5	6	6	6	
Serum							
Insulin, ng/mL [c, d]	0.39	0.52	0.46	0.38	0.43	0.39	0.04
IGF-I, ng/mL [d]	691	728	734	698	764	902	80
Plasma							
Urea, mM [b, d]	2.95	2.65	2.20	2.63	2.29	1.90	0.24
Glucose, mM	4.66	4.89	4.92	4.71	4.86	4.80	0.15
β-hydroxybutyrate, mM [e]	0.21	0.25	0.19	0.24	0.23	0.16	0.02
Amino acid, μM							
Methionine [b]	9.2	9.1	9.9	14.7	14.2	11.0	1.4
Valine [b]	334	267	270	251	215	264	31.0
Leucine [b, d]	224	217	200	189	167	170	8.9
Isoleucine [b]	126	124	121	107	97	102	5.0
Threonine	107	117	124	113	100	117	6.7
Phenylalanine [b]	57	59	50	54	45	49	3.5
Tyrosine	25	32	25	23	23	17	4.9
Lysine [b, e]	189	140	180	171	125	149	12.5
Histidine	83	81	92	96	85	71	8.9
Tryptophan [b]	51	55	55	50	50	37	4.5
Glutamate [c, d]	79	94	89	71	86	106	7.4
Glutamine [d]	205	240	259	222	219	237	15.6
Alanine	207	195	201	211	185	211	12.8
Glycine [c, e]	318	316	436	311	301	341	24.9
Serine [b, c, e]	79	87	124	68	64	75	6.0
Proline [b]	65	65	69	63	59	63	3.3
Aspartate [e]	6.6	5.6	6.6	6.2	5.2	6.5	0.5
Asparagine [b, e]	31.6	31.1	34.6	30.3	27.2	30.3	1.4
Ornithine [b, e]	100	77	94	86	70	74	6.0

[a] For n = 6.

[b] Effect of methionine ($P < 0.05$).

[c] Effect of interaction methionine × energy ($P < 0.05$).

[d] Linear effect of energy supplementation ($P < 0.05$).

[e] Quadratic effect of energy supplementation ($P < 0.05$).

CHAPTER 3 - EFFECTS OF ENERGY SOURCE ON METHIONINE UTILIZATION BY GROWING STEERS[2]

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ABSTRACT: We evaluated the effects of different supplemental energy sources on methionine (**Met**) utilization in growing steers. Ruminally cannulated Holstein steers were used in two 6×6 Latin squares, with data pooled for analyses. In Exp. 1, steers (148 kg) were fed 2.3 kg/d of DM of a diet based on soybean hulls. Treatments (2×3 factorial) were abomasal infusion of 0 or 3 g/d of L-Met, and supplementation with no energy or with glucose (360 g/d) or fat (150 g/d) continuously infused into the abomasum. In Exp. 2, steers (190 kg) received 2.6 kg/d of dietary DM and were provided (2×3 factorial) with 0 or 3 g/d of L-Met and with no supplemental energy or with acetate (385 g/d) or propionate (270 g/d) continuously infused into the rumen. Energy sources supplied 1.3 Mcal/d of GE. In both experiments, all steers received basal infusions of 400 g/d of acetate into the rumen and a mixture (125 g/d) of all essential AA except Met into the abomasum. Nitrogen balance (18.8 vs. 23.5 g/d, $P < 0.01$) and whole-body protein synthesis (2.1 vs. 2.3 kg/d, $P < 0.07$) were increased by Met supplementation, indicating that Met limited protein deposition. Supplemental energy reduced ($P < 0.01$) urinary N excretion and increased ($P < 0.01$) N retention, without differences among energy sources. Increases in N retention in response to Met were numerically greater when energy was supplemented. Efficiency of supplemental Met utilization was 11% when no energy was supplemented, but averaged 21% when 1.3 Mcal/d of GE was provided. Whole-body protein synthesis and degradation were not affected by energy supplementation. Serum insulin concentrations were increased by glucose and propionate supplementation. Serum IGF-I concentrations were increased by supplementation with Met or glucogenic sources of energy. In growing steers, N retention was increased by energy supplementation even though Met limited protein deposition, suggesting that energy supplementation improves the efficiency of AA utilization. These responses were independent of the source of energy.

Key words: Methionine, Energy, Growth, Utilization

INTRODUCTION

Schroeder et al. (2006) observed that growing steers increased protein deposition in response to increased energy supplementation, even when Met limited protein accretion. In preruminant calves, it was similarly observed that increasing energy intakes improved protein retention at both high and low protein intakes, suggesting that increasing energy supply increases

the efficiency of AA utilization (Donnelly and Hutton, 1976; Gerrits et al., 1996). These results indicate that the assumption of a single efficiency of AA utilization by growing cattle is unlikely to be correct, and that energy level should be considered to more precisely estimate AA requirements.

It is unknown if the source of energy also affects AA utilization because most researchers have used mixtures of energy sources (Gerrits et al., 1996; Schroeder et al., 2006) when studying interaction between energy and post-absorptive utilization of protein or AA. In growing pigs, similar increases in N retention in response to energy supplied as carbohydrate or fat have been observed (Reeds et al., 1981). Fasting N excretion in sheep was reduced by both glucose and fat supplementation, although the effect of glucose was greater (Asplund et al., 1985). In addition, in steers wholly nourished by intragastric infusion, supplementation with glucose or its precursors seemed to be more important than the total amount of energy supplied in improving N retention (Ørskov et al., 1999), suggesting that the source of energy provided should be considered when evaluating the effects of energy supplementation.

The objective of our study was to determine the effects of energy source on Met utilization for whole-body protein retention in growing steers. We hypothesized that energy supplementation would increase protein deposition, and that the effect would be greater for glucogenic than for nonglucogenic energy sources.

MATERIALS AND METHODS

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee. Management of cattle, sample collections, laboratory analyses, and calculations followed the procedures described in the companion paper (Schroeder et al., 2006).

Ruminally cannulated Holstein steers were used in 2 balanced 6×6 Latin squares. In Exp. 1, 6 steers (148 ± 25 kg) were fed 2.3 kg/d of DM of a diet based on soybean hulls (Table 1) at 12-h intervals. Treatments (2×3 factorial) were abomasal infusion of 0 or 3 g/d of L-Met, and supplementation with no energy or with 360 g/d of glucose or 150 g/d of fat continuously infused into the abomasum. Fat was provided as a mix of 20% stearic acid, 50% oleic acid, and

30% corn oil, resulting in a fatty acid composition of 4.2% C16:0, 21.3% C18:0, 54.7% C18:1, 19.1% C18:2, and 0.7% C18:3 measured as described by Sukhija and Palmquist (1988).

In Exp. 2, the same 6 steers used in Exp. 1 plus an extra steer (190 ± 19 kg) received 2.6 kg/d of dietary DM (Table 1) and were provided in a 2 × 3 factorial with 0 or 3 g/d of L-Met, and with no supplemental energy or with 385 g/d of acetic acid or 270 g/d of propionic acid continuously infused into the rumen. The extra steer (seventh) was provided treatments in a sequence identical to one of the other steers. Four observations were not obtained due to reasons not related to treatments.

Methionine was the AA studied because it is the first-limiting AA for cattle when metabolizable protein is supplied primarily by ruminal bacteria (Richardson and Hatfield, 1978; Greenwood and Titgemeyer, 2000). The amounts of Met were selected to be in the range of linear response (0 to 6 g/d) for our experimental model (Campbell et al., 1997; Lambert et al., 2004). The energy supplements provided about 1.3 Mcal/d of GE without increasing microbial protein synthesis in the rumen. This amount of energy was within the range of linear response to energy supplementation (0 to 2.6 Mcal/d of GE) observed by using a mixture of energy sources (Schroeder et al., 2006).

