



## Net Portal Fluxes of Nitrogen Metabolites in Holstein Steers Fed Diets Containing Different Dietary Ratios of Whole-crop Corn Silage and Alfalfa Hay

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**ABSTRACT :** The objectives of the present study were to investigate the effects of different dietary ratios of whole-crop corn silage and alfalfa hay on nitrogen (N) digestion, duodenal flow and metabolism across the portal-drained viscera (PDV) of growing beef steers, and to elucidate their relationships. Four steers (236±7 kg BW) fitted with duodenal cannulae and chronic indwelling catheters into the portal and mesenteric veins and abdominal aorta were used in a 4×4 Latin square design. Animals were fed (at 12-h intervals) the 4 diets consisting of whole-crop corn silage (C) and alfalfa hay (A) in 80:20 (C8A2), 60:40 (C6A4), 40:60 (C4A6) and 20:80 (C2A8) ratios of which dietary crude protein (CP) was 10.5, 12.0, 13.5 and 15.0% of dry matter (DM), respectively. Feeding level was restricted to 95% of *ad libitum* intake to measure N digestion, blood flow and net flux of N across the PDV. Digestibility of DM and neutral detergent fiber and digestible energy intake linearly increased as the ratio of alfalfa hay increased. The N intake, duodenal flow and intestinal disappearance increased linearly with increasing alfalfa hay. Arterial and portal concentrations of  $\alpha$ -amino N showed a quadratic response to increasing levels of alfalfa hay and were the highest in steers fed the C6A4 diet. The net PDV release of  $\alpha$ -amino N and ammonia N increased linearly with increasing alfalfa hay, but urea N uptake by PDV did not differ among diets. As a percentage of apparently digested N in the total gut, net PDV release of  $\alpha$ -amino N linearly decreased from 66 to 48% with increasing alfalfa hay. Conversely, net PDV recovery of  $\alpha$ -amino N to intestinal N disappearance varied with increasing alfalfa hay accounting for 49, 50, 58 and 61% on C8A2, C6A4, C4A6 and C2A8 diets, respectively. Net PDV uptake of urea N, relative to apparently digested N, linearly decreased from 81 to 25% as alfalfa hay increased from 20 to 80% of DM intake. Considering PDV uptake of urea N, microbial efficiency and conversion of total tract digested N to PDV  $\alpha$ -amino N net supply, a diet consisting of 80% whole-crop corn silage and 20% alfalfa hay (10.5% CP) was the best, while considering the quantities of intestinal N disappearance and  $\alpha$ -amino N absorption, a diet of 20% whole-crop corn silage and 80% alfalfa hay (15% CP) would be preferred. The proportion of  $\alpha$ -amino N recovered by PDV relative to the intestinal N disappearance may vary with energy intake level of mixed forage diets. (**Key Words :** Growing Steer, Whole-crop Corn Silage, Alfalfa Hay, Digestion, Nitrogen Metabolism, Portal-drained Viscera)

### INTRODUCTION

Nutritive value of feeds has been evaluated from the chemical composition and digestibility of nutrients. However, the patterns of nutrients available for absorption are modified by the site and extent of nutrient digestion. Furthermore, the nutrients absorbed from the gut lumen are altered by portal-drained visceral (PDV) tissue metabolism. Therefore, measuring net nutrient flux across the PDV is an important tool to evaluate the dietary nutrient value. Major nitrogen (N) metabolites released by PDV, either as ammonia or amino acid, are a key component for ruminant

animal production and an important aspect in reducing nutrient outflow into the environment (Bach et al., 2005). Previous works have examined the effect of feed intake level (Lapierre et al., 1999), gut fermentation and digestion site of starch and protein (Taniguchi et al., 1995), and forage to concentrate ratios (Seal et al., 1992) on N metabolism and net flux by PDV of growing beef steers. However, studies investigating N digestion and PDV metabolism simultaneously in beef steers fed all-forage diets are rare. Formulating diets for ruminants from homegrown, protein- and/or energy-rich forages will be more economical particularly after the great expansion of grain-based biofuels production. Whole-crop corn silage, which is an important feed for intensively managed ruminants, has a high energy but a low crude protein (CP)

