Duodenal Bacterial and Nonbacterial Protein Supply in Steers Fed Forage and Grain Diets

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DUODENAL BACTERIAL AND NONBACTERIAL PROTEIN SUPPLY IN STEERS FED FORAGE AND GRAIN DIETS[1]

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ABSTRACT

Four beef steers (avg wt 300 kg) fitted with duodenal re-entrant cannulae were used to study the effect of dietary concentrate to forage ratio on bacterial and nonbacterial N flow in the duodenum. According to a change-over design, the steers were designated to receive an all forage (83% alfalfa hay and 17% wheat straw) and an 80% sorghum grain diet. Lignin (ADL) and chromium oxide (Cr_2O_3) ratio techniques were compared with automated total collection (ATC) of digesta for quantitating duodenal protein flow and efficiency of bacterial N yield in the rumen. Estimates of bacterial protein synthesis and ruminal escape of feed protein based on Cr_2O_3 and lignin tended to be higher by 8 to 16% than those obtained by ATC. Efficiency of ruminal bacterial protein yield estimated by these two markers tended to be greater than that based on ATC (16 vs 12 g of bacterial protein/100 g ruminal true digestion of dry matter corrected for bacterial cell synthesis). Efficiency values did not differ between diets. Crude protein flow into the duodenum was about 33% greater ($P<.01$) for the grain than the forage diet, although protein intake was about 10% less on the grain diet. Duodenal bacterial protein, rather than feed protein escaping ruminal degradation, accounted for most of this difference. Average duodenal flow of N, expressed as g/Mcal metabolizable energy (ME) intake, was 11.9 for the forage diet and 10.3 for the grain diet. Grams of duodenal bacterial N per Mcal ME intake were similar ($P>.10$) for the diets (5.1 vs 5.0), but duodenal nonbacterial N (g/Mcal ME intake) was greater ($P<.01$) for the forage than the grain diet (6.9 vs 5.3). With the grain diet, microbial N production exceeded the amount of feed N degraded in the rumen, presumably by utilizing extensive amounts of recycled N for ruminal bacterial synthesis. Recycled N may be of greater magnitude in grain than forage diets. Dietary concentrate to forage ratios showed a marked effect on ruminal protein digestion and duodenal flow of protein.
(Key Words: Duodenum, Protein Digestion, Microbial Protein, Forage, Concentrates.)

Introduction

Bacterial protein synthesized in the rumen and feed protein that passes through the rumen are the sources of amino acids available to ruminant animals. Several dietary factors greatly influence microbial protein synthesis and the amount of feed protein passing nondegraded through the rumen, thus altering amounts and kinds of amino acids available for absorption from the small intestine (Theurer, 1979). These dietary factors include: level of feed intake (Weller et al., 1971; Tamminga et al., 1979); dietary protein source (Ling and Buttery, 1978; Zinn et al., 1981); dietary N concentration (Sutton et al., 1975; Amos et al., 1976); forage or grain processing methods (Tamminga, 1975; Prigge et al., 1978) and forage-to-concentrate ratios (Cole et al., 1976; Tamminga, 1979; Oldham and Tamminga, 1980; Wanderley and Theurer, 1983; Zinn and Owens, 1983b). However, accurrate in vivo measurement of bacterial and feed protein reaching the small intestine is difficult and, according to Theurer (1979, 1982), part of the difficulty is caused by the methods used to quantitate bacterial and feed protein in digesta samples, as well as digesta flow rates throughout the gastrointestinal tract.

The objectives of this study were: 1) ascertain the effect of a forage vs concentrate diet on the duodenal bacterial and nonbacterial (feed) protein flow to the small intestine of steers and 2) compare automated total collection of digesta with chromium oxide (Cr_2O_3) and lignin as digesta markers for estimating duodenal protein flow rates of bacterial and feed origin.

Experimental Procedures

Procedures, diets and animals are outlined

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by Wanderley et al. (1985). Four beef steers (avg wt 300 kg) fitted with duodenal re-entrant cannulae were used during two collection periods (3 to 6 d each). Two steers received an all-forage diet (83% alfalfa hay and 17% wheat straw) and two an 80% sorghum grain diet in the first period; the diets were switched for the second period. Animals were fed their respective diets (about 4 kg/d), twice daily, for at least 21 d before collection. Automated total collection of duodenal digesta was conducted for 6 d (continuously measuring and sampling). Samples were pooled on a 24-h basis. Days with missing periods of recorded measurements were deleted, resulting in data from 3- to 6-d collection periods.

