Lysine flux in dry and lactating dairy goats

D.L. Brown

Department of Animal Science, University of California, Davis, CA, USA

The objective of this experiment was to test the hypothesis that the physiological state of lactation is accompanied by both an increase in total plasma lysine flux (rate of loss and replacement of lysine) and a net reduction in flux through the plasma lysine pool after accounting for lysine secreted in the milk. Eight lactating French Alpine does were primed and infused for three hours with solutions of $\alpha^{15}N$ L-lysine HCl in 0.9% saline through indwelling jugular vein catheters. Enrichment of circulating plasma lysine by continuous intravenous infusion of $\alpha^{15}N$ L-lysine was used to estimate whole body lysine flux. This procedure was repeated one month after cessation of milking. Total plasma lysine flux was similar for dry and lactating does (116.6 and 123.0 mmol/d, SEM 16.6 mmol), but 54.2 mmol/d lysine was secreted as milk protein during lactation. Direct measurement of lysine absorption from the lower tract and independent measurement of lysine degradation are needed to provide a more complete portrait of caprine lysine kinetics.

Keywords: lysine flux; lactation; goats

Introduction

The deposition of body protein is a dynamic process in which specific tissue proteins are synthesized and degraded at the same time. Because of the energetic and nutrient costs involved in both anabolic and catabolic processes, the efficiency with which an animal withdraws body protein (as in periods of high milk production) or deposits protein (e.g., during growth) depends on how much effort the animal is "wasting" by carrying out the opposite process.

If the same net deposition or mobilization can be carried out at several rates of turnover (and nutrient utilization), then the ability to select the rate of turnover could provide an optimum balance between efficient production and the need of the animal to maintain enough dynamism and flexibility to respond to stress. Such control would provide both scientists and producers with the tools needed to expand greatly the capacity of livestock to produce food and basic biological information. The ability to develop this level of control will depend first on the ability to monitor these complex processes.

Address reprint requests to Dr. Brown, Department of Animal Science, University of California, Davis, CA 95616, USA.
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Radioactive amino acids have been used for several years to study protein turnover.[1] The protein turnover of rodents,[2] humans,[3] and sheep[4] have been studied utilizing amino acids containing stable isotopes. The costs of disposing of food animals containing radioactive tracers and the related perceived hazards now make the application of safe, non-radioactive ^{15}N labeled tracers necessary to solve a number of modern biological problems. These include determining the effects of growth-promoting substances (somatotropin, steroids, etc.) and dietary factors (high and low protein feeding, seasonal undernutrition) on both body tissue deposition and overall animal health.

Investigations carried out in our laboratory have shown that lactating goats have substantial abilities to withhold nitrogen from excretion and to withdraw protein from their bodies to continue lactation during periods of protein deficiency.[5] Unfortunately, one cannot tell from balance studies and in vivo body composition techniques if resetting the rate of overall body protein turnover is part of normal animal strategic response to increased demand for protein. The objective of this experiment was to test the hypothesis that the physiological state of lactation is accompanied by an increase in total plasma lysine flux and by a net reduction in plasma lysine flux to non-mammary tissues after accounting for lysine secreted in the milk.

Materials and methods

The experimental animals averaged 63 ± 12 kg, were offered 1.84 feed dry matter* (DM), and consumed an average 1.76 ± .09 kg DM for the week prior to the lactation infusion and 1.60 ± .15 kg DM during the week prior to the dry period infusion.

Eight lactating (six months after freshening, milking 2×/d) French Alpine does were primed and infused with solutions of α^{15}N L-lysine HCl (ICN Biomedicals, Cambridge, MA, USA) in 0.9% saline through indwelling jugular vein catheters for three hours. Two does each received a 60 ml priming dose followed by a 1 ml/min infusion of 4 mM α^{15}N lysine, while the other six received 2.89 mM α^{15}N lysine as a 24 ml prime and 1 ml/min infusion. Preliminary investigations had shown that both procedures achieved a plateau in 2 to 2½ hours (*Figure 1*) with a slope of 0.00085 ± .003654 mol % excess/min which was not significantly different from 0 (P = .9635). These subjects were infusesd after their morning feeding, but were not offered feed during the infusion itself. Blood samples were drawn by venipuncture into 10 ml evacuated tubes containing 500 mg Na citrate from the contralateral jugular vein a few minutes before the end of the infusion. These samples were centrifuged immediately, and the plasma was frozen for further analysis. The does were dried off immediately after this test period. After a month had passed without milking, this entire procedure was repeated on the same goats.

Free amino acids were extracted from thawed plasma by a modification of Bier and Christopherson's method[3] in which glass Pasteur pipettes and glass wool were used instead of Isolab disposable columns. Extracted lysine was then converted to tert-butyldimethylsilyl derivatives by a modification of Schwenk *et al.*[6] which utilized the t-butyldimethylsilylation agent MTBSTFA and reaction vials with Teflon-lined caps (both from Pierce Chemical Company, Rockford, IL, USA).