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**Table 1.** Chemical composition of dietary treatments consisting of whole-crop corn silage (C) and alfalfa hay (A)

Item	Dietary treatments			
	C8A2	C6A4	C4A6	C2A8
DM %	40.0	52.1	64.3	76.4
OM % of DM	91.7	91.4	91.1	90.8
CP % of DM	10.5	12.0	13.5	15.0
NDF % of DM	55.4	54.7	54.0	53.3

content. Conversely, alfalfa hay has high CP with high rates of degradation. Therefore, whole-crop corn silage and alfalfa hay are complementary forages. We hypothesized that feeding optimal proportions of whole-crop corn silage and alfalfa hay would increase N utilization through increasing microbial protein flow and consequently increasing the absorption of amino acids. Therefore, the objectives of this study were to investigate the effects of feeding growing steers forage diets consisting of whole-crop corn silage and alfalfa hay on N digestion, duodenal N flow and net flux of N metabolites across the PDV, and to elucidate the relationships between site and extent of N digestion and N net flux by PDV on all-forage diets.

## MATERIALS AND METHODS

### Animals and diets

All animal procedures were approved by the Animal Care and Use Committee of Hiroshima University. Four Holstein steers ( $236 \pm 7$  kg BW) were surgically fitted with duodenal cannulae and chronic indwelling catheters within portal and mesenteric veins and abdominal aorta. Procedures for implantation and maintenance of catheters were as described by Huntington et al. (1989). Steers were allowed at least 4 wk to recover from surgery, and then randomly allocated to a  $4 \times 4$  Latin square design and fed four diets containing the following ratios of whole-crop corn silage (C) and alfalfa hay (A): 80:20 (C8A2), 60:40 (C6A4), 40:60 (C4A6) and 20:80 (C2A8) on a dry matter (DM) basis (Table 1). Corn silage was made from corn plants harvested at dough stage (110 to 114 days after planting), chopped at 20 to 30 mm, put in a baler and wrapped with 8 to 10 layers of white plastic film, and then stored as roll-baled silage. Alfalfa hay used was a commercial product and was manually chopped to 100 mm before feeding. Steers were housed in individual tie stalls and had free access to water and trace-mineralized salt blocks. Each experimental period consisted of a minimum 14 d for adaptation to diets, 4 d for collection of fecal and duodenal samples, and a final day for blood sampling. Diets were offered twice daily at 12-h intervals (0800 and 2000). Chromic oxide ( $\text{Cr}_2\text{O}_3$ ) and acid detergent lignin (ADL) were used as indigestible flow markers. Two grams of  $\text{Cr}_2\text{O}_3$  were dosed orally immediately before feeding. For at least 7 d before start of sampling in each period, steers were

fed at 95% of *ad libitum* intake recorded for the adaptation period. Orts were collected daily at 0730 h to calculate dry matter intake (DMI). Silage DM was determined daily by the rapid drying method (Kett, FD-610), and diet formulations (as-fed basis) were adjusted accordingly to account for change in the DM content.

### Sampling

Feces were collected and weighed daily for 4 d, and aliquots were retained and frozen for later analysis. Duodenal fluid (200 ml) was collected via the duodenal cannula at 8 h-intervals for 4 d to measure duodenal N flow. The samples were collected at 0800 and 1600 on d 1; 0000, 1000 and 1800 on d 2; 0200, 1200 and 2000 on d 3; 0400, 1400 and 2200 on d 4; and 0600 on d 5, and were composited across sampling times within a steer and a sampling period and stored frozen ( $-20^\circ\text{C}$ ). Samples of feed and refusals were also collected daily and composited for each collection period. On d 19 of each experimental period, sets of twelve blood samples (20 ml each) were simultaneously collected hourly from the artery and the portal vein, from 0800 to 1900. All samples were collected into heparinized syringes, immediately placed on ice, and kept chilled until processed, analyzed or frozen ( $-20^\circ\text{C}$ ). Fresh blood samples were analyzed for packed cell volume (PCV) by micro-centrifugation. Blood flows for the PDV was determined using *p*-aminohippuric acid (PAH; 10% wt/vol; pH 7.4; Katz and Bergman, 1969) infused continuously (1 ml/min) into the mesenteric vein catheter, following a priming dose (15 ml). The PAH infusion was initiated at least 40 min before the collection of the first set of blood samples.

