

Intestinal Disappearance and Mesenteric and Portal Appearance of Amino Acids in Dairy Cows Fed Ruminally Protected Methionine¹

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ABSTRACT

An experiment was conducted to compare the rates of disappearance of amino acids (AA) from the small intestine and their net appearance in the blood draining only the small intestine (mesenteric-drained viscera) and the whole gastrointestinal tract (portal-drained viscera) of cows fed a silage-based diet supplemented or not with ruminally protected Met. Five lactating dairy cows (118 ± 4 DIM) equipped with duodenal and ileal cannulae ($n = 2$) or a duodenal cannula only ($n = 3$), two of which were multicatheterized, were fed a TMR top dressed with 0 or 72 g of ruminally protected Met per day. The addition of ruminally protected Met to the diet increased the duodenal flux of Met leading to a higher apparent digestibility of Met in the small intestine. Sixty-six percent of Met from ruminally protected Met bypassed the rumen and 82% of that Met disappeared from the small intestine. Arterial plasma Met concentrations numerically increased with ruminally protected Met (45 vs. 18 μM), while total AA concentration decreased. Feeding ruminally protected Met resulted in higher concentrations of urea-N and glucose in arterial plasma. Milk production and milk composition were unaffected. The disappearance of essential AA across the small intestine was equivalent (101%) to their flux through the mesenteric-drained viscera while the portal:mesenteric-drained viscera flux ratio for each essential AA varied from 38% for Thr to 76% for Phe. The portal:mesenteric-drained viscera flux ratio for Met was 66%. These results confirm observations made with pigs and sheep.

(Key words: methionine, ruminally protected amino acids, absorption)

Abbreviation key: BCAA = branched-chain amino acids, GIT = gastrointestinal tissues, MDV = mesen-

teric-drained viscera, PAH = *p* - aminohippurate, PCV = packed cell volume, PDV = portal-drained viscera, RPM = ruminally protected Met, VA = venous-arterial concentration differences.

INTRODUCTION

Although the use of ruminally protected Met (RPM) has been proved to increase the amount of Met flowing to the duodenum and disappearing from the small intestine of dairy cattle (4), results of lactation studies remain inconsistent (27). However, it has been shown that dietary provision of rumen-protected AA believed to be limiting did not necessarily result in an increased supply to the peripheral tissues following splanchnic metabolism (20). The AA supply available to fulfill requirements has usually been defined from an estimation of AA appearing at the duodenum (32). Experiments with sheep have shown that the duodenal profile is in fact quite different from the profile of AA reaching portal blood circulation. Tagari and Bergman (38) demonstrated that the amounts of AA appearing in portal blood varied from 30 to 80% of that disappearing from the gut lumen. The authors suggested that the gastrointestinal tissues (GIT) exerted a selective and preferential use of essential AA during absorption resulting in an imbalance in the profile of essential AA delivered to the liver and peripheral tissues. Recently, this concept has been reexamined by MacRae et al. (19) using lambs prepared with intestinal cannulae and catheters in the mesenteric and portal veins to compare the disappearance of essential AA from the small intestine with their appearance in the blood vessels draining only the small intestine (mesenteric vein) and the whole GIT (portal vein). Considering the similarity between the rates of disappearance of essential AA from the small intestine and their appearance in the mesenteric vein, these authors concluded that the differential fluxes of these essential AA in the mesenteric and portal veins could be due to the use of arterial essential AA by the forestomach and hindgut.

To our knowledge no such studies have been reported with lactating dairy cows fed diets comparable to those

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used on commercial dairy farms. Consequently, the objective of our study was to compare the rates of disappearance of individual AA from the small intestine with their net appearance in the blood draining only the small intestine (mesenteric vein) and the whole GIT (portal vein) of cows fed a silage-based diet supplemented or not with RPM to determine if the addition of RPM would have an effect on the metabolism of Met and other AA across the mesenteric-drained viscera (MDV) and portal-drained viscera (PDV).

MATERIALS AND METHODS

Animals and Treatments

Surgical preparation. Five lactating dairy cows were used for this trial. Cannulation is generally performed when cows are not lactating (R. Berzins, 1997, personal communication), whereas catheterization is usually done a few weeks before the beginning of experimentation to decrease the risk of losing catheter patency associated with calving. So, in the case of early lactating cows, catheters are usually implanted between 4 and 8 wk of lactation. Therefore, for the purpose of this trial, surgery was performed in early lactation. All procedures were approved in accordance with the Canadian Council on Animal Care guidelines (8). Cows #440 and #5073 (543 ± 56 kg and 32 ± 19.5 DIM) were fitted with closed T-shaped cannulae made of Teflon (31) in the proximal duodenum (first 25 cm) and terminal ileum (>1 m before the ileocaecal valve), and with chronic indwelling catheters in the portal vein, mesenteric vein, and caudal aorta via a mesenteric artery. Two mesenteric infusion catheters were sited in the distal branches of the mesenteric vein to allow proper mixing of p-aminohippurate (PAH) before sampling catheters. Placement of catheters in the artery and the portal vein for blood sampling and of catheters in the distal mesenteric vein for PAH infusion was done as reported by Huntington et al. (15). Another catheter for blood sampling was inserted in the cranial mesenteric vein (3). This catheter was made of Tygon and was inserted into a branch of the mesenteric vein, pushed in until the tip was located before the opening of the gastric veins and sutured in place. The carotid artery was elevated to provide an alternative source of arterial blood. Cows recovered well, but occasional blockage of the ileal cannula caused a significant drop in feed intake and, therefore, milk production. After a thorough examination of all the surgical procedures, considering that early lactation is a particularly demanding period and that the placement of this type of cannula in the ileum is considered as challenging (12), we decided to abandon it and use the information generated by cows #440 and

Table 1. Ingredient composition of diet.

