Animal Nutrition 13 (2023) 116–125

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Responses in splanchnic and mammary amino acid metabolism to short-term graded removal of methionine in lactating goats

Yantao Li [a], Mark D. Hanigan [b], Xueyan Lin [a], Zhiyong Hu [a], Zhengui Yan [a], Qiuling Hou [a], Yun Wang [a], Zhonghua Wang [a, *]

[a] Ruminant Nutrition and Physiology Laboratory, College of Animal Science and Technology, Shandong Agricultural University, Tai'an 271018, China
[b] Department of Dairy Science, Virginia Tech, Blacksburg 24061, USA

ARTICLE INFO

Article history:
Received 8 March 2022
Received in revised form
8 January 2023
Accepted 15 January 2023
Available online 21 January 2023

Keywords:
Methionine
Lactating goat
Milk protein
Amino acid
Splanchnic tissue

ABSTRACT

Four multi-catheterized lactating goats were used in a 4 × 4 Latin square experiment to investigate the responses of amino acid metabolism in portal-drained viscera (PDV), liver, and mammary glands to short-term varying supplies of methionine (Met). During the last 45 h in each experimental period, goats were fasted for 12 h and then abomasally infused with an amino acid (AA) mixture plus glucose for 33 h. Treatments consisted of graded removal of Met from an infused AA mixture to achieve Met content in the infusate of 100% (complete), 60%, 30%, or 0% that in casein. Graded Met removal decreased the production of milk, milk protein, lactose, and fat linearly whilst also decreasing arterial Met concentration linearly ($P < 0.05$). Meanwhile, net PDV uptake and liver removal of Met decreased linearly ($P < 0.05$) due to decreased Met affinity of PDV and liver ($P < 0.05$). Net mammary uptake of Met ($P > 0.1$) was maintained as Met supply declined. This was achieved through increased mammary affinity ($P < 0.05$) and increased mammary blood flow ($P < 0.05$) totally offsetting the negative effect of decreased circulating Met concentration. Graded removal of Met from the infusate linearly decreased mammary uptake-to-milk output ratios of Met ($P < 0.05$) and tended to decrease essential amino acid (EAA) linearly ($0.05 < P < 0.1$). Treatments also linearly decreased circulating concentration of prolactin and linearly increased insulin concentration ($P < 0.05$). In conclusion, results of the present study indicated there were several mechanisms used to mitigate a Met deficiency, including reduced catabolism of Met in PDV, liver, and peripheral tissue (including mammary glands) and a linear increase in mammary blood flow. The observed decreases in milk protein production as Met supply decreased appear to be a result of regulatory events which may have been driven by decreased circulating prolactin, rather than as a result of decreased mammary Met uptake.

1. Introduction

The nitrogen conversion efficiency of lactating ruminants is much lower than that of growing animals. Because of the low efficiency in converting dietary total nitrogen, dairy products produced by ruminants have a significant impact on the environment (Tamminga et al., 1995; Howarth et al., 2002). A portion of this inefficiency is a mismatch between individual amino acid (AA) and the needs of the animals (Arriola Apelo et al., 2014). Balancing dietary amino acids to improve lactational conversion efficiency of absorbed AA in lactating ruminants is a major industry problem. An accurate representation of AA metabolism in lactating ruminants will allow construction of diets that more closely match absorbed AA supply to animal needs, thus improving lactation conversion efficiency and decreasing nitrogen excretion (Fleming et al., 2019).

Absorbed AA are mainly metabolized in the portal-drained viscera (PDV), the liver, and the mammary glands. There is generally a linear relationship between total AA metabolism by the PDV

* Corresponding author.
E-mail address: zhwang@sdau.edu.cn (Z. Wang).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2023.01.006

and metabolizable protein (MP) supply, with a slope of 0.65 resulting in a 35% metabolic loss of total AA flux in the PDV (Lobley and Lapierre, 2003). Studies have shown that the metabolic loss rates of different AA in PDV are different. For example, the metabolic loss of His in PDV tissue is very small and the recovery in the portal vein can be as high as 95% (MacRae et al., 1997). In contrast, Leu is oxidized heavily in the PDV of dairy cows (Lapierre et al., 2002) and Thr can be oxidized by the pancreas (Le Floc'h et al., 1997). Net portal vein recovery of Leu and Thr were only 60% and 40% of the absorbed supply respectively (Berthiaume et al., 2001) with most of the loss being from arterial supplies (MacRae et al., 1997). The differences among AA in PDV use affect the profile of absorbed AA, and thus it is important to consider not only total loss but also the impact of that use on the composition of the net AA supply. In this case, altered use by peripheral tissues can also affect the supply and apparent composition of AA released by the splanchnic tissues, so the relationship between individual essential amino acid (EAA) supply and metabolic loss in PDV needs further investigation.

The liver contains a wide range of enzymes for AA catabolism. EAA have been classified into two distinct categories based on the distribution of the enzymes degrading AA, which are found in many tissues or predominantly in the liver. The first category involves branched-chain AA (BCAA) and lysine (Lys) that exhibit limited hepatic oxidation and can be catabolized in many tissues. For example, BCAA can be catabolized in muscle, adipose, mammary gland, and intestinal tissues (Goodwin et al., 1987; DeSantiago et al., 1998); Lys can be catabolized in intestinal mucosa and muscle of pigs (Pink et al., 2003) plus mammary glands of sheep (Mabjeesh et al., 2000). The second category involves Met, histidine (His), and phenylalanine (Phe), which are mainly catabolized in the liver and their degradative enzymes are restricted predominantly to the liver (Lobley and Lapierre, 2003). According to a meta-analysis of related research, the liver removes about 45% of the total AA appearing in the portal vein, but the proportion varies widely, ranging from 16% to 69% (Lapierre et al., 2005). It is not clear whether the mechanism regulating AA catabolism by the liver is active or passive (Lobley and Lapierre, 2003). Due to the frequent returns of AA to the liver with low marginal removal, regulatory effects can be achieved by linear fractional removal facilitated through slight activity in tissue affinity (Fleming et al., 2019). However, it is necessary to verify the meta-analyses through further exploration on the regulation of tissue affinity.

The primary net users of post-splanchnic AA supply in lactating ruminants are the mammary glands. For those AA catabolized predominantly within the liver, net splanchnic flux appears to be completely captured by the mammary gland and secreted quantitatively into milk protein (Lapierre et al., 2005). Therefore, the capture of AA by the mammary glands is a critical determinant of not only milk protein synthesis but also post-absorptive AA efficiency. This critical function appears to be more highly regulated than that of the splanchnic tissues. For example, when His was limited in dairy goats, mammary blood flow increased by 33% and the capacity to remove plasma histidine increased by 43-fold which reduced the impact of the deficiency (Bequette et al., 2000). In lactating goats fed basal diets meeting 77% of MP requirements, jugular infusion of mixed AA lacking Met did not cause decreased mammary uptake of Met (Lin et al., 2014). The AA, such as BCAA and Lys, which are not extensively removed by the liver, are obviously catabolized by other tissues of the body because the amount of post-liver supply is higher than that of mammary gland uptake, itself higher than that of milk output. The extra-splanchnic oxidation of these AA is affected by post-liver supply and there is a strong correlation between the amount of non-lactation use and splanchnic flux (Lapierre et al., 2005).

The objective of this study was to observe the response of PDV, liver, and mammary gland to short-term varying supply of Met in lactating animals using a multi-catheterized dairy goat model, and to evaluate the relationship between these responses and milk protein production. It was hypothesized that splanchnic tissues actively regulate the catabolism of AA in response to varying Met supply.

2. Materials and methods

2.1. Animal ethics statement

This study was conducted according to the Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University (protocol NO. 2019-DG-0524).

