Influence of Dietary Protein and Amino Acids on Reproduction in Dairy Cows

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▪ Take Home Messages

- The amino acids that make up dietary protein are important because they provide the building blocks for synthesis of proteins by the cow AND because specific amino acids are used to synthesize other molecules important for biological function.
- Overfeeding of protein can reduce energy availability and reduce dairy cow fertility. Lactating cow diets should contain less than 19% crude protein with ruminally degradable protein no more than 10%.
- There is less risk to reproduction of feeding diets low in protein although milk yield could be reduced.
- New products are being developed to increase delivery of specific amino acids to cows by bypassing utilization by microbes in the rumen. Rumen-protected methionine has been reported to increase lactation performance and reproductive function. Further studies are warranted.

▪ Protein – Can't Live Without Them

Protein is a class of nutrient consisting of individual protein molecules, each of which is composed of specific chains of nitrogen-containing amino acids. As a nutrient, protein provides amino acids for the animal to build its own proteins. Each particular protein has a unique sequence of amino acids. The stringing together of amino acids in the right sequence to produce a specific protein is directed by individual genes in the nucleus of the cell. Proteins play roles in every biological process — they are enzymes, (for example, thrombin involved in blood clotting), hormones (follicle stimulating hormone and somatotropin), the major structural component of muscle (actin and myosin) and are secreted into milk to feed the offspring (caseins, lactalbumin).

WCDS Advances in Dairy Technology (2016) Volume 28: 209-216

However, as shown in Figure 1, amino acids are used by animals for more than protein synthesis. Amino acids can be burned as fuel when other energy substrates are limiting. This is one reason why cows in negative energy balance experience weight loss; both fat and protein are mobilized to provide energy.

Besides being used for protein synthesis, specific amino acids are used to make other molecules that have their own biological function. Arginine, for example, is used to synthesize nitric oxide, which, among other things, plays a role in regulating blood flow to tissues. Methionine and lysine are used to synthesize L-carnitine, which functions to utilize fatty acids for energy production in the mitochondria of the cell. Another role of methionine is to participate in export of fat molecules from the liver to prevent fatty liver.

Methionine also plays an important role in the process of DNA methylation. Methylation of DNA can silence specific genes for a short period of time or permanently. One reason cells in the liver don’t produce milk proteins, for example, is because the genes for the proteins are shut off by DNA methylation. Feeding rumen-protected methionine has been shown to alter DNA methylation in the early embryo (Peñagaricano et al., 2013). It may be possible, therefore, to regulate specific physiological processes by using amino acids to regulate DNA methylation.

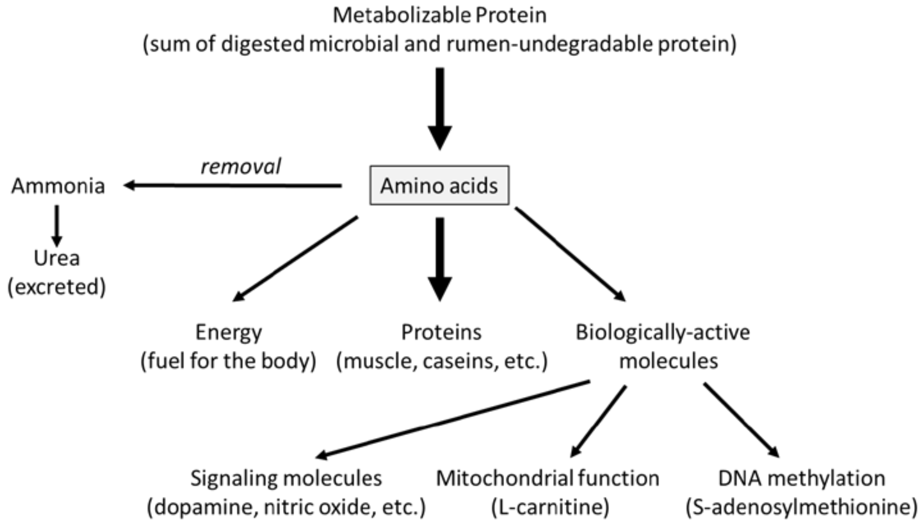

Figure 1. Source and uses of amino acids in the dairy cow.

Animals can synthesize amino acids from other nutrients. Of the 20 amino acids used to make proteins, only 10 of these can be synthesized in sufficient quantity to meet the animal's demands for amino acids. The other 10, which are termed essential amino acids, must be obtained from the diet. The essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The most limiting in dairy cattle are lysine, methionine and histidine.

In dairy cattle and other ruminants, there are two main sources of amino acids. Much of the protein in the diet is used by the microbes in the rumen for synthesis of their own proteins. When the microbes pass to the small intestine, microbial proteins are broken down and are used by the cow. This fraction of dietary protein is called microbial protein. The dietary protein not used by rumen microbes is available for digestion in the small intestine and is called rumen undegradable protein. The percent of dietary protein that is not degraded in the rumen can vary greatly depending on the feedstuff and ranges from 0-80%.