All steers received continuous abomasal infusion of a mixture of AA and vitamins described by Schroeder et al. (2006). In Exp. 1, glucose was dissolved in the AA mixture such that the total weight of the abomasal infusate did not differ among treatments. All steers received 400 g/d of acetate (mixed with water to a total weight of 4 kg) infused continuously into the rumen as an energy source. In Exp. 2, the acetate and propionate treatments were mixed with the basal acetate infusate such that the total weight of the daily ruminal infusate remained 4 kg for all treatments. In Exp. 1, an additional infusion line was used to continuously infuse the fat into the abomasum.

Each experimental period consisted of 2 d for adaptation and 4 d for sample collection. This adaptation period was validated during the first 2 periods of Exp. 2, in which N balance was measured during the 3 d before starting the treatments, while all steers were adapted to the same basal infusions, and during the first two 6-d periods in which steers received their assigned treatments. During these two 6-d periods, N balance was measured over 6 consecutive 2-d blocks of time. Differences between values for each of the 2-d measures and the average value for the last 4 d of each period were calculated and analyzed by using a Student *t*-test for paired

means. To allow lack of adaptation, if present, to be fully expressed, data from steers that demonstrated increases or decreases in N retention in response to changes in dietary treatment from one period to the next were analyzed separately.

Data from the 2 experiments were analyzed together as a double Latin square, with a 2 × 5 arrangement of treatments (2 amounts of Met and 5 energy sources). Statistical analyses were performed with the MIXED procedure of SAS System for Windows 8.1 (SAS Inst. Inc., Cary, NC), with the model including fixed effects for Met, source of energy, Met × energy, experiment, and period nested within experiment. Animal nested within experiment was included as a random variable. The following orthogonal contrasts were used to evaluate the effects of energy supplementation and its interactions with methionine: 1) effect of energy supplementation (control vs. all sources of energy), 2) glucogenic (propionate and glucose) vs. lipogenic (acetate and fat), 3) propionate vs. glucose, and 4) acetate vs. fat. Treatment means were determined by using the LSMEANS option.

RESULTS AND DISCUSSION

Changes in N balance over time during the first 2 periods of Exp. 2 are presented in Figure 1. Steers that increased, as well as those that decreased, N balance in response to the change in treatment presented a steady-state response after the first 2 d of treatment infusion (Figure 1). Thus, under the conditions of our model, 2 d of adaptation is fully adequate to allow stabilization of N retention responses to treatments. Adequate adaptation periods of 2 d or less for N balance studies were observed in lambs after changes in the amounts of VFA infused into the rumen and of protein into the abomasum (Hovell et al., 1983). Moloney et al. (1998) also observed that Holstein steers presented a steady-state response in N balance, plasma urea N, and IGF-I mRNA abundance in muscle within the first day of receiving continuous infusion of casein into the abomasum. Those previous studies, along with our results, suggest that ruminants adapt rapidly to changes in VFA infusion into the rumen and changes in postruminal nutrient supply.

In Exp. 1, as expected, infusion of glucose or fat into the abomasum had little impact on ruminal pH (6.0 ± 0.3), total VFA concentrations (112 ± 13 mM), or concentrations of the individual VFA (data not shown). In Exp. 2, the molar ratio of acetate:propionate in the rumen was 6.6:1 for control, greater for acetate (9.9:1), and less for propionate infusion (3.1:1), but total

VFA concentrations (109 ± 7 mM) and pH (6.1 ± 0.1) were similar among treatments. The average NH_3 concentrations (5.6 ± 1.5 mM) were sufficient to maximize microbial growth and rumen digestion (Satter and Slyter, 1974). Ruminal measures represent observations from only one steer for each treatment, and they are presented not as a means to discriminate among treatments, but rather to provide an indication of the ruminal environment.

Nitrogen balance and diet apparent total tract digestibilities are shown in Table 2. Ruminal infusion of acetate ($P < 0.05$) and propionate ($P < 0.08$) reduced the apparent total tract OM digestion (Table 2), likely due to reductions in NDF digestion, as was observed when increasing amounts of VFA infused into the rumen linearly reduced NDF digestion (Schroeder et al., 2006). Although these reductions in digestibility could decrease slightly the planned differences in energy supply, it is not a concern for the interpretation of the results. Rather, a reduction of Met supply in those treatments (due to less microbial protein synthesis) would strengthen our conclusions regarding to the effects of energy supplementation on the efficiency of Met utilization. In Exp. 1, fat supplementation increased the apparent total tract digestion of those fatty acids (C16:0, C18:0, C18:1, C18:2, and C18:3) present in the supplement (Table 2). If similar endogenous fatty acid losses and similar digestion of dietary fatty acids relative to the control treatment are assumed, the total tract digestion of supplemental fatty acids was 96.7%, indicating that the supplemental fatty acids were well digested.

The infusion of Met increased N retention (18.8 vs. 23.5 g/d), indicating that this AA limited protein accretion. This increase in N retention was related to decreased urinary N excretion (Table 2), as has been observed in previous studies when the most limiting AA was infused into the abomasum (Campbell et al., 1997; Lambert et al., 2004; Schroeder et al., 2006). The average increase in N retention (4.7 g/d) by Met supplementation would represent a 149-g increase in the ADG if empty BW of Holstein steers contains 3.15% N (Fortin et al., 1980). Increased energy supply also decreased urinary N excretion ($P < 0.01$) and improved N retention ($P < 0.01$; Table 2). These increases in N retention in response to energy supplementation were numerically greater when the steers received 3 g/d of L-Met, with no significant differences among energy sources (Table 2). Although the contrast for [control vs. energy] × Met interaction was not statistically significant ($P = 0.16$), the greater effects of energy supplementation on N retention when the steers received Met supplementation indicate that increasing energy supply increased the efficiency of supplemental Met utilization. In other

studies with preruminant calves (Donnelly and Hutton, 1976; Gerrits et al., 1996) and growing steers (Schroeder et al., 2006), however, the responses in N retention to energy supplementation were similar at different rates of protein intake. More research is needed to explain the apparent lack of consistency among studies with respect to interactions between supplies of protein and energy.

The efficiency of Met utilization was estimated by assuming that retained N is directly related to protein deposition and that protein in the whole empty body contains 2.0 g of Met/100 g of protein (Ainslie et al., 1993). The efficiency of supplemental Met utilization was 11% when no supplemental energy was provided, but increased to 28, 18, 19, and 21% when 1.3 Mcal/d of GE was provided as glucose, fat, acetate, and propionate, respectively. There were no differences among the energy sources for N retention, and those improvements in N balance in response to increased energy supply would correspond to increases in ADG of 37 and 114 g at 0 or 3 g/d of Met, respectively. The basal dietary Met supply was estimated to be 2.5 g/d (Campbell et al., 1997). Calculated from the basal Met supply and the increase in Met retention ($0.02 \times 6.25 \times$ N retention; Table 2), the estimated efficiency of utilization of dietary Met was increased from 91 to 97% by the supplemental energy ($P < 0.01$), without differences among sources of energy. Although these efficiencies of dietary Met utilization may be overestimated by overestimations of N retention (Gerrits et al., 1996), the treatment differences are likely to be appropriate. The magnitude of this improvement in the efficiency of Met utilization was similar to that observed when a mixture of supplemental energy sources was provided at the same energy level (Schroeder et al., 2006). These results were in agreement with those of previous studies (Donnelly and Hutton, 1976; Gerrits et al., 1996; Schroeder et al., 2006), indicating that increasing energy supply increased the efficiency of Met utilization, even when Met was clearly limiting for protein accretion. These results also suggest that the assumption of a constant efficiency of AA utilization across different energy levels is unlikely to be correct for growing ruminants.