2.2. Animals and diet

Four multiparous Laoshan dairy goats, averaging 60 ± 10 days in milk (DIM) and body weight (BW) 50 ± 5 kg were surgically implanted with vascular catheters across the splanchnic tissues (Mineo et al., 1991). Briefly, medical silicone catheters (1 mm × 2 mm, Chensheng Medical, China) were implanted into the portal vein, hepatic vein, and one mesenteric vein. The mesenteric catheter was used for para-amino hippuric acid (pAH) infusion in order to determine PDV and hepatic blood flows. A T-type Tygon tube (8 mm × 10 mm, Weili, China) was surgically implanted into the abomasum and the right carotid artery was also raised to a subcutaneous position to provide alternative access to arterial blood. Surgeries were performed at least 1 month before the start of the experiment. Anti-inflammatory drugs were given intravenously after surgery for 7 d and rectal temperature was recorded twice a day. All the catheters were surgically removed after the experiment. The goats were fed a pelleted diet, which was calculated to provide 10.65 MJ ME and 110.7 g MP per kilogram dry matter (Table 1). Daily amounts offered allowed for about 5% orts. Goats were allowed free access to water throughout the experiment. This experiment was carried out at the Animal Husbandry Experimental Center of Shandong Agricultural University.

2.3. Experimental design and abomasal procedure

Goats were randomly allocated to a 4 × 4 Latin square experimental design. The AA infusion was formulated according to the profile of casein (Table 2). Treatments were graded substitutions of Met by equal moles of glutamate (Glu) from the mixture with 100%, 60%, 30%, or 0% of Met remaining in the four treatments respectively. Because of solubility limitations, all the tyrosine (Tyr) was replaced by Phe. Daily amounts of AA infusion were calculated to match the MP requirements according to AFRC (1993) based on three consecutive day milk yield records before the start of infusion in each period. Glucose was co-infused with the AA mixture to supply energy. The infusion rate of glucose was calculated to provide 3.6 mg glucose/kg of BW per minute according to the metabolic rate of glucose measured in lactating goats using isotopic tracing technology (Annison and Linzell, 1964). In each experimental period, the goats were moved to individual metabolic cages and fasted for 12 h before start of the infusion in order to mitigate possible interference by Met absorbed from remaining digesta. The infusion began at 08:00 on the first day and lasted for 33 h to ensure a steady blood glucose and urea concentration, which need about 15 h for glucose and 20 h for urea to establish according to the result of our pre-experiment (Fig. 1). Samples were collected on the 2nd day. After stopping the infusion, goats were allowed a 12-d rest in individual free stalls before start of the next period.

Table 1
Ingredients, nutrients, and EAA compositions of the pelleted diet (DM basis, %).

Item	Content
Ingredients	
Corn	14.00
Soybean meal	10.55
Wheat bran	12.44
Alfalfa hay	28.94
Peanut vine	32.05
$NaHCO_3$	0.400
4% Premix[1]	1.600
Nutrients	
ME[2], MJ/kg DM	10.65
MP[2]	11.07
CP[3]	16.02
NDF[3]	33.83
ADF[3]	24.33
EAA	
Lys	0.810
Met	0.251
His	0.387
Arg	0.957
Thr	0.702
Val	0.904
Ile	0.652
Leu	1.22
Phe	0.764
Trp	0.214
Lys, % of MP	7.32
Met, % of MP	2.27

ME = metabolizable energy; MP = metabolizable protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EAA = essential amino acid.
[1] Contained the following (per kilogram of DM premix): 150 kIU vitamin A, 250 kIU vitamin D_3, 2,400 mg vitamin E, 2,000 mg nicotinic acid, 2,000 mg Fe, 2,500 mg Mn, 1,000 mg Cu, 3,600 mg Zn, 100 mg Se, 180 mg I, and 40 mg Co.
[2] Calculated according to NRC models.
[3] Laboratory determined values.

Table 2
Amino acid profile of the complete amino acid mixture (%).[1]

Item	Content
EAA	
Lys	7.49
Met	2.82
Leu	8.89
Ile	4.61
Val	6.24
Thr	3.86
Phe	9.22
His	2.82
Arg	3.57
Trp	1.49
NEAA	
Ala	2.82
Gly	1.78
Glu	9.95
Gln	9.80
Asn	3.40
Asp	6.83
Ser	5.05
Pro	8.32
Cys	0.59

EAA = essential amino acid; NEAA = non-essential amino acid.
[1] Adapted from Bequette et al. (1996).

The infusate was freshly made each day by dissolving the AA mixture and glucose in 1,920 mL of saline and adjusting the pH to 7.4 with HCl and NaOH. A peristaltic pump (Type H-L2, Huxi Analytical Instrument Factory, Shanghai, China) was used for abomasal infusion at a rate of 80 mL/h.

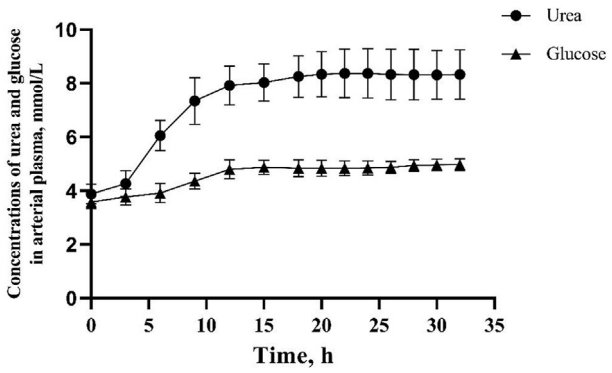

Fig. 1. Concentrations of urea and glucose in arterial plasma during infusion of glucose and amino acid into abomasum in a preliminary experiment.

During the resting periods, goats were milked twice daily at 08:00 and 18:00 and allowed free access to the pelleted diet and water.

2.4. Sampling and analysis

Samples were collected on the 2nd day during each infusion period. Milk samples collected between 08:00 and 17:00 were divided into two sub-samples: one was used for general composition analysis using an infrared milk composition analyzer (Type 76110, FOSS, Denmark) and the other for AA determination after hydrolysis using an AA analyzer (Type L-8900, Hitch, Japan). *Para*-amino hippuric acid (pAH, 1% wt/vol) was infused into a mesenteric vein catheter from 10:00 to 16:00 to determine plasma flow across the splanchnic tissues. The pAH infusion (0.48 g/h) began 1.5 h before the initiation of blood sampling and was preceded by a priming dose (2.4 g/h) for 5 min. Starting at 11:30, 4 blood samples were collected simultaneously into heparinized tubes from the arterial, hepatic venous, and portal catheters every 90 min. Mammary venous blood samples were taken by venipuncture immediately after withdrawal of the splanchnic blood samples. Blood samples were also divided into two samples, one was placed on ice and centrifuged (10 min, 1,800 × *g* at 4 °C) within 30 min of collection to yield plasma. The other one was for analysis of packed cell volume by an automatic blood cell analyzer (Mindray, BC-6100Plus). A total of 4 samples were pooled together and kept at −80 °C for later analysis of free AA concentration using an AA analyzer (Type L-8900, Hitch, Japan). Arterial plasma was subsampled for total protein, urea-N, and glucose analysis by an automatic biochemical analyzer (Type 7020, Hitch, Japan). The hormones such as insulin, glucagon, and prolactin were determined by radioimmunoassay using commercial assay kits (Tianjin Xiehe Medical Technology Co. Ltd.).

