

Amino acid utilization by growing cattle

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The NRC (1996) predicts that the incremental efficiency of metabolizable protein utilization for growth by cattle is dependent upon body weight with the efficiency defined as $83.4\% - (0.114\% \times \text{empty body weight, kg})$ for cattle weighing less than 300 kg, and as 49.2% for cattle weighing more than 300 kg. The same efficiency is applied to each essential amino acid as well as to metabolizable protein. Gerrits et al. (1996) demonstrated that this equation overestimates the efficiency of protein utilization for growing preruminant calves fed liquid diets, and we have observed that this equation overestimates efficiencies of methionine and leucine utilization in ruminating cattle. Moreover, our work, as well as that of Gerrits et al. (1996), showed that the efficiencies of amino acid use for growth are dependent on factors besides body weight.

Research model

To measure the incremental efficiency of amino acid use in growing cattle, we have used a research model where Holstein steers (130 - 230 kg) are ruminally cannulated to allow nutrient supply to the rumen and abomasum to be directly modified by infusions. To create a limitation in an amino acid, metabolizable amino acids are maintained in low supply by limit feeding a diet that is low in ruminally undegradable protein. Energy supply is increased by ruminal infusion of volatile fatty acids and abomasal infusion of glucose; neither of these infusions increase microbial protein production and thus they increase the energy:protein ratio supplied to the cattle. To make a single amino acid limiting, a mixture of amino acids containing all essential amino acids, except the amino acid under study, is infused abomasally. Increases in nitrogen retention (converted to increases in amino acid deposition) in response to supplementation of the limiting amino acid are then used to estimate the efficiency of amino acid use.

One observation that is consistent across a number of nitrogen balance experiments conducted to evaluate amino acid utilization in cattle is that the efficiency of amino acid utilization is strikingly less than that predicted by NRC (1996). Averaged across 11 trials with efficiencies calculated across 22 treatments, incremental methionine utilization for growth averaged 26% (Campbell et al., 1996, 1997; Lambert et al., 2002, 2004; Loest et al., 2002; Schroeder et al., 2006a, b; Awawdeh et al., 2004, 2006). Averaged across 4 trials with efficiencies calculated across 10 treatments, leucine utilization for growth averaged 38% (Awawdeh et al., 2005, 2006; Schroeder et al., 2007; Titgemeyer et al., 2012). For similarly sized cattle (132 to 228 kg), the NRC (1996) would predict efficiencies of amino acid utilization for growth to be 57 to 68%.

Effects of energy supply on amino acid utilization

With the research model described above, energy supplies can be changed without altering the amino acid supply. Schroeder et al. (2006a) studied the effect of energy supply in growing steers that were receiving limiting amounts of methionine. Cattle received 0 or 3 g/d of supplemental L-methionine in combination with supplemental energy in amounts of 0, 1.3, or 2.6 Mcal/d of gross energy. The energy was provided as a mixture of ruminally infused acetate, propionate, and butyrate along with abomasally infused glucose and fat. Given the sites where they were provided, these energy sources should not increase ruminal production of microbial

protein. Methionine was limiting, as demonstrated by increases in nitrogen retention in response to supplemental methionine, but the cattle also demonstrated increased nitrogen retention when energy was provided in the absence of supplemental methionine. Thus, the efficiency of methionine utilization, at least for maintenance purposes, was improved as energy supply was increased. The effects of energy and methionine supplementation were additive, such that the incremental efficiency of methionine utilization remained constant at 18%.

