

Regulation of Amino Acid Metabolism in Dairy and Beef Cattle

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Summary

Dairy cattle

- In cows fed well-balanced rations, mammary uptake of amino acids is influenced more by metabolic demand than by arterial supply.
- Changes in mammary blood flow accommodate changes in metabolic demand for amino acids but are unlikely to be the primary regulator of mammary uptake and utilization of amino acids or other substrates.
- Capacity of Na⁺-dependent and Na⁺-independent systems for membrane transport of amino acids is unlikely to be limiting for mammary amino acid supply.
- Several hormones directly or indirectly regulate mammary uptake and utilization of amino acids. Specific roles and mechanisms remain to be elucidated.
- The bovine mammary gland has greater metabolic capacity for milk protein synthesis than is normally expressed under optimal nutrition and management conditions. Management strategies to take advantage of this knowledge have yet to be developed.

Beef cattle

- Muscle protein deposition in growing steers may be limited by postabsorptive supply of amino acids even in animals fed to predicted energy and protein requirement.
- Membrane transport capacity is unlikely to be limiting for muscle uptake of amino acids and protein deposition.
- Net protein accretion in growing muscle can be substantially altered by subtle changes in protein synthesis and/or degradation.
- Muscle protein synthesis is positively regulated by somatotropin via the insulin-like growth factor system; muscle protein degradation is negatively influenced by insulin and, therefore, energy status.
- Metabolic modifiers such as the anabolic steroids and β -adrenergic agonists exert their positive effects on lean tissue growth via direct and, possibly, indirect influences on muscle protein turnover that have yet to be fully elucidated.

Introduction

Dairy and beef cattle have the important ability to convert relatively low quality dietary nitrogen sources into valuable milk and meat protein. However, the overall efficiency of capture of dietary nitrogen is relatively low and varies widely with animal genotype and environment, especially nutrition. This appears to offer significant opportunity for improvement in both the dairy and beef industries, with major potential benefits for profitability and reduction of the environmental impact of excreted nitrogen. Other papers in these proceedings are focused on important effects of feeding

management, ration quality (including amino acid balances), and ruminal and postruminal digestion on the quantity and composition of absorbed amino acids. However, once the postabsorptive supply is optimized, further improvement in efficiency of utilization of amino acids can be realized only if the capacity for protein deposition in mammary or muscle tissues is altered by genetics or use of metabolic modifiers. This paper will focus first on selected aspects of the control of mammary utilization of amino acids for milk protein synthesis and then will similarly consider the regulation of amino acid metabolism and protein deposition in skeletal muscle.

Regulation of mammary amino acid metabolism and milk protein synthesis

Importance Protein has become the most valuable component of milk produced by dairy cows. Milk caseins, which account for about 80% of bovine milk protein, are especially valuable because of their high nutritional value and ideal properties for cheese manufacturing. Also, new procedures for separation and reconstruction of milk proteins offer the promise of novel dairy products that will significantly add value to milk protein. Therefore, optimization of milk protein production has become a major objective of dairy producers and their advisors.

Arterial supply of amino acids Most amino acids supplied to the bovine mammary gland in arterial blood are in free form and are taken up by membrane transport systems similar to those described in many other mammalian cell types (Shennan et al., 1997). However, circulating peptides account for 10-30% of the total concentration (free plus peptide) of several amino acids in lactating goats (Backwell et al., 1996) and these authors were unable to fully account for the secretion in milk protein of at least one EAA, phenylalanine, by measurement of its mammary net uptake in free form. On the other hand, Mackle et al. (2000b) were able to fully account for mammary output of all EAA by measured values of net uptake of free amino acids. No evidence for transport of intact peptides into bovine mammary tissue has been found (Chen et al., 1999) but transport of free amino acids after local peptide hydrolysis is likely. For example, bovine mammary tissue has γ -glutamyl transferase activity (Baumrucker and Pocius, 1978), the substrate of which is the relatively abundant tripeptide, glutathione. Inhibition of this enzyme decreased milk protein synthesis in isolated ovine mammary cells (Johnston et al., 2004). The mRNA for other peptidases has been detected in caprine mammary tissue (Mabjeesh et al., 2005). Use of indirect tracer methods suggested that, in lactating goats, at least 10% of the mammary amino acid supply is from circulating small peptides (Backwell et al., 1996).

