Evaluation of Fishmeal Supplement with Net Nitrogen Flux by the Portal-drained Viscera and the Liver in Mature Sheep

T. Fukuma[1], K. Taniguchi* and T. Obitsu

Graduate School of Biosphere Science. Hiroshima University. Higashihiroshima-shi 739-8528. Japan

ABSTRACT : The objective of this study was to evaluate the net flux response of nitrogen compounds (alpha-amino N, ammonia N, urea N, essential amino acids) across the portal-drained viscera (PDV), liver and total splanchnic tissues of mature wethers to increasing level of dietary fishmeal (FM) supplementation. Four wethers (average body weight, 64 kg) with chronic indwelling catheters into the portal, hepatic and mesenteric veins and the abdominal aorta were used in a 4×4 Latin square design. A basal diet consisting of 0.7 hay and 0.3 concentrate was fed twice daily with a fixed amount at 1.4 times maintenance energy (1.3 kg/day on a dry matter basis). The supplementation proportion of FM as treatment was 0, 0.03, 0.06 and 0.09 to the amount of the basal diet to contain 119, 137, 154 and 170 g crude protein per kg dietary dry matter, respectively. Blood flows through PDV and liver did not differ ($p>0.05$) among the treatments. Both net PDV release and hepatic uptake of alpha amino acid N increased linearly ($p<0.05$) in response to increased dietary FM, which resulted in similar total splanchnic release of alpha-amino N among the treatments. Similarly, increased dietary FM increased net PDV absorption and hepatic removal of ammonia N linearly ($p<0.05$). Hepatic synthesis and total splanchnic release of urea N increased linearly ($p<0.01$) with increased dietary FM, but PDV uptake of urea N did not respond to increased dietary FM. Linear regression equations between the increases in FM N intake and PDV net flux indicated that 0.34 and 0.30 of FM N was absorbed in the form of alpha-amino N and ammonia N, respectively. The results demonstrated that FM supplementation provides more alpha-amino N than ammonia N to the liver, but the alpha-amino acid N absorption is less than the expected metabolizable protein N from FM supplementation. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1255-1261)***

Key Words : Fishmeal, Absorption, Amino Acid, Ammonia, Urea, Sheep

INTRODUCTION

Fishmeal (FM) is characterized as a low ruminally degradable protein source for feeding of ruminants, and is abundant in methionine and lysine, two amino acid which are in low concentration in most of plant protein sources. There are many published data on ruminal degradability of FM under various feeding conditions, and on the production response with FM supplementation (Hussein and Jordan, 1991). Ruminally undegraded protein ranges from 0.51 to 0.66 of total protein in FM; this proportion varies in response to the feed intake and forage level, and the digestibility in the small intestine is estimated to be 0.90 of the duodenal flow (NRC, 2001). This means that FM could provide 0.46 to 0.60 of the ingested nitrogen (N) as metabolizable protein in addition to the contribution of microbial protein synthesis from ruminally degraded protein, although there are few data available for FM on absorption of nitrogenous compounds from the gastrointestinal tract.

Researchers who measured the net flux of alpha-amino nitrogen (AAN) by PDV did not detect significant differences among protein supplements with different ruminal degradability when those sources were incorporated into one level of dietary crude protein (CP) per kg dry matter (120 g. Huntington, 1987; 112 g. Ferrell et al., 2001). When a protein supplement is added to a basal diet at several levels, the incremental response of net flux of N compounds by the splanchnic tissues may reflect the ruminal degradability and digestibility in the small intestine of the supplement.

Our objectives were to evaluate protein of FM from the perspective of net absorption response of ammonia N and AAN by the PDV, and to elucidate the metabolism of the N compounds by the liver of sheep fed four levels of FM. In order to obtain clear responses to the increase of FM *per se*, we used mature wethers given the basal diet containing sufficient degradable protein.

MATERIALS AND METHODS

Animals

Four Suffolk wethers (64 (SE = 14.6) kg live weight at the beginning of the experiment) were surgically fitted with chronic indwelling catheters in the hepatic and hepatic portal vein, mesenteric vein, and caudal aorta by the procedures adapted from Katz and Bergman (1969) and Huntington et al. (1989). All catheters consisted of specific tips inside the blood vessel and Tygon Microbore tubing outside the blood vessel. The hepatic venous catheter was constructed from a Teflon tubing tip (0.96 mm i.d., 1.56 mm

* Corresponding Author: K. Taniguchi. Tel: +81-82-424-7954, Fax: +81-82-424-7955, E-mail: kohzo@hiroshima-u.ac.jp

[1] Hiroshima Prefectural Livestock Technology Research Center, Syobara-shi, 727-0023, Japan.

Received September 26, 2004; Accepted February 26, 2005

Table 1. Ingredient and chemical composition of the basal diet and supplemented fishmeal on dry matter basis (%)

	Basal diet	Fishmeal
Ingredient		
Italian ryegrass hay	68.8	-
Flaked barley	23.0	-
Defatted rice bran	5.3	-
Calcium carbonate	1.1	-
Sodium chloride	0.5	-
Vitamin-mineral pre-mix[1]	0.5	-
Urea	0.8	-
Chemical composition		
Dry matter[2]	87.0	89.9
Organic matter	93.2	81.7
Nitrogen	1.91	11.49
Ether extract	1.64	9.54
Neutral detergent fiber	46.9	-

[1] Declared composition of vitamin-mineral pre-mix (per kg): retinol, 1.8 g; cholecalciferol, 17.5 mg; alpha-tocopherol, 1 g; Fe, 5 g; Co, 20 mg; Zn, 10 g; Cu, 0.5 g; Mn, 20 g.
[2] Air dry matter basis.

o.d. or 1.07 mm i.d., 1.67 mm o.d., 7 cm long) connected to Tygon Microbore tubing (0.13 mm i.d., 0.23 mm o.d., 76 cm long). The catheter was inserted into the hepatic vein by puncturing the diaphragmatic surface of the liver with a 15-gauge needle, inserting a wire guide (18-gauge, Cook, Bloomington, IN, USA) through the needle into a hepatic vein, and threading the catheter over the wire guide. The portal venous catheter was constructed from a silicone rubber tubing tip (1.0 mm i.d., 2.0 mm o.d., 2 cm long) connected to Tygon Microbore tubing (0.10 mm i.d., 0.18 mm o.d., 60 cm long). The catheter was inserted into the lateral surface of portal vein by procedures described for insertion of the hepatic vein catheter. The mesenteric venous catheter was constructed from silicone rubber tubing tip (1.0 mm i.d., 1.5 mm o.d., 3 cm long) connected to Tygon Mirobore tubing (0.10 mm i.d., 0.18 mm o.d., 120 cm long). The catheter was inserted into the major vein, 25 cm caudal from the portal hepatis. Before puncturing the portal and mesenteric vein wall with a needle, a purse-string suture (Nespolen, 3-0 and 6-0 size, non-absorbable monofilament, Azwell, Osaka) was incorporated onto the venous wall. After inserting the catheter, the suture was ligatured at the connecting point of the two types of tubing. The arterial catheter was constructed from polyurethane tubing tip (Argyle, soft type, 0.97 mm i.d., 1.5 mm o.d., 20 cm long, Japan Schawood, Tokyo) connected to Tygon Microbore tubing (0.13 mm i.d., 0.23 mm o.d., 110 cm long). The catheter was inserted into the arterial branch close to the anterior mesenteric artery and directed toward the caudal aorta. A guide wire (18-gauge) threaded inside the mesenteric and arterial catheters was used facilitate insertion of the catheters, and to avoid kinks or doubling over of the catheter tip inside the vessels.

Surgical preparation, post-surgical care and management were conducted in accordance with the guideline of the animal use regulation of Hiroshima University and also with the 'Guide for the Care and Use of Agricultural Animals in Agricultural research and Teaching' (Federation of Animal Science Societies, 1999). Sheep were allowed at least 4 wk to recover from surgery. To maintain patency and function, all catheters were filled with sterile, heparinized-saline solution (500 IU/ml) that was exchanged every week, but one sheep lost patency of the hepatic venous catheter.

