

Balance rations for all essential amino acids

The dairy cow must have all amino acids available to her in order to synthesize protein. This means she needs a supply of essential amino acids as well as carbon and nitrogen sources to meet the synthetic needs for non-essential amino acids.

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EACH amino acid has specific metabolic and structural roles to play, and no amino acid can be assumed to be more important than another.

As a matter of convenience, W.C. Rose (1938) divided amino acids into two categories: essential and non-essential. He defined an essential amino acid as one that "cannot be synthesized by the animal organism out of materials ordinarily available at a speed commensurate with the demands for normal growth."

This simple statement, written at a very early stage in the understanding of amino acid nutrition, reveals that qualifiers were needed to assign essential and non-essential status to amino acids.

This situation has not changed. All of the known amino acids are important and must be present, whether from absorption or synthesis, for an organism to thrive.

The amino acid composition of each and every protein is genetically programmed without the possibility for change. For protein synthesis to occur, DNA instructs messenger RNA of the sequence of amino acids required. Messenger RNA then transfers the code to transfer RNA, which is used as a template for the synthesis of the protein in question. Amino acids are added one at a time until the protein is complete and releases from the template. The amino acid chain then folds into specific proteins.

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If an amino acid is not available, the synthesis of a protein is halted. The amino acid that limits protein synthesis is called the first-limiting amino acid. Any amino acid can theoretically be limiting.

The amino acids least likely to be limiting are, by definition, the non-essential amino acids. These are readily synthesized within the animal's system if the precursor carbon skeletons and ammonia are present and, under normal conditions, are not of concern.

The essential amino acids are dietary (or post-absorptive) essentials. They cannot be synthesized at a rate equivalent to their rate of utilization and must be absorbed on a continuing basis.

Cow requirements

Cows, like non-ruminant animals, require amino acids for milk, for tissue maintenance and accretion and for

fetal growth. Each function relies on a unique set of amino acid requirements as dictated by that function. The relative proportion of the total of each function determines the appearance of the final set of requirements.

Also, there are some additional uses of amino acids that affect the amounts actually available for the required functions.

Milk proteins. Milk consists of two major protein groups: the caseins and the whey proteins. The caseins account for approximately 80% of the protein in milk, and beta-casein dominates. Whey proteins are made up of several individual proteins but, in the case of dairy cows, primarily two: approximately 50% beta-lactoglobulin and 25% alpha-lactalbumin.

Because there is very little variation in the proteins that make up milk, there is, likewise, very little variation in the amino acid composition of milk and, theoretically, in the amino acids that are ultimately required for the synthesis of milk proteins.

Amino acids are transported into the udder via the blood. The mammary gland has the ability to alter the extent of extraction based upon need (McGuire, 1998).

Varvikko et al. (1999) infused graded



levels of lysine or methionine in lactating dairy cows. In both cases, plasma concentrations were elevated. The proportion — but not the absolute amount — extracted by the mammary gland declined in concurrence.

Similarly, Thivierge et al. (2002) infused amino acids into the jugular vein or abomasally and, likewise, saw no effects on mammary uptake.

The mammary gland appears to have the flexibility to regulate its own supply of nutrients through a combination of altering blood flow rates and altering percentage extraction to meet the target requirement (Maas et al., 1998).

Interestingly, the amounts of amino acids extracted in relation to the amounts that appear in milk are reasonably consistent (Bequette et al., 1998; Evans, 2003) over a wide range of feeding conditions, nutritional statuses and production levels.

Studies of amino acid uptake-to-output ratios dating back as far as 1965 (Verbeke and Peeters, 1965; Mephram and Linzell, 1966; Clark, 1975) have shown that total uptake by the mammary gland is consistently higher than output into milk for arginine, lysine and the branched-chained amino acids.

On the other hand, mammary uptake of the non-essential amino acids glutamate, glutamine, aspartate and proline was considerably less than the quantities in milk.

Doepel et al. (2007) determined that glutamine uptake by the mammary gland was not enhanced by glutamine infusion. Infusion of essential amino acids increased milk protein output, whereas infusion of non-essential amino acids did not, indicating that the supply of non-essential amino acids to the mammary does not reduce the need for essential amino acids to produce them (Whyte et al., 2006).

Evans (1999) demonstrated that the uptake of total amino acids is only slightly higher than total output, indicating that the particular essential amino acids extracted in excess are needed to synthesize the non-essential amino acids found in milk.

A proportion of some essential amino acids is catabolized in the sow mammary gland rather than being available for the direct synthesis of milk protein.