Numerous studies have demonstrated that insufficiency in the supply of total amino acids or individual essential amino acids (EAA) will cause a decrease in milk protein synthesis and secretion (e.g. Guinard and Rulquin, 1994). However, in cows fed well-balanced rations adequate in rumen degradable and undegradable protein, attempts to increase milk protein synthesis by increasing the arterial supply of amino acids have been largely unsuccessful. For example, Cant et al. (2001) used unilateral mammary close-arterial infusion to boost the local mammary supply of a physiological mixture of amino acids by 15 or 30 g/h in well-fed dairy cows. These doses resulted in a systemic spillover effect such that both the infused and uninfused udder halves received a substantially increased supply of amino acids. Nevertheless, there was no effect on milk yield and only a very modest increase in milk protein concentration. These results are consistent with those of Mackle et al. (2000b) who increased mammary amino acid supply by abomasal

infusion of casein and branched-chain amino acids but saw no effect of arterial concentration or mammary arteriovenous concentration difference of EAA on milk protein yield (**Figure 1**).

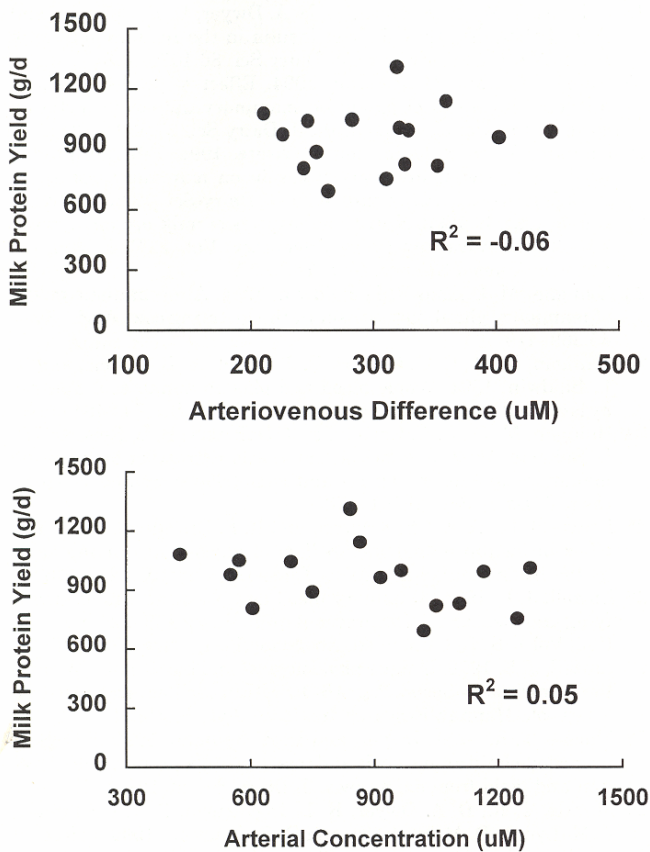


Figure 1. Relations between arterial concentrations of EAA (bottom panel) or arteriovenous differences for EAA (top panel) and milk protein yield. Each point represents a single cow ($n = 4$) during each of four treatments that caused wide variations in arterial concentrations of EAA. Reproduced with permission of the American Dairy Science Association (Mackle et al., 2000b).

Role of mammary blood flow There is no doubt that mammary blood flow (MBF) is a major determinant of the rate of supply of amino acids for milk protein synthesis. However, debate continues as to whether changes in MBF are compliant responses to changes in mammary metabolism or a direct regulator of milk synthesis. This question is difficult to address experimentally because of the close quantitative and temporal relations between MBF and milk yield but recent evidence suggests that mammary metabolism controls MBF rather than vice versa (Lacasse and Prosser, 2003). The importance of adaptations in MBF to metabolic need was demonstrated by Bequette et al. (2000) who observed a 35% increase in MBF in lactating goats fed a diet grossly deficient in histidine. This, together with a 3-fold increase in mammary fractional

extraction, resulted in a relatively moderate decrease in net uptake of histidine despite a 90% reduction in its arterial concentration. Also, MBF was increased in proportion with increases in milk protein yield elicited by chronic infusion of insulin in dairy cows, which helped to offset effects of concomitant decreases in arterial concentration of some EAA (Mackle et al., 2000b).

Membrane transport systems for mammary amino acid uptake As already noted, the bovine mammary gland possesses an array of Na^+ -dependent and Na^+ -independent amino acid transport systems similar to that described in other mammalian tissues (Shennan et al., 1997). However, we are unaware of any compelling evidence that capacity for (mostly) active transport is a limiting factor for the mammary acquisition and utilization of circulating amino acids. Rather, it seems likely that any association between level of expression or activity of transport proteins and amino acid uptake simply represents the ability of transport capacity to respond to mammary metabolic demand for amino acids (Bequette et al., 2000).