On a dry matter (DM) basis, the forage diet contained 14.9% crude protein and the grain diet, 12.6%. Metabolizable energy (ME) intake was estimated from daily DM intake, according to the diet composition and based on the NRC (1984) ME values for each feed. Chromium oxide (Cr_2O_3) and acid detergent lignin (ADL) were used as digesta markers and compared with automated total collection (ATC) for estimating duodenal DM and protein flow (Wanderley et al., 1985).

Samples of bacteria from each steer, for each collection period, were obtained by ATC and strained through six layers of cheese-cloth and glass wool. These samples were separated by differential centrifugation of duodenal digesta (initially at 3,000 x g and then the supernatants at 18,000 x g) following the procedure of Smith and McAllan (1974), and analyzed for protein and dry matter. Ruminal true digestion of DM (DMc) was estimated by correcting for de novo bacterial cell synthesis. The latter was estimated from the relationship between amounts of bacterial N reaching the duodenum and the DM and N content of bacterial samples collected from steers. Bacterial protein in duodenal digesta was estimated by the diaminopimelic acid method (DAP; Rahnema and Theurer, 1986).

Chromium oxide was determined by the perchloric acid method of Kimura and Miller (1957), while Kjeldahl N and DM analyses were according to AOAC (1975); ADL determinations were by the procedure of Goering and Van Soest (1970). Dates were analyzed by analysis of variance (Steel and Torrie, 1960) as a factorial arrangement, with diet and flow measurement methods as main effects; animal and interaction effects also were considered.

It was assumed that the adaptation period (minimum of 21 d) was adequate to eliminate any potential diet carry-over effect between collection periods. Because of the small number of animals, collection period effects was not considered in the analysis. The model used was:

$$Y_{ijk} = \mu + M_i + D_j + A_k +$$

$$MD_{ij} + MA_{ik} + DA_{jk} + e_{ijk},$$

where M = methods, D = diets and A = animals.

Results and Discussion

Average total crude protein flow into the duodenum was about 33% greater ($P<.01$) for the grain than the forage diet (table 1), even though protein intake was about 10% less on the grain diet. Bacterial protein, rather than feed protein escaping ruminal degradation, accounted for most of the differences between diets, since bacterial protein was 55% greater ($P<.01$); nonbacterial protein reaching the duodenum was only 20% greater ($P<.05$) for the grain than for the forage diet. This indicates that more bacterial protein was synthesized in the rumen of the grain-fed steers, which might be attributed to a higher intake of ruminal available energy (Oldham and Tamminga, 1980). The marked net gain (from diet to duodenum) for total protein on the grain diet, shown in table 1, has been reported previously by Wanderley and Theurer (1983). Greater amount of protein of feed and(or) bacterial origin reaching the small intestine for concentrate than for forage diets is, however, the overall tendency (Zinn and Owens, 1983b).

Although the differences were not significant ($P>.10$), chromium or lignin techniques yielded 6 to 18% higher estimates of fractional flow of protein in the duodenum than that based on ATC (table 1). These higher estimates of duodenal flow are consistent with incomplete recovery of digesta markers from the duodenum, which often have been reported (Tamminga, 1975; Sutton et al., 1976; Zinn et al., 1980), and result in underestimates of ruminal digestibilities (Wanderley et al., 1985). No interaction for main effects was detected ($P>.10$).

Bacterial DM entering the duodenum was 69% greater ($P<.01$) for the grain than for the forage diet (table 2), whereas duodenal flow of nonbacterial DM was lower ($P<.01$) for the

grain diet compared with the forage diet. Estimates based on chromium or lignin techniques tended to be greater than those based on ATC. Based on these fractional flow rates in the duodenum, disappearance of DMc in the rumen was estimated. Ruminal disappearance of DMc was greater ($P<.01$) when the steers were fed the grain than when fed the forage diet, regardless of the method used to estimate digesta flow (table 3). No interactions for main effects were detected ($P>.10$).