Lack of differences among the sources of energy on N balance was also observed in growing pigs, in which the increases in N retention were similar when energy was supplied as carbohydrate or as fat (Reeds et al., 1981). However, studies using sheep (Chowdhury et al., 1997) or steers (Ørskov et al., 1999) nourished intragastrically demonstrated that the infusion of glucose or glucogenic energy sources was more important to increase N balance than was the

total amount of energy infused. Moreover, when glucose requirements were met, N retention in ruminants was not affected by energy supply (Chowdhury et al, 1997; Ørskov et al., 1999). In addition, steers nourished intragastrically had reduced N balance when acetate was more than an 80 to 86 molar percentage of total VFA supplied, regardless of total amount of energy infused (Ørskov et al., 1991; Ørskov and MacLeod, 1993). This negative effect on N retention when acetate provided the preponderance of energy was associated with a deficiency of glucose precursors and subsequent increases in AA oxidation and urinary N excretion (Ørskov et al., 1991; Ørskov and MacLeod, 1993). Those results suggest a sparing effect of glucose that allows AA, which would otherwise be used for glucogenesis, to be available for protein deposition. In contrast, in our study, N retention was increased by energy supplementation, regardless of the energy source (Table 2), although there was not an evident glucose deficiency (plasma glucose averaged 4.7 mM; Table 4). Moreover, acetate was 79 ± 1.1 molar percentage of total ruminal VFA for control steers. Although this proportion of acetate in the rumen was close to those values reported as detrimental for N retention, ruminal infusion of acetate increased N balance, compared with the control (Table 2). In our experimental model, the steers received supplemental AA in addition to the basal microbial protein supply, resulting in greater total metabolizable protein supply than for steers nourished intragastrically (Ørskov and MacLeod, 1993), which may have prevented a deficiency of glucose precursors. The different experimental conditions (i.e., intragastrically maintained vs. normally fed animals) also may explain the apparent disagreements between our results and those from previous studies.

The greater N retention when steers were supplemented with 3 g/d of L-Met (Table 2) was associated with a tendency for increased ($P = 0.07$) WBPS, as well as WBPD ($P = 0.11$; Table 3). Similar results were reported for growing steers when the most limiting AA was infused abomasally (Wessels et al., 1997; Schroeder et al., 2006). In addition, the amount of protein retained as percentage of WBPS was increased by Met supplementation (5.7 vs. 6.3%, $P < 0.04$), with no differences among energy sources. These values, which were similar to that (6%) observed for growing steers by Lobley et al. (2000), indicate that the amount of protein retained is a small proportion of the protein synthesized. Although energy supplementation increased N retention (Table 2), there were no significant effects on WBPS or WBPD. Similar results were observed in growing pigs, from which it was concluded that the increase in WBPS was related more to changes in protein than to changes in energy intake (Reeds et al., 1981).

Plasma urea N concentrations were decreased by both Met and energy supplementation (Table 4), in agreement with the reduction in urinary urea N excretion observed for those treatments (Table 2). Plasma glucose concentrations were increased by the infusion of glycogenic sources of energy. Plasma glucose concentrations were lower when the steers received Met supplementation, however, suggesting greater tissue utilization or less synthesis due to tissue utilization of glucogenic AA for protein deposition. Supplementation with glucogenic energy sources increased serum insulin concentrations, and these effects were greater for glucose than for propionate (Table 4). Matsunaga et al. (1999) also observed increases in insulin and glucose concentrations in wethers infused with a VFA mixture. Serum IGF-I concentrations were increased by both Met and glucose supplementation, but were decreased by acetate infusion. In ruminants, the changes in blood IGF-I concentrations in response to nutrient intake were highly variable (Gluckman et al., 1987). In preruminant calves, plasma IGF-I concentrations increased with increasing protein supply, but not with increasing energy intake, even when energy supplementation resulted in greater protein retention (Gerrits et al., 1998). In our companion study; however, serum concentrations of IGF-I were increased linearly by increasing energy supply, but it was not affected by Met supplementation, though both energy and Met supplementation increased N retention (Schroeder et al., 2006). In growing steers, casein infusion increased N balance and IGF-I mRNA abundance in muscle, but did not affect circulating levels of IGF-I (Moloney et al., 1998). These results indicate that it is difficult to determine a relationship between responses in N balance and blood concentrations of IGF-I, and suggest that other factors may affect its concentrations and/or that serum IGF-I concentrations does not completely reflect the regulatory effects of this hormone at the tissue level.

Plasma AA concentrations are presented in Table 4. Plasma concentrations of Met were slightly increased ($P < 0.05$) by Met supplementation, although not to concentrations that would indicate that Met supply exceeded Met requirements. Supplementation with Met, the most limiting AA in our model, increased the uptake and utilization of other AA such as valine, leucine, isoleucine, and serine, as indicated by reduced concentrations in plasma. These results correspond to the greater N retention (Table 2) in response to Met supplementation. Previous studies also observed that the supplementation with the most limiting AA increased protein retention and decreased plasma concentrations of other AA (Campbell et al., 1997; Wessels et al., 1997; Schroeder et al., 2006). Energy supplementation similarly decreased plasma

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concentrations of valine, leucine, isoleucine, phenylalanine, lysine, tryptophan, alanine, and ornithine, indicating that energy supplementation also increased AA uptake for protein synthesis. Plasma concentrations of valine, leucine, and isoleucine were lower for glucogenic than for lipogenic energy sources; plasma concentrations of glutamine were lower, and lysine concentrations tended to be greater, for lipogenic sources of energy supplementation, although the importance of these changes is not clear.

IMPLICATIONS

Energy supplementation improved the efficiency of Met utilization. Thus, the use of a single efficiency is not appropriate for growing cattle, and the estimation of amino acids requirements may require consideration of the amount of energy supplied. Although the inclusion of energy intake may represent an increase in complexity in models for estimating amino acid requirements, our results also indicate that the source of energy does not greatly affect amino acids utilization.

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Table 3-1. Composition of diet fed to steers

Ingredient	% of DM
Pelleted soybean hulls	82.9
Wheat straw	7.6
Cane molasses	4.1
Dicalcium phosphate	1.9
Sodium bicarbonate	1.2
Calcium carbonate	1.0
Urea	0.42
Magnesium oxide	0.41
Trace mineralized salt [a]	0.20
Vitamin premix [b]	0.22
Sulfur	0.10
Bovatec-150 [c]	0.01
Nutrient	
OM	90.1
N	1.95

[a] Composition (g/kg, minimum guarantee): NaCl (960 to 990); Mn (>2.4); Fe (>2.4); Mg (>0.5); Cu (>0.32); Zn (>0.32); I (>0.07); and Co (>0.04).

[b] Provided 4,410 IU of vitamin A; 2,205 IU of vitamin D; and 45 IU of vitamin E per kg diet DM.

[c] Provided 35 mg of lasalocid per kg of diet DM.