Treatments

The sheep were housed indoors in individual metabolic crates, and were used in a 4×4 Latin square design balanced for residual effects. All sheep were fed a basal diet consisting of 0.70 chopped Italian ryegrass hay and 0.30 concentrate (Table 1). The diet contained urea N to provide sufficient ruminally degradable N for microbial protein synthesis inferred from NRC (1985). The amount of the basal diet was calculated to provide 1.4 times the maintenance energy requirement of the sheep, 1.3 kg of dry matter (DM) per day. The supplementation level of FM as treatment was equivalent to be 0 (Control), 0.03 (45 g/day, FM3), 0.06 (90 g/day, FM6) and 0.09 (135 g/day, FM9) of the amount of the basal diet. As the FM supplement contained 0.718 of DM as crude protein (CP), the dietary CP for Control, FM3, FM6 and FM9 was 119, 137, 154 and 170 g/kg DM, respectively. Sheep received their dietary treatments in two equal portions daily at 08:00 and 20:00 h. All ingredients of concentrate including FM were pre-mixed just before feeding. In order to ascertain complete consumption of FM, the sheep were fed the concentrate mixture before they were fed hay. Water was available at all times.

Sampling

Experimental periods lasted 14 days. Days 1 to 13 were for dietary adaptation. Diet samples and blood samples for measurement of blood flow and net flux of nutrients across the splanchnic tissues were collected on day 14. At 07:20 h on day 14, a priming dose (15 ml) of 0.02 (w/v) para-aminohippurate (PAH: pH 7.4) was infused through a sterile 0.45 μm-filter into the mesenteric venous catheter of each sheep followed by continuous infusion of 0.02 PAH (0.50 ml/min). About 6 ml of hepatic venous, portal venous and mesenteric arterial blood were collected simultaneously into heparinized syringes at 08:00 h (before the morning feeding) and then blood sampling continued every hour until 19:00 h. The blood samples were transferred immediately into test tubes, placed on ice, and brought to the laboratory.

Table 2. Dry matter and nitrogen intake by sheep fed the basal diet and fishmeal supplement (g/d)

Item	Treatment[1]			
	Control	FM3	FM6	FM9
Dry matter	1,303	1,344	1,384	1,417
Nitrogen	24.8	29.5	34.1	38.2

[1] Control was basal diet only, and FM3, FM6 and FM9 was supplemented 3, 6 and 9% fishmeal to the basal diet as fed-basis, respectively.

Chemical analysis

In the laboratory, blood was analyzed for packed cell volume (PCV) by centrifugation of capillary tubes filled with blood. An aliquot of blood (2 ml) was deproteinized with an equal volume of sodium tungstate (0.10 w/v) and 1 N sulfuric acid, mixed, left at room temperature (15 min), and then centrifuged (1,600×g, 15 min). The supernatant was analyzed for ammonia N (Okuda et al., 1965). The remaining blood was centrifuged (1,600×g, 15 min) and plasma was harvested and retained. A plasma aliquot (0.25 ml) was deproteinized by adding sodium tungstate (0.10 w/v, 0.25 ml) and 0.083 N sulfuric acid (2 ml), mixed, left at room temperature (10 min), and then centrifuged (1,600×g, 10 min). The supernatant was used for AAN analysis (Goodwin, 1968). The rest of plasma was analyzed for urea N (Ceriotti, 1971), glucose (Kabasakalian, 1974) and PAH (Harvey and Brothers, 1962). Composited samples were created by combining equal volumes of plasma from each of the 12 times for arterial, portal, and hepatic sites. The composited plasma samples were used for the analysis of glucose. All supernatant and plasma samples were kept in the freezer (-20°C) until analysis. Diet samples were dried at 55°C for 48 h, left at room temperature for 24 h and then ground through a 1-mm screen. The diet samples were analyzed for DM, Kjeldahl N and neutral detergent fiber (Van Soest et al., 1991).

Calculation and statistics

Plasma flow (L/h) was calculated by dividing PAH infusion rate (mg/h) by PAH venoarterial concentration difference (mg/L). Plasma flow was divided by 1-(0.01× PCV) to calculate blood flow. Venoartical concentration differences were multiplied by blood (or plasma) flows to calculate net nutrient fluxes across PDV, liver and total splanchnic (TS) tissues. Net hepatic fluxes of nutrients were calculated by subtracting PDV fluxes from TS fluxes. The average hourly net flux rates were extrapolated to daily rates by multiplying by 24. Because one sheep lost the patency of hepatic venous catheter, the data on whole blood (or plasma) flow, nutrient concentration in blood (or plasma), venoarterial concentration difference, and net flux of nutrients were analyzed by analysis of variance using the mixed linear models procedure (PROC MIXED) of Statistical Analysis System Institute (SAS, 2000). Fixed effects were period and level, and the random effect was sheep. Least square means of the treatment were computed from least-squares estimates of the parameter (LSMEANS option of SAS). Treatment effects were analyzed using orthogonal contrasts into single degree of freedom comparisons that included linear and quadratic components of the response to supplementary FM level. Effects were deemed significant at $p<0.05$, and tendencies were noted for $p<0.15$ otherwise declared.