No differences were observed ($P>.10$) between diets in the amount of bacterial protein reaching the duodenum per 100 g of DMc (table 4). According to the most recent NRC (1984), bacterial protein values range from 8 to 27 g/100 g of organic matter fermented (mean of 15), with higher values usually associated with forage diets. Mathison and Milligan (1971), using lignin and ^{15}N as digesta and microbial markers, found that microbial growth resulted in the assimilation of 1.7 and 1.9 g N/100 g DM digested in the rumen of sheep fed high-protein hay and barley diets, respectively. The data of McMeniman et al. (1976), as summarized by Theurer (1979), suggest that microbial protein yield is greater on forage than concentrate diets. Cole et al. (1976) reported an increase from 7 to 13 g of microbial protein per 100 g DM fermented in the rumen of steers that were fed high grain diets, as a result of increasing the roughage level from 0 to 21% in the diet. Data from Zinn and Owens (1983b) did not show any clear effect of the dietary roughage to concentrate ratio on the efficiency of microbial synthesis; however, their data suggest lower ruminal escape values (percentage of feed protein escaping ruminal degradation) for protein supplements fed with forage diets than when fed with high concentrated diets.

Tamminga (1979) concluded that an optimum ratio between nonstructural and structural

TABLE 1. FRACTIONAL FLOW OF PROTEIN IN THE DUODENUM OF STEERS RECEIVING FORAGE AND GRAIN DIETS AND ESTIMATED BY DIFFERENT METHODS[a]

Item	Forage		Grain	
	Mean	SD[b]	Mean	SD[b]
	g/d			
Protein intake	522	27	481	56
Total duodenal protein				
ATC[c]	461	57	623	46
Cr_2O_3	515	138	671	106
Lignin	537	99	725	100
Avg	504[f]		672[g]	
Bacterial duodenal protein[d]				
ATC[c]	195	44	307	66
Cr_2O_3	216	71	326	53
Lignin	227	57	351	38
Avg	213[f]		328[g]	
Nonbacterial duodenal protein[e]				
ATC[c]	266	61	316	67
Cr_2O_3	299	94	345	100
Lignin	309	84	374	117
Avg	291[h]		345[i]	

[a]Average of four steers and 3 to 6 d of total collection.

[b]Standard deviation of observations.

[c]ATC = automated total collection.

[d]Based on diaminopimelic acid concentrations.

[e]Differences between total duodenal protein and bacterial protein (assumed to be largely feed protein escaping ruminal degradation).

[f,g]Means within rows not having a common superscript differ ($P<.01$).

[h,i]Means within rows not having a common superscript differ ($P<.05$).

TABLE 2. FRACTIONAL FLOW OF BACTERIAL AND NONBACTERIAL DRY MATTER IN THE DUODENUM OF STEERS RECEIVING FORAGE AND GRAIN DIETS AND ESTIMATED BY DIFFERENT METHODS[a]

Item	Forage		Grain	
	Mean	SD[b]	Mean	SD[b]
	g/d			
Dry matter intake	3,500	200	3,825	450
Bacterial dry matter				
ATC[c]	482	97	824	160
Cr_2O_3	529	134	877	140
Lignin	559	120	944	96
Avg	523[d]		882[e]	
Nonbacterial dry matter				
ATC[c]	1,718	357	1,261	328
Cr_2O_3	2,033	649	1,414	393
Lignin	1,946	376	1,561	720
Avg	1,899[d]		1,412[e]	

[a]Average of four steers and 3 to 6 d of total collection.

[b]Standard deviation of observations.

[c]ATC = automated total collection.

[d,e]Means within rows not having a common superscript differ (P<.01).

TABLE 3. DRY MATTER (DMc) DISAPPEARANCE IN THE RUMEN OF STEERS RECEIVING FORAGE AND GRAIN DIETS AND ESTIMATED BY DIFFERENT METHODS[ab]

Item	Forage		Grain	
	Mean	SD[c]	Mean	SD[c]
DMc disappearance, g/d				
ATC[d]	1,782	258	2,564	606
Cr_2O_3	1,467	731	2,410	624
Lignin	1,554	296	2,276	969
Avg	1,600[e]		2,417[f]	
DMc disappearance, %				
ATC[d]	51	9	67	10
Cr_2O_3	41	19	63	12
Lignin	45	9	59	22
Avg	46[e]		63[f]	

[a]Average of four steers and 3 to 6 d of total collection.

[b]Corrected for bacterial dry matter synthesized in the rumen.

[c]Standard deviation of observations.

[d]ATC = automated total collection.

[e,f]Means within rows not having a common superscript differ (P<.01).

