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Influence of Site of Casein Infusion on Voluntary Feed Intake and Digestive Function in Steer Calves Fed a Sudangrass-Based Growing Diet

[1]E.G. Alvarez and [2]R.A. Zinn
[1]Instituto de Ciencias Agrícolas-UABC, Mexicali, México
[2]Desert Research and Extension Center, University of California, El Centro 92243

Abstract: Four medium-frame steer calves (269 kg BW) with cannulas in the rumen, abomasum and proximal duodenum were used in a 4 H 4 Latin square experiment. Steers were allowed ad libitum access to the basal diet, offering 120% of the previous days DMI. Treatments consisted of infusing sodium caseinate (300 g d^{-1}) into: 1) rumen, via the ruminal cannula; 2) abomasum, via ruminal cannula; 3) abomasum, via the abomasal cannula and 4) proximal duodenum, via the duodenal cannula. Dry matter intake averaged 97 g kg^{-1} $BW^{.75}$ and was not affected ($p>0.20$) by treatments. There were no treatment effects ($p>0.20$) on flow of OM, NDF and ADF to the small intestine. As expected due to the high ruminal degradability of casein (86%), flow of nonammonia N to the small intestine was greater ($p<0.01$) when casein was infused postruminally then when it was infused ruminally. Casein infusion did not affect ($p>0.20$) ruminal degradability of dietary N. Observed ruminal degradability of dietary N was in close agreement with expected (49 vs 51%, respectively) based on the National Research Council's Level 1 model. There were no treatment effects ($p>0.20$) on ruminal NDF digestion. However, ruminal ADF digestion was greater (5%, $p<0.05$) when casein was infused postruminally then when it was infused ruminally. As expected, postruminal casein infusion increased postruminal digestion of the OM (21%, $p<0.05$) and N (12%, $p<0.01$). There were no treatment effects ($p>0.20$) on total tract digestion of OM, N, ADF, NDF and GE. Casein infusion did not influence ($p>0.20$) flow of chyme to the small intestine or ruminal turnover. Flow of chyme to the small intestine was primarily a function of DMI (chyme, L = 5.3 + 12.1DMI; $R^2 = 0.50$). Postruminal casein infusion increased (75%, $p<0.01$) the soluble N content of duodenal chyme, but it did not affect ($p>0.20$) its tonicity, averaging 264 mOsm. The relationship between tonicity and passage rate of chyme from the abomasum was small ($R^2 = 0.05$). Casein infusion did not affect ($p>0.20$) ruminal DM content or liquid volume, averaging 17.5 g DM kg^{-1} $BW^{.75}$ and 471 g kg^{-1} $BW^{.75}$, respectively. There were no treatment effects ($p>0.20$) on indigestible NDF intake (averaging 26 g kg^{-1} $BW^{.75}$) or ruminal NDF fill (averaging 41.1 g kg^{-1} $BW^{.75}$). Casein infusion did not affect ($p>0.20$) ruminal pH, but ruminal acetate:propionate molar ratio was greater ($p<0.10$) when casein was infused ruminally. Ruminal acetate:propionate molar ratio was also greater ($p<0.05$) when casein was infused into the abomasum versus the proximal duodenum. We conclude that independently of N status, increasing protein supply to the small intestine will not facilitate an increase in DM intake. Instead, limits to maximal DM intake in cattle fed forage-based diets appears to be largely a function of ruminal NDF fill capacity and rates of passage and digestion of NDF.

Key words: Casein, infusion, intake, metabolism, cattle, NDF

INTRODUCTION

Nitrogen supplementation to ruminants has been considered essential to optimize forage usage. But, its role in the regulation of forage intake remains unclear. Zinn *et al.* (1981) observing that flow of chyme from the abomasum was closely associated with N flow, proposed that protein supplementation may indirectly enhance DM intake, by increasing the rate of ruminal turnover. Consistent with this, increasing dietary N level or infusing N into the rumen has increased rate of passage of DM from the rumen (Köster *et al.*, 1996; Olson *et al.*, 1999; Mathis *et al.*, 2000). Following a series of trials, Egan (1970) proposed that increasing N status may increase set points for ruminal fill, thus allowing for greater DM intakes. In those studies where postruminal protein infusions enhanced DM intake (Egan and Moir, 1965; Garza *et al.*, 1991), casein was infused into the proximal duodenum. In contrast, where postruminal protein infusion failed to enhance DM intake (Johnson *et al.*, 1981; Clark *et al.*, 1977; Titgemeyer and Merchen, 1990; Swanson *et al.*, 2004), casein was infused into the

Corresponding Author: E.G. Alvarez, Instituto de Ciencias Agrícolas-UABC, Mexicali, México

abomasum. Alvarez and Zinn (1998) observed that when casein was infused into the abomasum via the ruminal cannula, DM intake was actually depressed. The objective of this study was to further evaluate the role of postruminal protein supply on voluntary DM intake and digestive function in cattle fed a forage-based diet in which the postruminal protein supply of the basal diet is not limiting growth.