Recently, Li et al. (2009) demonstrated that milk output of branched-chain amino acids was substantially greater than uptake and that these amino acids were utilized in the synthesis of glutamine and aspartic acid — amino acids extracted by the mammary in quantities less than their output in milk, even when their supply is plentiful. The researchers proposed a mechanism whereby the branched-chain amino acids are preferentially channeled to the mammary gland and promote milk protein synthesis.

Trottier (1997) found that the uptake:output ratio of amino acids in swine is remarkably consistent and differs substantially from milk. Most notable was an elevated level of arginine. Trottier concluded that the mammary amino acid uptake pattern represents independent mammary metabolic needs plus milk amino acid output and that this pattern should be used to determine the amino acid needs for milk protein synthesis in sows.

Additionally, small quantities of amino acids may be used for regulatory purposes.

For example, Lacasse et al. (1996) demonstrated that secretory epithelial cells in proximity to arterioles produce nitric oxide from arginine. Nitric oxide is a vasorelaxant, increasing local blood flow.

Polyamines may also be involved in the regulation of protein synthesis (Kim and Wu, 2009).

Because of these influential factors, the pattern of amino acids needed for milk is different from the composition of milk, but consistently so. This pattern allows for supplementation that exclusively supports this function.

Somatic tissues. Tissue proteins can be considered those required for maintenance, protein accretion and pregnancy.

Muscle protein mass is maintained by balancing the amount of protein synthesized with the amount of protein degraded. Protein is degraded and remodeled in order to form a labile pool of amino acids to serve as a ready supply for critical functions. The portion irreversibly lost in the degradation and resynthesis of tissue is the maintenance requirement.

At maintenance, fractional synthesis rates and fractional degradation rates are at equilibrium. However, tissues within the body have different fractional synthesis rates. Waterlow (2006) reported that the fractional synthesis rates (percentage of the total per day) averaged about 2% for muscle but 22% for liver and 50% for mucosa. Thus, the relative proportion of gut tissue to total tissue can influence total protein turnover.

Hormones and additives influence protein synthesis but may or may not impart an effect on maintenance. Bovine somatotropin has been shown to increase the fractional synthesis rate but without changing the degradation rate (Boisclair et al., 1994) and would, therefore, not be expected to alter maintenance.

On the other hand, repartitioning agents such as ractopamine and clenbuterol reduce the fractional degradation rate (Walker, 2008) and can, therefore, be responsible for a lower maintenance use of amino acids.

Traditionally, models have used the

amino acid composition of bodily tissues to represent maintenance requirements (Evans and Patterson, 1985 and 2004), and these values may or may not be accurate.

Lapierre et al. (2007) recommended using the amino acid profile of metabolic fecal nitrogen as this represents the largest portion of maintenance amino acid losses.

Additional uses

Amino acids may further be used for other functions and, depending on the physiological priority, can impinge on their availability for milk.

For example, Emmanuel and Kennelly (1984) and Lobley et al. (1996) demonstrated that up to one-third of the total methionine supply could be used for the synthesis of choline, a function not considered in most requirement models.

Lysine can also be used for the synthesis of acetyl Co-A for entry into the tricarboxylic acid cycle (Encarnacao and Bureau, 2003).

Metabolic pathways are important convergence points in the metabolism of proteins, lipids and energy. Gluconeogenesis and lactose synthesis rely on the availability of amino acids to make up the deficit between the need for glucose and the availability of propionate (Bequette et al., 1998).

Glucose infusion studies have revealed lower somatic tissue turnover of amino acids (Ku Vera et al., 1989), further indicating the interaction between glucose and amino acids.

The gut, *per se*, may alter the amino acids that remain available for use by other tissues. Thivierge et al. (2002) infused a mixture of amino acids either into the abomasum or into the jugular vein. All circulating levels of essential amino acids were numerically higher, and leucine and isoleucine were statistically higher after jugular infusion.

Until such factors are taken into account when assessing requirements and in diet formulation, models will continue to have some inaccuracies.

Non-essential amino acids may not fare as well. Glutamine is a preferred energy source for the gut and may be utilized prior to absorption (Lobley et al., 2001), thereby reducing the available supply.

Efficiency concept

Efficiency generally relates to an input compared to an output relationship. In the case of amino acids, the input is absorbed amino acids and the output is milk proteins.