Mammary amino acid metabolism The fractional extraction ($[\text{A}-\text{V}]/[\text{A}]$) of most amino acids by the mammary gland is very high compared to that of other tissues, such as muscle (see below). Also, the overall efficiency of mammary utilization of amino acids for milk protein synthesis, assessed by mammary uptake to output ratios, exceeds 80% (Clark et al., 1978; Mackle et al., 2000b). The relatively low rate of amino acid catabolism is associated with similarly low rates of protein turnover, no doubt because the great majority of proteins synthesized in the mammary gland are quickly exported in milk. However, some EAA, most notably arginine and the branched-chain amino acids (BCAA), are taken up well in excess of their output in milk protein. Transamination of these acids must contribute to the intracellular pool of non-essential amino acids (NEAA), exogenous supply of which is clearly inadequate to account for the needs of milk protein synthesis (Clark et al., 1978; Mackle et al., 2000b). Mammary oxidation of leucine increased from 8% of net uptake in early lactation to 34% in late lactation in goats (Oddy et al., 1988). Additional metabolic fates of amino acids taken up by the mammary gland but not secreted in milk protein must include export in milk fat globule membrane protein.

Endocrine regulation The regulation of mammary amino acid metabolism and milk protein synthesis by hormones and local cytokines is not well understood but several endocrine systems have been implicated. Chronic, exogenous administration of prolactin had no effect on milk protein yield, consistent with its general lack of effect on galactopoiesis during established lactation in dairy cows (Plaut et al., 1987). However, increased mammary protein synthesis is part of the array of bovine mammary responses directly or indirectly elicited by prolactin during lactogenesis (Akers, 1985).

In contrast, treatment of cows with exogenous somatotropin (ST) causes dramatic increases in rates of mammary uptake of amino acids and milk protein synthesis (Etherton and Bauman, 1998). This is associated with increased protein synthetic capacity as indicated by increased RNA:DNA ratio in mammary tissue of cows chronically treated with bST (Baldwin and Knapp, 1993). The mechanism of action of ST is probably indirect, involving the insulin-like growth factor (IGF) system. Mammary close arterial infusion of ST had no effect on yield of milk or milk protein (McDowell et al., 1987) but similar application of IGF-1 stimulated milk yield and, presumably, protein synthesis in goats (Prosser et al., 1990). Also, IGF-1 stimulated casein synthesis in cultured bovine mammary cells (Collier et al., 1993).

Earlier studies found little effect of short-term treatment with insulin on mammary uptake of amino acids or milk protein yield in goats (Tesseraud et al., 1992) or cows (Metcalf et al., 1991). However, moderate elevation of plasma insulin for several days using the hyperinsulinemic, euglycemic clamp technique caused 25-30% increases in milk protein yield in lactating cows (**Table 1**; Griinari et al., 1997; Mackle et al., 1999; Mackle et al., 2000b) and goats (Bequette et al., 2001). These responses were optimized by postprandial supplementation with amino acids to offset the substantial insulin-induced decrease in circulating levels of some amino acids, especially the BCAA. Insulin treatment not only increased MBF but also the fractional extraction of the BCAA by 48% and arginine and lysine by 20% (Mackle et al., 2000b). The uptake to output ratios were reduced for the BCAA and increased for several NEAA.

Table 1. Effects of abomasal infusion of casein plus branched-chain amino acids (CB) and insulin on dry matter intake (DMI), milk yield, and milk protein concentration and yield in well-fed dairy cows (Mackle et al., 2000b).

Variable	Treatment ¹				SEM	P ² INS
	Water	CB	Water + I	CB + I		
DMI, ³ kg/d	26.2	27.6	25.1	25.2	1.2	0.09
Milk yield, kg/d	26.5 ^a	27.5 ^b	28.3 ^{ab}	29.8 ^a	2.4	0.02
Milk protein						
%	3.29 ^b	3.31 ^b	3.52 ^a	3.66 ^a	0.185	0.001
kg/d	0.867 ^c	0.895 ^c	0.995 ^b	1.080 ^a	0.073	0.001

^{a,b,c}Least square means within rows with different superscripts differ ($P < 0.05$).

¹Treatments were: 1) abomasal infusion of water, 2) abomasal infusion of casein plus branched-chain amino acids (CB), 3) water infusion plus insulin (Water + I), CB infusion plus insulin (CB + I).