Ingredient	% ¹
Grass silage	58.4
Corn silage	15.2
Ground shelled corn	11.3
Soybean meal	13.0
Cr ₂ O ₃	0.2
Mineral and Vitamin mix ²	1.15
Dicalcium phosphate	0.597
Binder ³	0.132
MgO	0.044

¹Dry matter basis.

²Composition: CP (2%); NE_L (0.77 Mcal/kg); Fat (2.3%); ADF (1.5%); Na (15.6%); Ca (15.3%); P (0.1%); Mg (3.5%); K (0.087%); Co (1210 mg/kg); Mn (4060 mg/kg); Zn (6090 mg/kg); Cl (24 mg/kg); Se (24 mg/kg); Fe (2500 mg/kg); vitamin A (548,000 IU/kg); vitamin D (185,000 IU/kg); vitamin E (1,800 IU/kg).

³Lignosol.

#5073 to determine disappearance in the small intestine.

Thus, 2 mo after the first surgeries, cows #308, #5002, #5056, and #5067 (605 ± 41 kg) were fitted with a closed T-shaped cannula (31) made of Teflon-coated stainless steel in the proximal duodenum and the same catheters as previously described. Surgery was performed when cows were in midlactation (118 ± 4 DIM). Unfortunately, cow #5067 had to be slaughtered during period 1 after developing acute enteritis. Finally, because of the loss of catheter patency, only two cows (#308 and #5056) had all catheters patent to measure mesenteric and portal fluxes.

Postsurgical care. Following surgery, animals were placed in straw bedded pens to recover, with free access to hay, a small amount of TMR, and fresh water. They usually started eating within a few hours after the completion of surgery and had stood up within 3 h. Normally, cows were transferred from the recovery pens to the animal wing 24 h after surgery. They were housed in conventional tie stalls equipped with rubber mats and individual water bowls and milked twice daily at 0600 and 1800 h.

Dietary treatments. Cows were used in a randomized complete block design with two treatments. A control diet (Table 1), with a 74:26 forage to concentrate ratio, was formulated according to NRC (22) recommendations for cows in mid to late lactation and fed alone or top dressed with 72 g of Mepron M85 (Degussa Hüls Corp., Allendale, NJ) per cow per day. Mepron® M85 contained 94.3% Met (DM basis). The amount of RPM added to the diet was chosen based on results from a previous trial (4) conducted in this laboratory. Nutrient compositions of ingredients are listed in Table 2. The complete diet was mixed once daily in a horizontal mixer and fed twice daily during surgical recovery or 12

Table 2. Chemical composition of feedstuffs.

	Grass silage	Corn silage	Grass hay ¹	Concentrate ²	TMR ³
DM, %	313	313	873	882	384
OM, g/kg DM	896	959	908	875	904
NDF, g/kg DM	523	443	570	100	440
ADF, g/kg DM	319	248	318	44	273
CP, g/kg DM	117	90	121	284	167
NH ₃ -N, g/kg N	70	66			
Soluble N, g/kg N	475	369			
AA, g/kg DM					
Arg	3.05	2.19	5.41	18.38	6.71
His	1.74	1.42	1.97	7.25	2.95
Ile	5.07	3.28	5.59	12.76	7.03
Leu	8.60	8.68	9.98	23.97	12.72
Lys	4.69	2.56	5.74	16.59	7.30
Met	2.02	1.86	2.19	4.32	2.47
Phe	5.34	3.82	6.32	12.01	7.57
Thr	4.85	3.82	5.52	11.78	6.17
Val	6.81	5.57	7.78	16.30	8.75
Ala	7.74	6.93	8.41	14.89	9.29
Glu	8.49	10.80	12.68	54.98	20.34
Gly	5.61	4.36	6.83	12.44	7.14
Pro	6.21	6.11	8.99	16.74	10.09
Ser	4.09	4.04	4.75	14.58	6.33
Cys	0.88	1.86	1.57	4.91	1.88
Asp	9.26	7.64	11.51	33.50	14.82
Trp	1.47	0.71	1.97	3.53	1.82

¹Grass hay (1 kg) was fed once a day.

²Composition, DM basis; ground shelled corn (43%); soybean meal (49%); Cr₂O₃ (1%); minerals and vitamins (7%).

³Based on chemical analysis of TMR samples.

times daily, using automated feeders (Ankom, Fairport, NY), during experimental periods to achieve steady state. The RPM was also fed 12 times daily. One kilogram of grass hay was fed once daily. Orts were taken daily, prior to the 1300-h feeding. Dry matter offered was fixed throughout the trial and the amount of fresh feed offered was adjusted twice weekly to account for silage DM variations. Cr₂O₃ was mixed with ground shelled corn and included (2 kg/tonne) in the pelleted concentrate. Chromium was used as an indigestible marker to assess DM flow in the gastrointestinal tract.