Efficiency can be variable if amino acids are consumed in reactions other than protein synthesis, such as gluconeogenesis, anaplorotic flux

Evaluation of milk production and milk components when diets are balanced for amino acids

Location	Cows	Treatment	-----Milk composition-----								-----Amino acid, % of requirement-----					
			Days in milk	Fat, %	Protein, %	150-day milk	Corr. fat	Corr. protein	Fat, lb.	Protein, lb.	Methio.	Lysine	Arginine	Leucine	Isoleuc.	Valine
Indiana	40	Control	145	3.70	3.00	76.67	3.71	3.01	2.85	2.31	121	107	97	106	106	105
		Test	186	3.88	3.02	79.02	3.78	2.96	2.99	2.34	115	108	99	110	110	110
Michigan*	109	Control	174	3.84	3.29	75.02	3.78	3.25	2.83	2.44	125	103	89	102	102	94
		Test	202	4.10	3.24	75.24	3.96	3.15	2.98	2.37	122	100	85	97	98	91
Michigan	112	Control	125	3.23	2.91	82.79	3.30	2.95	2.73	2.44	121	112	94	106	108	107
		Test	178	3.34	3.04	92.56	3.26	2.99	3.02	2.77	126	117	99	115	110	115
Michigan	50	Control	146	4.60	2.95	71.62	4.61	2.96	3.30	2.12	108	115	88	96	101	97
		Test	191	3.85	3.04	75.1	3.74	2.97	2.81	2.23	118	114	99	108	107	111
Minnesota	805	Control	161	3.75	3.18	86.76	3.72	3.16	3.23	2.74	106	106	94	100	98	102
		Test	189	3.79	3.41	90.29	3.69	3.34	3.33	3.02	108	103	95	103	100	103
Minnesota	271	Control	171	3.55	3.00	82.21	3.49	2.96	2.87	2.44	127	109	100	106	108	107
		Test	198	3.62	3.04	84.24	3.49	2.96	2.94	2.49	122	115	103	115	113	117
Minnesota 3	126	Control	167	3.50	3.00	95.42	3.45	2.97	3.30	2.84	126	107	95	108	97	100
		Test	199	3.58	3.05	101.27	3.45	2.97	3.49	3.01	130	113	100	111	100	103
Minnesota 5	278	Control	165	4.42	3.60	65.82	4.38	3.57	2.88	2.35	150	117	102	105	105	102
		Test	165	4.91	3.67	73.37	4.87	3.64	3.57	2.67	150	118	103	110	103	109
Minnesota 6	64	Control	154	3.90	3.10	83.44	3.89	3.09	3.25	2.58	120	104	86	94	99	92.4
		Test	189	3.43	3.06	87.23	3.33	2.99	2.90	2.61	133	118	99	111	108	111
New York	46	Control	166	3.82	2.93	86.10	3.78	2.90	3.25	2.50	136	106	93	110	101	99
		Test	226	3.88	3.02	90.05	3.68	2.89	3.31	2.61	141	115	100	118	104	113
Pennsylvania	44	Control	150	4.09	3.22	71.64	4.09	3.22	2.93	2.31	127	110	96	107	109	101
		Test	180	3.95	3.49	74.97	3.87	3.44	2.90	2.58	128	112	98	111	107	108
S. Dakota	270	Control	161	3.50	3.00	89.47	3.47	2.98	3.10	2.67	125	104	92	108	107	99
		Test	190	3.58	3.05	106.56	3.47	2.98	3.70	3.18	132	112	98	118	111	111

and transamination reactions. These functions all reduce the efficiency by reducing the amounts of amino acids available for protein synthesis, or input values.

In contrast, efficiency can decline if one or more amino acids are in limiting supply, which results in less protein being produced, or lower output.

Although an optimal efficiency can be theorized, such targets are difficult to attain. Available protein sources have differing profiles from the proteins being synthesized, which deem it necessary to oversupply all amino acids except the most limiting, being it intentional or unintentional.

Synthesis of non-essential amino acids from essential amino acids reduces the overall efficiency of the essential amino acids.

Energy balance — or glucose precursor balance — is also a closely related key factor. Animals losing weight contribute either glucose or proteins to the amino acid pool that are then available for synthetic processes. It has long been known that many amino acids are glucogenic and may be used for glucose synthesis as a priority over protein.

Vik-mo et al. (1974) found that post-ruminal infusion of glucose resulted in both higher milk yields and milk protein percentages. This has been confirmed by more recent studies (Huhtanen et al., 2002). The use of amino acids for functions other than protein synthesis reduces efficiency.

