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Effects of graded removal of lysine from an intravenously infused amino acid mixture on lactation performance and mammary amino acid metabolism in lactating goats

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ABSTRACT

To investigate responses of milk protein synthesis and mammary AA metabolism to a graded decrease of postruminal Lys supply, 4 lactating goats fitted with jugular vein, mammary vein, and carotid artery catheters and transonic blood flow detectors on the external pudic artery were used in a 4 × 4 Latin square experiment. Goats were fasted for 24 h and then received a 9-h intravenous infusion of an AA mixture plus glucose. Milk yield was recorded and samples were taken in h 2 to 8 of the infusion period; a mammary biopsy was performed in the last hour. Treatments were graded decrease of lysine content in the infusate to 100 (complete), 60, 30, or 0% as in casein. Lysine-removed infusions linearly decreased milk yield, tended to decrease lactose yield, and tended to increase milk fat to protein ratio. Milk protein content and yield were linearly decreased by graded Lys deficiency. Mammary Lys uptake was concomitantly decreased, but linear regression analysis found no significant relationship between mammary Lys uptake and milk protein yield. Treatments had no effects on phosphorylation levels of the downstream proteins measured in the mammalian target or rapamycin pathway except for a tended quadratic effect on that of eukaryotic initiation factor 2, which was increased and then decreased by graded Lys deficiency. Removal of Lys from the infusate linearly increased circulating glucagon and glucose. Removal of Lys from the infusate linearly decreased arterial and venous concentrations of Lys. Treatments also had a significant quadratic effect on venous Lys, suggesting mechanisms to stabilize circulating Lys at a certain range. The 2 infusions partially removing Lys resulted in a similar 20% decrease, whereas the 0% Lys infusion resulted in an abrupt 70% decrease in mammary Lys uptake compared with that of the full-AA mixture infusion. Consistent with the abrupt decrease, mammary Lys uptake-to-output ratio decreased from 2.2 to 0.92, suggesting catabolism of Lys in the mammary gland could be completely prevented when the animal faced severe Lys deficiency. Mammary blood flow was linearly increased, consistent with the linearly increased circulating nitric oxide by graded Lys deficiency, indicating mechanisms to ensure the priority of the mammary gland in acquiring AA for milk protein synthesis. Infusions with Lys removed increased mammary clearance rate of Lys numerically by 2 to 3 fold. In conclusion, the decreased milk protein yield by graded Lys deficiency was mainly a result of the varied physiological status, as indicated by the elevated circulating glucagon and glucose, rather than a result of the decreased mammary Lys uptake or depressed signals in the mTOR pathway. Mechanisms of Lys deficiency to promote glucagon secretion and mammary blood flow and glucagon to depress milk protein synthesis need to be clarified by future studies.

Key words: goat, lactation, lysine, milk protein

INTRODUCTION

Based on present knowledge of AA nutrition, postabsorptive efficiency of N use for milk or tissue proteins is determined by the amount of MP supplied relative to animal needs, the profile of absorbed EAA, and the rate of protein synthesis. Postabsorptive N efficiency would then be maximized if we could match an animal's MP needs exactly with a diet well balanced in absorptive EAA profile. By maximal utilization of the strategy, Baker (1996) demonstrated that postabsorptive N efficiency in pigs could be increased to 85%, which is in agreement with the theoretical efficiency of the mammary gland to convert an ideal EAA profile into milk protein proposed by AFRC (1993).

The ideal EAA profile and requirements for EAA are at present evaluated according to the theory of limiting AA, which predicates that protein synthesis is limited

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by the most deficient EAA relative to requirement (Mitchell and Block, 1946). The predication is obviously violated by observations that suggest the supply of individual EAA has independent and additive effects on protein synthesis (Hanigan et al., 2000), which implies that the theory may be an oversimplification and is inappropriate to predicate the responses of milk protein synthesis to EAA supply (Arriola Apelo et al., 2014a).