TABLE 4. EFFICIENCY OF BACTERIAL PROTEIN SYNTHESIS IN THE RUMEN OF STEERS RECEIVING FORAGE AND GRAIN DIETS AND ESTIMATED BY DIFFERENT METHODS[a]

Item	Forage		Grain		Avg
	Mean	SD[b]	Mean	SD[b]	
	g/100 g DMc[c] disappearance				
Bacterial protein					
ATC[d]	11	1	12	2	12
Cr_2O_3	18	12	14	1	16
Lignin	15	4	18	9	16
Avg	15		15		

[a]Average of four steers and 3 to 6 d of total collection.

[b]Standard deviation of observations.

[c]DMc = corrected for bacterial dry matter synthesized in the rumen.

[d]ATC = automated total collection.

carbohydrate in the diet must exist for maximum microbial synthesis in the rumen. According to Kaufmann and Lupping (1982), based on duodenal measurements in dairy cows, high levels of either forage (>80%) or concentrate (>70%) in the diet are associated with lower rates of bacterial protein synthesis in the rumen.

Efficiency of bacterial protein yield in the rumen estimated by Cr_2O_3 and lignin was 17 to 64% greater than that estimated from ATC (table 4), and supports the assumption that these two markers may overestimate duodenal flow and underestimate ruminal digestibilities. Moreover, bacterial yields estimated from lignin ratio for the grain and Cr_2O_3 ratio for the forage diet showed great variation. Probably due to this variation, difference between methods were not significant (P>.10). Coefficients of variation were 50% for bacterial yields estimated by lignin ratio for the grain diet and 68% for yields estimated by Cr_2O_3 ratio for the forage diet. These high variations reflect similar observations for digesta flow (Wanderley et al., 1985). These differences in variation might be considered as an indication of a marker method vs diet interaction, but the statistical analysis did not verify an interaction (P>.10). Theurer (1979) suggested that flow markers may have a more profound effect on calculation of microbial synthesis than microbial markers.

Flow of duodenal N per megacalorie of estimated ME intake averaged 10.3 g for the grain diet and 11.9 for the forage diet (P>.10; table 5). Partitioning this ratio, bacterial N/ME intake was similar (P>.10) for both diets; however, nonbacterial N/ME intake was greater (P<.01) for the forage than for the grain diet. Mathison and Milligan (1971) found microbial growth resulting in the assimilation of 4.1 and 4.3 g N/Mcal energy fermented in the rumen of sheep receiving barley and high-protein hay diets, respectively.

Oldham and Tamminga (1980) state that the "limiting value" of duodenal supply of non-ammonia N appears to be close to 2.5 to 3.0 g/MJ ME intake (or 10.5 to 12.6 g/Mcal) for both forage or concentrate diets. Their values agree with estimates from the equation of Journet and Verite (1979). Thus, ME intake would be a major determinant of duodenal nonammonia N supply. The higher intake of nonstructural carbohydrate in the grain diet apparently increased ruminal microbial synthesis as well as microbial protein reaching the small intestine, and may be proportional to the estimated increases in ME intake. However, response to various grains may differ (Oldham and Tamminga, 1980). Using the data of Spicer et al. (1986), grams of duodenal N/Mcal ME intake were 9.2, 7.4 and 9.0 respectively, for sorghum-, corn- and barley-based diets.

A high rate of fermentation leading to efficient capture of ruminal degraded N and(or) of N cycled into the rumen, may be the dominating factor to be considered in describing extent of microbial synthesis and efficiency of microbial yield in the rumen. Oldham and

Tamminga (1980) described N efficiency of microbial synthesis as the ratio of microbial N synthesized to feed N degraded in the rumen. In our studies, these values differed (P<.01) between diets and were .8 to 1.2 for the forage and 2.1 to 2.5 for the grain diet (table 6), considering feed N degraded as the difference between N intake and duodenal nonbacterial N. This ratio indicates greater microbial N yield in relation to feed N degraded for the grain vs the forage diet, suggesting a large influx of endogenous N which was utilized for bacterial synthesis in the rumen of steers receiving the grain diet.

Duodenal N flow, expressed as a percentage of N intake, was highly correlated with ratio of bacterial N to degraded feed N in the rumen for both diets (r=.97) using flow values estimated by ATC, Cr_2O_3 and lignin). The regression equation was: Y = 24.93x + 75.33, (P<.01), where Y = duodenal N flow as percent of intake and x = bacterial N/feed N degraded.

