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Intrauterine bacterial inoculation and level of dietary methionine alter amino acid metabolism in nulliparous yearling ewes

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ABSTRACT: Using an intrauterine bacterial inoculation method, our objective was to determine the effects of acute sepsis and level of dietary metabolizable Met (MM) on splanchnic metabolism of AA in ewes. Twenty-four nulliparous yearling Rambouillet-cross ewes (initial BW = 65.1 ± 0.6 kg), surgically fitted with chronic-indwelling catheters in hepatic and portal veins, a mesenteric vein and artery, and the uterine lumen, were assigned to a 2 × 2 factorial arrangement of treatments. Factors were intrauterine bacterial inoculation (noninoculated vs. inoculated) and level of MM [low (2.28 g/d) vs. high (3.91 g/d)]. Beginning 12 h before sampling, inoculated and noninoculated ewes received 10-mL intrauterine infusions of Escherichia coli (9.69 × 10^11 cfu) + Arcanobacterium pyogenes (2.76 × 10^12 cfu) and of sterile saline, respectively. Uterine infection was induced in ewes that received intrauterine bacterial inoculations, but not in ewes infused with sterile saline. Bacterial inoculation resulted in increased hepatic release and plasma concentrations of aromatic AA used for acute-phase protein synthesis, increased hepatic removal and decreased plasma concentrations of AA used for glutathione synthesis, and decreased plasma concentrations of some gluconeogenic and acetogenic AA used for glucose recycling and anaerobic energy production, respectively (P < 0.05). In ewes fed high-MM diets, compared with low-MM diets, a consistent net hepatic uptake of Phe occurred throughout the sampling period, more Asp was released from the portal-drained viscera, and hepatic vein glucose concentrations were greater (P < 0.05). We conclude that Met seemed to be limiting in low-MM ewes, and as such, would continue to be limiting during sepsis. However, additional MM, in excess of the dietary requirement, would not necessarily result in a benefit to ewes experiencing acute sepsis.

Key words: methionine, amino acid, sepsis, sheep, uterine infection

INTRODUCTION

Disease alters AA metabolism (for review, see Wannemacher, 1977). Site-specific use of AA may shift from one tissue to another during a host’s defense against sepsis. Anabolism of skeletal-muscle protein shifts to catabolism and makes AA available for acute-phase protein synthesis, cellular proliferation, and energy. However, AA composition of skeletal muscle may not complement the AA requirement for the host’s immune system to mitigate sepsis (Reeds and Jahoor, 2001).

Thus, gram for gram, more muscle protein must be mobilized to support synthesis of acute phase proteins (Reeds et al., 1994). When considering AA metabolism in diseased hosts, Reeds and Jahoor (2001) astutely surmised, “amino acids..., which are not traditionally regarded as essential, become, at least potentially, limiting”.

Endotoxin infused into the artery of sheep decreased venous plasma concentrations of Met and other AA (Hofford et al., 1996). When considering that Met is limiting in growing ruminants (Storm and Ørskov, 1984; Greenwood and Titgemeyer, 2000), acute sepsis may further antagonize the availability of Met. In rats given Escherichia coli injections (i.v.), Cys utilization for protein synthesis doubled (Malmezat et al., 2000a), with a substantial portion of Cys derived from Met transsulfuration (Malmezat et al., 2000b). Additionally, the key role of S-adenosylmethionine in cytokine production and acute-phase protein synthesis (Arias-Díaz et al., 1996; Song et al., 2002, 2005) suggests that poten-
tial interactions between Met and acute sepsis may affect splanchnic metabolism of AA in sheep.

Therefore, using an intrauterine bacterial inoculation method (Lewis and Wulster-Radcliffe, 2006), our objective was to determine the effects of acute sepsis and level of dietary metabolizable Met (MM) on splanchnic metabolism of AA in nulliparous yearling ewes.

MATERIALS AND METHODS

Experimental Ewes and Design

An Institutional Animal Care and Use Committee (USDA, ARS, Dubois, ID) reviewed and approved all experimental and husbandry procedures for this study.

Twenty-four nulliparous yearling Rambouillet-cross ewes (initial BW = 65.1 ± 0.6 kg), surgically fitted with chronic-indwelling catheters in hepatic and portal veins, a mesenteric vein and artery, and uterine lumen, were housed individually (80 × 240-cm pens with slatted floors) in an enclosed facility (temperature = 22.3 ± 2.5°C) and allowed ad libitum access to water and sugar beet-pulp pellets. In a randomized complete block design, ewes were assigned (as described below) to a 2 × 2 factorial arrangement of randomized treatments. Treatment factors were intrauterine bacterial inoculation (noninoculated vs. inoculated) and level of metabolizable Met (low MM [2.28 g/d] vs. high MM [3.91 g/d]). A timeline of the experimental events is presented in Figure 1.