Sample Collection

Experimental periods lasted 14 d: 6 d for diet adaptation followed by a 5 d total collection of feces and urine, 1 d for blood sampling, and 2 d for collection of digesta. Milk weights were recorded from d 5 to 14. Milk samples from a.m. and p.m. milkings were collected from d 5 (p.m.) to 10 (a.m.) and pooled on a daily basis according to milk production. Samples of TMR, individual feedstuffs and Orts were collected on d 5 to 14. On d 4, cows # 308, #5002, and #5056 were fitted with harnesses and tubes allowing the collection of feces and urine separately. Total collection of feces and urine proceeded

from d 5 to 10. Urine was collected in stainless steel containers with concentrated sulfuric acid to maintain pH < 2.0. Feces were voided in preweighed plastic lined plywood boxes. Feces and urine were weighed daily and a representative sample (2%) was taken and frozen immediately.

Blood sampling took place on d 12. Twelve sets of blood samples (15 ml) were simultaneously collected from the artery and the mesenteric and portal veins every 30 min, from 08:30 to 14:00. Blood flow in the portal and cranial mesenteric vein was determined by measuring dilution of PAH (10% wt/vol; 144 ml/h) that was infused continuously into one of the mesenteric vein infusion catheters (15). Infusion of PAH began at least 40 min before the first set of blood samples, after a priming dose (20 ml) and continued through samplings. Duodenal digesta (250 ml: all five cows), ileal digesta (150 ml: cows #440 and #5073) and fecal grab samples (approximately 200 g: all five cows) were collected at 0900, 1100, 1300 and 1500 h on d 13 and at 0800, 1000, 1200, and 1400 h on d 14 for a total of eight samples collected during the same period as blood. Duodenal and ileal digesta pH were determined immediately with a pH meter (Accumet 1001, Fischer) on the last samples collected at 1400 h.

Analytical Methods

Samples of feed and digesta (duodenal, ileal, and fecal) were lyophilized and ground to pass a 1-mm screen. Subsamples were ashed at 550°C for 12 h in a muffle furnace. Nitrogen was determined by thermal conductivity (LECO model FP-428 Nitrogen Determinator, LECO, St. Joseph, MI) except for acidified urine samples, where N was measured by micro-Kjeldhal analysis (1). Subsamples of urine were analyzed for urea nitrogen with the Technicon AutoAnalyser System (14) and purine derivatives (2). Fiber fractions (NDF and ADF) were determined on feed samples according to Van Soest et al. (40). Fresh silage extracts were analyzed for pH, soluble N and NPN (37), while NH₃-N was determined on silage extracts and fresh duodenal and ileal digesta (24). The Cr content of feed and digesta (duodenal, ileal, and fecal) samples was determined by a spectrophotometric procedure (10). For AA determination, samples of feed and digesta were predigested with performic acid to stabilize Met and Cys, treated with hydrobromic acid to destroy the performic acid and then were acid-hydrolyzed with 6N-HCl (1): (method # 994-12) and AA were quantified by ion-exchange chromatography (Beckmann 6300, Palo Alto, CA). A separate acid hydrolysis (6 N-HCl) digestion procedure was conducted for Phe, Tyr, and His, because those AA are destroyed during the oxidation process and by reaction

with bromine. Finally, Trp was determined by alkaline hydrolysis (NaOH), since it is destroyed by acid hydrolysis.

Blood samples were kept on ice until processed. Blood aliquots (2 ml) were treated with 100 μ l of aprotinin (Bayer Co; 500 trypsin inhibitor unit/ml) immediately after sampling, for glucagon analysis. The packed cell volume (PCV) of each blood sample was determined by filling a hematocrit capillary tube followed by centrifugation at 5000 rpm for 5 min. Concentrations of blood urea, PAH, $\text{NH}_3\text{-N}$, and α -amino-N were determined on fresh samples with the Technicon AutoAnalyzer System (14). $\text{NH}_3\text{-N}$ concentrations were corrected for an α -amino-N reaction in the ammonia assay, using the relative response of a leucine standard and measured α -amino-N concentrations (7). The remainder of the samples were then centrifuged at 3000 rpm for 12 min, and plasma samples were kept frozen until analyzed. An enzymatic method (kit #166391, Boehringer Mannheim, Dorval, Qc, Canada) was used to determine the concentration of plasma glucose. Plasma PAH was analyzed as described earlier for blood. Plasma hormone concentrations were determined by double antibody radioimmunoassay as described by Lapierre et al. (17) for insulin and by Herbein et al. (13) for glucagon (30 K antibodies). Inter and intraassay coefficients of variation for insulin and glucagon were 6.6, 13.2 and 9.1, 6.4 %, respectively. For AA determination, the 12 samples were pooled for every 2-h sampling period (three pooled samples), the day of sampling. Then, 200 mg of a dithioerythritol (5 mM)-norleucine (1 mM) solution was added to 1 g of blood and vortexed. Then, this mixture was deproteinized by adding 150 μ l of sulfosalicylic acid (48%) and centrifuged through a filter (0.22 μ ; Sigma Chemical Co., St Louis, MO) at 13,000 rpm for 12 min. The supernatant was decanted and centrifuged again as described above. The pH of the filtrate was adjusted to 2.0 to 2.5 with 20 to 50 μ l of NaOH (10%) and kept frozen. Samples were analyzed for individual free AA using an Amino Acid Analyzer (Pharmacia Alpha Plus II; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England).