2.5. Calculations and statistical procedures

Blood and plasma flow were calculated from downstream dilution of pAH infused into mesenteric catheters (Katz and Bergman, 1969). Daily averages of blood flows were used to calculate the net flux of AA. Mammary plasma flow was estimated according to the Fick principle using Phe and Tyr as internal markers, with allowance for a 3.5% contribution from blood-borne proteins: mammary plasma flow = [(milk Phe + Tyr) × 0.965]/(arterial and venous difference Phe + Tyr) (Cant et al., 1993). The net flux of AA across the portal drained viscera (PDV), liver, total splanchnic tissues (TSP), and mammary gland (MG) was calculated for each goat period as the product of the average plasma venous−arterial

concentration difference and the average blood flow. A negative flux indicates utilization or removal, whereas a positive flux indicates net production or release of the nutrient across the tissue (Huntington et al., 1996). Mammary uptake to milk output ratios (U:O) for individual AA were calculated as mammary uptake of the AA divided by the amount secreted in milk during the 9 h between milkings. PDV clearance (K_P), liver clearance rates (K_H), and mammary clearance rates (K_M) were calculated to assess tissue affinity for blood metabolites (Hanigan et al., 1998):

$$K_P\ (L/h) = [Abs + (C_A - C_p)\ F_P]/C_p,$$

$$K_H\ (L/h) = (F_AC_A + F_PC_P - F_HC_H)/C_H,$$

$$K_M\ (L/h) = [(C_A - C_M)\ F_M]/C_M,$$

where Abs refers to the absorbed metabolite; F_A, F_P, F_H, and F_M refer to arterial, portal venous, hepatic venous, and mammary blood flows (L/h), respectively; C_A, C_P, C_H, and C_M refer to arterial, portal venous, hepatic venous and mammary venous AA concentrations (μmol/L), respectively.

The experimental unit was an individual dairy goat ($n = 4$). The Dixon Test was used to check the data for outliers and no data was removed. Data were subjected to mixed procedure analysis by SAS (version 9.2, SAS Institute Inc., Cary, NC). The statistical model used was:

$$Y_{ijk} = \mu + G_i + P_j + T_k + e_{ijk},$$

where Y was the dependent variable; μ was the overall mean; G_i and P_j were the random effects of animal and period, T_k was the fixed effect of Met dose effects; and e_{ijk} is the random error associated with Y_{ijk}. The results are expressed as least square means (LSM) with stand error of the mean (SEM). Significance was declared at $P < 0.05$ and tendency was declared at $P < 0.1$. Multiple comparisons between treatment means were made using the Tukey method.

3. Results

3.1. Milk production and composition

Removal of Met from the infusion decreased production of milk, milk protein, milk fat, and milk lactose linearly ($P < 0.05$, Table 3). Production of milk and milk protein in the 100% group was greater than the 30% and 0% treatments.

3.2. Arterial and venous free AA

Met removal decreased arterial Met concentration linearly ($P < 0.05$, Table 4). Removing all Met from the infusate decreased arterial Met to less than one half that of full mixture infusion. Arterial concentrations of individual AA, BCAA, EAA, non-essential amino acids (NEAA), and total amino acids (TAA) were unaffected by Met removed from the infusions.

Venous concentrations of Met decreased linearly with decreased content of Met in the infusate ($P < 0.05$; Table 5). The 0% Met infusate decreased venous Met to about one third that of the complete mixture infusion. Venous concentrations of individual AA, BCAA, EAA, NEAA, and TAA were unaffected by Met removal from the infusions.

3.3. Arterial metabolites, hormones, and splanchnic blood flow

Treatments increased insulin linearly and decreased prolactin linearly ($P < 0.05$, Table 6). Insulin concentrations of 30% and 0% treatments were significantly higher than the 100% and 60% treatments ($P = 0.005$) and the prolactin concentrations of 30% and 0% treatments were lower than the 100% treatment ($P = 0.048$). Apart from these, arterial concentrations of all the measured metabolites and hormones were unaffected.

Graded removal of Met from the infusate linearly decreased hepatic blood flow and linearly increased mammary blood flow ($P < 0.05$, Table 7). The mammary blood flow of the 0% treatment was higher than the 100% and 60% treatments ($P = 0.041$).

3.4. Net fluxes of individual AA across tissues

The linear decrease in Met infusion rate was accompanied by a tendency for a linear increase in the Glu infusion rate because the missing Met was replaced by equal moles of Glu. Net fluxes of individual AA across the PDV, HEP, TSP, MG, and in milk are presented in Table 8. Graded removal of Met from the infusate linearly decreased the net flux of PDV, HEP, and TSP ($P < 0.05$). Mammary uptake of Met and the secretion of Met in milk were unaffected by graded removal of Met. Met deficiency tended to decrease the PDV net flux of Tyr linearly ($P < 0.1$). The TSP net fluxes of leucine (Leu), alanine (Ala), and Glu decreased linearly ($P < 0.05$) and that of Phe, threonine (Thr), and glycine (Gly) tended to decrease by the graded removal of Met ($0.05 < P < 0.1$). Met deficiency tended to increase mammary uptake of Thr and valine (Val) linearly ($0.05 < P < 0.1$) and caused a linear decrease of Gly ($P < 0.05$). All the other net fluxes of AA were unaffected by Met removed from the infusate.

3.5. PDV, Liver and mammary clearance rates of EAA

The PDV clearance rate of Met decreased linearly ($P < 0.05$) while PDV clearance for Ile tended to decrease linearly ($0.05 < P < 0.1$, Table 9). Numerically, PDV clearance rate of Met decreased about 15-fold for the 0% Met treatment compared with that of complete mixture infusion. Graded removal of Met from the

Table 3
Effects of graded removal of Met from the amino acid mixture infused into abomasum on lactation performance of lactating goats (totally 9 h).

Item	Percentage of Met				SEM	*P*-value		
	100%	60%	30%	0%		Treatment	Linear	Quadratic
Milk, g	347.6[a]	317.1[ab]	269.6[b]	262.5[b]	34.9	0.033	0.005	0.763
Milk protein, %	4.098	3.870	3.945	3.892	0.26	0.716	0.376	0.554
Milk protein, g	14.26[a]	12.28[ab]	10.56[b]	9.994[b]	1.19	0.049	0.004	0.620
Milk fat, %	4.951	4.562	4.965	4.475	0.41	0.945	0.698	0.889
Milk fat, g	16.46	14.94	12.92	11.50	2.54	0.089	0.009	0.850
Milk lactose, %	4.904	5.112	5.075	4.672	0.29	0.721	0.629	0.300
Milk lactose, g	17.14	16.05	13.53	12.17	1.72	0.077	0.001	0.604
Fat to protein ratio	1.176	1.206	1.203	1.150	0.14	0.974	0.866	0.662

[a, b] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

Table 4
Effects of graded removal of Met from the amino acid mixture infused into abomasum on amino acid concentrations in carotid artery plasma of lactating goats (μmol/L).

Item	Percentage of Met				SEM	*P*-value		
	100%	60%	30%	0%		Treatment	Linear	Quadratic
Met	34.29[a]	26.72[ab]	20.20[bc]	14.83[c]	3.36	0.004	<0.001	0.643
Lys	139.0	121.3	132.4	123.0	30.1	0.971	0.806	0.912
Val	303.3	255.7	337.4	294.6	83.9	0.921	0.897	0.921
Leu	130.9	128.7	131.9	128.7	22.2	0.994	0.968	0.988
Ile	48.87	55.22	33.32	38.95	10.1	0.498	0.425	0.656
His	51.01	41.33	50.10	46.12	8.55	0.848	0.917	0.996
Phe	55.18	53.51	53.90	54.88	10.3	0.991	0.879	0.988
Thr	67.06	68.87	72.95	64.91	15.5	0.985	0.984	0.760
Trp	21.40	22.56	22.34	23.19	3.44	0.986	0.717	0.971
Arg	108.5	108.0	106.1	100.0	16.3	0.982	0.704	0.844
Gly	960.2	770.6	1,010	916.6	157	0.734	0.919	0.690
Glu	316.5	320.9	285.7	326.2	29.6	0.772	0.951	0.606
Ser	82.49	73.24	95.62	99.31	25.4	0.878	0.527	0.734
Tyr	55.66	53.20	52.97	61.55	8.86	0.892	0.677	0.520
Ala	155.9	132.6	149.6	138.2	22.7	0.883	0.684	0.770
Pro	90.49	116.1	60.98	83.73	33.4	0.714	0.636	0.868
BCAA	480.1	439.6	503.1	462.2	98.0	0.975	0.997	0.978
EAA	956.6	882.8	961.1	878.1	172	0.981	0.889	0.971
NEAA	1,662	1,467	1,655	1,626	247	0.938	0.865	0.700
TAA	2,618	2,349	2,616	2,513	408	0.962	0.871	0.821

BCAA = branched-chain amino acid. EAA = essential amino acid; NEAA = non-essential amino acid; TAA = total amino acid.
[a, b, c] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

Table 5
Effects of graded removal of Met from the amino acid mixture infused into abomasum on amino acid concentrations in mammary vein plasma of lactating goats (μmol/L).