Table 3-2. Effect of methionine supplementation and energy source[a] on nitrogen balance and apparent diet digestion in growing steers

Item	No methionine					3 g/d L-methionine					SEM [b]
	CON	GLC	FAT	AC	PR	CON	GLC	FAT	AC	PR	
No. of observations	13	6	6	7	6	13	6	6	5	6	
N, g/d											
Infused	19.0	19.0	19.0	19.0	19.0	19.3	19.3	19.3	19.3	19.3	-
Dietary intake	47.7	46.8	47.5	47.5	47.5	47.4	48.3	48.3	47.6	47.6	0.6
Total intake [c]	66.7	65.8	66.5	66.5	66.5	66.6	67.6	67.5	66.8	66.8	0.6
Fecal excretion	18.1	18.8	16.9	18.8	18.5	18.2	18.1	18.1	19.3	18.1	0.8
Urinary excretion [c, d]	30.7	28.5	30.1	28.6	28.7	27.8	24.3	25.9	23.5	24.7	1.4
Urea [c, d, e]	19.5	18.6	20.8	16.5	17.0	16.2	15.3	16.1	12.3	13.9	1.4
Ammonia [c, f]	0.8	0.8	0.8	1.4	1.0	0.9	0.7	0.5	0.8	0.6	0.2
Retained [c, d]	17.9	18.5	19.4	19.2	19.2	20.6	25.1	23.6	23.8	24.2	1.7
Diet apparent digestibility, %											
DM [g]	74.0	74.8	73.9	71.4	73.1	74.8	74.9	75.5	72.5	73.7	1.1
OM [g]	76.1	77.6	76.8	73.3	74.9	77.1	77.7	78.0	74.3	75.7	1.1
Fatty acids [h]	79.7	84.5	90.9	-	-	88.0	79.0	94.6	-	-	3.1

[a] CON = control, GLC = 360 g/d of glucose infused abomasally, FAT = 150 g/d of fat infused abomasally, AC = 385 g/d of acetate infused ruminally, and PR = 270 g/d of propionate infused ruminally.

[b] For n = 6.

[c] Effect of methionine ($P < 0.05$).

[d] Effect of energy (control vs. all others; $P < 0.05$).

[e] Effect of glucogenic (glucose plus propionate) vs. lipogenic (fat plus acetate) sources ($P < 0.05$).

[f] Effect of methionine × energy ($P < 0.05$).

[g] Effect of acetate vs. fat ($P < 0.05$).

[h] Fatty acid (16 and 18 carbon chain lengths) digestion was measured only in Exp. 1. Fatty acid digestibility was greater ($P < 0.05$) for fat-supplemented steers than for control or glucose-supplemented steers.

Table 3-3. of methionine supplementation and energy source[a] on whole-body protein turnover in growing steers

Item	No methionine					3 g/d L-methionine					SEM [b]
	CON	GLC	FAT	AC	PR	CON	GLC	FAT	AC	PR	
No. of observations	13	6	6	7	6	13	6	6	5	6	
Protein, kg/d											
Flux	2.15	1.93	2.36	2.43	2.51	2.52	2.32	2.53	2.48	2.63	0.25
Synthesis	1.96	1.75	2.17	2.25	2.33	2.34	2.17	2.37	2.32	2.48	0.24
Degradation	1.84	1.63	2.05	2.13	2.21	2.22	2.01	2.22	2.18	2.32	0.24
Retention, % of synthesis [c]	5.71	6.61	5.59	5.33	5.15	5.50	7.23	6.22	6.41	6.10	0.83

[a] CON = control, GLC = 360 g/d of glucose infused abomasally, FAT = 150 g/d of fat infused abomasally, AC = 385 g/d of acetate infused ruminally, and PR = 270 g/d of propionate infused ruminally.

[b] For n = 6.

[c] Effect of methionine ($P < 0.05$).

Table 3-4. Effect of methionine supplementation and energy source[a] on serum and plasma hormones and metabolites in growing steers

Item	No methionine					3 g/d L-methionine					SEM [b]
	CON	GLC	FAT	AC	PR	CON	GLC	FAT	AC	PR	
No. of observations	12	6	6	7	7	13	6	6	6	6	
Serum											
Insulin, ng/mL [c, d, e, f, g]	0.32	0.74	0.43	0.35	0.49	0.35	0.57	0.32	0.24	0.40	0.06
IGF-I, ng/mL [c, e, f, h]	308	406	312	259	259	330	444	377	266	380	37
Plasma											
Urea, mM [c, d]	1.71	1.55	1.62	1.46	1.62	1.34	1.13	1.22	0.94	1.22	0.14
Glucose, mM [c, d, e]	4.80	5.36	4.89	4.72	5.14	4.59	5.06	4.69	4.43	4.89	0.15
Amino acid, μM											
Methionine [c]	13.8	11.4	10.3	11.7	11.3	18.1	16.9	18.1	18.1	18.8	2
Valine [c, d, e, g]	333	255	277	315	227	269	207	267	283	234	18
Leucine [c, d, e, i]	266	229	230	256	180	223	171	216	227	186	16
Isoleucine [c, d, e, g]	154	107	126	144	97	123	90	121	131	103	10
Threonine	130	140	120	106	105	116	138	127	110	128	13
Phenylalanine [d]	74	59	58	64	56	64	57	61	65	58	5
Tyrosine	37	37	33	32	32	30	47	29	27	30	8
Lysine [d]	205	148	137	174	122	188	123	223	174	156	35
Histidine	99	101	100	80	90	80	113	99	77	97	15
Tryptophan [d]	53	42	34	47	44	48	36	42	45	47	5
Glutamate [f, g, h]	118	76	70	123	107	99	78	85	133	132	12
Glutamine [e, g]	283	302	276	246	249	233	304	265	250	292	21
Alanine [c, d, h]	174	169	160	135	139	174	172	187	135	176	14
Glycine [d, e, f]	445	581	415	419	407	399	540	467	408	451	37
Serine [c]	94	110	94	112	103	71	71	71	64	94	10
Proline	54	52	49	44	42	48	48	50	44	48	4

Aspartate	7.4	8.3	7.1	5.6	5.8	10.0	7.5	8.2	5.7	6.8	2
Asparagine	33	33	30	31	29	30	30	31	32	30	2
Ornithine [d]	108	83	71	94	77	97	50	96	84	82	16

[a] CON = control, GLC = 360 g/d of glucose infused abomasally, FAT = 150 g/d of fat infused abomasally, AC = 385 g/d of acetate infused ruminally, and PR = 270 g/d of propionate infused ruminally.

[b] For n = 6.

[c] Effect of methionine ($P < 0.05$).

[d] Effect of energy (control vs. all others; $P < 0.05$).

[e] Effect of glucogenic (glucose plus propionate) vs. lipogenic (fat plus acetate) sources ($P < 0.05$).

[f] Effect of glucose vs. propionate ($P < 0.05$).

[g] Effect of energy × methionine ($P < 0.05$).

[h] Effect of acetate vs. fat ($P < 0.05$).