### Chemical analyses

The composited samples of feed, refusals and feces were dried for 48 h at  $55^\circ\text{C}$  in a forced-air oven, while duodenal samples were freeze-dried, and then all samples were ground in a Wiley mill to pass a 1 mm screen and analyzed for DM, organic matter (OM) and N using the conventional procedures (AOAC, 1990). Ash-free neutral detergent fiber (NDF) and ADL were determined using the procedure described by Van Soest et al. (1991). Gross energy (GE) was determined by a bomb calorimeter (Shimadzu, 3A).  $\text{Cr}_2\text{O}_3$  was determined calorimetrically in fecal and duodenal samples (Yoshida et al., 1967). Bacterial cells were isolated from the duodenal contents (McAllan and Smith, 1969), freeze-dried, ground with a grinder, and pooled per animal and period before N, OM and purine content determinations. Purine content was determined in duodenal and bacterial samples following the method of Zinn and Owens (1986). Blood plasma samples were analyzed for PAH (Harvey and Brothers, 1962), urea N (Ceriotti, 1971) and  $\alpha$ -amino N (AAN; Goodwin, 1968)

**Table 2.** Intake and digestion of DM, OM, NDF, and DE in steers fed varying dietary ratios of whole-crop corn silage (C) and alfalfa hay (A)

Item	Dietary treatments				SEM <sup>1</sup>	p-value <sup>2</sup>	
	C8A2	C6A4	C4A6	C2A8		L	Q
<b>DM</b>							
Intake (kg/d)	4.49	4.94	4.90	5.44	0.46	0.032	0.860
Total tract digestibility (%)	57.6	60.8	61.0	62.7	0.66	0.002	0.273
<b>OM</b>							
Intake (kg/d)	4.12	4.51	4.47	4.94	0.43	0.039	0.871
Apparent ruminal digestion (kg/d)	1.37	1.93	1.83	2.05	0.11	0.005	0.140
True ruminal digestion (kg/d)	1.95	2.57	2.48	2.76	0.14	0.009	0.258
Total tract digestibility (%)	59.2	62.0	62.0	64.0	0.67	0.003	0.519
<b>NDF</b>							
Intake (kg/d)	2.48	2.70	2.67	2.91	0.27	0.013	0.954
Total tract digestibility (%)	50.8	54.5	54.9	56.7	1.07	0.009	0.416
DE intake (Mcal/d)	10.9	12.7	12.7	14.5	1.13	0.004	0.875

<sup>1</sup> Standard error of least squares means. <sup>2</sup> L = Linear effect; Q = Quadratic effect.

concentrations. Ammonia N was analyzed with whole blood (Okuda et al., 1965).

### Calculation and statistics

Nutrient flows at the duodenum and fecal output were calculated by reference to fecal excretion of Cr<sub>2</sub>O<sub>3</sub> and ADL. Duodenal bacterial N flow was estimated by multiplying duodenal purine flow (g/d) by the N: purine ratio in isolated bacterial cells (Zinn and Owens, 1986). 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. Venoarterial concentration differences were multiplied by blood (or plasma) flows to calculate net nutrient fluxes across the PDV. The average hourly net flux rates were extrapolated to daily rates by multiplying by 24. Positive values indicate net output whereas negative values indicate net uptake. Data were analyzed as a 4×4 Latin square using the MIXED procedure of SAS (SAS, 2000). The model included the treatment as the fixed effect and steer as the random effect. Period effect was not included in the model. Treatments means were compared with linear and quadratic contrasts. Results are expressed as least squares means with the lowest standard error of means because of unequal numbers of steers for blood metabolites within treatments. Because of catheter patency problems, only 11 measurements of portal net flux were obtained. Significance was declared at  $p \leq 0.05$ , and tendency at  $0.05 > p \leq 0.15$ .