The enrichment of circulating free lysine was measured with a Hewlett Packard 5790A gas chromatograph (Palo Alto, CA, USA) equipped with a 30 m/.25mm ID DB-1 capillary column (J & W, Folsom) and a VG ZAB mass spectrometer. The ratios of the 489 to 488 amu lysine derivative ion peaks were regressed on $^{15}N/^{14}N$ for lysine standards containing 0, 25, 50, and 75% ^{15}N at the α position. Rates of lysine flux were calculated by the method of Garlick[1] from known infusion rates and measured isotope excesses. Milk lysine excretion was determined by multiplying milk protein production by the lysine content of goat milk protein. Non-milk lysine flux was calculated as the difference between the total flux and the flow of lysine into the milk.

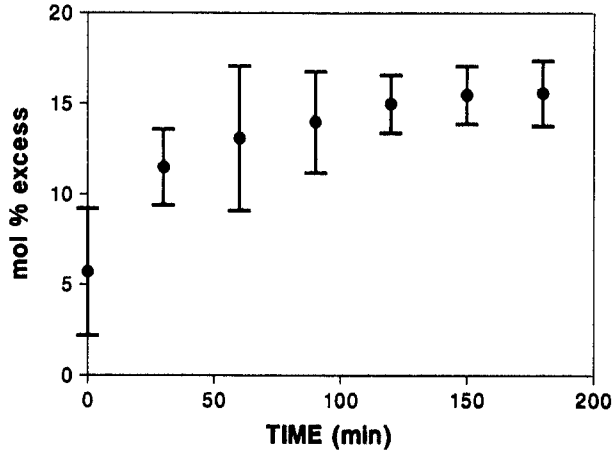

Figure 1 An example of enrichment of plasma lysine by infused α^{15}N L-lysine. N = 4. Slope from 120 to 180 minutes = 0.01088 ± 0.043598 mol % excess/min. P = 0.8081.

Data from this randomized block design and the SAS GLM procedure were used to make the calculations needed to test the following model:

$$y_{ij} = \mu + A_i + P_j + \epsilon_{ij}$$

where y_{ij} = each observation; μ = the true mean; A_i = the animal effect (block); P_j = the effect of physiological state (treatment); and ϵ_{ij} = the residual error not accounted for by animal and physiological state effects.

Results

Total lysine flux was not affected by drying off lactating goats, but flux not directed to milk protein synthesis was nearly doubled (*Table 1*).

Discussion

The average lysine flux rates of these dry (116 mmol/d) and lactating (123 mmol/d) goats are similar to the 179 mmol/d reported for a fasting man,[3] and 129 to 181 mmol/d for men fed two levels of dietary protein.[7] The goat figures are closer to human figures when calculated on a body weight basis since the goats averaged 68 kg and the men were about 75 kg. Although caprine lysine flux rates are of the same order of magnitude as those for humans, the microbial activity in

Table 1 Lysine flux in dry and lactating goats (mmol/d) n = 8

State	Dry	Lactating	S.E.M.	P
Total	116.6	123.0	16.6	0.79
Milk	0.0	54.2		
Non-milk	116.6	68.8	15.9	0.05

The goats averaged 1.78 ± .23 kg/d DM intake and 3.26 ± .59 kg/d milk output during lactation, and consumed 1.61 ± .14 kg/d DM when dry.

* This 1.84 kg DM was offered as 2 kg of pellets containing 69% alfalfa hay, 30% corn, and 1% salt. This diet contained 13.1% crude protein and 1.32 Mcal NEm/kg DM.

the rumen presents a problem for relating dietary lysine to the amount absorbed each day.

In order to obtain an approximate check on lysine flux rates and a rough idea of the magnitude of mobilized body lysine, we estimated that 68 mmol lysine/d/kg DM intake was absorbed from the small intestine. This estimate was based on the protein and lysine composition of microbial and diet ingredient protein[8] and assumed rumen undergradabilities of 25% and 65% for alfalfa and corn protein, respectively.[9] We further assumed that only 75% of lysine reaching the lower tract was absorbed, the balance being heat-damaged or part of totally indigestible compounds. If these assumptions are correct, then average lysine absorption for lactating goats was 120.9 mmol/d, and for dry goats was 109.4 mmol/d.

These estimates of lysine entry from the gut are very close to the estimated total flux measurements, indicating that 1) the circulating lysine pool received very little entry from body protein degradation†; 2) our estimates of intestinal lysine absorption are too high; or 3) our measurements of lysine flux are too low. Only direct measurements of lower gut lysine absorption made simultaneously with total flux measurements can tell us which alternative explanation to accept.

Assuming our absorption estimates and flux measurements are correct, we reject the hypothesis that lactation increases plasma lysine flux and accept our primary hypothesis that lactation represents a redirection, but not an increase in total lysine flux.

Previous experiments[5] have shown that this primary hypothesis might not hold for lactating goats subjected to diets which contain 7.2% crude protein. Those protein deficient animals did mobilize body nitrogen and probably exhibited increased lysine flux as a result. Caution must be exercised in extending the conclusions drawn in the current experiment from observations of well-fed does in late lactation to very productive or inadequately fed does in early lactation.

Direct measurements of lysine absorption by the lower gut and loss to oxidation are needed to complete a model of caprine lysine kinetic responses to lactation that would allow good estimates of whole body protein turnover, mobilization, and synthesis with a variety of diets and production levels.

† If the contribution of body protein degradation is taken as the difference between total flux and estimating absorption, then the body contributed 2.1 mmol/d lysine for lactating and 16.6 mmol/d lysine for dry does.

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