[i] Effect of (glucose vs. propionate) × methionine ($P < 0.05$).
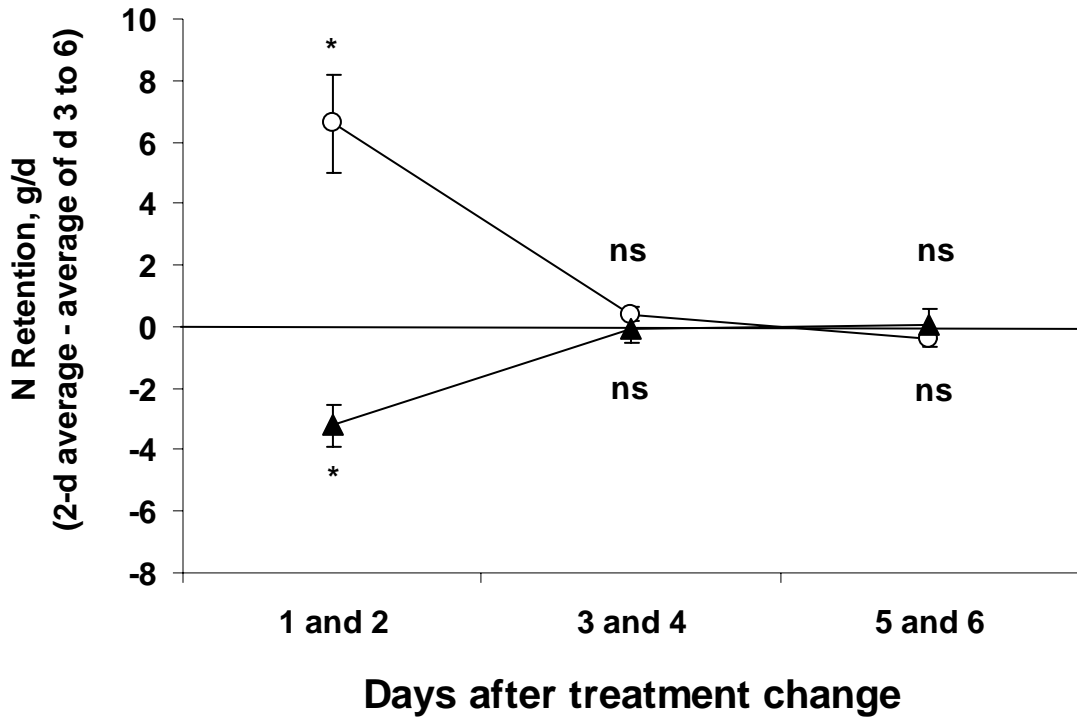
Figure 3-1. Differences in N retention between each 2-d measurement and the average of the last 4 d of each period in growing steers that demonstrated increases (▲; n = 4) or decreases (○; n = 8) in N retention in response to changes in dietary treatment from one period to the next. * = $P < 0.05$ for comparison to zero, ns = not significantly different from zero.

CHAPTER 4 - EFFECTS OF ENERGY SUPPLY ON LEUCINE UTILIZATION BY GROWING STEERS AT TWO BODY WEIGHTS[1]

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ABSTRACT: The effects of energy supplementation on leucine (Leu) utilization in growing steers were evaluated in 2 experiments using 6 ruminally cannulated Holstein steers. In Exp. 1, steers (initial BW = 150 ± 7 kg) were limit-fed (2.3 kg of DM/d) a diet based on soybean hulls and received a basal ruminal infusion of 100 g of acetate/d, 75 g of propionate/d, and 75 g of butyrate/d, as well as abomasal infusions of 200 g of glucose/d and a mixture (215 g/d) containing all essential AA except Leu. Treatments were arranged as a 3 × 2 factorial with 3 amounts of Leu infused abomasally (0, 4, and 8 g/d) and supplementation with 2 amounts of energy (0 and 1.9 Mcal/d of GE). The supplemental energy was supplied through ruminal infusion of 100 g of acetate/d, 75 g of propionate/d, and 75 g of butyrate/d, as well as abomasal infusion of 200 g of glucose/d to provide energy to the animal without affecting microbial protein supply. Nitrogen balance was increased linearly ($P < 0.01$) by abomasal supplementation of Leu as a result of linear ($P < 0.01$) decreases in urinary N excretion. Energy supplementation increased N balance ($P < 0.01$) as a result of decreased urinary N excretion ($P < 0.01$), indicating that energy supplementation improved the efficiency of Leu utilization. When additional energy was supplied, N retention increased linearly in response to Leu (25.6, 28.5, and 31.6 g/d for 0, 4, and 8 g/d of Leu). However, when no energy was supplemented, increases in N retention were similar for 4 and 8 g/d of Leu (24.5, 27.0, and 27.3 g/d for 0, 4, and 8 g/d of Leu). Experiment 2 was similar to Exp. 1, but steers had an initial BW of 275 ± 12 kg and were limit-fed at 3.6 kg of DM/d. Retention of N was not affected ($P = 0.22$) by Leu supplementation, indicating that Leu did not limit protein deposition. Energy supply increased N retention ($P < 0.01$) independent of Leu supplementation (33.0 vs. 27.8 g/d). Overall, energy supplementation improved Leu utilization by modestly increasing N retention when Leu was limiting and by increasing the ability of steers to respond to the highest amount of supplemental Leu. These results indicate that the assumption of a constant efficiency of AA utilization is unlikely to be appropriate in growing steers.

Key words: Energy, Growth, Leucine, Utilization

INTRODUCTION

It was observed in growing pigs that when energy intake was adequate, protein deposition increased linearly with supply of the most limiting AA, but when AA supply limited protein retention, increased energy did not affect protein deposition (Campbell et al., 1985; Chiba et al., 1991). This type of relationship between energy and protein supplies on protein deposition has been described as protein- and energy-dependent phases of growth (Gerrits et al., 1996; Titgemeyer, 2003), and it demonstrates that the efficiency of AA use is not affected by the level of energy intake. Although information is limited in growing ruminants, a constant efficiency of AA utilization has been assumed for growing cattle by nutrient requirements systems (Ainslie et al., 1993).

In previous studies with growing steers we observed that, even when Met limited protein accretion, N retention increased in response to increased energy supplementation, independent of the energy source supplemented (Schroeder et al., 2006a, b). These results suggest that the assumption of a constant efficiency of AA utilization by growing cattle is unlikely to be appropriate, and that energy level should be considered to more precisely estimate AA requirements.

It is unknown if the observed positive effects of energy supply on Met utilization are of similar magnitude for other AA which are metabolized differently. For instance, Met is primarily catabolized by transmethylation followed by transsulfuration, whereas Leu is catabolized throughout the body by transamination as the initial step. Due to these differences, their metabolism may be differently regulated, and energy supply could have different effects on their utilization. Moreover, it is unknown if BW (stage of maturity) affects AA utilization or the impact of energy on AA utilization in growing steers. Our objective was to determine effects of energy supplementation on Leu utilization by growing steers at 2 initial BW.

MATERIALS AND METHODS

Animals and Treatments

Procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Two experiments were carried out with the same 6 ruminally cannulated