Surgeries

For surgical placement of catheters, ewes were anesthetized (2,747 ± 105 mg of sodium pentobarbital administered i.v. throughout surgery [198 ± 6 min]) and placed in dorsal recumbency. The uterus was elevated through a midventral incision (~5 cm), and a catheter (130 cm in length × 1.02 mm i.d. × 1.78 mm o.d. sterile polyvinyl tubing; Tygon MicroBore Tubing, Formulation S-54-HL, VWR International, Bristol, CT) was inserted (~6 cm) into the uterine lumen via a puncture wound and anchored to the uterine body. The incision was closed, and ewes were rotated to a left lateral recumbency. A paracostal incision was made approximately 3 to 5 cm caudal to the last rib. Catheters were placed 10 to 15 cm into a hepatic vein (catheter = 130 cm in length × 1.27 mm i.d. × 2.29 mm o.d. polyvinyl tubing) of the left lobe; 3 to 4 cm into the portal vein (catheter = 130 cm in length × 1.27 mm i.d. × 2.29 mm o.d. polyvinyl tubing), with a final placement that was approximately 2 cm inside the body of the liver; and 13 and 5 cm into a mesenteric artery and vein (catheters = 150 cm long × 1.27 mm i.d. × 2.29 mm o.d. polyvinyl tubing), respectively, in the second and sixth accessible caudal vascular loops of the small intestinal vasculature. All catheters were anchored at the insertion sites and exteriorized, together, near the paracostal incision and subcutaneously along the last rib through a 1-cm incision above and near the first lumbar vertebra. Vascular catheter void volumes were filled with heparinized saline (100 U/mL), and 2 knots were tied at the exterior ends of each catheter. Ewes received 10 mL of Penicillin G Procaine i.p. (300,000 U/mL; Agri-Cillin, AgriLabs, St. Joseph, MO) before closure, and 10 mL s.c. the following morning. Surgeries were conducted over a 3-wk period (8 ewes/wk; surgery week = blocking factor). To encourage intake soon after surgery, chopped (1 cm length) alfalfa hay was fed with sugar beet-pulp pellets for 2 d (Figure 1; Table 1).

Treatment Delivery

Beginning 3 d after the surgeries, all ewes were fed a sugar beet-pulp pellet diet (Table 1) in equal portions daily (0700 and 1900) at 1.3 kg of DM/d (Figure 1) throughout the experiment. Ewes were assigned to MM treatments and received an estimated 2.28 and 3.91 g of MM/d for low-MM and high-MM treatments, respectively (Table 1). Ewes assigned to high-MM treatment were provided supplemental MM from Mepron M85 (Degussa Corporation, Kennesaw, GA), a rumen-protected feed product. High-MM ewes were bolused twice daily, at feeding, with preweighed gelatin capsules (9.9 × 26.1 mm; Torpac Inc., Fairfield, NJ) containing 2.4 g of Mepron M85, which was estimated to provide an additional 1.63 g of MM/d (Table 1).

Approximately 12 d after surgeries (Figure 1), ewes received 10-mL intrauterine infusions of either 9.69 × 1011 cfu of Escherichia coli + 2.76 × 1012 cfu of Arcanobacterium pyogenes or sterile saline, according to the inoculation treatment assignment. The bacteria were originally obtained from a dairy cow, purified, and stored (Seals et al., 2002). To ensure susceptibility to sepsis, ewes were injected (i.m.) with 5 mg of prostergone (2 mg/mL of canola oil, i.m.) twice daily at feeding, beginning 5 d before intrauterine infusions and continuing throughout the experiment (Lewis and Wulster-Radcliffe, 2006).

Sampling

Sampling began 12 h after intrauterine infusions (refer to Figure 1). A primed (15 mL) and constant infusion (0.57 mL/min; KDS 220 MultiSyringe Pump, KD Scientific, Holliston, MA) of paraaminohippurate (PAH; 3% wt/vol, pH 7.4) was administered, beginning 1 h before sampling, into the mesenteric vein for estimation of venous and arterial plasma flows. Before feeding (h 0) and every 2 h throughout the 12-h sampling period, simultaneous artery, portal vein, and hepatic vein blood samples were collected into 6-mL syringes. Blood was transferred (6 mL) immediately to tubes containing 12.15 mg of EDTA (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were centrifuged (1,500 × g, 30 min, 4°C), and plasma was removed and stored (~20°C). At h 0 and 6, simulta-
neous artery and hepatic vein blood samples were collected in 1-mL syringes tipped with ∼40 µL of heparinized saline (1,000 U/mL) and immediately analyzed for hemoglobin (Type B Roche Opti CCA cassettes, AVL OPTI Critical Care Analyzer; Osmetech Inc., Roswell, GA; inter- and intraassay CV < 0.01%). Neither frequency nor volume of sampling altered (P = 0.08 to 0.67) artery or hepatic-vein blood hematocrit (based on hemoglobin; data not shown). Rectal temperatures were measured immediately before intrauterine infusions (h −12) and then again at feeding (h 0) and every 4 h throughout the duration of the 12-h sampling period.

**Sample Analyses**

Plasma concentrations of PAH, AA (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Asn, Asp, Cys, Glu, Gln, Gly, Orn, Pro, Ser, and Tyr), glucose, and lactate were analyzed for all vessel samples from each sampling time. Plasma PAH (inter- and intraassay CV < 5.8%) was measured according to the method of Huntington (1982) and with modifications of Taylor (2000). Plasma glucose (inter- and intraassay CV < 4.0%; kit #G7517, Pointe Scientific Inc., Lincoln Park, MI, and based on the method of Trinder, 1969), and lactate (inter- and intraassay CV < 3.8%; kit #L7596, Pointe Scientific Inc., and based on the method of Noll, 1974) were measured using prepared kits. Plasma AA (CV < 8%) were measured according to the method of Chen et al. (2002) with the following modifications. Briefly, the amount of methanol, methanol:0.1 M HCl solution (vol/vol), and 0.1 M HCl used to prepare the SCX column was increased to 2.5 mL. The amount of sample and standard was 250 µL, with 5 µL of L-norvaline as the internal standard, and 900 µL of 1 M ammonium hydroxide was used to elute the AA. Due to difficulties (CV > 10%) in initial analysis attempts and depleted sample, plasma subsamples within ewe and vessel were pooled across sampling times for final ammonia and urea analyses. Urea (inter- and intraassay CV < 1.8%; kit #DIUR, BioAssay Systems, Hayward, CA, and based on the method described by Jung et al., 1975) and ammonia (inter- and intraassay CV < 7.1%; kit AA0100, Sigma-Aldrich Inc., Saint Louis, MO, and based on the method...
Cloudy uterine flushings, large amounts of sediment were signs that a uterus was not infected. Inability to culture dense colonies of bacteria from the cytes, no signs of endometrial inflammation, and the infection is consistent with a method used for cervical mucus from cattle (Sheldon et al., 2006).

Calculations and Statistics

Due to improper catheter placement and catheter patency complications, some sample analysis values were excluded from the data set or missing. For example, hepatic vein catheters were placed in the vena cava of 3 ewes, and artery, hepatic vein, and portal vein catheter patency were lost periodically in some ewes during sampling. The greatest incidence of patency complications occurred at h 6, resulting in removal of all data corresponding to h 6. These incidences resulted in decreased replication, especially when plasma flow rates and metabolite/AA fluxes were calculated. Therefore, for some measures, our ability to detect treatment effects or treatment interactions was limited. Experimental units for data corresponding to each vessel concentration, plasma flow, flux, and hepatic extraction ratio are presented in Table 2.

Table 1. Feedstuffs, diet composition (DM basis), and daily metabolizable Met (MM) intake

| Item                | Treatment            | Low MM | High MM |
|---------------------|----------------------|--------|---------|
| Feedstuffs, %       |                      |        |         |
| Sugar beet-pulp pellet | 99.3                | 99.3   |         |
| Trace mineral salt1 | 0.62                 | 0.62   |         |
| Vitamin E mix2      | 0.06                 | 0.06   |         |
| Diet composition    |                      |        |         |
| ADF,3 %             | 23.2                 | 23.2   |         |
| TTN,4 %             | 70.8                 | 70.8   |         |
| CP, %               | 10.7                 | 10.7   |         |
| UIP,5 % of CP       | 54.4                 | 54.4   |         |
| Total MM, g/d       | 2.28                 | 3.91   |         |
| MM supplemented7    | 1.89                 | 1.89   |         |
| MM supplemented7    | 0                    | 1.63   |         |

195.0% NaCl, 0.55% Ca, 0.0007% Cu, 0.002% I, 0.07% Fe, 0.09% Mg, 0.0007% Mn, 0.05% P, 0.12% K, and 0.13% S; DM basis (Redmond NTM salt, Redmond, UT).
26,287 IU of vitamin E/kg (E-5000, Vita-Flex Nutrition, Council Bluffs, IA).
3AOAC (1990) Method No. 973.18.
4TDN = 88.9 − (ADF, % × 0.779).
5Undegradable intake protein (Mortenson et al., 2005).
6MMUIP = Met provided by dehydrated sugar beet pulp (0.65%) × UIP, % of CP × 0.8 digestibility (NRC, 1996). MMUIP = microbial CP (13% of dietary TDN; NRC, 1996) × 0.64 g of digestible AA/g microbial CP × 0.0247 g of Met/g of AA (Storm and Ørskov, 1983).
7Supplemental MM provided as Mepron M85 (Degussa Corporation, Kennesaw, GA), which is a high-Met rumen-protected product.

of Neeley and Phillipson, 1988) concentrations were measured using prepared kits.

Postmortem Sampling and Examination

All animals were euthanized (captive bolt and exsanguination) after the last sampling (h 12; Figure 1), reproductive tracts were collected, and catheter placement was examined. To determine presence of infection, uterine contents were flushed with 15 mL of sterile saline (Lewis and Wulster-Radcliffe, 2006). Flushing turbidity (as determined by measuring the optical density at 560 nm) and sediment (centrifugation, 1,500 × g for 10 min) were measured. The uterus was opened, the luminal surface was scraped with a sterile-cotton swab, and swab contents were transferred to a culture plate (Columbia SB Agar, Becton, Dickinson, and CO., Sparks, MD) and incubated (12 h at 37°C). The plates were examined for bacterial growth (absent or present). Clear uterine flushings, small amounts of sediment (<5% by volume), which contained bacteria and leukocytes, no signs of endometrial inflammation, and the inability to culture dense colonies of bacteria from the flushings were signs that a uterus was not infected. Cloudy uterine flushings, large amounts of sediment (>5%, but usually >20%, by volume), an inflamed endometrium, and the ability to culture dense colonies of bacteria from the flushings were signs of infection. This method for determining whether a sheep uterus contained an infection and then classifying the severity of the infection is consistent with a method used for cervical mucus from cattle (Sheldon et al., 2006).
Table 2. Number of experimental units for data analyses corresponding to each vessel concentration, plasma flow, flux, and hepatic extraction ratio

| Metabolizable Met treatment¹ | Low   | High  |
|-----------------------------|-------|-------|
| Inoculation treatment²      |       |       |
| Item                        | No    | Yes   | No    | Yes   |
| Vessel                      |       |       |       |       |
| Artery                      | 4     | 4     | 5     | 5     |
| Hepatic vein                | 4     | 2     | 5     | 5     |
| Portal vein                 | 5     | 4     | 6     | 6     |
| Plasma flow                 |       |       |       |       |
| Artery                      | 4     | 3     | 4     | 4     |
| Hepatic vein                | 4     | 3     | 4     | 4     |
| Portal vein                 | 4     | 4     | 5     | 4     |
| Flux³                       |       |       |       |       |
| PDV⁴                        | 4     | 4     | 5     | 4     |
| Hepatic                     | 3     | 2     | 4     | 4     |
| TST⁴                        | 3     | 2     | 4     | 4     |
| HER⁵                        | 3     | 2     | 4     | 4     |

¹Low = 2.28 g metabolizable Met/d; High = 3.91 g metabolizable Met/d.
²No = intrauterine infusion of sterile saline; Yes = intrauterine bacterial inoculation (9.69 × 10¹¹ cfu of Escherichia coli and 2.76 × 10¹² cfu of Arcanobacterium pyogenes).
³For low-metabolizable Met × no inoculation ewes, experimental units for hepatic and TST ammonia and urea fluxes were 4.
⁴PDV = portal-drained viscera; TST = total-splanchnic tissue.
⁵HER = hepatic extraction ratio.

Figure 2. Rectal temperatures [least squares means ± SEM (bars)] of nulliparous yearling Rambouillet crossbred ewes that received an intrauterine sterile saline infusion (○) or bacterial inoculation (●; 9.69 × 10¹¹ cfu of Escherichia coli and 2.76 × 10¹² cfu of Arcanobacterium pyogenes). Sampling time × inoculation interaction was significant (P < 0.01). a,bMeans with unlike superscripts within each sampling time are different (P < 0.01).

RESULTS

Sepsis Verification and Ewe Performance

Neither the inoculation × MM interaction (P = 0.04 to 0.78) nor MM main effect for uterine-sepsis indicators were significant (P = 0.49 to 0.91). However, uterine sepsis was induced in ewes that received intrauterine-bacterial inoculations when compared with those that received intrauterine-saline infusions. Specifically, inoculated ewes had greater rectal temperatures (P < 0.05; Figure 2) following inoculation, and subsequent uterine flushings were greater in volume (P < 0.01), more turbid (P < 0.01), and resulted in larger pellets following centrifugation (P < 0.01; Table 3) compared with noninoculated. Uteri from inoculated ewes appeared inflamed and had apparent pustule-like sites throughout the uterine lumen. Bacteria were present only in cultures from uteri inoculated with E. coli and A. pyogenes. Neither the treatment main effects nor the inoculation × MM interaction was significant for ewe BW and DMI on the day of sampling (P = 0.59 to 0.93). The grand mean BW and DMI (for the 12-h sampling period) was 60.4 ± 0.6 and 0.60 ± 0.03 kg, respectively.

Plasma Flow Rates

Although the inoculation × MM interaction was significant (P = 0.03) for hepatic vein plasma-flow rates, no differences (P = 0.07 to 0.92) among factor-combination means were detected. The detected interaction seemed to be because of a numerically lower hepatic vein plasma flow rate (124 ± 11 L/h) for the low-MM, noninoculated ewes than for ewes from other factor-combination treatments (data not shown). Neither the sampling time × treatment interactions nor treatment main effects were significant for hepatic artery and portal-vein plasma flow rates (P = 0.06 to 0.99). Grand mean plasma-flow rates were: portal vein = 106.6 ± 2.9 L/h, hepatic vein = 137.2 ± 3.3 L/h, hepatic artery = 32.8 ± 1.7 L/h.

AA and Metabolite Concentrations

The inoculation × MM interactions were significant for artery plasma Trp and Gln concentrations (P < 0.05;
Table 3. Indicators (least squares means) of sepsis in nulliparous yearling Rambouillet crossbred ewes that received either an intrauterine sterile-saline infusion or intrauterine bacterial inoculation

| Item                          | Inoculation treatment¹ | SEM | P-value² |
|-------------------------------|------------------------|-----|---------|
| Number of uteri              | No                     | 12  |        |
|                               | Yes                    | 11  |        |
| Flush volume,³ mL            |                        | 10.4|        |
|                               |                        | 13.7|        |
| Turbidity,⁴ OD               |                        | 0.19|        |
|                               |                        | 2.22|        |
| Sediment depth,⁵ cm          |                        | 0.2 |        |
|                               |                        | 2.1 |        |
| Bacterial presence in uterine cultures⁶ |            | 0/12|        |

¹No = intrauterine infusion of sterile saline; Yes = intrauterine bacterial inoculation (9.69 × 10¹¹ cfu of *Escherichia coli* and 2.76 × 10¹² cfu of *Arcanobacterium pyogenes*). Inoculation treatments occurred 12 h before commencement of blood sampling.
²F-test probability for effect of inoculation.
³Flush volume (mL) collected from the uterus after an intrauterine flush (postmortem) with sterile saline (15 mL).
⁴OD = optical density (560 nm) of the uterine flushing.
⁵Sediment depth (cm) of flushings was measured after centrifugation (1.500 × g, 10 min).
⁶Visual presence of bacterial growth expressed as counts of positive cultures per total counted cultures.

Table 4. Mean (least squares) plasma AA concentrations and metabolite fluxes in nulliparous yearling Rambouillet-cross ewes that were fed diets low or high in metabolizable Met¹ and received an intrauterine sterile-saline infusion or intrauterine bacterial inoculation²

| Item                          | Inoculation treatment² | SEM | P-value³ |
|-------------------------------|------------------------|-----|---------|
| Artery concentration, mM     | Low                    |     |         |
| Trp                           | 0.045ab                | 0.041ab |       |
|                               | 0.036a                 | 0.047b  | 0.004  | 0.05 |
| Gln                           | 0.287                  | 0.212  |       |
|                               | 0.217                  | 0.269  | 0.040  | 0.03 |
| PDV⁴ flux, mmol/h             |                        |       |         |
| Ammonia                       | 21.8a                  | 13.7b  |       |
|                               | 14.7ab                 | 20.6ab | 2.8    | 0.01 |
| TST⁵ flux, mmol/h             |                        |       |         |
| Ammonia                       | −0.56a                 | −3.53b |       |
|                               | −2.00ab                | −1.49ab| 0.99   | 0.05 |

¹Low = 2.28 g of metabolizable Met/d; high = 3.91 g of metabolizable Met/d.
²No = intrauterine infusion of sterile saline; Yes = intrauterine bacteria inoculation (9.69 × 10¹¹ cfu of *Escherichia coli* and 2.76 × 10¹² cfu of *Arcanobacterium pyogenes*). Inoculation treatments occurred 12 h before commencement of blood sampling.
³F-test probability for the metabolizable Met × inoculation interaction.
⁴PDV = portal-drained viscera.
⁵TST = total splanchnic tissue.
Table 5. Mean (least squares) plasma AA concentrations and AA and metabolite fluxes in nulliparous yearling Ramboullet-cross ewes that received an intrauterine sterile saline infusion or intrauterine bacterial inoculation

| Item          | No     | Yes    | SEM  | P-value |
|---------------|--------|--------|------|---------|
| Artery concentration, mM |        |        |      |         |
| Leu           | 0.122  | 0.097  | 0.008| 0.04    |
| Met           | 0.018  | 0.012  | 0.002| <0.01   |
| Phe           | 0.046  | 0.052  | 0.003| 0.05    |
| Thr           | 0.144  | 0.093  | 0.016| 0.04    |
| Ala           | 0.185  | 0.139  | 0.011| <0.01   |
| Asn           | 0.056  | 0.026  | 0.005| 0.02    |
| Gly           | 0.511  | 0.371  | 0.029| <0.01   |
| Pro           | 0.095  | 0.065  | 0.005| <0.01   |
| Ser           | 0.102  | 0.059  | 0.020| <0.01   |
| Hepatic-vein concentration, mM |        |        |      |         |
| Ala           | 0.168  | 0.114  | 0.017| <0.01   |
| Glu           | 0.146  | 0.114  | 0.027| <0.01   |
| Gly           | 0.506  | 0.364  | 0.052| 0.02    |
| Pro           | 0.098  | 0.071  | 0.012| 0.04    |
| Portal-vein concentration, mM |        |        |      |         |
| Met           | 0.022  | 0.016  | 0.003| 0.03    |
| Phe           | 0.053  | 0.060  | 0.004| 0.05    |
| Ala           | 0.215  | 0.163  | 0.012| <0.01   |
| Gly           | 0.539  | 0.394  | 0.037| <0.01   |
| Pro           | 0.106  | 0.079  | 0.008| 0.02    |
| Ser           | 0.128  | 0.087  | 0.017| 0.02    |
| PDV<sup>3</sup> flux, mmol/h |        |        |      |         |
| Lactate       | 4.33   | 8.89   | 1.2  | <0.01   |
| Asp           | 0.331  | 0.051  | 0.17 | 0.04    |

<sup>1</sup>No = intrauterine infusion of sterile saline; Yes = intrauterine bacteria inoculation (9.69 × 10<sup>12</sup> cfu of *Escherichia coli* and 2.76 × 10<sup>12</sup> cfu of *Arcanobacterium pyogenes*). Inoculation treatments occurred 12 h before commencement of blood sampling.

<sup>2</sup>F-test probability for effect of inoculation.

<sup>3</sup>PDV = portal-drained viscera.

Systolic-Tissue Flows and Hepatic Extraction Ratio

The sampling time × inoculation × MM interaction was significant for PDV-glucose flux (Figure 3; P = 0.05). The interaction was partly because of noninoculated, low-MM ewes having a different (P = 0.02) and positive PDV-glucose flux compared with noninoculated, high-MM ewes at h 12. This response would not be expected, because similar differences did not occur at previous sampling times (P = 0.06 to 0.99) and because the PDV-glucose flux of noninoculated, low-MM ewes did not return to near 0 values. Based on PDV-glucose fluxes of noninoculated, high-MM ewes at h 0 and 12, additional MM, when uterine sepsis is not present, seems to result in a significant PDV uptake of glucose immediately before feeding. Neither a significant (P = 0.06 to 0.84) net uptake nor release of glucose from the PDV was detected in ewes from other factor-combination treatments. The sampling time × MM interaction was significant for PDV-Gly flux (Figure 4; P = 0.02). At h 10, Gly flux in low-MM ewes was negative and different (P < 0.01) from high-MM ewes. The inoculation × MM interaction was significant for PDV-ammonia flux (P = 0.01; Table 4). When MM was low, intrauterine bacterial inoculation, compared with no inoculation, resulted in less ammonia being released from the PDV (P = 0.04). The inoculation effect was significant for PDV fluxes of lactate and Asp (P < 0.04; Table 5). Intrauterine bacterial inoculation, compared with no inoculation, resulted in more lactate but less Asp being released from the PDV. Neither the sampling time × treatment interactions nor treatment main effects were significant for PDV flux of His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Asn, Cys, Glu, Gln, Orn, Pro, Ser, Tyr, and urea (P = 0.07 to 0.98); grand means are reported in Table 6.

The sampling time × inoculation interaction was significant for Gly hepatic flux (P = 0.05; Figure 5). At h 8, noninoculated ewes had greater Gly hepatic flux than inoculated ewes (P < 0.01), which indicated that intrauterine-bacterial inoculation suppressed the hepatic release of Gly (P < 0.01) at this sampling time. The sampling time × MM interaction was significant for Phe hepatic flux (P = 0.04; Figure 6); however, no treatment differences were detected within any sampling time (P = 0.10 to 0.87). Generally, there was a net uptake of Phe in both MM treatment groups, except at h 4 for high-MM and h 2 and 8 for low-MM ewes. The MM treatment effect was significant for hepatic Asp flux (P = 0.03). The Asp flux was greater in high-MM ewes, compared with low-MM (0.168 vs. −0.028 ± 0.061 mmol/h, respectively). Overall, additional MM resulted in a significant net release, as opposed to neither a net uptake nor release of Asp from the hepatic tissue. Neither the sampling time × treatment interactions nor treatment main effects were significant for hepatic fluxes of His, Ile, Leu, Lys, Met, Thr, Trp, Val, Ala, Asn, Cys, Glu, Gln, Orn, Pro, Ser, Tyr, ammonia, glucose, lactate, and urea (P = 0.06 to 0.99); grand means are reported in Table 6.

The sampling time × inoculation × MM interaction was significant for TST-Asp flux (P = 0.05). At h 0, TST-Asp flux was less in low-MM × inoculation treatment ewes than in ewes from all other factor-combination treatments (data not shown; P < 0.01; grand mean is reported in Table 6). The sampling time × inoculation interaction was significant for TST-Gln flux (P = 0.05), but no inoculation treatment differences were detected at any sampling time (P = 0.06 to 0.92; grand mean is reported in Table 6). The inoculation × MM interaction...
was significant for TST-ammonia flux ($P = 0.05$). When MM was low, the net uptake of ammonia (negative flux) was greater in inoculated compared with noninoculated ewes ($P = 0.03$), but when MM was high, this effect did not occur (Table 4). Neither the sampling time $\times$ treatment interactions nor treatment main effects were significant for TST fluxes of His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Asn, Cys, Glu, Gly, Orn, Pro, Ser, Tyr, glucose, lactate, and urea ($P = 0.09$ to $0.98$); grand means are reported in Table 6.

The sampling time $\times$ MM $\times$ inoculation interaction was significant for the Phe HER (Figure 7; $P = 0.03$). Phenylalanine HER was greatest and positive in inoculated, low-MM ewes at h 2 and in inoculated, high-MM ewes at h 4 ($P < 0.05$). Regardless of level of MM, there was a brief and early (after a meal) proportional contribution of Phe to the plasma from the hepatic tissue of inoculated ewes. This response is different in noninoculated ewes, in which a consistent hepatic consumption of Phe was maintained. Neither the sampling time $\times$

treatment interactions nor treatment main effects were significant for HER of ammonia, glucose, lactate, urea, or AA other than Phe ($P = 0.07$ to $1.00$); the grand means are reported in Table 6.

**DISCUSSION**

**Successful Bacterial Inoculation**

An experimental goal for this study was to coordinate measurement of AA metabolism during acute stages of sepsis. Based on body temperature, an apparent inflammatory response was noticeable in inoculated ewes. Based on body temperature, an apparent inflammatory response was noticeable in inoculated ewes. Based on body temperature, an apparent inflammatory response was noticeable in inoculated ewes.
Amino acid metabolism in septic ewes

Figure 3. Portal-drained viscera flux [least squares means ± SEM (bars)] of glucose in nulliparous yearling Rambouillet crossbred ewes that were: 1) fed low dietary metabolizable Met (2.28 g/d) and received an intrauterine saline infusion (○), 2) fed high metabolizable Met (3.91 g/d) and received an intrauterine saline infusion (□), 3) fed low dietary metabolizable Met (2.28 g/d) and received an intrauterine bacterial inoculation (●; 9.69 × 10¹¹ cfu of *Escherichia coli* and 2.76 × 10¹² cfu of *Arcanobacterium pyogenes*), or 4) fed high metabolizable Met (3.91 g/d) and received an intrauterine bacterial inoculation (■; 9.69 × 10¹¹ cfu of *E. coli* and 2.76 × 10¹² cfu of *A. pyogenes*). Sampling time × metabolizable Met × inoculation interaction was significant (*P* = 0.05). a,bMeans with unlike superscripts within each sampling time are different (*P* = 0.02).

Figure 4. Portal-drained viscera flux [least squares means ± SEM (bars)] of Gly in nulliparous yearling Rambouillet crossbred ewes that were fed either low (○; 2.28 g/d) or high (□; 3.91 g/d) metabolizable Met. Sampling time × metabolizable Met interaction was significant (*P* = 0.02). a,bMeans with unlike superscripts within each sampling time are different (*P* < 0.01).

Supporting Sepsis-Induced Anabolism

To support immune-system activation, anabolic and catabolic processes occur simultaneously. For example, AA from muscle-protein catabolism are used for immune-responsive synthesis of products such as acute-phase proteins and glutathione. In the current study, such sepsis-induced effects are evident based on 2 major observations for inoculated ewes: 1) sustained hepatic specifically, in inoculated ewes, vena caval-blood neutrophil, lymphocyte, monocyte, and eosinophil numbers were significantly altered (Lewis, 2003). Based on these immediate immune responses, repeatability, and ease of application, this method seemed appropriate for achieving the objectives of the present experiment. As a result, acute sepsis was successfully induced in inoculated ewes. Indicators of sepsis, in addition to elevated body temperatures, were alterations in AA metabolism (discussed below) and ability to culture bacteria from the inoculated uteri (postmortem).

Figure 5. Hepatic flux [least squares means ± SEM (bars)] of Gly in nulliparous yearling Rambouillet crossbred ewes that received an intrauterine sterile-saline infusion (○) or bacterial inoculation (●; 9.69 × 10¹¹ cfu of *Escherichia coli* and 2.76 × 10¹² cfu of *Arcanobacterium pyogenes*). Sampling time × inoculation interaction was significant (*P* = 0.05). a,bMeans with unlike superscripts within each sampling time are different (*P* < 0.01).
uptake of Gly over time and 2) greater proportional hepatic release of Phe.

In noninoculated ewes, hepatic tissue transitioned from net uptake (h 0 and 2) to net release of Gly (h 8 and 10). However, intrauterine bacterial inoculation seemed to inhibit net release of Gly, resulting in a sustained hepatic uptake throughout the 12-h sampling period. This response, coupled with decreased artery plasma Met and Ser and hepatic-vein plasma Glu, indicates increased glutathione synthesis. In rats receiving i.v. injection of *E. coli*, glutathione production increased 3.4-fold with the greatest response occurring in hepatic tissue (Malmezat et al., 2000a). This increased rate of glutathione synthesis accounted for 38% of Cys used for protein synthesis. In the current study, intrauterine bacterial inoculation did not influence Cys flux across splanchnic tissues or concentration in plasma. Thus, it seems that Cys was not limiting, regardless of the level of MM, in inoculated ewes. Compared with noninoculated ewes, the lower Met concentrations in artery and portal-vein plasma may have been, in part, because of increased transsulfuration of Met. Partial demand of Cys used for glutathione synthesis in septic rats (discussed above and by Malmezat et al., 2000b) was from increased Met transsulfuration. Although inoculation treatment did not alter Glu flux, it did decrease the concentration in hepatic-vein plasma when compared with noninoculated ewes. Taken together, we suggest that Gly was used to support sepsis-induced glutathione synthesis.

Regardless of level of MM, hepatic tissue in noninoculated ewes consistently and proportionally (based on the HER) released less Phe than was received from PDV and artery plasma. Conversely, intrauterine inoculation resulted in proportionally more Phe released from the hepatic tissue 14 (low MM) and 16 (high MM) h after inoculation. Increased plasma Phe during sepsis has been documented in humans (for review see Jeevanandam, 1995); it is a result of peripheral-protein catabolism, mainly skeletal muscle protein. In artery and portal-vein plasma of inoculated ewes, the Phe:Tyr ratio was 1.2 compared with 0.9 for noninoculated ewes (inoculation treatment effect, \( P = 0.08 \) and 0.01, respectively), which indicates that intrauterine bacterial inoculation resulted in movement of Phe from protein to plasma via catabolism, mainly skeletal muscle protein. In rats, presence of sepsis does not influence Cys flux across splanchnic tissues or concentration in plasma. Thus, it seems that Cys was not limiting, regardless of the level of MM, in inoculated ewes. Compared with noninoculated ewes, the lower Met concentrations in artery and portal-vein plasma may have been, in part, because of increased transsulfuration of Met. Partial demand of Cys used for glutathione synthesis in septic rats (discussed above and by Malmezat et al., 2000b) was from increased Met transsulfuration. Although inoculation treatment did not alter Glu flux, it did decrease the concentration in hepatic-vein plasma when compared with noninoculated ewes. Taken together, we suggest that Gly was used to support sepsis-induced glutathione synthesis.
ence renal clearance rate or hepatic oxidation of Phe, but impedes its incorporation into skeletal muscle (Wannemacher et al., 1976). In the current study, we suggest that sepsis-induced peripheral release of Phe (i.e., increased arterial Phe) was greater than the hepatic demand of Phe for sepsis-induced protein synthesis (e.g., acute phase proteins).

Supply of MM

The supply of additional MM from the commercial rumen-protected feed product, Mepron M85, did not alter net PDV flux of Met. This suggests that 1) the supply of MM was less than that estimated (Table 1) from product specifications, or 2) loss of experimental units due to catheter-patency complications (Table 2) limited our ability to detect differences in Met flux. An examination of the bioavailability of several different ruminally protected Met sources revealed that Mepron M85 was least effective at raising plasma Met concentrations (Südekum et al., 2004), which confirmed earlier findings (Blum et al., 1999). Regardless, altered fluxes of blood metabolites and altered arterial concentrations of other AA for the high-MM vs. low-MM treatment indicated that the rumen-protected product used in this study supplied some absorbable Met.

Need for MM

In the context of this study, it is important to decipher, first, if additional MM was needed, regardless of sepsis, in low-MM ewes. Two events that occurred indicate that Met may have been limiting in low-MM ewes: 1) sustained net hepatic uptake of Phe in high-MM ewes and 2) a net release of Asp accompanied with greater hepatic-vein plasma glucose in high-MM ewes. High-MM resulted in greater portal-vein plasma Phe. Additional MM may have been needed in low-MM ewes, which would have ensured use of Phe for protein synthesis in hepatic tissue and, possibly, elsewhere. If Met was needed, breakdown of “excess” AA would be expected to occur resulting in increased ureagenesis. In high-MM ewes, compared with low-MM, hepatic-vein glucose concentration was greater, and there was a net hepatic release of Asp. Thus, ureagenesis may have been greater in low-MM ewes, although differences in urea flux were not observed. Taken together, it seems that low-MM ewes needed additional dietary Met.

Still unanswered is whether the presence of an acute infection in inoculated ewes prompted a need for additional MM and whether Met was the most limiting AA during sepsis. In low-MM ewes, arterial plasma Trp remained unchanged regardless of inoculation treatment. But, when MM was increased in the diet, artery plasma Trp was less in noninoculated ewes than inoculated. As with Phe, Trp is required for synthesis of acute-phase proteins and its removal from the plasma is mainly either through incorporation into protein or breakdown in hepatic tissue (Reeds et al., 1994; Le Floc’h et al., 2004). Because high MM did not affect rises in artery plasma Trp in inoculated ewes, Met availability did not seem to be the limiting factor during sepsis. Human skeletal-muscle protein is rich in Met (Reeds et al., 1994). Assuming the same for ovine muscle, the fact that, gram for gram, more Met-rich muscle protein must be mobilized to ensure availability of aromatic AA for acute phase protein synthesis, Met would not necessarily be limiting in ewes experiencing acute sepsis. Therefore, when MM is sufficient for nulliparous yearling ewes, additional Met may not provide any benefit to a ewe experiencing acute sepsis.

Concluding Remarks

Although somewhat described in nonruminant organisms, the effects of acute sepsis on splanchnic AA metabolism in ruminants are largely unknown. The lack of comparative data in the literature presents challenges when attempting to link observed effects to physiological processes. Nevertheless, using an established intrauterine bacterial inoculation method, we induced acute sepsis in nulliparous yearling ewes, while simultaneously measuring AA metabolism in splanchnic tissues. For some measurements, the number of treatment experimental units available for statistical analyses were small; thus, some effects of inoculation may have occurred undetected. However, inoculation resulted in increased plasma concentrations of Phe, increased hepatic removal and decreased plasma concentrations of AA important for glutathione synthesis, and decreased plasma concentrations of some gluconeogenic and acetogenic AA. We also investigated the effects of feeding diets low or high in MM on splanchnic AA metabolism in septic ewes. Supplemental Met above what is physiologically needed would not necessarily result in a benefit to septic ewes.

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