## RESULTS AND DISCUSSION

### Apparent digestive flows

DM, OM, NDF and digestible energy (DE) intake and digestion increased linearly with high levels of alfalfa hay (Table 2). The energy intake level varied from 1.5 to 2.0 times maintenance energy requirements from diet C8A2 to diet C2A8, respectively. The increased DM and DE intake

and digestion with alfalfa hay was due to the high content of soluble fiber and a high rate of digestion of NDF in alfalfa hay compared with corn silage (Onetti et al., 2002; Min et al., 2007). Diets with high levels of alfalfa hay linearly increased N intake and N digestion in the total tract and intestine, and there was a linear tendency for fecal N excretion (Table 3). N intake increased from 75.6 g/d on diet C8A2 to 130.0 g/d on diet C2A8, and this increase was expected, as it followed the dietary CP concentration, in which diet C8A2 was the lowest (10.5%) and diet C2A8 was the highest (15.0%). Apparently digested N in the total tract increased from 41 g/d on diet C8A2 to 90 g/d on diet C2A8 and intestinal N disappearance increased from 55 g/d on diet C8A2 to 72 g/d on diet C2A8, respectively. Disappearance of N from the intestine, as a percentage of duodenal N flow, increased linearly and quadratically with high alfalfa hay and was the highest on diet C6A4. Apparent total tract digestibility increased linearly with high alfalfa hay and averaged 62.8% of N intake across treatments. The increased N digestion observed with increasing high alfalfa hay can be attributed to the increased intake of N in the form of rumen undegradable N (RUN) and rumen degradable N (RDN) or the increase in total duodenal N flow.

The apparent ruminal N disappearance was negative on diet C8A2, almost equal to zero on diets C6A4 and C4A6, but N loss from the rumen was significantly increased on diet C2A8 (Table 3). Regression analysis of ruminal N disappearance on dietary CP suggested that when a diet consists of 50% whole-crop corn silage and 50% alfalfa hay (12.8% CP), the amount of degraded N in the rumen was apparently equal to the amount used by the microorganisms. Microbial N flow to the duodenum was not affected by treatments, but microbial efficiency linearly decreased from 27.5 g of microbial N/kg of OM truly digested in the rumen on diet C8A2 to 22 g of microbial N/kg of OM truly digested in the rumen on diet C2A8 (Table 3). Efficiency of

**Table 3.** Nitrogen intake, duodenal N flows, N digestion, and microbial efficiency in steers fed varying dietary ratios of whole-crop corn silage (C) and alfalfa hay (A)

Item	Dietary treatments				SEM <sup>1</sup>	p-value <sup>2</sup>	
	C8A2	C6A4	C4A6	C2A8		L	Q
N intake (g/d)	75.6	95.6	104.0	130.0	9.7	<0.001	0.582
Ruminal N disappearance							
g/d	-14.1	-5.70	4.40	18.6	4.1	<0.001	0.235
% of intake	-17.1	-6.50	5.00	14.7	4.2	<0.001	0.880
Ruminal degraded N (g/d)	42.2	52.1	66.5	83.2	3.8	<0.001	0.384
Duodenal flow (g/d)							
Total N	89.6	101.3	99.6	111.4	13.1	0.059	0.996
Ammonia-N	1.70	2.40	2.80	3.80	0.4	<0.001	0.453
Non-ammonia-N	87.9	98.9	96.8	107.6	12.8	0.082	0.989
Undegraded N	33.4	43.6	37.5	46.8	7.6	0.028	0.890
Microbial N	54.5	55.4	59.3	60.7	6.6	0.392	0.956
Microbial efficiency <sup>3</sup>	27.5	21.9	23.9	22.0	1.4	0.031	0.169
Intestinal N disappearance							
g/d	55.1	65.0	62.8	71.5	9.0	0.068	0.902
% of intake	71.8	68.6	59.8	54.7	2.9	<0.001	0.622
% of duodenal flow	61.0	64.3	62.7	63.8	0.7	0.024	0.070
Fecal N flow (g/d)	34.6	36.3	36.7	39.9	4.6	0.122	0.757
Total tract N disappearance							
g/d	41.0	59.3	67.2	90.1	5.6	<0.001	0.534
% of intake	54.7	62.1	64.8	69.4	1.5	<0.001	0.249

<sup>1</sup> Standard error of least squares means. <sup>2</sup> L = Linear effect; Q = Quadratic effect.

<sup>3</sup> g of microbial N/kg of OM truly fermented in the rumen.

microbial protein synthesis obtained with diet C8A2 is close to the average value of 25 g of microbial N/kg of OM truly digested in the rumen for optimum microbial growth adopted for all diets by Czerwaski (1986) or the optimum value of 29 g of microbial N/kg of OM truly digested in the rumen assumed by the NRC (2001). From these results it seems that the dietary combination consisting of 80% whole-crop corn silage and 20% alfalfa hay improved N efficiency for microbial protein synthesis probably through a better balance in N and energy supply to the rumen.

The pattern of duodenal flow of non-ammonia N (NAN) and RUN (Table 3) followed the same pattern as DM, OM, NDF, N and DE intakes, where steers fed diet C2A8 showed the highest values and steers fed diet C8A2 showed the lowest values. Duodenal NAN flow was higher than N intake on diets C8A2 and C6A4 by 14 and 6 g/d, respectively, indicating that N was recycled to the rumen through the rumen wall or via saliva. Duodenal flow of ammonia N linearly increased from 1.7 g/d on diet C8A2 to 3.8 g/d on diet C2A8 (Table 3). The linear increase in duodenal flow of ammonia N paralleled the linear increases in RDN supply, and there was a positive correlation ( $r = 0.82$ ) between RDN and duodenal ammonia N flow.

#### Net PDV fluxes of AAN, ammonia N and urea N

Increasing N intake did not affect blood nor plasma flows across the PDV (Table 5). Thus, changes in net flux of N metabolites across the PDV largely reflected venous-arterial concentration differences for these metabolites

except for urea N (Table 4). Arterial and portal plasma concentrations of AAN showed a quadratic response with diet C6A4 being the highest, while the portal-arterial concentration difference (P-A) linearly increased with high levels of alfalfa hay (Table 4). The increased arterial concentration of AAN on diet C6A4 without a corresponding increase in PDV recovery may contribute to a higher sequestration rate of amino acids across the PDV (MacRae et al., 1997). These results also suggest that the arterial concentration of AAN does not necessarily reflect net release from the PDV (Fukuma et al., 2005). Net PDV release of AAN as grams/day increased linearly with high levels of alfalfa hay (Table 5). This could be attributed to the greater duodenal NAN flow and the increased N digestion in the intestine, as well as the increased DE intake. Based on the treatments means, net PDV absorption of AAN was positively correlated with duodenal NAN flow ( $r = 0.94$ ) and intestinal N disappearance ( $r = 0.93$ ).

Net PDV release of AAN was equivalent to 66, 55, 54 and 48% of N apparently digested in the total tract for C8A2, C6A4, C4A6 and C2A8 diets, respectively (calculated from Table 3 and Table 5). The higher values reported with diets C8A2 and C6A4 were probably due to the greater contribution of endogenous N to duodenal N flow: 14 and 6 g/d for diets C8A2 and C6A4, respectively. Through many studies, the net PDV of AAN/digested N in the total tract averaged 54 and 61% in steers fed all- and high-forage diets, respectively (Huntington and Archibeque, 1999). This implies a greater recovery of digested N as

**Table 4.** Nitrogen concentration (mM) and venous-arterial concentration difference (mM) in portal-drained viscera of growing steers fed varying dietary ratios of whole-crop corn silage (A) and alfalfa hay (A)

Item	Dietary treatments				SEM <sup>1</sup>	p-value <sup>2</sup>	
	C8A2	C6A4	C4A6	C2A8		L	Q
$\alpha$ -amino N							
Portal	2.220	2.520	2.420	2.370	0.067	0.302	0.079
Arterial	2.050	2.360	2.090	2.100	0.068	0.432	0.021
Portal-arterial	0.154	0.207	0.236	0.226	0.035	0.044	0.163
Ammonia N							
Portal	0.223	0.262	0.318	0.346	0.021	0.006	0.835
Arterial	0.076	0.072	0.066	0.051	0.009	0.179	0.637
Portal-arterial	0.157	0.190	0.254	0.317	0.015	<0.001	0.330
Urea N							
Portal	2.730	4.740	5.78	8.10	0.380	<0.001	0.646
Arterial	2.980	4.960	5.91	8.21	0.340	<0.001	0.472
Portal-arterial	-0.200	-0.212	-0.136	-0.097	0.037	0.190	0.680