In a companion study, Schroeder et al. (2006b) compared the effects of different energy sources on the efficiency of methionine utilization. In that work, the cattle receiving no supplemental energy demonstrated the expected increases in nitrogen retention when 3 g/d of supplemental L-methionine was provided, demonstrating the deficiency of methionine. These cattle were then supplemented with acetic or propionic acid ruminally or with glucose or a fat mixture abomasally. The four energy sources provided equal amounts of gross energy, and none of the energy supplements were expected to increase the ruminal production of microbial protein. Despite the observation that methionine was a limiting nutrient, supplementation of energy increased nitrogen retention slightly when no methionine was supplemented and to a greater extent when 3 g/d of methionine was supplemented. The efficiency of methionine use increased from 11% when no energy was provided to an average of 21% when any of the energy sources was provided. Thus, the efficiency of utilization of limiting amino acids by cattle is affected by energy supply. This will require consideration when estimates of amino acid requirements are made. The improvement in efficiency of amino acid use was not related to elevations in serum insulin or IGF-I, because supplemental glucose increased both of these hormones and propionate increased insulin, but responses in nitrogen retention were the same across all energy sources without increases in insulin or IGF-I for ruminal acetate or abomasal fat.

Similar to the work with methionine, Schroeder et al. (2007) evaluated the effect of energy supply on the efficiency of leucine utilization. Using an approach similar to that described for methionine, limiting amounts of leucine were supplemented with 0 or 1.9 Mcal/d of supplemental energy (provided as ruminal acetate, propionate, and butyrate and abomasal glucose). When leucine supply was limiting, increasing the energy supply led to only small increases in nitrogen retention, suggesting that the efficiency of leucine utilization is not as responsive to energy supply as is that of methionine. Similarly, Titgemeyer et al. (2012) observed that abomasal glucose supplementation or ruminal infusion of volatile fatty acids merely tended to improve nitrogen retention when leucine supply was limiting. This demonstrated both the inability of energy to greatly improve the efficiency of leucine use as well as the lack of difference between energy sources.

Effects of ammonia load on amino acid utilization

Work by Lobley et al. (1995) with sheep demonstrated that increases in portal vein ammonia supply might increase hepatic amino acid oxidation; it was considered that this might be a cost associated with the need to supply aspartic acid to support ureagenesis. Subsequent work (Milano and Lobley, 2001) suggested that the increased hepatic amino acid oxidation in response to the ammonia may have been related to shifts in acid-base balance rather than to the ammonia per se.

To better evaluate the effect of increased ammonia supply on amino acid utilization, Awawdeh et al. (2004) ruminally infused 0, 40, or 80 g/d of urea to steers as a means of increasing ruminal absorption of ammonia. These steers were maintained under conditions of a methionine limitation so that the effect of ammonia supply on the efficiency of methionine use

could be evaluated. For these methionine deficient steers, ammonia load had no effect on nitrogen retention, demonstrating no negative impact on whole-body methionine utilization. When a similar experiment was conducted with steers maintained under leucine-limiting conditions (Awawdeh et al., 2005), nitrogen retention was improved when 80 g/d of urea was supplemented ruminally as a means of increasing ammonia absorption. It was suggested that the ammonia load might reduce the transamination of leucine or increase the transamination of alpha-ketoisocaproate to leucine, thereby improving whole-body leucine utilization.

Effects of amino acid supply on amino acid utilization

Supplies of amino acids in amounts greater than their requirement for maximal whole-body protein deposition have the potential to affect metabolism. Nitrogen from the excess amino acids will need to be eliminated from the body (largely as urea). Moreover, amino acid supply plays an important role in regulating protein synthesis in muscle via mTOR-related signaling (Suryawan et al., 2012). In addition, amino acid supplies can impact amino acid degradation pathways. For example, branched-chain keto-acid dehydrogenase, the rate limiting step in the degradation of the branched-chain amino acids, is increased in activity when the supply of branched-chain amino acids (predominantly leucine) is increased (Block, 1989). Thus antagonisms among amino acids can occur with excesses of one amino acid increasing degradation of another.