Holstein steers. In Exp. 1, the steers (150 ± 7 kg initial BW) were allocated in a 6 × 6 balanced Latin square design. The steers were housed in individual metabolism crates with continuous lighting and controlled temperature (22°C). The animals had continuous access to fresh water and were limit-fed at 2.3 kg of DM/d (48.3 g of OM/kg BW$^{0.75}$ daily) a diet based on soybean hulls (Table 1) at 12-h intervals. Diet composition (Table 1) and feed restriction were designed to provide the estimated maintenance energy requirements (6.0 Mcal of ME/d) and to supply small amounts of dietary AA to create a limitation in Leu. All the steers received a basal infusion of a mixture of 20 g of L-lysine·HCl/d, 10 g of L-threonine/d, 10 g of L-histidine·HCl·H$_2$O/d, 10 g of L-phenylalanine/d, 5 g of L-tryptophan/d, 10 g of L-methionine/d, 10 g of L-isoleucine/d, 10 g of L-valine/d, 10 g of L-arginine/d, 100 g of L-glutamate/d, and 20 g of glycine/d continuously infused into the abomasum to provide all essential AA in excess of the steers' requirements to prevent limitations in protein synthesis by AA other than Leu, thereby allowing protein deposition until limited by energy or Leu supply. The profile of AA infused was based on the supplies and requirements of AA estimated for growing Holsteins steers fed with a diet similar to that used in our study (Greenwood and Titgemeyer, 2000). Amino acid solutions for each steer were prepared by dissolving the Ile and Val in 1.0 kg of water containing 40 g of 6 *M* HCl. Once these AA were dissolved, the remaining AA (except Glu) were added to the mixture. Glutamate was dissolved separately in 500 g of water containing 30 g of NaOH. After all AA dissolved, the 2 solutions were mixed together, and then Leu or 6 *M* HCl (see below) and water were added to bring the total weight of the daily infusate to 4 kg. All steers received 10 mg of pyridoxine·HCl/d, 10 mg of folic acid/d, and 100 µg of cyanocobalamin/d mixed with the abomasal infusate, to avoid deficiencies in those vitamins (Lambert et al., 2004). In addition, all steers received a basal infusion of 100 g of acetate/d, 75 g of propionate/d, and 75 g of butyrate/d into the rumen and 200 g of glucose/d into the abomasum as energy sources to provide an additional 1.9 Mcal of GE/d without affecting microbial protein synthesis. The VFA were mixed with water such that the total weight of the ruminal infusate was 4 kg/d, and the glucose was mixed with the basal AA infusate.

Treatments were arranged in a 3 × 2 factorial, with abomasal infusion of 3 amounts of L-Leu (0, 4, or 8 g/d) and supplementation with 2 amounts of energy (0 or 1.9 Mcal of GE/d). Leucine was dissolved in water containing 20 g of 6 *M* HCl and added to the basal AA mixture according to treatment. Additional solutions of 20 g of 6 *M* HCl and water were prepared to

equalize acid supply. The amounts of Leu supplemented were based on a previous study with the same experimental model (Awawdeh et al., 2005) that demonstrated that supplemental Leu requirements are close to 8 g/d. Energy supplementation (1.9 Mcal of GE/d) was within a linear range of response observed in previous studies using Met as the limiting AA (Schroeder et al., 2006a) and was achieved by continuously infusing additional amounts of VFA (100 g of acetate/d, 75 g of propionate/d, and 75 g of butyrate/d) into the rumen and of glucose (200 g/d) into the abomasum. Therefore, steers receiving the energy supplementation treatment received a total energy infusion of 3.8 Mcal of GE/d (1.9 Mcal/d from the basal infusion plus 1.9 Mcal/d from the treatment), whereas control steers received only 1.9 Mcal/d from the basal infusion. The supplemental energy sources were added to the basal infusions such that the total weight of ruminal and abomasal infusates was 4 kg/d for all treatments. A peristaltic pump and polyvinylchloride tubing (2.4 mm i.d.) were used to infuse solutions into the rumen and abomasum. Abomasal lines were placed through the reticulo-omasal orifice and into the abomasum, and were retained by a rubber flange (8-cm diameter) attached at the end of the line.

Sample Collection and Analyses

Each experimental period consisted of 2 d for adaptation and 4 d for sample collection. It has been demonstrated in our experimental model (Schroeder et al., 2006b) that ruminants adapt within the 2-d time frame to changes in nutrient supply when both protein and energy supply are varied and ruminal adaptation is not required. Feed samples were collected from d 2 through 5 of each period, composited by period, and stored (-20ºC) for later analysis. Feed refusals (if any) were fed through the ruminal cannula approximately 1 h before next feeding. Total urinary (into buckets containing 1.3 L of 1.38 M HCl to keep pH below 3) and fecal outputs were collected daily, with samples of urine (1%) and feces (10%) saved, composited by period within animal, and stored at -20ºC. Before analysis, samples were thawed at room temperature and homogenized. Feed and fecal samples were partly dried at 55ºC for 36 h, air-equilibrated for 36 h, and ground with a Willey mill to pass a 1-mm screen. Partly dried diet and fecal samples were analyzed for DM (105ºC for 24 h) and ash (450ºC for 8 h). Total N was determined on diet, wet fecal samples, and urinary samples with a LECO FP 2000 N Analyzer (LECO, St. Joseph, MI). Urine samples were analyzed colorimetrically for NH_3 (Broderick and Kang, 1980) and urea concentrations (Marsh et al., 1965).

On d 6 of each period, 4 h after the morning feeding, jugular blood was collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ). Blood collected in tubes containing sodium heparin was placed immediately on ice and then centrifuged for 20 min at $1,000 \times g$ to obtain plasma that was frozen (-20ºC) for later analysis. Plasma was analyzed for glucose and urea as described by Schroeder et al. (2006a). Blood samples were also collected in tubes without anticoagulant, left for 30 min at room temperature, and centrifuged for 20 min at $1,000 \times g$, and the serum was stored (-20ºC) for later analysis of insulin by a RIA kit (intra-assay CV of 9.1% and a sensitivity of 0.043 ng/mL; DSL-1600, Diagnostic Systems Laboratories, Webster, TX).

Statistical Analyses

One animal did not tolerate satisfactorily the energy supplementation as demonstrated by large feed refusals, and all data from 2 periods were not used for analyses. For the third assignment of energy supplementation to this steer, the energy supplementation was not provided, which yielded an additional observation for the treatment with no supplemental energy and no supplemental Leu. All data from 1 other observation (energy supplementation, no Leu) were lost due to problems not related to treatment. Statistical analyses were performed using the MIXED procedure of SAS System for Windows 8.1 (SAS Inst. Inc., Cary, NC). The model included the effects of Leu, energy, Leu × energy, and period. Steer was included as a random variable. Linear and quadratic effects of Leu supplementation, and their interactions with energy, were tested using single degree of freedom orthogonal contrasts. Treatment means were determined by using the LSMEANS option.

When Exp. 1 was finished, steers were housed in an outdoor pen for 105 d and fed a diet based on corn-silage for ad libitum consumption. Once the steers reached the target BW (275 ± 12 kg), Exp. 2 was conduced with housing, periods, diet (Table 1), basal infusions, treatments, sample collection, laboratory analysis, and statistical analysis similar to Exp. 1. The only adjustment was that feed intake was 3.6 kg of DM/d to provide a similar amount of feed on a metabolic BW basis (48.6 g of OM/kg $BW^{0.75}$ daily) in order to supply the estimated energy maintenance requirements (9.8 Mcal of ME/d). In Exp. 2, all data from 1 observation were lost due to problems not related to treatment.

RESULTS

Experiment 1

Nitrogen balance and diet apparent total tract digestibilities for Exp. 1 are presented in Table 2. Apparent digestibilities of DM and OM were similar among treatments and averaged 71.0 and 75.4%. Total N intake was not affected by energy supplementation, but was linearly increased by Leu ($P < 0.05$) due to the N provided by the Leu. Fecal N excretion was similar among treatments. Leucine supplementation linearly decreased ($P < 0.05$) excretion of total and urea N in urine. In addition, the interaction of the linear effect of Leu × energy was significant ($P < 0.05$), indicating that energy supplementation decreased urinary N excretion when 4 or 8 g/d of Leu was supplied, but not when Leu was not supplemented. The interaction of the linear effect of Leu × energy tended to be significant ($P = 0.06$) for N retention, indicating that the effect of increasing Leu supplementation was dependent on energy supplementation. When energy was not supplemented, N retention was increased by increasing Leu supplementation from 0 to 4 g/d, but there were no further changes by increasing Leu supplementation to 8 g/d. When the steers received additional energy, there was a linear increase in N retention in response to Leu supplementation.