<sup>1</sup> Standard error of least squares means. <sup>2</sup> L = Linear effect; Q = Quadratic effect.

absorbable AAN in our study especially from low-N diets. Conversely, as a percentage of apparent intestinal N disappearance, the net PDV release of AAN was 49, 50, 58 and 61% on C8A2, C6A4, C4A6 and C2A8 diets, respectively (calculated from Table 3 and Table 5). To our knowledge, such a response has not been reported with steers and this quantitative information could contribute to better understanding of the PDV metabolism of ruminants. The differences in proportional AAN recovery of intestinal N disappearance may be related to differences in energy intake level, site and extent of endogenous N secretion in the intestine, extent of metabolism of amino acids by the intestinal tissues and/or ammonia N absorption from the lower digestive tract.

Ammonia N concentrations in the portal vein and P-A difference (Table 4) and net PDV absorption as grams/d (Table 5) increased linearly with increasing alfalfa hay. The increased PDV absorption of ammonia N with alfalfa hay is attributed to increased N intake and the high rate of N degradation within the rumen. A considerable portion of the daily digestible N intake appeared across the PDV as net ammonia release: 92, 74, 89 and 86% on C8A2, C6A4, C4A6 and C2A8 diets, respectively. Our values are higher than reported in previous studies with steers fed forage diets (average 72%; Lappierre and Lobley, 2001) probably

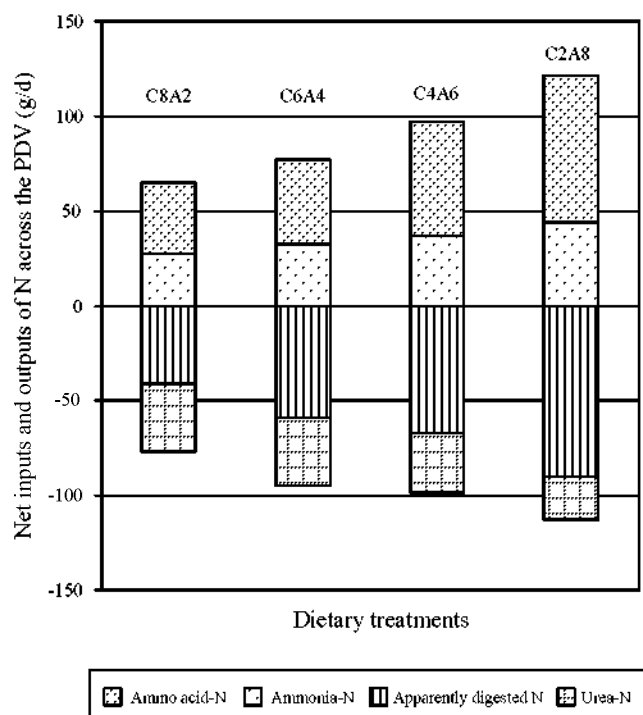
because of the higher rates of ruminal N degradation and recycled urea N in the current study. The ratio of absorbed ammonia N to absorbed AAN increased with high alfalfa hay representing 1.32, 1.33, 1.62 and 1.87 for C8A2, C6A4, C4A6 and C2A8A diets, respectively (calculated from Table 5). Other studies have indicated that N absorbed as ammonia exceeds N absorbed as AAN in steers fed all- or high- forage diets (Lappierre and Lobley, 2001). A substantial portion of ammonia N absorbed across PDV could be derived from degradation of urea N transferred into the gut lumen.

The portal and arterial plasma urea N concentrations increased linearly with high alfalfa hay, but P-A difference was not affected (Table 4). The PDV urea flux that provides the urea N recycled via the gut wall except for saliva decreased with nonsignificant response and was the highest numerically for C8A2 diet (Table 5). On a proportional basis, however, urea N transfer by PDV relative to apparent digestible N linearly ( $p < 0.05$ ) decreased with increasing N intake and represented 81, 54, 51 and 25% of total tract digestible N on C8A2, C6A4, C4A6 and C2A8 diets, respectively (calculated from Table 3 and Table 5). The greater PDV uptake of urea N on diet C8A2 is in agreement with other studies which reported an improved urea N recycling in ruminants fed low-N diets (Marini and Van