Milk samples were analyzed for DM and OM with a thermogravimetric analyser (model TGA 601, LECO, St. Joseph, MI), milk fat (1), total N was determined by thermal conductivity (LECO model FP-428 Nitrogen Determinator, LECO, St. Joseph, MI), nonprotein N and noncasein N were measured by micro-Kjeldhal analysis (1) while lactose was determined by difference.

Calculations

PDV include the total digestive tract, pancreas, spleen, and mesenteric fat. One of the main branches

of the portal vein, the cranial mesenteric vein, drains the majority of the small intestine, caecum, large intestine, mesenteric fat, and part of the pancreas (MDV). Therefore, measurements of venous-arterial concentration differences (VA) obtained for the portal and cranial mesenteric veins represent VA for the entire GIT and poststomach tissues, respectively. Blood flows in the portal and cranial mesenteric veins were measured by dilution of PAH (16). Net fluxes of nutrients and hormones were calculated as the product of blood or plasma flow and blood or plasma VA for the PDV and MDV. Negative fluxes denote a net removal by the tissue, whereas positive fluxes denote a net release from the tissue.

Nutrient flows at the duodenum, ileum, and rectum were calculated by reference to concentrations (g/g of DM) of Cr and nutrients. Thus, duodenal, ileal and rectal flows of AA were calculated by multiplying individual AA concentrations (g/g of DM) by the appropriate DM flow for each cow in each period.

Statistical Analysis

Analysis of the difference between treatment means was done by a two-way analysis of variance (34) with the effect of cow and treatment included in the model. Because of the different problems encountered (death of one animal, occasional blockage of the ileal cannula), data for duodenal and fecal fluxes are for all five cows, data for ileal fluxes are for two cows fitted with both cannulae while data for milk production, and composition and for nitrogen balance are for three cows with duodenal cannulae only. The model was as follows:

$$Y_{ij} = \mu + \text{COW}_i + \text{TRT}_j + \varepsilon_{ij},$$

where:

Y_{ij} is the observation for the i th cow and j th treatment, μ is the overall mean, COW_i is the fixed effect of the i th animal where $i = 1$ to 3 for N balance and milk production data, $i = 1$ to 5 for intestinal flows and digestibility data and $i = 1$ to 2 for arterial plasma data, TRT_j is the fixed effect of the j th treatment where $j = 0$ or 72 g/d RPM, and ε_{ij} is the residual effect associated with the Y_{ij} observation.

Data comparing apparent digestibility in the small versus the whole intestine were analyzed as a completely randomized design with repeated measures in space using Proc GLM (34). The model used was as follows:

$$Y_{ijk} = \mu + \text{COW}_i + \text{TRT}_j + \text{COW}(\text{TRT})_{i(j)} + \text{SITE}_k + \text{TRT}_j * \text{SITE}_k + \varepsilon_{ijk},$$

where:

Y_{ijk} is the observation for the i th cow, on the j th treatment and at the k th site,

μ is the overall mean,

COW_i is the effect of the i th animal where $i = 2$,

TRT_j is the effect of the j th treatment where $j = 0$ or 72 g/d RPM,

$COW (TRT)_{i(j)}$ is the effect of the i th cow within the j th treatment,

$SITE_k$ is the effect of the k th site where $k =$ duodenal or ileal,

$TRT_j * SITE_k$ is the interaction effect of the j th treatment with the k th site, and

ε_{ijk} is the residual effect associated with the Y_{ijk} observation.

$COW (TRT)_{i(j)}$ was used as the error term for the effect of COW and TRT,

ε_{ijk} was used as the error term for the effect of SITE and SITE * TRT.

Because of the loss of catheter patency, arterial concentrations were obtained for two cows (#308 and #5056). In addition, incorrect placement of the mesenteric catheter (#5056, period 2) and of the portal catheter (#308, period 1) resulted in the impossibility of performing any statistical analysis to test for the effect of RPM. However, the data comparing intestinal disappearance with MDV and PDV fluxes are provided as descriptive statistics due to the uniqueness of this data set. Parameters were considered unaffected by RPM if $P > 0.05$ unless stated otherwise.

RESULTS

Cannula Placement and Marker Recovery

Proper placement of the duodenal cannula was evaluated by measuring digesta pH (2.61 ± 0.12) for each cow in each period. A pH lower than 3.0 is considered indicative that the cannula was placed proximal to the pancreatic duct (39). Ileal digesta pH (7.9) was in close agreement with values reported in the literature (39) with different types of cannulae located in the ileum. The marker was fed through the whole experiment as it was included in the concentrate portion of the diet. During the 5-d total fecal collection periods, $98.6 \pm 5.2\%$ of the marker was recovered.

Intestinal Fluxes

The addition of RPM had no effect on intake, duodenal flows, fecal output, and apparent digestibility in the stomach, whole intestine and total GIT of OM and N (Table 3). As expected, the addition of RPM to the diet increased ($P = 0.05$) the duodenal flow of Met (Table 4), resulting in a higher level of digestibility in the whole

Table 3. Effect of ruminally protected Met on daily flow and digestion of OM and nitrogen in the gastrointestinal tract of dairy cows.¹

	Mepron M85 (g/d)		SEM	P
	0	72		
OM				
Intake, kg/d	12.6	12.2	0.2	0.28
Flow to duodenum, kg/d	7.9	7.8	0.4	0.88
Fecal output, kg/d	3.8	3.8	0.2	0.88
Apparent digestion in stomach, %	37.0	35.9	4.4	0.87
Apparent digestion in intestine, %	52.2	52.1	3.1	0.98
Apparent digestibility in GIT, ² %	70.1	69.9	1.7	0.93
Nitrogen				
Intake, g/d	363.4	359.6	10.9	0.82
Flow to duodenum, g/d				
Total	408.2	419.2	19.8	0.71
NAN ³	389.5	399.0	26.8	0.80
Fecal output, g/d	118.6	118.4	6.2	0.98
Apparent digestion in stomach, %	-12.8	-16.2	7.1	0.76
Apparent digestion in intestine, %	71.1	72.0	2.1	0.77
Apparent digestibility in GIT, %	67.8	68.0	1.6	0.93

¹Least squares means with SEM, $n = 5$.

²Gastrointestinal tissue.

³NAN = nonammonia N.

intestine ($P = 0.01$) and total GIT ($P = 0.0006$). The RPM had no effect on the duodenal appearance, or on the apparent digestibility of other AA in different segments of the GIT. Data from the two cows equipped with both duodenal and ileal cannulae averaged over

Table 4. Effect of ruminally protected Met on daily flow and digestion of Met, essential amino acids (EAA) without Met and nonessential amino acids (NEAA) in dairy cows.¹

Item	Mepron M85 (g/d)		SEM	P
	0	72		
Met, g/d				
Intake	33.8	99.6	0.5	0.001
Flow to duodenum	37.2	80.8	10.8	0.05
Fecal output	15.3	18.6	1.4	0.18
Digestibility in stomach, %	-10.5	19.5	12.8	0.17
Digestibility in intestine, %	59.4	75.7	2.6	0.01
Digestibility in GIT, ² %	55.5	81.7	1.9	0.0006
EAA without Met, g/d				
Intake	804.2	781.6	22.7	0.52
Flow to duodenum	860.1	861.5	47.9	0.99
Fecal output	247.2	230.9	11.6	0.38
Digestibility in stomach, %	-7.6	-9.1	6.6	0.88
Digestibility in intestine, %	71.5	73.5	2.0	0.51
Digestibility in GIT, %	69.7	71.4	1.4	0.46
NEAA, g/d				
Intake	946.9	923.7	30.7	0.62
Flow to duodenum	946.4	947.1	49.3	0.99
Fecal output	264.3	248.1	11.9	0.39
Digestibility in stomach, %	-0.7	-2.1	6.7	0.89
Digestibility in intestine, %	72.2	74.1	1.9	0.53
Digestibility in GIT, %	72.5	74.0	1.3	0.48

¹Least squares means with SEM, $n = 5$.

²Gastrointestinal tissue.

Table 5. Apparent digestibility of amino acids in the small intestine versus the whole intestine of lactating dairy cows.¹

AA	Site		SEM	P
	SI ²	WHOLE ³		
Arg	75.8	80.8	0.8	0.04
His	55.9	69.5	2.4	0.06
Ile	68.1	77.9	0.8	0.01
Leu	67.7	78.1	1.1	0.02
Lys	74.7	80.2	1.2	0.08
Met	65.7	73.5	2.1	0.12
Phe	62.6	72.8	1.8	0.06
Phe	62.6	72.8	1.8	0.06
Thr	59.8	75.8	1.6	0.02
Val	58.4	71.6	2.4	0.06
Ala	61.6	76.7	1.4	0.02
Asp	65.0	77.9	1.3	0.02
Asp	65.0	77.9	1.3	0.02
Cys	46.7	65.9	2.4	0.03
Glu	66.0	80.0	1.0	0.01
Gly	66.7	78.2	1.7	0.04
Pro	59.8	75.6	0.2	0.0005
Ser	61.0	77.3	1.7	0.02
EAA	66.5	76.5	1.4	0.03
NEAA	63.9	77.8	1.1	0.01
TAA	65.2	77.2	1.2	0.02

¹Least squares means with SEM, n = 2.

²SI = % disappeared between duodenum and ileum.

³Whole = % disappeared between duodenum and feces.

treatments indicated (Table 5) that apparent digestibility of the total AA in the whole intestine (77.2%) was higher ($P = 0.05$) than apparent digestibility in the small intestine (65.2%). The proportion that apparently disappeared from the small intestine (duodenum–ileum) compared with the apparent disappearance from the whole intestine (duodenum–feces) varied from one AA to the other. Cys was the lowest at 70.9% and Arg the highest at 93.8%, while Met disappearance in the small intestine amounted to 89.3% of Met disappearing in the whole intestine.

Blood Metabolites

Arterial plasma Met concentration (Table 6) increased with RPM (45 vs. 18 μM); however, this effect was not significant ($P = 0.16$). Total AA concentration decreased ($P = 0.01$) due to a numerical drop of all AA, except Met and Arg. This reduction reached significance in the case of Ala ($P = 0.02$) and Asp ($P = 0.04$) while it tended to reach statistical significance in the case of Thr, Val, Lys, and Phe ($P < 0.10$). Feeding RPM resulted in higher concentrations of urea-N and glucose ($P = 0.05$) in arterial plasma (Table 7). Circulating levels of insulin and glucagon were unaffected.

Nitrogen Balance and Milk Composition

The addition of RPM had no effect on the amount of N excreted in the feces and urine and in the amount

Table 6. Effect of ruminally protected Met on plasma arterial concentrations of amino acids (μM) in lactating dairy cows.¹

Amino acid ²	Mepron M85 (g/d)		SEM	P
	0	72		
TAA (mM)	1.68	1.55	0.002	0.01
EAA (mM)	0.77	0.73	0.02	0.31
NEAA (mM)	0.91	0.81	0.03	0.24
BCAA (mM)	0.43	0.38	0.01	0.21
Alanine	193	180	0.4	0.02
Arginine	67	67	3.7	0.96
Asparagine	32	28	0.6	0.16
Aspartate	8	6	0.1	0.04
Citrulline	86	74	2.4	0.19
Cysteine	45	35	7.0	0.50
Glutamate	70	64	1.9	0.26
Glutamine	226	217	7.1	0.54
Glycine	209	184	5.2	0.18
Histidine	31	18	5.2	0.34
Isoleucine	110	100	4.3	0.34
Leucine	114	105	4.5	0.38
Lysine	69	63	0.6	0.09
Methionine	18	45	4.8	0.16
Ornithine	38	35	2.2	0.43
Phenylalanine	49	43	0.7	0.10
Proline	73	66	14.7	0.80
Serine	83	69	2.8	0.18
Taurine	30	29	4.1	0.88
Threonine	89	74	1.0	0.06
Tyrosine	42	40	1.6	0.46
Valine	205	168	2.5	0.06

¹Least squares means with SEM, n = 2.

²TAA = Total AA; EAA = essential AA; NEAA = nonessential AA; BCAA = branched-chain AA.

of N secreted in milk (Table 8). Excretion of purine derivatives and urea N in the urine (data not shown) did not change. Milk yields and concentrations of fat, protein, and casein were unaffected by RPM (Table 9). However, milk lactose concentration was elevated ($P = 0.03$) with RPM.

Intestinal Disappearance versus MDV and PDV Appearance

Data comparing the disappearance in the small intestine of essential AA, their net flux across the MDV and

Table 7. Effect of ruminally protected met on arterial concentrations of metabolites and hormones in lactating dairy cows.¹

Item	Mepron M85 (g/d)		SEM	P
	0	72		
Blood				
Ammonia-N (mM)	0.11	0.12	0.02	0.76
Urea-N (mM)	9.23	10.19	0.05	0.05
Plasma				
Glucose (mM)	2.81	3.11	0.02	0.05
Insulin (ng/ml)	0.45	0.45	0.03	0.99
Glucagon (ng/ml)	0.13	0.14	0.01	0.50

¹Least squares means with SEM, n = 2.

Table 8. Effect of ruminally protected Met on nitrogen balance in dairy cows.¹

Item	Mepron M85 (g/d)		SEM	P
	0	72		
Intake, g/d	391.7	414.6	25.4	0.59
Fecal output, g/d	130.4	136.9	12.0	0.74
Urinary output, g/d	152.3	151.8	12.3	0.98
Milk output, g/d	87.5	86.9	4.3	0.93
Apparent digestion, %	66.7	67.2	1.1	0.81
Retained, g/d	21.4	38.9	10.7	0.37

¹Least squares means with SEM, n = 3.

PDV, and secretion in milk, are shown in Table 10. Considering that the small intestine is the site of absorption of AA (33), total intestinal disappearance was adjusted for each AA to represent the proportion that apparently disappeared in the small intestine only, with values presented in Table 5. Overall, the mean disappearance in the small intestine of essential AA represented 101% of MDV flux, while PDV flux averaged 62% of MDV flux (Table 10). Essential AA secreted in milk averaged 68% of PDV flux and 42% of MDV flux. When compared with the PDV flux, Arg had the lowest transfer efficiency (39%) while Val (93%) was the most efficiently transferred essential AA into milk. The same comparison with the MDV flux revealed again that Arg (24%) was the least efficiently transferred essential AA, while Lys (54%) was the most efficiently transferred essential AA.

In the case of nonessential AA, mean disappearance in the small intestine of Asp, Glu, Cys, Pro, and Gly was considerably higher than their respective MDV fluxes, while 116% of Ala and 123% of Ser that disappeared from the small intestine were recovered in the MDV. Intestinal disappearance of nonessential AA amounted to 131% of MDV flux, while PDV flux represented 50% of MDV flux. Negative PDV fluxes of Cys and Gln indicate a net removal of those AA by the PDV. Of all nonessential AA, Ala (70 g/d) was the AA that had the highest flux through the PDV.