²Main effect of insulin treatment. There were no significant main effects of abomasal infusions or interactions with insulin effects.

³Excludes abomasal infusions.

The stimulatory effect of insulin on milk protein synthesis may be indirect and mediated by IGF-1. Insulin treatment increases circulating IGF-1 in lactating cows (McGuire et al., 1995) in a temporal pattern that closely resembles the increase in milk protein yield (Mackle et al., 2000a). Intramammary infusion of neither insulin nor a potent IGF-1 analogue affected milk protein yield in well-fed cows (Mackle et al., 2000a). However, the lack of response may have been due to limitations in the intramammary infusion technique.

Amino acid metabolism and protein turnover in skeletal muscle

Importance Rapid rates of muscle protein deposition are important for efficient beef production because protein is the principal nutritional component of meat and also because lean growth rate is positively associated with overall feed efficiency in growing and finishing cattle. Fractional rates of muscle growth and protein deposition in beef cattle are influenced by genotype, physiological age, sex, present and previous levels of nutrition, and use of metabolic modifiers (National Research Council, 1994). The rest of this section will focus on the regulatory influences of amino acid supply, endogenous

hormones and growth factors, and the major classes of metabolic modifiers on amino acid uptake, protein turnover, and net protein deposition in bovine muscle.

Influence of amino acid supply Amino requirements for optimal muscle protein deposition have been difficult to determine in beef cattle compared to swine (e.g. Campbell, 1988) because of uncertainties in the prediction of quantity and composition of absorbed amino acids from dietary inputs. It is notable that abomasal infusion of casein significantly increased N balance in steers fed diets balanced to optimize rumen fermentation and provide protein in excess of NRC requirements (Houseknecht et al., 1992). This implies that muscle protein deposition was constrained by quantity and/or quality of the postabsorptive amino acid supply.

Amino acid uptake Fractional extraction of EAA by hindlimb tissues (mostly skeletal muscle) in growing steers is only 2-7%, consistent with the relatively slow rates of protein deposition in muscle (Boisclair et al., 1994). Analysis of the kinetics of amino acid transfer into ovine muscle indicated that carrier-mediated transport of amino acids is unlikely to be limiting for net protein deposition (Hoskin et al., 2003). In general, the composition of the net flux of EAA across hindlimb tissues was similar to that of muscle protein (Ainslie et al., 1993), except that the net uptakes of the BCAA were in relative excess. This is consistent with observations of extensive catabolism of BCAA in ruminant skeletal muscle (Harris and Lobley, 1990).

Protein turnover Net deposition of muscle protein represents the balance between protein synthesis and degradation, fractional rates of which are similar and relatively low in bovine muscle (**Table 2**; Lobley et al., 2000). The relation between rates of synthesis and degradation is such that only subtle changes in either flux rate can result in a relatively large change in the rate of protein deposition. For example, treatment of growing steers with bST caused only a 10% increase in synthesis which, with unaltered degradation, resulted in a 45% increase in muscle protein accretion (Boisclair et al., 1994). In contrast, increasing the plane of nutrition accelerated fractional rates of both muscle protein synthesis (K_s) and degradation (K_d) in Angus and Charolais steers, but to a greater extent for K_s , resulting in increased net protein accretion (**Table 2**; Lobley et al., 2000).

Table 2. Effects of breed and plane of nutrition on fractional rates of muscle protein synthesis K_s , degradation (K_d), and deposition (K_g) in Angus and Charolais steers¹ (adapted from Lobley et al., 2000).

Breed	Angus		Charolais		Significance (<i>P</i>) of:	
	Medium	High	Medium	High	Breed	Nutrition
K_s , %/d	1.29	1.46	1.04	1.36	0.067	0.006
K_d , %/d	0.88	0.94	0.90	0.98	NS	0.012
K_g , %/d	0.41	0.52	0.14	0.38	NA ³	NA

¹Mean values for 6 animals per group

²Medium, predicted gain of 1.0 kg/d; High, predicted gain of 1.4 kg/d

³NA, not applicable because values were calculated from means for separately measured values of K_s and K_d