Awawdeh et al. (2006) evaluated the effect of providing excess amino acids on the efficiency of methionine and leucine utilization. Steers were maintained under conditions where only methionine or leucine was limiting, and then nitrogen retention responses to supplementation with methionine or leucine were studied without or with supplementation of a mixture of amino acids that did not include the amino acid being studied. For methionine, supplementation with a methionine-free mixture of essential amino acids (200 g/d) led to large increases in nitrogen retention, even though methionine was clearly demonstrated to be first limiting. Thus, the supplementation of the excess amino acids improved the efficiency of methionine use, and this response appeared to be particularly important in reducing maintenance requirements. Improvements in nitrogen retention were associated with increases in serum insulin concentrations, but no changes in serum IGF-I were observed. The improvements in methionine utilization in response to amino acid supplementation were much larger than could be attributed to the increase in energy supplied by the amino acids (Schroeder et al., 2006a).

For leucine utilization, Awawdeh et al. (2006) observed that either a mixture of leucine-free essential amino acids (200 g/d) or a mixture of essential (100 g/d) and non-essential amino acids (100 g/d) could improve nitrogen retention when leucine was limiting, with the responses being somewhat better for the mixture of all amino acids rather than for the essential amino acids alone. However, the improvements in utilization of leucine in response to supplementation with excess amounts of amino acids were less than the improvements in methionine utilization. This difference between amino acids could have several causes. First, Schroeder et al. (2006a, 2007) demonstrated that methionine utilization was more responsive than leucine utilization to increases in energy supply, which could be a result of control mechanisms that are more responsive for the methionine than for leucine. Second, the profile of supplemental amino acids differed between the two trials. Of necessity, the amino acids supplemented during evaluation of leucine utilization did not contain leucine, whereas for the evaluation of methionine utilization leucine was included in the amino acid mixture. This could be important because leucine is a key amino acid involved in cell signaling via the mTOR pathway (Suryawan et al., 2012). Third,

there may be counteracting mechanisms acting in the case of leucine utilization. The amino acid mixture contained valine and isoleucine, which could increase activity of the branched-chain keto-acid dehydrogenase. This might increase leucine oxidation (i.e., decrease its efficiency of use) even in the face of generalized improvements in leucine utilization resulting from cell signals, such as mTOR, that increase protein synthesis.

In an experiment similar to that used by Awawdeh et al. (2006), McCuiston et al. (2004) evaluated the effect of excess amino acid supplies on histidine utilization. Supplementation with histidine-free mixtures of excess amino acids led to improvements in nitrogen retention under conditions where histidine was limiting. Thus, the efficiency of histidine use in cattle was improved by supplementation of excess amino acids.

Maintenance requirements

In the studies described throughout this paper, specific efforts were not made to evaluate maintenance requirements for amino acids. Surprisingly, the basal supplies of the amino acid under study supported positive nitrogen retentions (i.e., body protein deposition), even though the basal supplies of methionine and histidine were less than maintenance requirements predicted by NRC (1996). Moreover, in some studies under basal conditions, deposition of the limiting amino acid was estimated to exceed the supply of that amino acid. In part, these observations may be due to an overestimation of nitrogen retention due to incomplete recovery of excreta. For example, Gerrits et al. (1996) estimated in growing calves that nitrogen retention measured by difference was 10 to 17% greater than that measured by the serial slaughter technique. Even with adjustment for overestimation of nitrogen retention, maintenance requirements, predicted from extrapolation of the response surface to zero nitrogen retention, are typically predicted to be negative, which is unlikely to be a correct conclusion. The incongruity between the maintenance requirements of NRC (1996) and our empirical observations is lessened when one considers that our estimates of amino acid deposition, based on nitrogen retention measured by difference, would include scurf losses, which normally are considered as part of the maintenance requirement.

Frequently, cattle perform better than predicted when dietary protein supplies are limiting and respond less to protein supplementation than is predicted by NRC (1996). Part of these responses may be attributable to the buffering effect of ruminal fermentation on supply of metabolizable protein to the animal. However, overestimation of the maintenance requirements for amino acids as well as overestimation of the efficiency of amino acid utilization for growth are both likely contributors to these observations.

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