**Table 5.** Least squares means for arterial packed cell volume (PCV), blood flow and nitrogen fluxes across portal-drained viscera of growing steers fed varying dietary ratios of whole-crop corn silage (C) and alfalfa hay (A)

Item	Dietary treatments				SEM <sup>1</sup>	p-value <sup>2</sup>	
	C8A2	C6A4	C4A6	C2A8		L	Q
Arterial PCV (%)	25.2	27.4	27.6	26.2	0.68	0.326	0.079
Portal blood flow (L/h)	729	712	671	738	107	0.977	0.726
Portal plasma flow (L/h)	548	531	491	547	82	0.906	0.685
Net portal absorption (g/d)							
$\alpha$ -amino N	27.3	32.8	36.7	43.7	3.98	0.042	0.872
Ammonia N	37.9	44.1	60.1	77.9	8.39	0.023	0.549
Urea N	-35.8	-34.8	-31.4	-23.4	6.80	0.261	0.662

<sup>1</sup> Standard error of least squares means. <sup>2</sup> L = Linear effect; Q = Quadratic effect.



**Figure 1.** Portal-drained viscera inputs and outputs of nitrogen in growing steers fed varying dietary ratios of whole-crop corn silage (C) and alfalfa hay (A). Positive value indicates net output whereas negative value indicates net input.

Amburgh, 2003; Marini et al., 2004). In this study, however, PDV urea N uptake relative to digested N averaged 55%, which is higher compared to the values recorded for cattle but lower compared to the mean value for sheep. Lapiere and Lobley (2001) reported that urea returned to the gut represents, on average, between 30 and 40% of the digested N in cattle and up to 80% in sheep. Whitt et al. (1996) suggested that the twice-daily feeding cycle is associated with diurnal variation of urea N recycling, and this may explain the greater urea N recycling in our study compared to previous studies, which mostly were carried out under steady state conditions with frequent feeding. Such a large amount of urea N recycled into the digestive tract for the current forage diet usually seems to be a cause of a high ammonia N absorption under high forage diets. If ammonia N derived from urea N recycled into the rumen was always available for microbes, a shortage of dietary RDN could be quite small under high forage feeding except for low digestible N intake.

Overall, Figure 1 summarizes the extent of net inputs and outputs of N metabolites by the PDV across all forage diets. Measured PDV outputs (net ammonia N and AAN absorption) accounted for 1.59, 1.30, 1.44 and 1.35 of apparently digested N in the total tract on C8A2, C6A4, C4A6 and C2A8 diets, respectively. When we added the PDV removal of urea N to the apparently digested N, the PDV outputs accounted for 0.85, 0.81, 0.98 and 1.01 of

measured PDV inputs (apparently digested N and net PDV removal of urea N) on C8A2, C6A4, C4A6 and C2A8 diets, respectively. The differences in output:input ratios among the diets are probably due to differences in protein and energy intake as reflected in recovery of intestinal N disappearance as AAN. To improve the efficiency of N utilization by forage-fed ruminants, more research is necessary on the influence of energy intake on AAN recovery by PDV.

In conclusion, increasing alfalfa hay at the expense of corn silage increased nutrient intake and digestion, duodenal N flow and PDV N release, but decreased the transfer of plasma urea N to the gut. AAN absorption by PDV was highly correlated with intestinal N disappearance, but the recovery proportion varied with the feeding ratio of whole-crop corn silage and alfalfa hay. Net PDV release of AAN increased linearly with increasing N intake representing 66, 55, 54 and 48% of apparently digested N, and 49, 50, 58 and 61% of intestinal N disappearance for C8A2, C6A4, C4A6 and C2A8 diets, respectively. The recovery of AAN to N disappearance in the intestine may vary with the intake of mixed forage diets. The optimal ratio of whole-crop corn silage and alfalfa hay through all variables measured was unclear, but considering the microbial efficiency, PDV uptake of urea N and conversion of total tract digested N to PDV net AAN supply, a diet consisting of 80% corn silage and 20% alfalfa hay was the best. On the other hand, considering the quantities of intestinal N disappearance and AAN absorption, a diet of 20% whole-crop corn silage and 80% alfalfa hay would be preferred.

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