Urea, which is derived from ammonia, is also used as a dietary ingredient to increase amino acid availability. While not a protein, rumen microbes can utilize nitrogen from urea for synthesis of amino acids by rumen microbes. Urea contributes to the estimate of crude protein (CP) because CP is calculated based on the amount of nitrogen in the diet. Urea is not effective in young calves (less than 3 months old) because the rumen is not fully functional.

Most, but not all, of the microbial protein and rumen undegradable protein that passes into the small intestine is digested and absorbed into the blood as amino acids. Termed metabolizable protein, this represents the amino acid supply available to the cow for its needs (Figure 1).

▪ Too Much of a Good Thing Can be Bad

The nitrogen in amino acids is converted to ammonia during amino acid degradation. Ammonia is toxic to mammalian cells so it is removed from the cow by conversion to urea and excretion into the urine (Figure 1). Synthesis of urea requires energy so feeding protein in amounts higher than required by the cow can waste otherwise-needed energy. Additionally, urea itself can compromise reproductive function. Feeding high amounts of protein can reduce uterine pH (Elrod and Butler, 1993) and compromise the function of the oocyte or embryo (Rhoads et al., 2006). Given consequences of excess protein for energy metabolism and the function of the oocyte, embryo and uterus, feeding high protein diets have been reported to delay the resumption of estrous cycles after calving, reduce fertility and increase days from calving to conception (Lean et al., 2012; Tamminga, 2006).

Example of a typical experiment to demonstrate the negative effects of overfeeding of protein are shown in Table 1. Cows in this experiment were maintained on ryegrass pastures and began receiving various supplements at an average of 42 days in milk. Diet 1 had the highest estimated CP content (22.8%). Diets 2 and 3 had similar estimated CP (18.0%) but more of the protein was not degraded in the rumen for diet 3 than diet 2. Cows fed the diet with 22.8% CP experienced a delay in first breeding ($P \leq 0.05$) and fewer cows conceived to that breeding ($P \leq 0.05$) than cows fed the 18.0% CP diets. Moreover, there was a tendency for the total number of days non-pregnant to be longer for the cows fed the high CP diet ($P \leq 0.10$). There were no differences in any of these variables between the two 18% CP diets.

Table 1. Effect of protein feeding on reproductive function of lactating cows on ryegrass pastures and fed various supplements in Louisiana, USA (McCormick et al., 1999).

	Diet 1	Diet 2	Diet 3
Estimated crude protein (%)	22.8	18.0	18.0
Estimated rumen undegradable protein (%)	6.4	5.7	8.5
Number of cows	58	61	62
Days to first breeding	90	81	79
Percent pregnant to first service (%)	24	41	39
Days non-pregnant	129	114	114

As a practical matter, effects of excess protein on fertility can be limited by formulating diets so that CP is less than 19% and ruminal degradable protein is no more than 10% (Tamminga, 2006). It is often recommended to monitor urea concentrations in milk or blood to assess protein status. However, the actual correlation between urea concentrations and fertility can be low (reviewed by Sinclair et al., 2014). In a study using records from over 19,000 cows in Poland, the correlation between milk urea concentration and calving interval was significant but only 0.05 (Sawa et al., 2011). It is possible that errors in accurately estimating overall circulating urea status limits the precision of the relationship between urea concentrations in blood or milk and fertility.

▪ Slight Underfeeding of Protein Does Not Seem to Impair Reproductive Function

In several countries, there has been interest in reducing amounts of dietary protein in dairy cow rations so as to reduce feed costs and the discharge of nitrogen excreted by cows into the environment. Sinclair et al. (2014) recently evaluated results of 6 studies to determine whether cows fed diets low in CP experienced reductions in milk yield or reproductive function. Overall, there was a consistent reduction in milk yield for cows in the low CP group (range

12.7–14.5% CP) as compared to cows fed high CP diets (range 16.9–20.0%). The average reduction in milk yield in the low CP group was 1.2 kg/day. In contrast, there was no consistent effect on reproductive function (Figure 2). Thus, it is likely that while feeding too much protein can impair fertility, there is less concern about inadequate protein in the diet, at least with typical dairy cow rations.

