



## Changes in mammary metabolism in response to the provision of an ideal amino acid profile at 2 levels of metabolizable protein supply in dairy cows: Consequences on efficiency

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### ABSTRACT

The aim of this study was to compare the modifications in mammary gland metabolism by supplying an ideal versus an imbalanced essential AA (EAA) profile at low and high metabolizable protein (or PDIE, its equivalent in the INRA feeding system). Four lactating, multiparous Holstein cows received 4 treatments composed of 2 basal diets containing 2 levels of PDIE (LP or HP) and 2 different infusions of AA mixtures (AA– or AA+) in the duodenum. The AA+ mixture contained Lys, Met, Leu, His, Ile, Val, Phe, Arg, Trp, and Glu, whereas the AA– mixture contained Glu, Pro, and Ser. The infusion mixtures were iso-PDIE. The diet plus infusions provided 13.9 versus 15.8% of crude protein that corresponded to 102 versus 118 g/kg of dry matter of PDIE in LP and HP treatments, respectively. The treatments were designed as a 2 × 2 crossover design of 2 levels of PDIE supply (LP vs. HP) with 28-d periods. Infusions of AA in the duodenum (AA– vs. AA+) were superimposed to diet within each 28-d period according to 2 × 2 crossover designs with 14-d subperiods. Increasing the PDIE supply tended to increase milk protein yield; however, the efficiency of PDIE utilization decreased and the plasma urea concentration increased, indicating a higher catabolism of AA. The AA+ treatments increased milk protein yield and content similarly at both levels of protein supply. This was explained by an increase in the mammary uptake of all EAA except His and Trp. The mammary uptake of non-EAA (NEAA) was altered to the increase in EAA uptake so that the total AA uptake was almost equal to milk protein output on a nitrogen basis. The ratio between NEAA to total AA uptake decreased from 46% in LPAA– to 40% in LPAA+, HPAA–, and

HPAA+ treatments. The PDIE efficiency tended to increase in the AA+ versus the AA– treatments because the NEAA supply and the amount of NEAA not used by the mammary both decreased. Nevertheless, our AA+ treatments seemed not to be the ideal profile: the mammary uptake-to-output ratio for Thr was higher than one in LPAA–, but it decreased to one in all the other treatments, suggesting that Thr was deficient in these treatments. Conversely, an excess of His was indicated because its uptake was similar in AA+ and AA– treatments. In conclusion, balancing the EAA profile increased milk protein yield and metabolizable protein efficiency at both levels of protein supply by increasing the mammary uptake of EAA and altering the NEAA uptake, leading to less AA available for catabolism.

**Key words:** amino acid, metabolizable protein, mammary gland, efficiency, milk protein

### INTRODUCTION

Formulating diets that can increase the efficiency of dietary nitrogen (N) utilization is a challenging task in dairy cattle nutrition. Several dietary strategies can be combined to increase the efficiency of dietary N conversion to milk in lactating dairy cows. The first is to limit the dietary protein supply in excess of cow requirements for PDIE (protein digested in the small intestine supplied by RUP and by microbial protein from rumen-fermented OM, INRA, 1989) because efficiency decreases with an increasing N supply (Huhtanen and Hristov, 2009). The second strategy is to balance the dietary protein-to-energy ratio (Vérité and Delaby, 2000) to reduce urinary N excretion. In this context, a ratio of 58.8 g of PDIE per Mcal of NE<sub>L</sub> is reported to serve as a threshold where maximum milk protein yield can be obtained with minimal urinary N loss (Vérité and Delaby, 2000; INRA, 2007). The third strategy is to balance the EAA profile of dietary protein as reported for Met and Lys (Rulquin et al., 1993; Schwab, 1996; NRC, 2001). However, the fact that Met and Lys can increase milk protein yield was initially established at a high

Received July 24, 2014.

Accepted March 4, 2015.

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protein supply level; that is, at or above 65 g of PDIE per Mcal of  $NE_L$  (Rulquin et al., 1993), where urinary N excretion has been shown to increase more rapidly than milk protein yield (Vérité and Delaby, 2000). In a recent study, we have shown that balancing a complete EAA profile increased milk protein yield and the efficiency of dietary N utilization at both a low (<65 g of PDIE per Mcal of  $NE_L$ ) and a high PDIE supply (Haque et al., 2012), which provided an opportunity to combine the second and third dietary strategies to mitigate N wastage. Consequently, this raised the problem of understanding the underlying mechanism of AA metabolism in the mammary gland (MG) that contributed to this gain in N efficiency.