Plasma urea concentrations (Table 2) presented a pattern similar to that observed for urinary urea N excretion, being lower as Leu supplementation increased and when additional energy was supplied at the highest levels of Leu supplementation (4 and 8 g/d). Energy supplementation increased ($P < 0.05$) plasma glucose (6.1 vs. 5.6 mM) and serum insulin concentrations (1.02 vs. 0.58 ng/mL), but neither glucose nor insulin were affected by Leu supplementation.

Experiment 2

Nitrogen balance and diet apparent total tract digestibilities for Exp. 2 are presented in Table 3. Apparent DM and OM digestibilities were linearly increased by Leu supplementation ($P < 0.05$). In addition, apparent OM digestibility was negatively affected by additional energy supply (76.2 vs. 74.4%; $P < 0.05$). Changes in apparent OM digestion matched with the observed fecal N excretion, which was greater when steers received supplemental energy at the lowest levels of Leu supplementation (0 and 4 g/d), but was lower in response to energy

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supplementation at the highest level of Leu (Leu × energy interaction, $P < 0.05$). The experimental design led to linear increases in total N intake as the level of Leu infusion increased, although the magnitude of that increase was small (< 1 g of total N intake/d). Total and urea N excretions in urine were significantly reduced by additional energy supply, but they were not affected by Leu supplementation (Table 3). Retention of N was not affected by Leu supplementation, but was increased ($P < 0.05$) by energy supplementation (33.0 vs. 27.8 g/d) due to the reductions ($P < 0.05$) in urinary N excretion (31.3 vs. 37.9 g/d).

As expected from the results on urinary N excretion, plasma urea concentrations (Table 3) were lower (3.0 vs. 2.4 mM; $P < 0.05$) when additional energy was supplied, with no effect of Leu supplementation. As observed in Exp. 1, energy supplementation was associated with significant increases in plasma glucose (5.4 vs. 5.1 mM) and serum insulin concentrations (0.94 vs. 0.62 ng/mL).

DISCUSSION

Two experiments were conduced to evaluate how Leu utilization is affected by energy supplementation by growing steers at 2 different BW. Other than the initial BW (150 vs. 275 kg), both experiments were similar in terms of diets, housing, treatments, experimental design, etc. The only modification between experiments was the increased amount of diet offered in Exp. 2 to maintain a similar energy intake on a metabolic BW basis in order to provide the maintenance energy requirements from the dietary energy supply.

Diet digestibility was not affected by treatment in Exp. 1 (Table 2), but it was linearly increased by Leu supplementation in Exp. 2 (Table 3). Although changes in apparent OM digestion are not typically observed by the infusion of the most limiting AA, small increases have also been observed when Leu (Awawdeh et al., 2005) or Met (Schroeder et al., 2006a) were supplemented in growing steers. Nevertheless, in our study, the changes were not of large enough magnitude to alter the interpretation of our results. The moderate reduction in apparent OM digestion due to energy supplementation in Exp. 2 (Table 3) was also observed in previous studies when additional VFA (primarily acetate) were infused into the rumen (Schroeder et al., 2006a, b). As total VFA infusion was similar in both experiments, we can not explain why apparent OM digestion was only affected in Exp. 2, where total DMI was greater and the

amounts of VFA infused were lower if expressed on a metabolic BW basis. Although it is possible that the supply of Leu would be reduced by the energy supplementation in Exp. 2, total Leu supply was not limiting in Exp. 2 (see discussion below) and, therefore, it does not affect the interpretations regarding responses to Leu and energy supply.

In Exp. 1, when additional energy was not provided, N retention was increased by increasing Leu supplementation from 0 to 4 g/d, with no additional increases by increasing supplemental Leu from 4 to 8 g/d (Table 2). These results indicate that the supplemental Leu requirement was, at most, not much greater than 4 g/d. The potential for protein deposition in growing animals has been described as an energy-driven process (Gerrits et al., 1996; Titgemeyer, 2003), being greater with increased energy supply. Therefore, when steers received additional energy supply, the potential for protein deposition might be greater, increasing the ability of the steers to respond to higher levels of supplemental Leu supply and, thus, the Leu requirements. Consequently, when steers were provided with an additional 1.9 Mcal of GE/d, the supplemental Leu requirement was, at least, greater than 4 g/d and, based on linear increases in N retention, appeared to be at least 8 g/d. In agreement with our results, Awawdeh et al. (2005), using a similar diet and intake level (48 g of OM/kg $BW^{0.75}$ daily) and a basal energy supplementation of 3.1 Mcal of GE/d (intermediate value between the 1.9 and 3.8 Mcal of GE/d supplemented in our study), observed that supplemental Leu requirements were greater than 4 g/d, and likely close to 8 g/d. Assuming that retained N was deposited as tissue protein (N × 6.25) and that tissue protein gain contains 6.7% Leu (Ainslie et al., 1993), when Leu was limiting (from 0 to 4 g of Leu/d) the estimated incremental efficiency of supplemental Leu use was ≥ 26% for control and 30% for energy-supplemented steers. Those values were slightly lower than those (34 to 49%) observed in previous studies (Awawdeh et al., 2005, 2006) and much lower than the value predicted (66%) by the NRC (1996). Energy supplementation also increased N retention at the same amounts of Leu supply (Table 2), even at the lowest levels of Leu supplementation (0 and 4 g/d). By dividing the total Leu retention (N retention × 6.25 × 0.067) by the Leu supply from the diet (5.9 g of metabolizable Leu/kg DMI; Campbell et al., 1997), the estimated efficiency of use of dietary Leu was numerically increased from 75 to 79% by energy supplementation when Leu was not supplemented. At 4 g/d of Leu supplementation, the estimated efficiency of Leu utilization numerically increased from 64 to 68% due to additional energy supply. Although the absolute values for these efficiency are likely

overestimated by overestimations inherent with the N retention procedure (Gerrits et al., 1996), the relative changes suggest that the that additional energy supply improved the efficiency of Leu utilization. In previous studies, we observed that when Met limited protein deposition, energy supplementation increased the efficiency of Met utilization (Schroeder et al., 2006a, b), although the magnitude of the improvement in efficiency of use seems to be greater for Met than for Leu. Differences in the metabolism of these two AA, Met primarily catabolized via transmethylation followed by transsulfuration and Leu catabolized throughout the body via transamination, may partially explain the differences in the magnitude of the effects of additional energy supply. However, more research is needed to identify the specific tissues and mechanisms involved in changing the efficiency of AA utilization by energy supplementation.