RESULTS AND DISCUSSION

Both N and DM intakes increased with FM supplementation as planned, but sheep fed FM9 refused to

Table 3. Blood concentration of nitrogen compounds and glucose in artery, portal and hepatic veins of sheep fed the basal diet and fishmeal supplement (mM)[1]

Item	Treatments[2]				SE	Significance of	
	Control	FM3	FM6	FM9		Linear effect	Quadratic effect
Alpha-amino N							
Arterial	3.78	3.63	3.87	4.02	0.146	#	
Portal	3.94	3.93	4.22	4.38	0.174	*	
Hepatic	3.85	3.83	4.17	4.25	0.194	#	
Ammonia N							
Arterial	0.085	0.084	0.086	0.084	0.0046		
Portal	0.422	0.478	0.499	0.521	0.0135	**	
Hepatic	0.080	0.078	0.074	0.073	0.0045		
Urea N							
Arterial	10.6	13.2	15.8	16.6	0.58	***	*
Portal	10.3	12.9	15.5	16.3	0.56	***	*
Hepatic	11.5	13.8	16.3	17.4	0.58	**	
Glucose							
Arterial	3.41	3.56	3.48	3.52	0.088		
Portal	3.40	3.55	3.42	3.48	0.101		
Hepatic	3.71	3.89	3.84	3.88	0.125		

[1] Plasma concentration except ammonia N in whole blood.
[2] See Table 2 footnote.
Tending toward significance ($p<0.15$).

Table 4. Concentration difference of nitrogen compounds and glucose in the blood of artery, portal and hepatic vein of sheep fed the basal diet and fishmeal supplement (mM)[1]

Item	Treatments[2]				SE	Significance of	
	Control	FM3	FM6	FM9		Linear effect	Quadratic effect
Alpha-amino N							
Portal-arterial	0.258	0.305	0.351	0.376	0.0200	***	
Hepatic-arterial	0.132	0.139	0.147	0.135	0.0206		
Hepatic-portal	-0.139	-0.188	-0.214	-0.237	0.0122	**	
Ammonia N							
Portal-arterial	0.337	0.394	0.414	0.438	0.0187	***	
Hepatic-arterial	-0.004	-0.008	-0.005	-0.006	0.0023		
Hepatic-portal	-0.366	-0.415	-0.424	-0.452	0.0180	*	
Urea N							
Portal-arterial	-0.278	-0.282	-0.285	-0.306	0.0290		
Hepatic-arterial	0.265	0.291	0.312	0.446	0.0373	*	
Hepatic-portal	0.532	0.593	0.633	0.751	0.0134	***	#
Glucose							
Portal-arterial	-0.014	-0.016	-0.060	-0.046	0.0322		
Hepatic-arterial	0.282	0.267	0.260	0.287	0.0616		
Hepatic-portal	0.323	0.255	0.297	0.348	0.0663		

[1] Plasma concentration except ammonia N in whole blood.
[2] See Table 2 footnote.
Tending toward significance ($p<0.15$).

eat a small portion of their diet (6 g DM/day, 0.4 g N/day; Table 2). A large portion of FM in diets may be distasteful for sheep, but small refusals with FM9 in the present study was regarded as no serious influences on net flux of N compounds.

Blood concentration

Plasma concentrations of AAN in portal vein increased linearly as FM intake increased, and the concentration in arterial and hepatic plasma tended to be linear (Table 3). The concentration difference of AAN between portal and arterial plasma (P-A) increased as FM intake increased, but that between hepatic and portal plasma (H-P) decreased linearly (Table 4). Hepatic-arterial plasma concentration difference (H-A) of AAN was not affected by FM intake.