Item	Percentage of Met				SEM	*P*-value		
	100%	60%	30%	0%		Treatment	Linear	Quadratic
Met	23.27	17.76	12.97	8.081	4.13	0.111	0.011	0.909
Lys	99.47	87.37	108.9	99.63	29.9	0.972	0.886	0.856
Val	250.8	206.6	298.4	264.5	83.5	0.897	0.739	0.885
Leu	84.13	79.43	95.03	95.26	22.4	0.949	0.558	0.864
Ile	21.28	27.82	17.33	22.55	6.30	0.732	0.689	0.836
His	40.52	29.57	43.90	36.66	12.4	0.719	0.766	0.766
Phe	44.09	42.12	44.73	48.85	12.1	0.975	0.614	0.924
Thr	43.67	49.44	58.08	52.35	16.1	0.939	0.603	0.766
Trp	16.63	19.01	18.43	20.39	3.77	0.892	0.461	0.812
Arg	81.61	84.95	89.36	84.34	16.2	0.991	0.957	0.844
Gly	954.8	759.4	995.4	902.2	164	0.732	0.862	0.680
Glu	285.2	292.8	266.1	303.8	36.4	0.849	0.610	0.597
Ser	69.17	61.49	84.60	88.59	23.8	0.786	0.377	0.730
Tyr	48.03	45.56	44.91	56.07	9.95	0.869	0.541	0.542
Ala	125.0	110.4	132.6	124.6	24.3	0.935	0.852	0.981
Pro	70.44	95.20	41.63	69.73	33.9	0.769	0.633	0.846
BCAA	356.3	313.8	410.7	382.3	102	0.927	0.716	0.895
EAA	705.5	644.0	787.1	732.6	180	0.956	0.788	0.941
NEAA	1,553	1,365	1,593	1,545	265	0.945	0.942	0.717
TAA	2,258	2,008	2,353	2,277	436	0.954	0.919	0.798

BCAA = branched-chain amino acid; EAA = essential amino acid; NEAA = non-essential amino acid; TAA = total amino acid.

Table 6
Effects of graded removal of Met from the amino acid mixture infused into abomasum on concentrations of metabolites and hormones in the plasma of carotid artery in lactating goats.

Item	Percentage of Met				SEM	*P*-value		
	100%	60%	30%	0%		Treatment	Linear	Quadratic
Urea N, mmol/L	7.718	8.213	6.240	6.150	0.859	0.272	0.111	0.589
Glucose, mmol/L	4.313	4.070	4.158	4.180	0.315	0.958	0.788	0.665
Total Protein, g/L	80.70	80.78	75.73	77.65	4.09	0.780	0.429	0.910
Insulin, μIU/mL	48.26[b]	42.39[b]	80.85[a]	89.78[a]	9.27	0.005	0.006	0.169
Glucagon, pg/mL	153.5	168.7	179.7	166.8	12.6	0.273	0.346	0.285
Prolactin, ng/mL	3.920[a]	3.720[ab]	3.247[b]	2.922[b]	0.46	0.048	0.027	0.613

[a, b] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

Table 7
Effects of graded removal of Met from the amino acid mixture infused into abomasum on splanchnic blood flows in lactating goats (L/h).

Blood flow	Percentage of Met				SEM	*P*-value		
	100%	60%	30%	0%		Treatment	Linear	Quadratic
Portal	97.65	97.59	95.47	94.47	8.33	0.877	0.425	0.828
Hepatic	131.3	123.5	119.6	117.3	10.7	0.087	0.009	0.569
Mammary	20.26[b]	21.04[b]	24.90[ab]	26.45[a]	2.16	0.041	0.029	0.697

[a, b] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

infusate linearly decreased liver clearance rate of Met ($P < 0.05$) with the clearance rate declining by about 6-fold for the 0% Met treatment compared with that of complete infusion. Mammary clearance rates of Met linearly increased ($P < 0.05$) with the 0% Met infusion having a numerical increase of about 2-fold compared with that of complete mixture infusion. Graded removal of Met from the infusate had no significant effects on PDV, liver and mammary clearance rates of the other EAA measured ($P > 0.1$).

3.6. U:O of AA

Graded removal of Met from the infusate linearly decreased mammary U:O of Met and tended to linearly decrease U:O of the EAA ($P < 0.05$; Table 10). Treatments affected the U:O of serine (Ser) quadratically ($P < 0.05$) with the U:O of Ser decreased at 60% treatment of Met and then increased with decreased content of Met in the infusate. Treatments had no significant effects on mammary U:O of the other AA measured.

4. Discussion

4.1. Milk protein production

Met is essential for protein metabolism in all animals and increased supply generally results in greater protein production (Manjarin et al., 2014). It has been shown that the responses of milk protein production to graded doses of postruminally supplied Met may be linear (Pisulewski et al., 1996). The addition of postruminal Met in the basal diet significantly promoted the milk protein production of dairy cows (Guinard and Rulquin, 1995; Chen et al., 2011; Zhou et al., 2016). Decreasing the supply of Met using a mixed AA infusion also reduced the milk protein production of lactating goats and dairy cows (Weekes et al., 2006; Fu et al., 2013; Liu et al., 2019). Nevertheless, Met uptake by the mammary gland was not affected by decreasing postruminal Met supply (Fu et al., 2013; Liu et al., 2019). In this study, the mammary uptake of Met and all measured AA except for Thr and Val was not affected, although Met deficiency decreased the production of milk protein, indicating that under short-term supply of Met, the decrease of milk protein production did not appear to be caused by an effect of mass action. Altered circulating hormone concentrations and signaling pathway stimulation in mammary epithelial cells may cause a decrease in milk protein production.

4.2. Blood flow

Portal blood flow (96 L/h) was 79% of splanchnic blood flow (122 L/h), which was consistent with previous studies of lactating ruminants. The contribution of portal flow to splanchnic flow ranged from 75% to 85% (Reynolds et al., 1988; De Visser et al., 1997; Bach et al., 2000; Raggio et al., 2004). Graded removal of Met resulted in a linear decrease in splanchnic blood flow, which was consistent with prior observations in multiparous lactating cows (Berthiaume et al., 2006). Although prior work showed that splanchnic blood flow was regulated by energy supply rather than protein supply (Huntington, 1990), this work and that of Berthiaume et al. (2006) clearly demonstrate that splanchnic flow is also responsive to at least Met supply.

The mammary blood flow increased linearly with the graded removal of Met. This phenomenon was observed for Met removal (Liu et al., 2019), Lys removal (Guo et al., 2017), His deficiency (Bequette et al., 2000), Leu deficiency (Bequette et al., 1996), and Arg deficiency (Fu et al., 2013). Therefore, increased mammary blood flow in response to deficiencies in individual EAA appears to be a common response (Cant et al., 2003), which helps mitigate deficiency.

4.3. Splanchnic and mammary net fluxes of AA

The concentration of circulating EAA usually declines with decreased postruminal supply (Fu et al., 2013; Paz et al., 2013; Lin et al., 2014; Liu et al., 2019). In fact, the efficacy of ruminally protected EAA such as Lys and Met can be evaluated based on blood concentration responses (Whitehouse et al., 2017). Consistent with these expectations, arterial and venous concentrations of Met decreased linearly with the removal of Met in this study. Compared with the complete mixture of the 100% treatment group, removal of all Met reduced arterial and venous Met concentrations by 57% and 65% in the control, respectively. Arterial and venous concentrations of other EAA were not affected by treatment, indicating that the metabolism of individual EAA is generally independent of others and had an independent effect on protein synthesis (Hanigan et al., 2000).