In Exp. 2, when the experiment was repeated with the same steers, but weighing 275 kg BW, the lack of response in N retention to Leu supplementation (Table 3) indicated that Leu did not limit protein deposition. Although there was no previous information on Leu requirements for steers of that weight, the lack of response was unexpected. According to the equations used by the Cornell Net Carbohydrate and Protein System for estimating Leu requirements (O'Connor et al., 1993), 2 of the 3 determinants of maintenance requirements increase with BW, although with an exponent smaller than 0.75 (0.6 for scurf protein and 0.5 for urinary protein). By maintaining a similar DMI between Exp. 1 and Exp. 2 on a metabolic BW basis (using the exponent 0.75), we provided more Leu (g/d) in Exp. 2, and the increase in supply was slightly greater than the increase in maintenance Leu requirements. Basal supply of Leu was 161 and 195% of the estimated (O'Connor et al., 1993) maintenance requirement in Exp. 1 and Exp. 2, respectively. Comparing the estimated maintenance Leu requirements (8.4 and 12.7 g/d for Exp. 1 and 2; O'Connor et al., 1993) to the basal supply of 5.9 g of metabolizable Leu/kg of DMI (Campbell et al., 1997), Leu supply available for growth was 5.2 and 12.1 g of metabolizable Leu/d in Exp. 1 and Exp. 2, respectively. Based on the equation generated by INRA (1989) [efficiency = 0.834 − 0.0114 × equivalent shrunk weight (kg)] and adopted by several nutrient requirement models (Ainslie, 1993; O'Connor et al., 1993; NRC, 1996), the efficiency of absorbed Leu utilization for growth was predicted to decrease from 66 to 52% from Exp. 1 to Exp. 2. Although a lower efficiency for growth in Exp. 2 than in Exp. 1 could partially compensate for the greater basal Leu supply, the Leu available for growth was 2.3-fold greater in Exp. 2, allowing greater N retention and limiting the capacity of steers to respond to

supplemental Leu. Regardless, results indicated that during Exp. 2 the steers were in an energy-dependent phase of growth, with the potential for increased protein deposition in response to additional energy (Table 3).

In our experimental model, where severe deficiencies in glucose are unlikely, additional glucose and propionate supply were previously associated with increased plasma glucose concentrations (Schroeder et al., 2006b). In agreement, in both Exp. 1 and Exp. 2, significant increases in plasma glucose and serum insulin concentrations (Table 2 and 3) were observed when steers received additional VFA and glucose as supplemental energy. Insulin may play an important role in mediating the nutritional effects on muscle protein deposition, which affects the efficiency of AA utilization in growing animals (Davis et al., 2003). Although positive relationships between plasma insulin concentrations and increases in AA utilization has been observed (Rooyackers and Nair, 1997), we also found that increasing energy supply through lipogenic energy sources increased N retention without affecting serum insulin concentrations (Schroeder et al., 2006b), suggesting that insulin may not be the most critical regulator of protein deposition in our model. In a previous study with growing steers (Awawdeh et al., 2006), we observed that, when Leu was the most limiting AA, Leu supplementation increased serum insulin but did not affect plasma glucose levels. In the present study, Leu supplementation did not affect plasma glucose or serum insulin concentrations (Tables 2 and 3).

IMPLICATIONS

The present study, in conjunction with our previous studies, indicates that the assumption of a constant efficiency of amino acid utilization for all the essential amino acids and across different levels of energy supply may not be appropriate for estimating amino acid requirements of growing steers. Modeling of amino acid requirements in growing cattle may require consideration of the amount of dietary energy supplied. Moreover, the magnitude of the effect of energy supplementation on the efficiency of amino acid use may be different depending on which amino acid limits protein deposition.

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Table 4-1. Composition of diet fed to growing steers with 150 (Exp. 1) and 275 kg of body weight (Exp. 2)

Ingredient	% of DM
Pelleted soybean hulls	82.9
Wheat straw	7.6
Cane molasses	4.1
Dicalcium phosphate	1.9
Sodium bicarbonate	1.2
Calcium carbonate	1.0
Urea	0.42
Magnesium oxide	0.41
Trace mineralized salt[1]	0.20
Vitamin premix[2]	0.22
Sulfur	0.10
Bovatec-150[3]	0.01
Nutrient (Exp. 1 – Exp. 2)	
DM	86.8 – 86.1
OM	91.8 – 90.9
N	1.7 – 1.6

[1] Composition (g/kg): NaCl (960 to 990); Mn (>2.4); Fe (>2.4); Mg (>0.5); Cu (>0.32); Zn (>0.32); I (>0.07); and Co (>0.04).

[2] Provided 4,410 IU of vitamin A; 2,205 IU of vitamin D; and 45 IU of vitamin E per kg diet DM.

[3] Provided 35 mg of lasalocid per kg diet DM.

Table 4-2. Effects of energy and leucine supplementation on nitrogen balance, apparent diet digestion, and blood metabolites in growing steers weighing 150 kg (Exp. 1)

	Energy, Mcal/d						
	0			1.9			
	Leucine, g/d						
Item	0	4	8	0	4	8	SEM[1]
No. of observations	7	6	6	4	5	5	
N, g/d							
Infused	27.3	27.8	28.2	27.3	27.8	28.2	-
Dietary intake	39.2	39.2	39.2	39.2	39.2	39.2	0.04
Total intake [2]	66.6	67.0	67.3	66.5	66.9	67.4	0.04
Fecal excretion	16.6	16.6	17.2	15.5	18.0	17.0	0.8
Urinary [2,3,4]	25.4	23.4	22.8	25.5	20.4	18.9	1.2
Urea [2,4]	15.4	14.3	13.4	16.7	11.4	10.2	1.2
Ammonia [3]	1.8	1.6	1.3	2.5	1.9	1.2	0.37
Retained [2,3]	24.5	27.0	27.3	25.6	28.5	31.6	1.3
Diet apparent digestibility, %							
DM	70.5	70.6	71.3	72.5	70.5	70.4	1.7
OM	75.3	75.4	76.2	76.2	74.6	74.7	1.5
Serum							
Insulin, ng/mL [3]	0.63	0.59	0.51	0.97	1.02	1.07	0.15
Plasma							
Glucose, [3] mM	5.6	5.6	5.6	6.1	6.0	6.3	0.17
Urea, [2,3,4] mM	2.6	2.4	2.3	2.6	1.9	1.7	0.22

[1] For n = 6.

[2] Linear effect of leucine ($P < 0.05$).

[3] Effect of energy ($P < 0.05$).

[4] Linear effect of leucine × energy interaction ($P < 0.05$).

Table 4-3. Effects of energy and leucine supplementation on nitrogen balance, apparent diet digestion, and blood metabolites in growing steers weighing 275 kg (Exp. 2)

	Energy, Mcal/d						
	0			1.9			
	Leucine, g/d						
Item	0	4	8	0	4	8	SEM[1]
No. of observations	6	6	6	6	5	6	
N, g/d							
Infused	27.3	27.8	28.2	27.3	27.8	28.2	-
Dietary intake	58.9	58.9	58.9	58.9	58.9	58.9	0.01
Total intake [2]	86.3	86.7	87.1	86.3	86.7	87.1	0.01
Fecal excretion [3,4]	20.5	20.2	22.3	23.6	23.6	19.8	1.0
Urinary [5]	38.5	38.1	37.2	30.7	31.4	31.9	1.5
Urea [5]	24.0	21.6	21.4	17.0	16.5	16.9	2.2
Ammonia	3.8	6.7	5.2	4.2	4.4	4.1	1.8
Retained [5]	27.3	28.4	27.6	32.0	31.6	35.4	1.3
Diet apparent digestibility, %							
DM [2]	73.2	73.1	74.0	70.2	73.0	73.5	1.5
OM [2,5]	75.8	75.9	76.9	72.1	75.5	75.6	1.5
Serum							
Insulin, ng/mL [5]	0.66	0.65	0.55	1.04	1.01	0.77	0.13
Plasma							
Glucose, [5] mM	5.1	5.2	5.0	5.5	5.5	5.3	0.11
Urea, [5] mM	3.0	3.0	3.0	2.3	2.4	2.4	0.25

[1] For n = 6.

[2] Linear effect of leucine ($P < 0.05$).

[3] Linear effect of leucine × energy interaction ($P < 0.05$).

[4] Quadratic effect of leucine × energy interaction ($P < 0.05$).

[5] Effect of energy ($P < 0.05$).