Oldham et al. (1979), using total collection to measure digesta flow and either RNA or DAP as microbial markers, reported values of microbial N yield 20 and 25% greater than feed N degraded in the rumen of cows fed a 90% barley diet. Calculations from data presented in several studies (Cole et al., 1976; Prigge et al., 1978; Muntifering et al., 1981; Stern et al., 1983; Zinn and Owens, 1983a; Spicer et al., 1986) based on various bacterial and flow markers, indicate that yields of microbial N could be greater than accounted for by feed N degraded in the rumen of cattle fed high grain diets (ratios of microbial N to feed N degraded ranged from .6 to 2.6).

It appears that N recycled from post-ruminal areas to the microbial population in the rumen is of great magnitude in various feeding conditions, especially with high grain diets, and must play an important role in modifying the duodenal amino acid supply. According to Varady et al. (1979), endogenous urea transferred from the blood through the rumen wall may be in such quantities as to supply the total N requirements of ruminants, under certain conditions.

While there is some disagreement on the quantitative aspects of N sources entering the large intestine, it is generally agreed that ammonia is the main N compound absorbed from the hindgut. Hoover (1978) states that ammonia absorption for the hindgut is probably a major source of N recycled to the rumen, and that the major contribution of the large intestine of ruminants to N metabolism is in the recovery

TABLE 5. RATIOS BETWEEN FRACTIONAL FLOW OF N IN THE DUODENUM AND ESTIMATED METABOLIZABLE ENERGY (ME) INTAKE IN STEERS RECEIVING FORAGE AND GRAIN DIETS AND DETERMINED BY DIFFERENT METHODS[a]

Item	Forage		Grain	
	Mean	SD[b]	Mean	SD[b]
	g/Mcal ME			
Total duodenal N				
ATC[c]	10.9	1.0	9.5	.5
Cr_2O_3	12.3	3.9	10.2	.9
Lignin	12.7	2.1	11.1	1.7
Avg	11.9		10.3	
Bacterial duodenal N				
ATC[c]	4.6	1.0	4.7	1.0
Cr_2O_3	5.2	.5	5.2	2.0
Lignin	5.4	.7	5.4	1.3
Avg	5.1		5.0	
Nonbacterial duodenal N				
ATC[c]	6.3	1.3	4.8	1.0
Cr_2O_3	7.1	2.3	5.3	1.4
Lignin	7.3	1.8	5.7	1.9
Avg	6.9[d]		5.3[e]	

[a]Average of four steers and 3 to 6 d of total collection.

[b]Standard deviation of observations.

[c]ATC = automated total collection.

[d,e]Means not having a common superscript differ (P<.01).

TABLE 6. RATIO OF BACTERIAL N SYNTHESIZED TO FEED N DEGRADED IN THE RUMEN OF STEERS RECEIVING FORAGE AND GRAIN DIETS AND ESTIMATED BY DIFFERENT METHODS[a]

Item	Forage		Grain	
	Mean	SD[b]	Mean	SD[b]
Duodenal bacterial N (BN), g/d				
ATC[c]	31	7	49	10
Cr_2O_3	35	11	52	9
Lignin	36	9	56	7
Avg	34[e]		52[f]	
Feed N degraded[d] in the rumen (FND), g/d				
ATC[c]	41	8	26	11
Cr_2O_3	36	17	22	14
Lignin	34	12	26	11
Avg	37[e]		24[f]	
Ratio BN/FND[g]				
ATC[c]	.76	.16	2.06	.72
Cr_2O_3	1.21	.87	2.39	.97
Lignin	1.20	.73	2.48	.92
Avg	1.06[e]		2.31[f]	

[a]Average of four steers and 3 to 6 d of collection.

[b]Standard deviation of observations.

[c]ATC = automated total collection.

[d]Feed N degraded = difference between N intake and duodenal nonbacterial N (derived from table 1).

[e,f]Means within rows not having a common superscript differ ($P<.01$).

[g]Ratios of BN to FND are based on individual steer values and not on mean treatment values.

and transfer of N to body fluids, where it may be used for the synthesis of nonessential amino acids in the liver or be returned to the rumen for microbial protein production. Studies are needed to understand better the mechanisms and elucidate the significance of the recycling-N process in ruminants.

For achieving a maximum yield of microbial protein, an optimum ratio between dietary structural and nonstructural carbohydrates seems to exist even though this ratio is not clearly understood (Tamminga, 1979). Further studies to elucidate the importance of this ratio vs source of protein would be useful for manipulating diets and optimizing the supply of protein in the small intestine, particulary for high-producing animals.

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