As FM intake increased, ammonia N concentration in portal venous blood increased linearly, but arterial and hepatic venous concentrations were not affected (Table 3). The P-A and H-P of ammonia N increased linearly as FM intake increased (Table 4). The concentrations of urea N in arterial, portal and hepatic plasma increased linearly as FM intake increased (Table 3). The H-A and H-P of urea N increased linearly as FM intake increased, but FM intake did not change P-A (Table 4). The FM intake did not affect any plasma concentrations and concentration differences of glucose (Tables 3 and 4)

Blood flow

Blood flow of the portal and hepatic veins did not show clear changes with increased FM (Table 5). Ferrell et al. (1999) also reported that blood flow of the portal vein did not change when sheep consuming low-quality forage were supplied with ruminal undegraded protein (RUP). On the other hand, in the studies of protein sources that differed in ruminal degradability with beef steers (Huntington, 1987) or sheep (Ferrell et al., 2001), plasma flows of the portal vein were greater when low RUP diets fed than when high RUP diets were fed. Taniguchi et al. (1995) observed that portal and hepatic blood flows of beef steers were greater with ruminal infusion of casein than with abomasal infusion. These results suggest that digestion of dietary protein in the small intestine compared with the rumen does not affect or increase PDV blood flow. Hepatic arterial blood flow did not differ among the treatments. The average proportion of blood flow in portal vein to hepatic vein in all treatments was 0.865. This figure agrees with that from other experiments with sheep (0.771 to 0.865; Bohnert et al., 1999; Ferrell et al., 1999, 2001).

Nitrogen fluxes

Net PDV flux of AAN increased linearly with increased dietary FM. When the ruminal degradability and the small intestine digestibility of FM is assumed to be 0.32 and 0.74, respectively (Titgemeyer et al., 1989), ingested FM can provide 0.50 of the its protein as metabolizable protein (protein that is digested and absorbed as peptides or amino acids that are available to support metabolism). Based on these values, FM supplementation to the basal diet (Control) in the current study is calculated to increase AAN absorption by 2.37, 4.68, and 6.74 g/d for the three FM diets, respectively. However, the actual increases (1.63, 3.82, and 4.35 g/d, respectively) averaged about 0.70 of the calculated amounts. Remond et al. (2000) observed that amino acids N net flux by PDV of adult sheep was 0.70 of

Table 5. Blood flow and net flux of nitrogen compounds and glucose across the visceral tissues of sheep fed a basal diet and fishmeal supplement[1]

Item	Treatments[2]				SE	Significance of	
	Control	FM3	FM6	FM9		Linear effect	Quadratic effect
Blood flow (L/h)							
Portal venous	149	147	150	136	7.7		
Hepatic venous	167	172	171	162	15.3		
Hepatic arterial	21	33	24	31	12.3		
Plasma flow (L/h)							
Portal venous	115	115	117	107	6.5		
Hepatic venous	130	134	132	127	12.0		
Hepatic arterial	16	26	18	24	9.5		
Alpha-amino N flux (g/d)							
Portal-drained viscera	9.9	11.5	13.7	14.3	0.61	***	
Hepatic	-4.1	-5.9	-7.7	-7.8	0.98	*	
Splanchnic	5.9	6.4	6.4	6.2	1.14		
Ammonia N flux (g/d)							
Portal-drained viscera	16.9	19.4	20.8	20.9	0.90	**	#
Hepatic	-18.0	-19.9	-20.7	-21.4	1.07	**	
Splanchnic	-0.19	-0.49	-0.27	-0.36	0.123		
Urea N flux (g/d)							
Portal-drained viscera	-10.9	-10.9	-11.2	-11.7	1.26		
Hepatic	22.2	24.6	25.9	31.3	1.39	**	
Splanchnic	12.0	13.2	13.8	20.0	1.91	***	**
Glucose flux (g/d)							
Portal-drained viscera	-8	-11	-29	-23	16.4		
Hepatic	181	152	167	195	37.4		
Splanchnic	159	156	149	166	39.8		

[1] Positive values indicate net release or output; negative values indicate net uptake or remove.

[2] See Table 2 footnote.

Tending toward significance ($p<0.15$).

the mesenteric-drained viscera flux. This proportion is consistent with our calculation of recovery of AAN from dietary FM presented above. The linear increase in hepatic uptake of AAN counterbalanced the increased PDV of AAN in response to increased dietary FM, so TS release of AAN did not change among FM treatments (Table 5).