Although AA metabolism by the PDV tissue affects the net supply and composition of AA entering the bloodstream (Lapierre et al., 2005), it is also clear that the vast majority of this use is from arterial supplies, and thus represents use of recycled AA (MacRae et al., 1997). Gut use of AA also varies by AA with the loss of His being very small, while the loss of Leu, Thr, and some NEAA can be much greater (MacRae et al., 1997). Oxidation of Leu and Met was also reported in sheep (Lobley and Lapierre, 2003). The same phenomenon occurred in this study where net recovery ratio of Lys and BCAA in blood averaged about 60%, but net entry of His and Phe were the greatest at about 80%. If the PDV flux of the 0% Met treatment is taken as the basal absorption, the metabolic loss of Met in the PDV is 48.4%, 43.1% and 25.8% for the 100%, 60%, and 30% treatments, respectively. Thus, decreases in postruminal Met supply resulted in decreased utilization by the digestive tract and increased relative availability of Met for other tissues. The reduction in net use was due to a decrease in PDV clearance rates for Met from 12.14 to 0.845 L/h. There were no significant changes in the PDV flux of other AA, which indicated that the metabolic loss rate of PDV could be changed by adjusting the supply of a single EAA.

Met is largely removed when it passes through the liver. The net removal ratio of the liver ranged from 0.22 to 0.70 (Lapierre et al., 2005). Data from pregnant cattle (Wray-Cahen et al., 1997) and sheep (Lobley et al., 2001) showed that the extraction of AA by the liver was a function of the total internal flow of the liver (that is, the

Table 8
Effects of graded removal of Met from the amino acid mixture infused into abomasum on splanchnic and mammary net fluxes of amino acids in lactating goats (μmoL/h).

Item		Percentage of Met				SEM	*P*-value		
		100%	60%	30%	0%		Treatment	Linear	Quadratic
Met	Rate	797.6[a]	527.1[a]	226.6[b]	0[b]	88	<0.001	<0.001	0.789
	PDV	496.0[a]	393.2[a]	286.8[b]	159.9[b]	53	0.008	<0.001	0.501
	HEP	−231.8[b]	−164.7[b]	−79.81[a]	−14.68[a]	24	<0.001	<0.001	0.557
	TSP	264.2[a]	228.5[a]	207.8[ab]	145.2[b]	34	0.045	0.011	0.498
	MG	−206.1	−207.0	−186.1	−177.3	33	0.721	0.261	0.738
	Milk	176.9	158.8	163.2	157.1	25	0.879	0.468	0.761
Lys	Rate	2,140	2,156	2,145	2,138	437	0.910	0.646	0.873
	PDV	1,433	1,508	1,316	1,359	251	0.763	0.604	0.875
	HEP	−112.6	−128.0	−179.1	−174.1	35	0.463	0.129	0.894
	TSP	1320	1380	1140	1185	227	0.597	0.429	0.849
	MG	−764.8	−692.7	−582.7	−620.5	91	0.528	0.174	0.641
	Milk	469.3	474.1	419.2	463.4	76	0.710	0.273	0.618
Val	Rate	2,249	2,266	2,221	2,243	453	0.910	0.646	0.873
	PDV	1,531	1,394	1,346	1,364	213	0.688	0.456	0.469
	HEP	112.3	156.0	191.4	121.9	25	0.210	0.264	0.287
	TSP	1,643	1,550	1,537	1,485	209	0.799	0.597	0.538
	MG	−1,012	−988.7	−930.4	−793.3	86	0.099	0.067	0.431
	Milk	588.5	574.5	533.4	534.8	75	0.494	0.146	0.508
Ile	Rate	1,483	1,494	1,465	1,479	299	0.910	0.646	0.873
	PDV	934.4	970.6	878.7	920.9	196	0.950	0.865	0.778
	HEP	−107.1	−108.4	−119.3	−104.6	51	0.678	0.298	0.494
	TSP	827.3	862.2	759.0	815.4	182	0.970	0.905	0.912
	MG	−460.5	−556.4	−420.4	−433.7	97	0.753	0.652	0.596
	Milk	275.7	284.6	273.1	268.0	50	0.783	0.425	0.493
Leu	Rate	2,859	2,881	2,825	2,852	576	0.910	0.646	0.873
	PDV	1,881	1,894	1,744	1,671	286	0.817	0.950	0.775
	HEP	−152.9	−223.7	−185.0	−272.1	38	0.625	0.838	0.265
	TSP	1,728	1,670	1,559	1,399	265	0.091	0.048	0.637
	MG	−938.3	−1024	−918.0	−892.7	128	0.895	0.693	0.612
	Milk	627.6	615.2	642.6	607.2	115	0.652	0.365	0.444
His	Rate	766.8	772.6	757.6	765.3	154	0.910	0.646	0.873
	PDV	580.2	624.2	506.7	597.2	93	0.830	0.878	0.875
	HEP	−272.3	−319.3	−273.1	−311.3	76	0.955	0.812	0.929
	TSP	307.9	304.9	233.6	285.9	27	0.244	0.295	0.473
	MG	−208.6	−239.8	−192.5	−230.0	19	0.316	0.771	0.324
	Milk	183.5	184.6	155.4	174.4	22	0.681	0.399	0.437
Phe	Rate	2,355	2,373	2,327	2,349	475	0.910	0.646	0.873
	PDV	1,678	1,652	1,349	1,549	282	0.823	0.749	0.947
	HEP	−686.3	−756.6	−510.5	−857.9	147	0.433	0.865	0.936
	TSP	991.6	895.7	839.0	693.9	191	0.076	0.058	0.974
	MG	−720.4	−687.5	−630.6	−661.0	41	0.561	0.354	0.276
	Milk	681.3	640.2	658.4	683.1	50	0.747	0.597	0.406
Thr	Rate	1,368	1,378	1,351	1,364	276	0.910	0.646	0.873
	PDV	877.8	901.7	763.0	834.3	175	0.946	0.724	0.945
	HEP	−323.3	−428.3	−409.8	−407.9	125	0.933	0.633	0.672
	TSP	554.5	473.4	353.2	426.4	66	0.233	0.098	0.346
	MG	−460.2	−403.7	−362.6	−332.5	55	0.422	0.082	0.903
	Milk	359.3	376.0	363.3	341.3	60	0.752	0.437	0.430
Trp	Rate	308.1	310.4	304.4	307.4	62	0.910	0.646	0.873
	PDV	197.2	185.7	146.1	183.7	31	0.692	0.547	0.511
	HEP	−89.88	−88.57	−62.88	−89.71	19	0.703	0.742	0.539
	TSP	107.3	97.15	83.24	93.96	16	0.752	0.409	0.562
	MG	−99.89	−69.05	−94.33	−73.88	15	0.407	0.407	0.688
	Milk	73.82	67.55	78.23	69.88	12	0.516	0.492	0.665
Arg	Rate	864.9	871.4	854.5	862.9	174	0.910	0.646	0.873
	PDV	649.0	688.3	612.8	605.5	120	0.958	0.706	0.799
	HEP	−233.6	−272.0	−233.7	−167.8	54	0.606	0.369	0.299
	TSP	415.3	416.3	379.1	437.7	79	0.962	0.941	0.736
	MG	−533.2	−466.3	−406.2	−408.8	62	0.454	0.111	0.663
	Milk	187.0	161.8	186.7	198.4	28	0.717	0.459	0.842
Gly	Rate	1,000	1,008	988.2	998.5	202	0.910	0.646	0.873
	PDV	810.6	826.9	895.9	793.5	142	0.958	0.962	0.699
	HEP	−956.2	−1127	−1270	−1070	191	0.512	0.328	0.259
	TSP	−145.53	−300.0	−374.1	−276.5	82	0.072	0.055	0.058
	MG	−300.3[ab]	−233.0[a]	−353.6[b]	−377.6[b]	53	0.011	<0.001	0.478
	Milk	173.7	183.0	207.8	180.7	38	0.926	0.763	0.671
Glu	Rate	1,420	1,455	1,506	1,619	368	0.698	0.076	0.948
	PDV	466.0	575.5	223.6	218.3	158	0.327	0.155	0.583
	HEP	763.7	398.4	626.3	465.7	262	0.761	0.528	0.675
	TSP	1,230	974.0	849.9	684.1	180	0.063	0.032	0.907
	MG	−630.9	−445.1	−505.2	−598.1	122	0.697	0.869	0.246
	Milk	1105	974.8	1,055	1,086	244	0.803	0.691	0.740
Ser	Rate	2,028	2,043	2,003	2,023	409	0.910	0.646	0.873