The relationship of EAA supply with protein synthesis is much more complex than what the limiting AA theory describes. Though it cannot be excluded, mass action may not be the main mechanism for the supply of individual EAA to affect protein synthesis. It has long been recognized that the deficiency of an AA must be severe to have any effect on the concentrations of intracellular aminoacylated transfer RNA, the actual substrate for protein synthesis (Rogers, 1976; Flaim et al., 1982a,b). In respect to the mammary glands, they seem to have the ability to modify AA extraction efficiencies to meet a preset need of AA utilization (Mackle et al., 2000). For instance, removal of individual EAA (Arg, His, Lys, or Met) from an intravenously infused AA mixture had no significant effects on their respective mammary uptake in lactating goats restrictively fed a basal diet meeting only maintenance MP requirements (Ying et al., 2013). The liver modulates AA supply for peripheral tissues, including the mammary glands, but largely in response to recycling of the excess not removed by peripheral tissues (Arriola-Apelo et al. 2014a). The endocrine system also plays a role in mediating the effects of EAA supply on protein synthesis. Insulin (Bequette et al., 2001; Lemay et al., 2007; Bionaz and Loor, 2011), prolactin, glucocorticoid (Doppler et al., 1989), and hormones in the somatotropic axis (Cant et al., 1999) have all been shown to affect milk protein synthesis. However, these effects may not be all direct as the supply of some EAA affect the secretion of insulin (Davis et al., 2003; Bolster et al., 2004, Xiao et al., 2014), glucagon (Tovar et al., 2002), IGF-I (Wheelhouse et al., 1999; Noguchi, 2000; Stubbs et al., 2002), and parathyroid hormone (Conigrave et al., 2004). Supply of individual EAA may also affect protein synthesis through the mechanistic target of rapamycin complex 1 (**mTORC1**), which is an important intracellular signaling pathway that integrates environmental and intracellular signals to regulate cell growth and proliferation (Kim et al., 2013). A recent in vitro study using mammary tissue slices from lactating cows demonstrated that Ile, Leu, Met, and Thr affected casein synthesis through the mTORC1 pathway (Arriola Apelo et al., 2014b,c); the respective supply of these 4 EAA may also affect milk protein synthesis through mTORC1 pathway.

The relative importance of individual mechanisms mediating the effects of EAA supply on milk protein synthesis has not been fully investigated. The aim of the present study was to observe the effects of a graded deficiency of Lys on milk production, mammary AA metabolism, circulating hormone levels, and phosphorylation status of selected agents in the mTORC1 pathway in lactating goats.

MATERIALS AND METHODS

Animals and Diet

Four multiparous Laoshan dairy goats averaging 110 ± 10 DIM and 45 ± 5 kg of BW were used. Surgery was performed about 1 mo before the start of the experiment to elevate the right carotid artery to a subcutaneous level and fit a blood flow detector (6 mm, Transonic Systems Inc., Ithaca, NY) to the right external pudic artery. Goats were housed in individual stalls before and after each experimental period, and fed and milked twice daily at 0800 and 1800 h. The diet was pelleted and predicted to provide 49.44 g of MP/kg of DM and 10.86 MJ of ME/kg of DM according to AFRC (1993; Table 1). Feed refusals were determined daily and the amount of pelleted diet offered was adjusted to maintain a 5% refusal rate. Goats were allowed free access to water throughout the experiment. All animal-handling and surgical procedures were approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University.

Experimental Design and Procedure

The experimental design was a 4 × 4 Latin square. Treatments were intravenous infusion of an AA mixture containing 100 (complete), 60, 30, or 0% of the Lys present in casein. Temporal catheters were introduced into the left side jugular vein, right side carotid artery, and right side mammary vein of each goat 2 d before each experimental period, and goats were moved into individual cages. After fasting for 24 h, goats received a 9-h infusion of an AA mixture and glucose. Feeding was restored after completion of each infusion. Goats were moved into individual stalls and rested for 10 d between each experimental period. Similar starvation-refeeding procedures have been used in lactating goats (Chaiyabutr et al., 1983) and lactating cows (Toerien et al., 2010). No carryover effects on milk production were observed in those experiments.

The complete AA mixture was formulated according the profile of casein (Table 2). Lysine was substituted by equal moles of glutamate to achieve the Lys deficient infusates. The infusion rate was set to match the MP