Endocrine regulation The dominant role of ST in regulation of protein synthesis and deposition in muscle and other lean tissues during postnatal growth is unquestioned (Etherton and Bauman, 1998). Stimulation of muscle protein synthesis by ST is believed to be mediated at least in part by the IGF system. Plasma concentrations of IGF-1 are increased in growing cattle treated with bST (Boisclair et al., 1994) and IGF-1 stimulated muscle protein synthesis in vivo (Douglas et al., 1991). However, treatment with ST was far more efficacious than infusion of IGF-1 when comparable plasma levels of IGF-1 were achieved. Possible explanations include direct actions of ST on muscle protein turnover and/or the beneficial effects of reduced amino acid catabolism in liver and, possibly, other tissues (Etherton and Bauman, 1998). Interestingly, abomasal infusion of casein caused an appreciable increase in abundance of IGF-1 mRNA in muscle of growing steers, suggesting an additional, local mechanism for modulation of muscle protein synthesis (Moloney et al., 1998).

It has been postulated that part of the effect of increased nutrient intake on muscle protein synthesis is mediated by insulin. However, recent, careful experiments on growing lambs found no evidence that insulin stimulates muscle K_s , with or without additional supplementation of glucose or BCAA (Wester et al., 2004). Thus, this group's previous observation that close-arterial infusion of insulin increased net protein deposition in the hind limb (Wester et al., 2000) was probably due to decreased muscle protein degradation.

Actions of metabolic modifiers Mechanisms of action of exogenous bST on muscle protein metabolism are discussed above. This recombinantly-derived product, marketed as Posilac® for use in lactating dairy cows, is not approved for use in growing beef cattle in the US, despite its undoubted efficacy in improving lean growth (National Research Council, 1994). Likely reasons are its low benefit-cost advantage in promoting growth versus milk production and disadvantages in its delivery system compared to those for anabolic steroids (long-term implant) or phenethanolamines (feed additive).

For many years, several estrogenic and androgenic compounds have been widely used alone and in combination to promote muscle protein gain and feed efficiency in US beef cattle (National Research Council, 1994). Combinations of estradiol and an androgen, trenbolone acetate, are especially efficacious for use in steers. Mechanisms of action of these compounds on muscle protein metabolism are still a matter for debate. Early studies attributed the positive effect of trenbolone acetate to an overall slowing of protein turnover, with the reduction in K_d being greater than that in K_s (Buttery and Dawson, 1990). Other studies have failed to detect convincing effects of estradiol and/or trenbolone on protein synthesis or degradation (Hayden et al., 1992). Recently, steers implanted with an estradiol-trenbolone combination exhibited marked, time-related increases in plasma concentration and muscle mRNA abundance of IGF-1 (Pampusch et al., 2003), reviving earlier suggestions that at least part of the anabolic response to steroids is mediated by the ST-IGF system.

The phenethanolamines or β -adrenergic agonists are orally active, catecholamine-like compounds with varying ability to increase protein deposition in skeletal muscle and decrease lipid deposition in adipose tissue (National Research Council, 1994). Of several compounds that have been studied extensively, only ractopamine (Optaflexx®) has been approved for commercial use in beef cattle in the US. Close arterial infusion of another compound, cimaterol, into the hind limb of steers for 21 d substantially but transiently

increased net extraction of EAA by the infused limb (Byrem et al., 1998). This was accompanied by increases in rates of muscle protein deposition in the infused limb of 61% on d 7 and 130% on d 14 of treatment, resulting in increases in protein mass of semitendinosus (10%) and semimembranosus (15%) muscles over those in the uninfused limb at 21 d. These dramatic responses occurred in the absence of systemic endocrine changes, adding support for a direct mode of action involving binding of the agonist to specific muscle receptors (Beermann, 2002). The mode of action of β -adrenergic agonists on muscle protein turnover remains to be clarified, with incomplete evidence for both enhanced protein synthesis (ractopamine) and reduced protein degradation (cimaterol and several other phenethanolamines) having been obtained (Beermann, 2002).

Conclusions

Studies of the regulation of amino acid utilization for deposition of milk and muscle proteins have revealed opportunities, challenges, and unanswered questions. In dairy cows, the demonstration of an unprecedented milk protein response to insulin offers an opportunity that now requires translation of new knowledge of mammary synthetic capacity into feasible management strategies. In beef cattle, the greatest challenge continues to be development of schemes to optimize the postabsorptive supply of energy and amino acids, including better definition of the varying amino acid requirements for processes other than muscle protein deposition. Several classes of metabolic modifiers have clear efficacy in promoting rate and efficiency of growth in cattle but, despite decades of study and, in the case of anabolic steroids, practical application, the mechanisms by which these compounds alter muscle protein turnover and thence, deposition, are not well understood.

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