The MG is the site where 96.5% of the milk proteins are synthesized (Cant et al., 1993) and is the largest net user of AA in the body of lactating cows (Clark et al., 1978). In terms of protein metabolism in mid-lactating dairy cows, the gross efficiency of PDIE utilization can be translated into the difference between the AA taken up by the MG for milk protein synthesis and that catabolized to produce urea (Lapierre et al., 2002). One of the possible variations in dietary N efficiency could be due to the partitioning of AA uptakes between the MG and other tissues. The MG appears to regulate the uptake of AA based on the whole-body supply of AA and the requirement for milk protein synthesis. Such studies, investigating the deficit of a single EAA, for example, His (Bequette et al., 2000) or Met (Guinard and Rulquin, 1995), showed a large modification in the mammary uptake of several AA. An increase in the mammary blood flow and the extraction rate of the deficient EAA were observed in parallel with a decrease in the extraction rate of other EAA in these studies (Guinard and Rulquin, 1995; Bequette et al., 2000). However, very few studies reported the effect of balanced diets with a complete EAA profile on milk protein synthesis (Fraser et al., 1991; Haque et al., 2012) and on mammary uptake (Bach et al., 2000; Doepel and Lapierre, 2010). In addition to the partitioning of AA uptake by the MG versus other tissues, intramammary AA metabolism could also contribute to the explanation of whole-body N-utilization efficiency. First, some EAA can stimulate milk protein synthesis or regulate mammary protein signaling pathways (Arriola Apelo et al., 2014), and second, some NEAA, for example, Pro, Ser, and Gly, are synthesized in the MG from EAA (Wohlt et al., 1977; Mephram, 1982).

Interestingly, increasing the PDIE supply has been shown to increase milk protein synthesis principally because of a higher uptake of EAA and a decreased uptake of NEAA (Lemosquet et al., 2010; Lapierre et al., 2012b). Moreover, the mammary uptake of Ile, Leu,

and Val are reported to be higher than their output in milk protein, and their mammary uptake-to-output ratio increases with increasing PDIE supply (see the meta-analysis by Lapierre et al., 2012b). The N and carbon (C) from these EAA, which are not used directly for milk protein synthesis, could be used for NEAA synthesis (Doepel and Lapierre, 2010). This indicates that the mammary AA requirements could be different at different PDIE supply levels.

These observations emphasize the need to understand how the MG adjusts its uptake of individual AA in response to different AA profiles (balanced vs. imbalanced) at different PDIE supply levels (low vs. high). Previously, this type of experiment was conducted in early-lactating cows; however, the variation in AA profile did not greatly modify mammary AA uptake (Bach et al., 2000). The first objective of this experiment was to analyze the changes in mammary AA uptake and metabolism by supplying a balanced AA profile to the EAA profile at 2 levels of protein supply as in Haque et al. (2012). The second objective was to explain potential changes in the PDIE efficiency by analyzing the AA partition between utilization in MG and other tissues. The third objective was to review utilization of individual EAA to better define EAA requirements for dairy cows.

## MATERIALS AND METHODS

### *Cows and Surgical Preparations*

The experiment was conducted at the INRA UMR 1348 experimental farm of Méjusseume (1.71°W, 48.11°N; Brittany, France) in the winter of 2010 to 2011, in accordance with the National Legislation on Animal Care (certified by the French Ministry of Agriculture—Agreement No. C35-275-23). Four multiparous Holstein cows in their second lactation averaging  $622 \pm 48$  kg and  $76 \pm 12$  DIM at the beginning of the experiment were used in this study. One week before the start of the experiment, the average daily milk yield of the cows was  $36 \pm 1.9$  kg/d, with a protein and fat content of  $3.1 \pm 0.06\%$  and  $4.0 \pm 0.45\%$  of milk, respectively. The cows were housed in individual tie stalls and had free access to fresh water. The cows were fitted with T-shaped duodenal cannulas 10 to 15 cm distal to the pylorus a year before the experiment. At  $49 \pm 13$  DIM they were surgically prepared for the implantation of an ultrasonic A probe (Transonic System Inc., Ithaca, NY) on the left external pudic artery as described by Rigout et al. (2002) and 2 permanent catheters were implanted in the left carotid artery and in the left subcutaneous abdominal vein (Guinard and Rulquin,

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