In the present study, the least supplemented diet (FM3) appeared to supply sufficient amino acids to the visceral and peripheral tissues of adult sheep consuming 1.4 times of maintenance energy. However, arterial plasma concentration of AAN increased with increasing FM (Table 3). Similarly, in growing beef steers receiving abomasal infusions of casein, AAN concentration in arterial blood increased without increasing net TS release of AAN (Guerino et al., 1991). These results suggest that arterial concentration of amino acids does not necessarily reflect the net TS release. Amino acids released with the changes in the peripheral protein turnover besides TS release could affect the arterial concentration of amino acids (Hoskin et al., 2003). In the current work, even though TS release of AAN was similar among the treatments, the essential amino acids concentration in arterial plasma might be different. The proportion of glutamate in AAN released by TS provides some insight into potential changes in supply of essential amino acids, as well as priority of glutamate supply for tissue growth or interorgan N shuttle (Huntington and Archibeque, 1999).

Net PDV release and net liver removal of ammonia N increased linearly and quadratically ($p = 0.08$) as FM increased (Table 5). Ammonia N released by PDV mainly derives from degraded dietary N in the rumen and urea N transferred into the lumen of the gastrointestinal tract via the gut wall or saliva. Urea N removal by PDV in this study was almost constant among treatments, and the net liver release of urea N increased linearly and quadratically as FM increased. Although urea N transfer via saliva secretion is increased with the increased dietary N, total entry rate of urea N including gut wall transfer is not affected by the dietary N content due to the small portion of saliva urea N transfer (Marini and Van Amburgh, 2003). Thus, the treatment difference for urea entry into the gastrointestinal tract might be small in the present study. In addition, because the basal diet contained sufficient ruminal degradable protein, the major parts of increased ammonia N by PDV could be attributed to the increase of ruminally degraded N with FM.

All fluxes of glucose by PDV and TS did not respond to increased dietary FM (Table 5). Negative PDV flux of

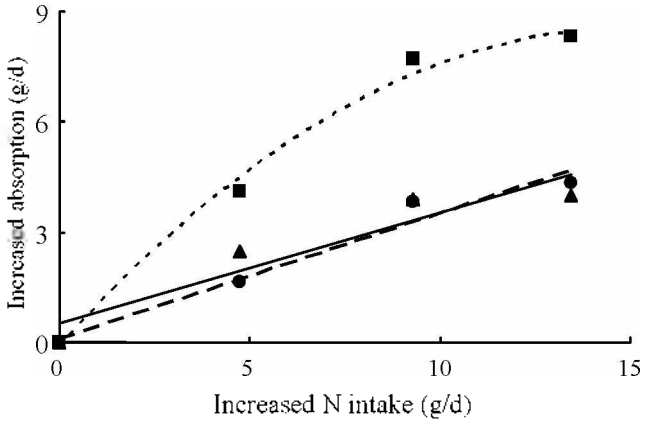

Figure 1. Response of the increase in the net flux of alpha-amino acid (AAN) and ammonia nitrogen (NH_3N) by the portal-drained viscera to the increased intake of fishmeal nitrogen (FMN). Regression equation to FMN: Y (AAN, ●) = 0.106+0.341X, R^2 = 0.964; Y (NH_3N, ▲) = 0.525+0.301X, R^2 = 0.881; Y (AAN+ NH_3N, ■) = -0.120+1.155X-0.038X^2, R^2 = 0.992.

glucose means that more use of arterial glucose by PDV than glucose absorption. In general, the starch in rolled barley used in the present study is readily fermentable in the rumen, so starch flow to the small intestine would be little, if any. Although PDV uptake of AAN increased with increased dietary FM, hepatic glucose net flux did not increase, ostensibly due to rigid glucose homeostasis or low demand by the peripheral tissues of mature sheep used in this study. Guerino et al. (1991) also observed that an increase in AAN uptake by the liver with casein infusion into the abomasums of growing steers was accompanied with no changes in hepatic glucose net flux, but with an increase in oxygen consumption. Energy derived from amino acid degradation in hepatocyte may be used for protein synthesis, ureagenesis and/or heat through mitochondrial proton leak (Rolfe and Brown, 1997).

Estimation of nitrogen net flux derived from fishmeal

AAN release by PDV increased linearly with increased dietary FM as mentioned above. The linear and quadratical contrast in ammonia N release by PDV with FM supplement was significant, and urea N entered the gastrointestinal tract was almost constant among the diets. The basal diet must have supplied sufficient ruminal degradable N for microbial protein synthesis. Therefore, the increase in AAN and ammonia N by PDV from dietary FM increase relative to the control diet can be regarded as N derived from FM by itself. Linear regression equations for AAN and ammonia N absorption by PDV based on the increase to the Control were calculated with means for Table 5 for the evaluation of FM protein (Figure 1). Fitted equations were as follows. Y = 0.341X+0.109 (R^2 = 0.966) for AAN, and Y = 0.301X+0.527 (R^2 = 0.880) for ammonia N, where Y is an average increase of PDV release (g/d), and X is average intake of FM N (g/d) at four points ranging 0 (Control) to 13.4 g/d (FM9). The equations imply that 0.34 and 0.30 of FM N are absorbed by PDV in the form of AAN and ammonia N, respectively. Ruminal degradability of CP of FM ranges from 0.30 to 0.70 due to the several factors in the manufacturing process (Hussein and Jordan, 1991). The calculated, the net absorption of ammonia N (0.30) is similar to 0.32 of the ruminal degradability of protein in FM reported by Titgemeyer et al. (1989). In addition, assuming the digestibility of FM N in small intestine is 0.74 of the duodenal N flow (Titgemeyer et al., 1989), and assuming the uptake of AAN by the other segments of the gastrointestinal tract including the forestomach is equal to 0.30 of AAN absorbed from the small intestinal lumen (Reynolds and Huntington, 1988; Remond et al., 2000), one can estimate that the net absorption of AAN by PDV is 0.35 of FM N. This calculated figure is also very close to our calculated recovery of FM N as AAN discussed above. Furthermore, the linear regression of AAN absorption related to FM N intake in the present study suggest that PDV of sheep may consume AAN at a constant proportion to the metabolizable protein in the gastointestinal lumen.

The sum (0.64) of the increased absorption by PDV for ammonia N and AAN with FM supplement almost accounted for the apparent digestibility (0.65) of FM protein in the total digestive tract (NRC, 2001). Nonetheless, the response of N absorption by PDV to FM protein may vary with the level of N intake. In deed, the sum of AAN and ammonia N absorption by PDV responded quadratically to increased N intake due to little difference of ammonia N absorption between FM6 and FM9 (Figure 1). Ammonia N absorbed by PDV mainly derives from feed protein degraded in the rumen and urea N entered into the gastrointestinal tract (recycled urea N). Rooke and Armstrong (1987) reported that apparent dietary N degradability in the rumen decreased as FM intake increased. The urease activity of the ruminal wall also decreases with increasing level of N intake, but may not restrict to hydrolyze the recycled urea N even for high-N diets (Marini et al., 2004). More researches on other protein supplements for determining the absorption response of N compounds by ruminant PDV are desirable for development of the metabolizable protein concept, because feeding systems should define in quantitative the responses of animals to their feed supplies (Corbett and Freer, 2003).

In conclusion, FM supplement provides 0.34 and 0.30 of the ingested N to the liver as alpha amino acid and ammonia nitrogen, respectively, but the liver modifies the supply of amino acids to the production tissues according to the animal's amino acid requirements. Measuring net flux of nitrogen compounds by PDV with increasing protein supplement seems to be an effective method for the evaluation of metabolizable protein.

ACKNOWLEDGEMENTS

The authors would like to thank G. B. Huntington, PhD., North Carolina State University, for the suggestion to refine the manuscript.

REFERENCES

Bohnert, D. W., B. T. Larson, S. J. Lewis, C. J. Richards, K. C. Swanson, D. L. Harmon and G. E. Mitchell Jr. 1999. Net nutrient flux in visceral tissues on lambs fed diets differing in supplemental nitrogen source. J. Anim. Sci. 77:2545-2553.

Ceriotti, G. 1971. Ultramicrodetermination of plasma urea by reaction with diacetylmonoxime-antipyrine without deproteinization. Clin. Chem. 17:400-402.

Corbett, J. L. and M. Freer. 2003. Past and present definitions of the energy and protein requirements of ruminants. Asian-Aust. J. Anim. Sci. 16:609-624.

Federation of Animal Science Societies. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching. 1st Ed. Federation of Animal Science Societies, Savoy, Ilinoy.

Ferrell, C. L., K. K. Kreikemeier and H. C. Freetly. 1999. The effect of supplemental energy, nitrogen, and protein on feed intake, digestibility, and nitrogen flux across the gut and liver in sheep fed low-quality forage. J. Anim. Sci. 77:3353-3364.

Ferrell, C. L., H. C. Freetly, A. L. Goetsch and K. K. Kreikemeier. 2001. The effect of dietary nitrogen and protein on feed intake, nutrient digestibility, and nitrogen flux across the portal-drained viscera and liver of sheep consuming high-concentrate diets *ad libitum*. J. Anim. Sci. 79:1322-1328.

Goodwin, J. F. 1968. On the measurement of urinary nitrogen with 1-fluoro-2,4-dinitrobenzene. Clin. Chim. Acta. 21:231-240.

Guerino, F., G. B. Huntington and R. A. Erdman. 1991. The net portal and hepatic flux of metabolites and oxygen consumption in growing beef steers given postruminal casein. J. Anim. Sci. 69:387-395.

Harvey, R. B. and A. J. Brothers. 1962. Renal extraction of para-aminohippurate and creatinine measured by continuous *in vivo* sampling of arterial and renal-vein blood. Ann. N. Y. Acad. Sci. 102:46-54.

Hoskin, S. O., I. C. Savary-Auzeloux, A. G. Calder, G. Zuur and G. E. Lobley. 2003. Effect of feed intake on amino acid transfer across the ovine hindquarters. Br. J. Nutr. 89:167-179.

Huntington, G. B. 1987. Net absorption from portal-drained viscera of nitrogenous compounds by beef heifers fed on diets differing in protein solubility or degradability in the rumen. Br. J. Nutr. 57:109-114.

Huntington, G. B. and S. L. Archibeque. 1999. Practical aspects of urea and ammonia metabolism in ruminants. Proceeding of the American Society of Animal Science, E23: 1-11. http://www.asas.org./jas/symposia/proceedings/0939.pdf

Huntington, G. B., C. K. Reynolds and B. H. Stroud. 1989. Techniques for measuring blood flow in splanchnic tissues of cattle. J. Dairy Sci. 72: 1583-1595.

Hussein, H. S. and R. M. Jordan. 1991. Fish meal as a protein supplement in ruminant diets: a review. J. Anim. Sci. 69:2147-2156.

Kabasakalian, P., S. Kalliney and A. Westcott. 1974. Enzymatic blood glucose determination by colorimetry on N,N-diethylaniline-4-aminoantypyrine. Clin. Chem. 20:606-607.

Katz, M. L. and E. N. Bergman. 1969. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. Am. J. Physiol. 216:946-952.

Marini, J. C. and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545-552.

Marini, J. C., J. D. Klein, J. M. Sands and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lams. J. Anim. Sci. 82:1157-1164.

National Research Council. 1985. Ruminant Nitrogen Usage. National Academy Press, Washington, DC.

National Research Council. 2001. Nutrient Requirements of Dairy cattle. 7th Ed. National Academy Press, Washington, DC.

Okuda, H., S. Fujii and Y. Kawashima. 1965. A direct colorimetric determination of blood ammonia. Tokushima Journal of Experimental Medicine 12:11-23.

Remond, D., L. Bernard and C. Poncet. 2000. Free and peptide amino acid net flux across the rumen and the mesenteric- and portal-drained viscera of sheep. J. Anim. Sci. 78:1960-1972.

Reynolds, C. K. and G. B. Huntington. 1988. Partition of portal-drained viscera net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. Br. J. Nutr. 60:539-551.

Rolfe, D. F. S. and G. C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol. Rev. 77:732-758.

Rooke, J. A. and D. G. Armstrong. 1987. The digestion by cattle of silage and barley diets containing increasing quantities of fishmeal. J. Agric. Sci. (Cambridge) 109:261-272.

SAS Institute. 2000. SAS/STAT User's Guide, Version 8 Edition SAS Institute Inc., Cary, North Carolina.

Taniguchi, K., G. B. Huntington and B. P. Glenn. 1995. Net nutrient flux by visceral tissues of beef steers given abomasal and ruminal infusions of casein and starch. J. Anim. Sci. 73:236-249.

Titgemeyer, E. C., N. R. Merchen and L. L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262-275.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Method for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.