Table 9. Effect of ruminally protected Met on milk yield and milk composition.¹

Item	Mepron M85 (g/d)		SEM	P
	0	72		
Milk, kg/d	17.2	16.9	0.60	0.76
Fat, %	4.88	4.78	0.24	0.79
Crude Protein, %	3.29	3.30	0.11	0.93
Casein, %	0.308	0.309	0.01	0.97
Lactose, %	4.97	5.24	0.03	0.03

¹Least squares means with SEM, n = 3.

Table 10. Intestinal disappearance, mesenteric (MDV) and portal (PDV) drained viscera fluxes and milk output of amino acids (g/d).

AA	Intestinal disappearance	MDV Flux	PDV Flux	Milk Output
Arg	79.6	81.8	50.1	19.5
His	23.6	29.9	22.4	15.0
Ile	81.7	83.5	50.9	32.8
Leu	127.7	117.6	79.6	54.0
Lys	107.9	82.5	59.7	45.1
Met	36.3	36.7	24.3	14.5
Phe	69.6	69.6	53.1	27.3
Thr	65.2	74.8	28.2	25.6
Val	77.9	86.4	40.0	36.7
EAA	669.5	662.8	408.2	270.5
Ala	88.0	102.5	70.0	18.9
Asp	153.4	4.1	13.0	23.4
Cys	15.1	4.3	-24.4	4.5
Glu	191.1	20.7	16.2	67.4
Gly	88.8	50.7	37.5	11.1
Pro	64.5	31.8	5.5	55.1
Ser	58.2	71.6	43.6	31.2
Asn		56.1	29.9	18.9
Cit		32.9	16.1	
Gln		37.7	-35.6	52.3
Orn		16.0	21.7	
Tau		0.6	10.4	
Tyr		72.2	44.6	28.4
NEAA	659.0	501.3	248.5	311.2
TAA	1328.5	1164.1	656.7	581.7

DISCUSSION

The diet used in this experiment was designed to cover energy and protein requirements (22), except for Met as a percentage of essential AA (35). Based on calculations made with the Mepron Dairy Ration Evaluator (21), it was estimated that Met represented 3.3% of essential AA absorbed in the small intestine, which represented 66% of the recommended level (> 5%) (35). Lysine supply (16.6% of essential AA) was higher than the recommended level (> 15%). The addition of RPM (72 g/d) more than doubled (7.1%) the proportion of Met as a percentage of essential AA, while Lys dropped to 15.8% of essential AA absorbed in the small intestine.

Apparent digestibilities of OM, N and AA determined in this trial are in agreement with reported data, in which Met was included in the diet as RPM (6) or infused in the abomasum as D,L Met (41). Duodenal non-ammonia N flux was numerically higher than N intake (107 to 111%), indicating that N was recycled to the rumen either through the rumen wall or via saliva (39).

The addition of 72 g of RPM translated into an increase of 65.8 g of Met ingested per day (Table 4). This resulted in an elevation of the duodenal flow of Met (43.6 g/d). Assuming that RPM had little effect on microbial protein synthesis, such that all the increment in the duodenal flow originated from RPM intake, 66% of Met in RPM would have bypassed the rumen. Hence, considering the small increase in Met fecal excretion

(3.3 g/d), it appears that 92% of the extra Met delivered at the duodenum with RPM disappeared in the whole intestine. When corrected for apparent losses of Met in the large intestine (Table 5), 82% of the extra Met was apparently digested in the small intestine. This is in the same range as the disappearance in the small intestine (75%) of cannulated (duodenum and ileum) nonlactating cows estimated in vivo (4).

Data from the two cows (#440 and #5073) equipped with both duodenal and ileal cannulae (Table 5) confirmed that for all AA, disappearance in the whole intestine overestimates ($P < 0.05$) the disappearance in the small intestine (30). Disappearance in the small intestine of TAA, 65.2%, was in close agreement with values (57 to 76%) reported in the literature (30). Essential AA disappearance in the small intestine was numerically higher than nonessential AA (66.5 vs. 63.9%). The opposite was true for the disappearance in the whole intestine, indicating that nonessential AA tend to be digested more in the hindgut. As previously reported (30), intestinal disappearance of Met (on the control diet), His, and Cys were low relative to other AA. In this trial, the addition of RPM increased the apparent disappearance of Met, due to the high digestibility of Met in RPM.

Arterial plasma Met concentration numerically increased after feeding RPM as observed previously (6, 25, 26). Circulating levels of Met were similar on the basal diet (18 μM); however, the two cows responded differently to the addition of RPM (37 vs. 52 μM). Considering the very small number of observations, this might explain why the treatment effect did not reach statistical significance ($P = 0.16$). A similar increase was observed when 20 g/d of D,L-Met were infused in the abomasum of lactating dairy cows fed grass silage and grain (41). When RPM was fed, the small numerical increase in Met concentration was accompanied by a significant decline in Ala and Asp and a numerical decrease in all AA except Arg, resulting in a significant decline in circulating total AA ($P = 0.01$). Similar observations were made with cows in midlactation fed 60 g of RPM per day (6) or abomasally infused incremental amounts of Met (11, 41). Since the quantitative uptake of AA by the mammary gland remained similar with increasing supply of Met (11, 41), it can be hypothesized that the reduction in plasma AA concentrations would be due to 1) a decrease in absorption of AA, 2) an increase in hepatic removal of AA, 3) an effect on AA utilization by other tissues (e.g., muscle). Results from studies with dairy cows (5) and steers (20) are contrary to the first hypothesis and tend to support the second hypothesis. However, since metabolism of AA by the muscle was not determined, the hypothesis that high levels of Met would enhance muscle uptake of branched-

chain AA (BCAA) for catabolism or protein synthesis (6) cannot be ruled out.

The observed increase in blood urea-N and glucose ($P = 0.05$) with no effect on insulin contrasts with previously published data on RPM (6). However, when Met was infused in the abomasum, plasma glucose tended to increase linearly (41). Differences in the physiological state of the cows (mid vs. late lactation) and energy balance (28) might explain such discrepancies. Considering that high concentrations of Met can be toxic to cells and that Met interacts with other AA such as the BCAA by using the same dehydrogenase complex in the transamination pathway (18), it could be hypothesized that in this trial, AA such as Ala and Asp, were oxidized, producing glucose and leading to the synthesis of urea (42). Thus, the elevation in circulating glucose translated into an increased content of lactose in the milk, which contrasts with previous observations where the same RPM source had no effect (6, 25, 26) on lactose.

Considering the very small number of observations, MDV and PDV fluxes must be interpreted cautiously. Nevertheless, it is interesting to note that overall (Table 10), the apparent disappearance of essential AA from the small intestine was equivalent (101%) to their net appearance in the MDV as observed previously in sheep (19). This similarity does not rule out utilization of AA by the intestinal wall since apparent digestion in the small intestine underestimates true digestibility due to endogenous losses. A study conducted with the same cows revealed that endogenous N secretions represented between 10 and 20% of duodenal N flow (9). As a substantial fraction of the endogenous secretions originate from viscera drained by the portal vein but not by the mesenteric vein (e.g., stomach), this fraction would provide an additional supply of AA to the mesenteric vein that is unaccounted for in terms of net utilization. In the case of nonessential AA, despite the very large intestinal disappearance of Glu and Asp (Table 10), there was little net appearance of these AA in the portal vein, confirming that Glu and Asp are important fuels for gastrointestinal metabolism (30). As previously observed with rats and ruminants (30), Ala was the nonessential AA released in the greatest amount by the PDV. Ala is a carrier of N and C from the gut to the liver for the synthesis of glucose and urea (30).

Net PDV fluxes of total AA averaged 56% of net MDV fluxes. Based on α -amino N measurements, Reynolds and Huntington (29) reported an average PDV:MDV ratio of 80%, while Seal and Parker (36) observed an average ratio of 64% based on individual AA analysis. Net PDV fluxes of each essential AA varied from 38%, for Thr, to 76%, for Phe, of MDV fluxes. In the present trial, net apparent stomach utilization of total AA, essential AA, and nonessential AA averaged respectively,

44, 38, and 50% of MDV fluxes, which is contrary to the suggestion made by others (36) that venous blood from the stomach and large intestine may have a relatively lower ratio of nonessential AA to essential AA compared with blood draining the MDV reflecting a reduced demand for AA by these sections of the gut. Nevertheless, these observations confirm the high metabolic activity of the whole GIT, which uses, on a net basis, more than 30% of AA absorbed. Such close agreement between data was observed although mesenteric blood flow represented only 12% of portal blood flow in this trial, while published data with steers (29, 36) and sheep (19) indicate that MDV blood flow accounts for 40% of PDV blood flow. The reason for this apparent discrepancy is not known; however, considering the anatomy of the portal vein in bovine (3), it can be hypothesized that the tip of the catheter was located in a branch of the cranial mesenteric vein where most of the absorption took place; therefore, compensating for the underestimation of blood flow. Reported data with sheep (23) indicated that depending on the position of the mesenteric vein catheter, mesenteric blood flow can represent as little as $18.7 \pm 1.2\%$ of portal blood flow and give biologically reasonable estimates of AA absorption.

CONCLUSIONS

The addition of RPM to the diet did increase the amount of Met delivered to the duodenum and apparently absorbed in the small intestine due to the high level of digestibility (82%) of Met from RPM. Increased absorption of Met was reflected in arterial plasma. However, numerically increased arterial concentrations of Met had no effect on milk protein production. When averaged across treatments, the amount of Met that apparently disappeared from the small intestine was almost equal (99%) to the amount of Met flowing through the MDV while the amount of Met flowing through the PDV represented 66% of that absorbed through the MDV. The PDV:MDV flux ratio of other essential AA varied between 38% for Thr and 76% for Phe. Net portal fluxes of Glu and Asp were close to zero, while net portal fluxes of Gln were negative, confirming the importance of those nonessential AA as metabolic fuels for the GIT. More research is needed to confirm these observations and quantify the impact of AA metabolism by the MDV and PDV.

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