Table 8 (*continued*)

Item		Percentage of Met				SEM	*P*-value		
		100%	60%	30%	0%		Treatment	Linear	Quadratic
	PDV	1,397	1,246	1,128	1,258	189	0.929	0.756	0.669
	HEP	−911.8	−868.2	−770.0	−967.9	162	0.834	0.922	0.466
	TSP	385.2	377.0	357.8	281.1	108	0.899	0.487	0.719
	MG	−288.5	−244.6	−277.7	−287.6	66	0.960	0.945	0.654
	Milk	427.2	451.0	498.6	455.4	86	0.705	0.699	0.353
Tyr	Rate	0	0	0	0	–	–	–	–
	PDV	523.3	406.8	303.3	351.6	75	0.245	0.071	0.360
	HEP	−292.1	−188.7	−131.6	−164.6	61	0.324	0.105	0.327
	TSP	231.2	218.2	171.7	186.9	27	0.408	0.140	0.730
	MG	−158.7	−155.5	−207.3	−146.5	47	0.799	0.928	0.600
	Milk	161.5	167.1	195.4	185.5	38	0.744	0.799	0.419
Pro	Rate	3,050	3,073	3,013	3,043	615	0.910	0.646	0.873
	PDV	1,612	1,926	1,425	1,684	345	0.783	0.882	0.860
	HEP	−606.3	−655.3	−429.3	−620.1	149	0.715	0.780	0.725
	TSP	1,005	1,270	995.6	1,064	234	0.826	0.968	0.623
	MG	−380.2	−422.9	−491.4	−466.8	86	0.736	0.902	0.366
	Milk	735.8	746.8	746.8	765.2	131	0.714	0.398	0.442
Ala	Rate	1,336	1,346	1,319	1,333	269	0.910	0.646	0.873
	PDV	1,609	1,581	1,255	1,253	259	0.641	0.228	0.934
	HEP	−849.0	−961.3	−709.9	−884.3	186	0.811	0.873	0.945
	TSP	759.7	620.1	545.0	369.1	115	0.069	0.022	0.763
	MG	−571.5	−455.6	−431.1	−363.2	109	0.608	0.168	0.879
	Milk	400.5	340.6	321.5	260.7	66	0.539	0.132	0.931

Rate = infused rate of AA; PDV = portal-drained-viscera; HEP = hepatic tissues; TSP = splanchnic tissues; MG = mammary gland; Milk = milk output.
[a, b] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

Table 9
Effects of graded removal of Met from the amino acid mixture infused into abomasum on PDV, liver and mammary amino acid clearance rates in lactating goats (L/h).

Item		Percentage of Met				SEM	*P*-value		
		100%	60%	30%	0%		Treatment	Linear	Quadratic
PDV	Met	12.14[a]	10.04[a]	4.307[b]	0.845[b]	1.71	0.002	<0.001	0.306
	Lys	5.608	5.378	5.693	6.312	2.32	0.993	0.816	0.838
	Val	3.814	4.631	4.638	4.557	2.84	0.996	0.842	0.878
	Leu	8.326	7.696	7.123	6.291	3.38	0.977	0.643	0.952
	Ile	10.64	8.463	8.334	7.018	1.50	0.428	0.096	0.830
	His	4.207	3.325	2.733	2.494	1.85	0.914	0.461	0.900
	Phe	15.18	11.08	10.16	8.159	4.72	0.761	0.272	0.866
	Thr	7.883	7.192	6.373	7.588	3.04	0.986	0.881	0.769
	Trp	5.878	5.716	5.321	4.797	2.62	0.991	0.747	0.926
	Arg	1.812	1.614	1.616	2.348	1.10	0.959	0.752	0.660
Liver	Met	6.653[a]	6.430[a]	3.758[b]	1.086[b]	1.21	0.007	0.002	0.557
	Lys	1.168	1.121	1.541	1.698	0.56	0.857	0.417	0.790
	Val	−0.354	−0.720	−0.723	−0.797	0.21	0.489	0.151	0.533
	Leu	1.149	1.912	1.371	1.377	0.47	0.704	0.882	0.392
	Ile	2.173	2.109	2.837	5.331	1.67	0.508	0.191	0.397
	His	6.029	8.444	6.112	8.235	2.67	0.867	0.692	0.923
	Phe	11.82	17.03	9.508	13.48	4.55	0.700	0.937	0.811
	Thr	5.791	7.602	9.814	9.579	4.89	0.928	0.508	0.876
	Trp	4.730	4.400	3.065	4.483	1.49	0.865	0.757	0.530
	Arg	2.260	2.839	2.628	2.009	0.92	0.919	0.845	0.497
Mammary	Met	11.04[b]	16.68[ab]	17.05[ab]	20.58[a]	3.67	0.042	0.027	0.971
	Lys	27.18	11.64	7.280	12.55	11.1	0.625	0.299	0.385
	Val	9.844	8.404	6.021	5.908	4.36	0.899	0.444	0.930
	Leu	13.93	20.45	11.80	14.43	6.23	0.787	0.833	0.686
	Ile	39.18	22.69	28.19	27.11	13.1	0.834	0.551	0.552
	His	6.323	12.50	3.745	9.386	3.41	0.339	0.935	0.828
	Phe	6.429	8.087	5.881	4.290	2.43	0.746	0.455	0.449
	Thr	18.74	15.07	11.76	11.69	7.38	0.891	0.430	0.853
	Trp	5.942	4.646	5.759	4.557	1.56	0.883	0.635	0.953
	Arg	8.287	6.055	5.431	5.580	2.18	0.782	0.317	0.707

PDV = portal-drained viscera.
[a, b] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

sum of absorption and peripheral blood recirculation from the portal vein and hepatic artery). Whether in late lactation or in non-lactating cows, the clearance of AA in the liver was closely related to the total liver inflow (Lobley and Lapierre, 2003; Hanigan et al., 2004; Fleming et al., 2019). In this experiment, the net removal ratio of Met by the liver in the 100% treatment group was 46.7%. As the supply of Met decreased, the net removal rates of the remaining 3 groups were 41.7%, 27.8% and 9.18%, respectively. The proportion

Table 10
Effects of graded removal of Met from the amino acid mixture infused into abomasum on the ratio of mammary uptake-to-milk output (U:O) in lactating goats.

Item	Percentage of Met				SEM	*P*-value		
	100%	60%	30%	0%		Treatment	Linear	Quadratic
Met	1.271	1.287	1.135	1.123	0.07	0.054	0.036	0.994
Lys	1.736	1.484	1.400	1.767	0.17	0.338	0.862	0.079
Val	1.831	1.704	1.768	1.624	0.36	0.960	0.712	0.731
Leu	1.535	1.692	1.537	1.455	0.11	0.273	0.170	0.314
Ile	1.798	1.888	1.569	1.618	0.24	0.562	0.687	0.471
His	1.185	1.299	1.238	1.325	0.10	0.320	0.225	0.777
Phe	0.964	1.014	0.910	0.968	0.06	0.419	0.206	0.389
Thr	1.516	1.044	1.034	1.169	0.21	0.374	0.244	0.175
Arg	2.892	2.878	2.471	2.790	0.34	0.808	0.629	0.703
Gly	0.792	1.367	1.796	2.047	0.30	0.057	0.005	0.749
Glu	0.545	0.522	0.492	0.550	0.04	0.113	0.332	0.223
Ser	0.673	0.538	0.571	0.797	0.08	0.074	0.027	0.038
Tyr	0.954	0.904	1.042	1.091	0.08	0.425	0.172	0.457
Ala	1.436	1.351	1.350	1.425	0.24	0.989	0.954	0.735
Pro	0.520	0.581	0.664	0.689	0.10	0.604	0.164	0.947
BCAA	1.836	1.907	1.817	1.629	0.18	0.559	0.310	0.474
EAA	1.567	1.546	1.430	1.397	0.10	0.245	0.068	0.305
NEAA	0.708	0.703	0.731	0.715	0.05	0.246	0.862	0.273
TAA	1.185	1.155	1.103	1.091	0.08	0.339	0.435	0.159

BCAA = branched-chain amino acid; EAA = essential amino acid; NEAA = non-essential amino acid; TAA = total amino acids.
[a, b] Least square means within a row with a different superscript indicate a significant difference ($P < 0.05$).

of net removal of liver to total internal flow also decreased gradually (4.7%, 4.2%, 3.0% and 0.9%, respectively). The clearance rate of Met by the liver also decreased significantly with the decreased Met supply (Table 9). This suggested that the liver is an active regulatory organ, which can not only remove excess AA, but also provide anabolic AA for peripheral tissue according to the supply of amino acids. For other EAA, net removal of His, Phe, Thr, Trp and Arg by the liver was relatively large, between 35% and 50%, while the removal of Lys and BCAA by the liver was very small.

The graded removal of Met resulted in a linear decrease in the amount of Met delivered to the peripheral tissue (net TSP flux, Table 8). In previous work, the addition of ruminally protected Met also resulted in an increase of net TSP flux of Met (Bach et al., 2000; Berthiaume et al., 2006). Unexpectedly, the TSP net flux of Leu, Glu and Ala decreased linearly and that of Phe and Thr tended to decrease with the graded removal of Met in the current experiment. This phenomenon also occurred in Berthiaume's experiment, which may be explained by the splanchnic tissue trying to rebalance the supply of AA to the peripheral tissue in the case of AA supply imbalance (Berthiaume et al., 2006).

Changes in mammary blood flow and mammary affinity compensated for part of the varied supply of individual EAA, thereby mitigating the responses. In this study, although the yield of milk protein decreased by about 30% from the 100% group to 0% group, there was no significant change in net removal of Met by the mammary glands despite large declines in the arterial concentrations of Met. This was due to the increased affinity of the mammary gland for Met and increased mammary blood flow.

4.4. Postruminal Met supply and mammary Met uptake

In this study, because dairy goats adapted to the lack of Met by reducing net use by the PDV, liver and peripheral (non-mammary) tissues and increasing mammary affinity and blood flow in order to maintain net Met uptake by mammary tissue, the mammary supply of Met as a precursor does not appear to be the sole reason for the decrease in milk protein production. This phenomenon was supported by previous experiments (Bequette et al., 2000; Guo et al., 2017). Met supply may exert a portion of its effects on milk protein synthesis through prolactin and insulin. Prolactin plays an important role in the mammary development and lactation (Lacasse et al., 2012). Inhibiting the release of prolactin significantly reduced milk yield in dairy goats and cows (Akers et al., 1981a, 1981b; Knight et al., 1990; Forsyth and Lee, 1993). In this experiment, the concentration of prolactin decreased linearly with the graded deletion of Met, which may be the main reason for the decrease in milk protein production. However, the concentration of insulin increased as Met supply decreased in this study, and this should have stimulated production of milk protein (Mackle et al., 2000; Ranga et al., 2010; Bionaz and Loor, 2011). It is possible the stimulatory effects of insulin were overwhelmed by the negative prolactin effects, or possibly low prolactin resulted in reduced insulin receptor or cell signaling.

5. Conclusions

Lactating ruminants utilize several mechanisms to mitigate Met deficiency, including reduced utilization of Met by the PDV and liver, linear increases in mammary blood flow and mammary affinity for Met. This results in a shift in post-absorptive use away from the splanchnic tissues to the mammary tissues. The increases in mammary affinity and blood flow maintained mammary removal rates equal to the non-deficient state. Therefore, the decrease in milk protein production caused by deficiency was not the result of limited mammary Met uptake, and suggests that other factors such as prolactin may contribute to the deficiency response. Further work is required to delineate external hormonal effects and intracellular cell signaling effects.

Author contributions

Yantao Li: Data curation, Formal analysis, Methodology, Investigation, Writing-Original draft preparation. **Mark D. Hanigan:** Methodology, Writing-Review and Editing. **Xueyan Lin:** Project administration, Supervision. **Zhiyong Hu:** Writing-Review and Editing. **Zhengui Yan**: Methodology, Surgery. **Qiuling Hou:** Resources. **Yun Wang:** Resources. **Zhonghua Wang:** Conceptualisation, Project administration, Writing-Reviewing and Editing, Supervision, Funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

The work is funded by National Natural Science Foundation of China under project number 31772623 and by China Agriculture Research System of MOF and MARA.

References

Akers RM, Bauman DE, Capuco AV, Goodman GT, Tucker HA. Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. Endocrinololgist 1981a;109:23–30.
Akers RM, Bauman DE, Goodman GT, Capuco AV, Tucker HA. Prolactin regulation of cytological differentiation of mammary epithelial cells in periparturient cows. Endocrinololgist 1981b;109:31–40.
Annison EF, Linzell JL. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over-all metabolism and to milk formation. J Physiol 1964;175:372–85.

Arriola Apelo SI, Knapp JR, Hanigan MD. Invited review: current representation and future trends of predicting amino acid utilization in the lactating dairy cow. J Dairy Sci 2014;97:4000–17.
Bach A, Huntington GB, Calsamiglia S, Stern MD. Nitrogen metabolism of early lactation cows fed diets with two different levels of protein and different amino acid profiles. J Dairy Sci 2000;83:2585–95.
Bequette BJ, Backwell FR, MacRae JC, Lobley GE, Crompton LA, Metcalf JA, et al. Effect of intravenous amino acid infusion on leucine oxidation across the mammary gland of the lactating goat. J Dairy Sci 1996;79:2217–24.
Bequette BJ, Hanigan MD, Calder AG, Reynolds CK, Lobley GE, MacRae JC. Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. J Dairy Sci 2000;83:765–75.
Berthiaume R, Dubreuil P, Stevenson M, McBride BW, Lapierre H. Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. J Dairy Sci 2001;84:194–203.
Berthiaume R, Thivierge MC, Patton RA, Dubreuil P, Stevenson M, McBride BW, et al. Effect of ruminally protected methionine on splanchnic metabolism of amino acids in lactating dairy cows. J Dairy Sci 2006;89:1621–34.
Bionaz M, Loor JJ. Gene networks driving bovine mammary protein synthesis during the lactation cycle. Bioinf Biol Insights 2011;5:83–98.
Cant JP, Berthiaume R, Lapierre H, Luimes PH, Mcbride BW, Pacheco D. Responses of the bovine mammary glands to absorptive supply of single amino acids. Can J Anim Sci 2003;83:341–55.
Cant JP, DePeters EJ, Baldwin RL. Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. J Dairy Sci 1993;76:762–74.
Chen ZH, Broderick GA, Luchini ND, Sloan BK, Devillard E. Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. J Dairy Sci 2011;94:1978–88.
De Visser H, Valk H, Klop A, Van der Meulen J, Bakker JG, Huntington GB. Nutrient fluxes in splanchnic tissue of dairy cows: influence of grass quality. J Dairy Sci 1997;80:1666–73.
DeSantiago S, Torres N, Suryawan A, Tovar AR, Hutson SM. Regulation of branched-chain amino acid metabolism in the lactating rat. J Nutr 1998;128:1165–71.
Fleming AJ, Lapierre H, Martineau R, White RR, Hanigan MD. Modeling portal-drained viscera and liver fluxes of essential amino acids in dairy cows. J Dairy Sci 2019;102:10964–82.
Forsyth IA, Lee PD. Bromocriptine treatment of periparturient goats: long-term suppression of prolactin and lack of effect on lactation. J Dairy Res 1993;60:307–17.
Fu Y, Lin XY, Ma WM, Chi HL, Yan ZG, Song YF, et al. Metabolic responses to the deficiency of Lys, Arg, Met, or His in the mammary gland of lactating goats. Small Rumin Res 2013;113:219–30.
Goodwin GW, Gibboney W, Paxton R, Harris RA, Lemons JA. Activities of branched-chain amino acid aminotransferase and branched-chain 2-oxo acid dehydrogenase complex in tissues of maternal and fetal sheep. Biochem J 1987;242:305–8.
Guinard J, Rulquin H. Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. J Dairy Sci 1995;78:2196–207.
Guo CL, Li YT, Lin XY, Hanigan MD, Yan ZG, Hu ZY, et al. Effects of graded removal of lysine from an intravenously infused amino acid mixture on lactation performance and mammary amino acid metabolism in lactating goats. J Dairy Sci 2017;100:4552–64.
Hanigan MD, Crompton LA, Reynolds CK, Wray-Cahen D, Lomax MA, France J. An integrative model of amino acid metabolism in the liver of the lactating dairy cow. J Theor Biol 2004;228:271–89.
Hanigan MD, France J, Crompton LA, Bequette BJ. Evaluation of a representation of the limiting amino acid theory for milk protein synthesis: modeling Nutrient Utilization in Farm Animals. 2000.
Hanigan MD, France J, Wray-Cahen D, Beever DE, Lobley GE, Reutzel L, et al. Alternative models for analyses of liver and mammary transorgan metabolite extraction data. Br J Nutr 1998;79:63–78.
Howarth RW, Boyer EW, Pabich WJ, Galloway JN. Nitrogen use in the United States from 1961–2000 and potential future trends. AMBIO 2002;31:88–96.
Huntington GB. Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. Reprod Nutr Dev 1990;30:35–47.
Huntington GB, Zetina EJ, Whitt JM, Potts W. Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. J Anim Sci 1996;74:908–16.
Katz ML, Bergman EN. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. Am J Physiol 1969;216:946–52.
Knight CH, Foran D, Wilde CJ. Interaction between autocrine and endocrine control of milk yield: thrice-daily milking and bromocriptine-treated goats. 1990.
Lacasse P, Lollivier V, Dessauge F, Bruckmaier RM, Ollier S, Boutinaud M. New developments on the galactopoietic role of prolactin in dairy ruminants. Domest Anim Endocrinol 2012;43:154–60.
Lapierre H, Berthiaume R, Raggio G, Thivierge MC, Doepel L, Pacheco D, et al. The route of absorbed nitrogen into milk protein. Anim Sci 2005;80:11–22.
Lapierre H, Blouin JP, Bernier JF, Reynolds CK, Dubreuil P, Lobley GE. Effect of supply of metabolizable protein on whole body and splanchnic leucine metabolism in lactating dairy cows. J Dairy Sci 2002;85:2631–41.
Le Floc'h N, Thibault JN, Sève B. Tissue localization of threonine oxidation in pigs. Br J Nutr 1997;77:593–603.
Lin XY, Wang JF, Su PC, Wang Y, Wang ZH. Lactation performance and mammary amino acid metabolism in lactating dairy goats when complete or met lacking amino acid mixtures were infused into the jugular vein. Small Rumin Res 2014;120:135–41.
Liu W, Xia F, Hanigan MD, Lin XY, Yan ZG, White RR, et al. Short-term lactation and mammary metabolism responses in lactating goats to graded removal of methionine from an intravenously infused complete amino acid mixture. J Dairy Sci 2019;102:4094–104.
Lobley G, Lapierre H. Post-absorptive metabolism of amino acids. European Association for Animal Production; 2003. p. 737–56.
Lobley GE, Bremner DM, Brown DS. Response in hepatic removal of amino acids by the sheep to short-term infusions of varied amounts of an amino acid mixture into the mesenteric vein. Br J Nutr 2001;85:689–98.
Mabjeesh SJ, Kyle CE, Macrae JC, Bequette BJ. Lysine metabolism by the mammary gland of lactating goats at two stages of lactation. J Dairy Sci 2000;83:996–1003.
Mackle TR, Dwyer DA, Ingvartsen KL, Chouinard PY, Ross DA, Bauman DE. Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. J Dairy Sci 2000;83:93–105.
MacRae JC, Bruce LA, Brown DS, Calder AG. Amino acid use by the gastrointestinal tract of sheep given lucerne forage. Am J Physiol 1997;273:G1200–7.
Manjarin R, Bequette BJ, Wu G, Trottier NL. Linking our understanding of mammary gland metabolism to amino acid nutrition. Amino Acids 2014;46:2447–62.
Mineo H, Oyamada T, Akiyama M, Yasuda T, Kato S. A new method of catheterization of the hepatic and portal veins in sheep for long-term blood sampling. Small Rumin Res 1991;5:293–300.
Paz HA, de Veth MJ, Ordway RS, Kononoff PJ. Evaluation of rumen-protected lysine supplementation to lactating dairy cows consuming increasing amounts of distillers dried grains with solubles. J Dairy Sci 2013;96:7210–22.
Pink DRE, Dw T, Br O. Lysine catabolism in the neonatal piglet during postnatal stages of growth and development. FASEB (Fed Am Soc Exp Biol) J 2003;17:A702 [abstr.].
Pisulewski PM, Rulquin H, Peyraud JL, Verite R. Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. J Dairy Sci 1996;79:1781–91.
Raggio G, Pacheco D, Berthiaume R, Lobley GE, Pellerin D, Allard G, et al. Effect of level of metabolizable protein on splanchnic flux of amino acids in lactating dairy cows. J Dairy Sci 2004;87:3461–72.
Ranga J, Appuhamy N, Hanigan M, Akers C, Escobar JE. Regulatory roles of essential amino acids, energy, and insulin in mammary cell protein synthesis. Virginia Tech; 2010.
Reynolds CK, Huntington GB, Tyrrell HF, Reynolds PJ. Net portal-drained visceral and hepatic metabolism of glucose, L-lactate, and nitrogenous compounds in lactating holstein cows. J Dairy Sci 1988;71:1803–12.
Tamminga S, Schulze H, Van Bruchem J, Huisman J. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch Tierernahr 1995;48:9–22.
Weekes TL, Luimes PH, Cant JP. Responses to amino acid imbalances and deficiencies in lactating dairy cows. J Dairy Sci 2006;89:2177–87.
Whitehouse NL, Schwab CG, Brito AF. The plasma free amino acid dose-response technique: a proposed methodology for determining lysine relative bioavailability of rumen-protected lysine supplements. J Dairy Sci 2017;100:9585–601.
Wray-Cahen D, Metcalf JA, Backwell FR, Bequette BJ, Brown DS, Sutton JD, et al. Hepatic response to increased exogenous supply of plasma amino acids by infusion into the mesenteric vein of Holstein-Friesian cows in late gestation. Br J Nutr 1997;78:913–30.
Zhou Z, Vailati-Riboni M, Trevisi E, Drackley JK, Luchini DN, Loor JJ. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. J Dairy Sci 2016;99:8716–32.