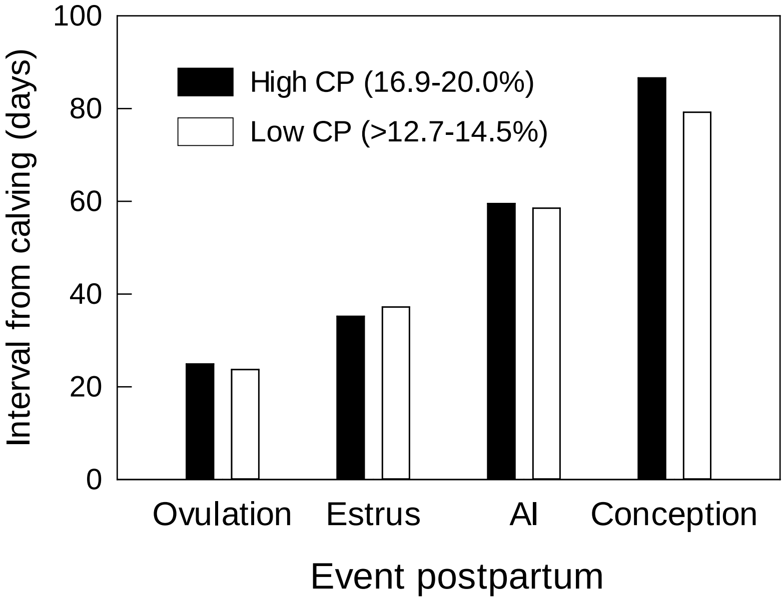

Figure 2. Lack of differences in intervals between calving and ovulation, estrus, artificial insemination (AI), and conception for cows receiving diets higher (high CP) or lower in crude protein (low CP). Sinclair et al. (2014) obtained data from 6 studies in the literature and calculated the average of each interval across studies after adjusting for number of cows in the study.

▪ Prospects for Changing Reproductive Function by Providing Specific Amino Acids in a Rumen-Protected Form

Methionine, which is often the first limiting amino acid in dairy cows, is not only required for milk protein synthesis but also is metabolized into other molecules that play important functions in the animal including those involved in export of lipids from the liver and gene expression (by providing methyl groups used in DNA methylation). A variety of products are available that provide rumen-protected methionine for dairy cattle. Examples include coated pellets of methionine (for example, Smartamine® M from Adisseo and Mepron® from Evonik) and a chemical precursor of methionine called 2-hydroxy-4-methylthiobutanoic acid (examples are Alimet® and MFP® from Novus and, as an isopropyl ester, MetaSmart® from Adisseo).

Recently, Zanton et al. (2014) performed a meta-analysis on 64 studies that examined effects of feeding rumen-protected methionine. The most consistent effect was increased protein percent and yield. Milk fat percent and yield was increased in some studies. While there was not a significant effect of supplementation on milk yield, there was a trend for a positive effect.

There have been few studies on consequences of feeding rumen-protected methionine for reproductive function. Reliable data on reproduction require larger number of cows that are often used in feeding studies. Effects on reproduction have been evaluated in one study in Iran with Smartamine M (Nikkhah et al., 2013). In that study, which involved 24 animals, cows receiving supplemental methionine experienced, among other things, increases in dry matter intake, milk yield and yield of fat and protein (Table 2). Additionally, there was also improvement in several aspects of reproductive function, including a reduction in days to estrus, AI, and conception. Further research is warranted into whether such positive effects of feeding rumen-protected methionine occur widely.

Table 2. Effect of feeding rumen-protected methionine on function of lactating Holstein cows in Iran during the summer (Nikkhah et al., 2013).

	Treatment	Control	P value
Dry matter intake, kg/day	21.9	19.1	0.01
Milk yield, kg/day	42.4	37.4	0.06
Milk fat yield, kg/day	1.40	1.04	0.002
Milk protein yield, kg/day	1.25	1.02	0.006
Days to first estrus	30.0	52.7	<0.01
Days to first AI	50.5	78.0	0.01
Days to conception	137.0	173.0	0.06
Services per conception	2.8	3.1	0.30

Effectiveness of feeding rumen-protected methionine may vary between products although direct experimental comparisons are lacking. It is also likely that benefits of feeding will be greater for high-yielding cows, cows fed low amounts of metabolizable protein and cows fed diets that are adequate for other important essential amino acids like lysine.

Arginine is another amino acid that can be converted to a variety of biologically-active molecules including nitric oxide and various polyamines. Feeding supplemental arginine in pregnant pigs has been reported to increase placental weight, litter size and litter birth weight (Bazer et al., 2014; Chacher et al., 2013). Intravenous administration of arginine increased fetal survival and growth in sheep and milk yield in dairy cattle (Chacher et al., 2013). It is possible to increase concentrations of arginine in plasma of dairy cows by feeding N-cabamoyl glutamate, a molecule that can provide glutamate for arginine biosynthesis (Chacher et al., 2013). Perhaps there are opportunities for using N-cabomoyl glutamate for improving milk production and reproduction in dairy cattle. One note of caution — arginine can also adversely affect ovulation and secretion of the pregnancy hormone progesterone. When fed at the beginning at estrus in pigs, supplemental arginine reduced ovulation rate, growth of the embryo, and litter size (Bazer et al., 2014). The need for precise timing of administration of supplemental arginine may limit its effectiveness in dairy cattle systems.

▪ Conflict of Interest

Some of the research from the author's laboratory was funded by Adisseo.

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