MATERIALS AND METHODS

Four crossbred steers (269 kg BW) fitted with cannulas in the rumen (7.5 cm i.d.), abomasum (fundic region, .7 cm i.d.) and in the proximal duodenum (6 cm from the pyloric sphincter, 1.9 cm i.d.) were used in a 4 H 4 Latin square experiment. Steers were housed in slotted-floor pens (1.42×2.74) with automatic drinkers. Ambient temperature in the metabolism unit was maintained between 21 and 26°C. The protocol for surgical preparation and maintenance and handling of steers was approved by the University of California, Animal Care Administrative Advisory Committee. Steers were fed the basal diet (Table 1) during a 4-wk adaptation period, prior to beginning the experiment. Steers were allowed ad libitum access to the basal diet, offering 120% of the previous days DMI, providing 70% at 1800 and 30% at 0800 the following day. Orts were weighed and re-included in the morning feeding allowance. The casein infusate solution was prepared daily by dissolving 1,200 g sodium caseinate (ICN Biomedicals, Dawn OH 44202) in 16 L of a 1% Na_2HPO_4 solution at 45EC. Each steer was infused with 4 L d^{-1} of the casein (167 mL h^{-1} at room temperature, 27°C). Infusion tubing (3.1 mm i.d. H 5.1 m Tygon®; Norton, Akron OH 44309-3660) was suspended using constant tension from the roof beams above each pen. This allowed free movement of steers during the infusion period. The casein solution was infused using a variable flow peristaltic pump (Variable Flow Mini-Pump II, VWR Scientific Products, West Chester, PA). Steers were infused with their respective casein treatments on d 7 through 14 of each experimental period. All steers were fed the same basal diet. Treatments consisted of infusing casein (300 g d^{-1}) into: 1) rumen, via the ruminal cannula; 2) abomasum, via ruminal cannula; 3) abomasum, via the abomasal cannula; 4) proximal duodenum, via the duodenal cannula. Abomasal infusion via the ruminal cannula was accomplished using a temporary catheter (3 mm i.d. Tygon tube) passed through the ruminal cannula and into the abomasum via the reticular-omasal orifice (Clark *et al*., 1977; Titgemeyer and Merchen, 1990; Alvarez and Zinn, 1998). Verification that the cannula was properly placed was accomplished by introducing 1 g of vegetable dye (diluted in 60 mL of distilled water) into the abomasum via the cannula and looking for the appearance of the coloring in duodenal chyme in less than 10 min. The trial consisted of four 14-d experimental period. Each period was divided into two phases: the first 6 d was the non-infusion phase. The infusion phase began on d 7 and continued through d 14. Samples were taken twice daily on d 11 to 14 as follow: d 1, 1000 and 2200, d 2, 0700 and 1900, d 3, 0400 and 1600 and d 4, 0100 and 1300. Infusion was in progress while sampling. Individual samples consisted of approximately 700 mL of duodenal chyme and 200 g (wet basis) of fecal material. Fecal samples represented a composite of material fecal that accumulated on floor slots during the collection interval. At 1000 on d 1 of the collection period (just before the first collection) 150 mL of a Li-Co-EDTA stable solution (Uden *et al*., 1980) was pulse dosed into the rumen of each steer via the ruminal cannula to estimate ruminal volume, fluid flow rate and retention time. A 100-mL aliquot of duodenal chyme obtained at each sampling time was centrifuged at 20.000×g for 20 min. Cobalt concentration (atomic absorption photometry, Perkin-Elmer 403, Norwalk CT; adjusted to 241 nm, with flow of air and acetylene in 12 and 40 psi, respectively, with limit of detection of .15 ppm), tonicity (Micro Osmometer Osmete®, Precisión Systems), and Kjeldahl N (AOAC, 1975) concentration of supernatant fluid was determined. During the final day of each collection period, ruminal samples were obtained from each steer 4 h after feeding, via the ruminal cannula. