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MAIN LECTURE

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INTRODUCTION – Because more than 60% of milk produced in Italy is transformed into cheese, milk economical value strongly depends on cheese yield. Among the factors that influence cheese yield, milk casein and fat content plays a major role: when milk is converted into Grana Padano and Parmigiano Reggiano, three grams of seasoned cheese are produced from one gram of milk casein. Because casein and, to a lower extent, fat determine the economic value of milk, increasing casein production is an essential goal for dairy farmers. The importance of this subject is confirmed by the fact that several related reviews have been published in the past (Mordenti and Formigoni, 1986; Jenkins and McGuire, 2006).

In general, there is a strict correlation between milk production and cheese yield and this is the reason why, in the past, research mainly aimed at increasing milk production. Still, in order to increase cheese yield, dairy farmers have to increase also casein and fat milk content. In fact, despite the fact that milk casein and fat are mainly influenced by genetic factors, proper management and feeding strategies can increase their concentration in milk. It is well known that casein concentration as well as milk production are higher during the winter season; furthermore, different farms hosting genetically similar animals can achieve different milk yields, showing that paratypic factors can strongly influence milk production.

The present paper presents a review of factors that influence casein production in dairy cows.

MILK CASEIN SYNTHESIS – Caseins are synthesized by the mammary gland and account for about 77-79% of total milk nitrogen, the remaining nitrogen being in the form of soluble protein and non-protein nitrogen. Casein synthesis follows the steps of a typical protein synthesis pathway: amino acids are transported to ribosomes where they are converted to proteins following RNA genetic information. The extent of protein synthesis depends on number and activity of secreting cells, and the latter is mainly influenced by the availability of energy and amino acids (especially the essential ones). Because amino acids are taken up by secreting cells from the blood stream, all factors that increase blood flow through the mammary gland are likely to increase the availability of milk precursors. Despite the fact that the mammary gland is the organ with the highest amino acid requirements, in dairy cows, amino acids are needed also for maintenance and growth, and, during the first weeks after calving, when the energy balance is negative, as a substrate for the synthesis of glucose. Because these alternative utilizations of amino acids can lead to the deficiency of some nutrients thus limiting casein synthesis, they should be contolled to assure that sufficient amounts of amino acids are available to the mammary gland.

Several scientific studies have shown that strategies aimed at improving milk quality give different results depending on lactation stage. In particular,

- during early lactation, it is possible to increase production of milk and, as a consequence, milk solids, but casein concentration is hard to increase;
- in a later stage of lactation (more than 100-150 days after calving), daily milk production can still be increased but casein concentration is more likely to show significant improvements.

These differences, although not fully explained, are likely to be the consequence of hormonal changes that take place during the lactation; in fact, hormonal factors regulate the availability of nutrients to the mammary gland (Chiesa et al., 1991) as well as number, duration, and activity of secreting cells (including milk synthesis; Molento et al., 2002). Several trials have investigated the effect of somatotropic hormone, prolactin, somatomedins, and insulin on milk production but there is still a lack of knowledge regarding how nutrients affect hormones secretion and function, activity of their peripheral receptors, and mammary gland activity. Recently, it has been shown that the infusion of somatotropin in cultures of mammary cells increased the concentration of messenger RNA for casein by 35% (Yang *et al.*, 2005).

NUTRIENTS THAT MIGHT AFFECT CASEIN SYNTHESIS – As already mentioned, availability of energy and essential amino acids is the most important factor influencing casein synthesis.

ENERGY SUPPLY – It is well known that increasing the energy density of the diet (as long as energy is not provided in form of lipids) is the more efficient feeding strategy to increase casein secretion. Energy should be provided to dairy cows in form of digestible carbohydrates. In general, reducing forages and increasing concentrates, cereals in particular, in the diet of dairy cows leads to higher casein synthesis (Mordenti and Formigoni, 1986; Formigoni and Piva, 1996; Formigoni *et al.*, 2001; Jenkins and McGuire, 2006). Possible explanations for this effect include:

- increase of energy available to the animal because of the higher feed intake due to reduction of dietary NDF;
- higher ruminal passage rate that enhances intestinal availability of dietary nutrients;
- increased ruminal bacterial growth with higher intestinal availability of bacterial protein and amino acids;
- higher blood flow through the mammary gland (also through direct stimulation of the synthesis of some hormones) and higher amounts of nutrients available to the mammary cells;
- stimulation of mammary cells synthetic activity by hormones that are influenced by animal energy balance, such as GH and IGF-I, or availability of some nutrients (glucose, amino acids, peptides, ...);
- reduced amount of amino acids that are utilized for the synthesis of glucose, which leads to higher availability of amino acids to the mammary gland;
- higher availability of glucose for the synthesis of lactose which accounts for the most osmotic pressure of milk; to this regard, it is interesting to observe how lactose and amino acids syntheses in mammary cells seem to be connected. This might explain why strategies that increase milk yield also determine higher casein production (if enough amino acids are available) showing that synthetic capacity of the mammary gland is not the main limiting factor during early lactation. Conversely, a limiting factor for the mammary gland might be represented by the availability of energy substrates, both in form of glucose and volatile fatty acids produced in the rumen.

The influence determined by glucose availability on milk production and casein synthesis has been the object of several studies (Huhtanen *et al.*, 2002). Recently, Rulquin *et al.* (2004) have investigated the correlation between glucose intestinal availability and casein synthesis by the mammary gland in a trial with high producing dairy cows (100 days after calving). Duodenal infusion of 963 g of glucose increased milk and casein yield by 9% and 12%, respectively, whereas higher amounts of glucose determined lower milk and casein production (presumably due to the inhibition of lactose synthesis). The authors concluded that the best results in terms of milk and casein yield were achieved when glucose was available in the intestine at the concentration of 8% of dry matter.
The source of glucose that is used is also important and several researches have investigated this aspect. In general, ruminal highly fermentable starch carbohydrates increase casein production and concentration but decrease milk fat, probably due to lower acetate availability and altered biohydrogenation of fatty acids (the latter leads to higher concentrations in the mammary gland of isomers that inhibit *de novo* fat synthesis).
Availability of highly fermentable carbohydrates, especially starch, has a dramatic effect on bacterial growth in the rumen (Cannas *et al.*, 2006; Formigoni and Fusaro, 2007). The higher is the amount of starch that is fermented in the rumen, the higher is the availability of propionate which is efficiently utilized as a glucose precursor by the body. From the studies conducted by several authors (Broderick *et al.*, 2002; Krause *et al.*, 2003), it can be concluded that the ideal concentration of dietary starch is between 16-17% and 21-22%.
Dietary starch strongly influences casein yield and several related issues have to been considered:

- the need for reliable and efficient methods to estimate ruminal degradability of different starch sources (also in relation to technological treatments and preservation techniques);
- the need for a better knowledge of ruminal rate of passage of feedstuffs to achieve a better estimate of nutrient ruminal degradability and by-pass;
- the need for a better estimate of animal requirements, especially in relation to environmental conditions, to avoid the deleterious effect of ruminal acidosis.

Starch can not be replaced by structural carbohydrates even if they are highly fermentable. When starch is replaced with citrus pulp or soybean hulls, milk protein decreases; conversely, when highly fermentable fiber replaces less degradable fiber, milk yield and quality are increased. The substitution of corn silage with pressed sugar beet pulp increases milk yield without affecting milk quality, if dietary starch content is not decreased (Formigoni *et al.*, 1991). In conclusion, dietary starch should be as high as possible and highly neutral detergent fiber degradable sources (mainly forages) should be fed.

NITROGEN SOURCES – The feeding of nitrogen sources to dairy cows should be aimed at:

- meeting nitrogen requirements of the ruminal microflora to enhance its growth;
- meeting animal requirements of metabolizable protein;
- ensuring quantitative and qualitative supply of those essential amino acids that might limit milk yield;
- improving conversion rate of dietary nitrogen into milk protein;
- reducing nitrogen environmental pollution.

It is well known that feeding excessive protein, as such exceeding animal protein requirements, does not increase milk protein but can increase the amount of nitrogen that is excreted in form of urea, as a result of lower conversion efficiency of dietary nitrogen.

The definition of protein animal requirements is a very important step in practical animal feeding. Despite the amount of milk that is produced by the herd, the quantity of dietary metabolizable protein that has to be supplied depends on the amount of milk protein that is secreted; nonetheless, because milk protein depends on supply of dietary protein, the expected protein yield influences the amount of protein that must be supplied with the diet. According to Recktenwald and Van Amburgh (2006), meeting the requirements of metabolizable protein is essential to enhance milk protein yield, which is strictly correlated with milk production.

Requirements of metabolizable protein can be met by enhancing ruminal fermentation and using high biological value nitrogen sources that are able to escape ruminal degradation. Unfortunately, estimating the rate of degradation of nitrogen sources in the rumen, ruminal rate of passage, intestinal digestibility, and amino acid composition of feedstuffs is a very difficult task; moreover, the high variability of nitrogen-rich supplements that can be found on the market (due to the use of different feedstuffs and technological treatments) makes an estimation of their biological value very difficult. Furthermore, only few laboratories seem to be able to provide reliable results in reasonable time. For this reason, the use of low-degradable nitrogen sources has often lead to inconsistent results (NRC, 2001). The best improvements in milk yield and quality are obtained when the nitrogen requirements of the ruminal microflora are met. According to NRC (2001), the feeding of 10.2-12.2% of degradable protein allows the best results (about 16-17% of crude protein on a dry matter basis; Colmenero and Broderick, 2006a,b,c). Provided the diet contains enough degradable carbohydrates, the mentioned protein concentration is able to maximize milk yield and nitrogen retention (whereas nitrogen retention decreases if excessive protein is fed).

AMINO ACIDS – Milk protein is synthesized by the mammary gland from amino acids that are taken up from the blood stream. In dairy cows, amino acids are mainly of bacterial origin and amino acid composition of bacterial protein is constant and well balanced for casein synthesis. Conversely, amino acids that are obtained from digestion of escape protein show a much higher variability, which makes difficult to predict protein amino acid composition in the intestine. However, it has been demonstrated that milk protein yield is maximized when methionine and lysine are supplied in a 1:3 ratio and make up for 2.3-2.5% and 6.8-7.5% of metabolizable protein, respectively (NRC, 2001; Doepel *et al.*, 2004). Recently, Rulquin and Pisulewski (2006) have concluded that the quantity of leucine needed to optimize milk protein yield is between 8 and 9% of intestinal protein. According to Huhtanen *et al.* (2002), histidine is the limiting amino acid when diets contain high amounts of grass silage and barley. It has also been hypothesized that glutamine, a non essential amino acid, in specific metabolic conditions, might limit milk production; in fact, glutamine is used by the body for different purposes, such as gluconeogenesis. Under specific circumstances, proline and glutamine might limit milk protein yield (NRC, 2001). Because glutamine makes up for 30% of casein amino acids (Meijer *et al.*, 1995), it can not be excluded that the beneficial effects that are observed when rumen-protected casein is fed to dairy cows might be the consequence of a better coverage of glutamine requirements (Formigoni *et al.*, 1991; Khalili and Huhtanen, 2002). In practical feeding of dairy cows, methionine seems to be the most frequently limiting amino acid, while lysine can become limiting when dietary protein is mainly obtained from cereals, and corn in particular (corn gluten feed and meal, distiller). The benefits that can be derived from the use of rumen-protected amino acids have been clearly demonstrated (Formigoni *et al.*, 1993; Martelli *et al.*, 1993; Robinson *et al.*, 1998; Schwab and Ordway, 2004; Loest, 2006; Schwab *et al.*, 2002); such benefits are maximized when proper sources of escape protein of high biological value and digestibility are used (Noftsger and St-Pierre, 2003). In general, it can be concluded that diets with an optimal amino acid composition

- maximize milk yield and quality
- improve conversion of dietary nitrogen
- increase farmers' profits

CONCLUSIONS – Based on present knowledge, milk protein yield can be improved in dairy cows by meeting animal requirements of metabolizable protein and feeding protein sources with the proper amino acid composition. Means to increase production of milk solids, in particular casein, include better nutritional characterization of feedstuffs (laboratories and methods of analysis), better understanding of animal nutritional requirements, and improved animal welfare (in order to reduce nutritional expenditures).

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