Response options. Short term, the cow may mobilize tissue to provide a pool of amino acids for synthesis of labile proteins. The mammary gland is in competition with other tissues for amino acids (Bequette et al., 2000) and can respond by increasing blood flow and the extent of extraction of amino acids that have been mobilized.

Champredon et al. (1990) demonstrated the ability to reduce fractional protein synthesis rates in muscle, which can help spare maintenance losses in amino acids.

Weekes et al. (2006) and Cant et al. (2001) found that infusing mixtures of amino acids in which key amino acids were missing resulted in higher butterfat in milk. This would suggest that tissue is being mobilized to supply the deficit and that the fat was of tissue origin.

Longer term, a marginal deficiency or imbalance may result in tissue mobilization. There have been cases when an imbalance has resulted in higher milk fat for longer feeding periods, and that might be related to greater fatty acid availability from tissue sources (Varvikko et al., 1999; Kim et al., 2001).

Apparent correction of an imbalance in lysine resulted in a decline in milk fat, presumably in association with an increase in tissue protein deposition (Swanepoel et al., 2009).

Amino acids frequently serve as sources of carbon for glucose synthesis. Orskov et al. (1999) demonstrated that the infusion of glucose or propionate in animals supported by intragastric

nutrition lowered nitrogen excretion, with greater quantities available for tissue use. The study suggested that fractional protein degradation remained the same but that net protein synthesis was higher.

Challenges

As mentioned, the cow must have all amino acids available to her in order to synthesize protein. This means the cow needs a supply of essential amino acids as well as carbon and nitrogen sources to meet the synthetic needs for non-essential amino acids.

There has been a significant amount of interest in using rumen-protected amino acids. Because these amino acids are available in such a concentrated form, when an imbalance occurs, it is possible to supply the amino acid and increase overall protein efficiency.

However, the additives also need to be cost effective, so that eliminates all but methionine and lysine, which, fortuitously, are considered to be the most limiting in many dairy rations.

There are innumerable studies suggesting that the addition of rumen-protected methionine will improve milk production, milk protein yield or milk fat yield. There are also many studies showing that no improvements in performance occurred with amino acid supplementation.

Currently, the National Research Council (2001) does not recommend balancing diets for amino acids except to

recommend a lysine-to-methionine ratio of 3:1 in metabolizable protein.

This model does not take into account the likelihood that other essential amino acids could be limiting animal performance or productive efficiency. It is not unusual for other amino acids to be first limiting and for cows to respond to dietary changes that result in the removal of the limitation.

One example is histidine. Vanhatalo et al. (1999) found that histidine was the first limiting amino acid when cows receive grass-based diets. These researchers reported increases in milk protein with an abomasal infusion of histidine. No further increases occurred when methionine, lysine or both were additionally supplied, indicating that lysine or methionine were not even the second limiting amino acids.

The researchers suggested that glucose was most likely the nutrient most limiting after histidine with these particular diets, and this was confirmed in a later study (Huhtanen et al., 2002).

Other studies have also shown that histidine can be limiting. Cant et al. (2002) demonstrated that milk protein synthesis was compromised after only 10 hours if histidine was limiting. Supplementation with this amino acid improved milk yield and decreased milk fat in early-lactation cows (Doelman et al., 2008).

Another example is arginine. In addition to the arginine in milk, the mammary gland uses arginine for the production of nitric oxide (Lacasse et al., 1996) and ornithine (Bequette, 1998), and arginine also is used for the synthesis of the non-essential amino acid proline. Infusions of proline have been demonstrated to increase milk production (Bruckental, 1991), presumably through the sparing of arginine.

Older studies (Hertelendy et al., 1970; Gow et al., 1979) demonstrated that arginine can increase milk production.

The Table shows results from 12 field studies where amino acids were balanced using the CPM model. In all cases, the diets being offered contained sufficient methionine and lysine, and the diets were determined by the program to be balanced.

Upon closer inspection, it was revealed that the original diets were not balanced for amino acids beyond lysine and methionine, most notably either for arginine or branched-chain amino acids. The diets were rebalanced, resulting in improved performance in most cases. Milk production increased in 11 of the trials, and milk fat yield was improved in nine trials, while milk protein yield was higher in 10 trials.

Gemini Protein is a protein supplement that was formulated to supply an array of essential amino acids similar to those required for milk protein synthesis based on uptake:output. This mixture replaced

an equal or greater amount of dietary plant protein. The product did not elevate protein in any of the trials, nor did the product supply additional energy.

Clearly, there is a need to go beyond formulation of diets strictly for two amino acids when cows have a need for all amino acids.

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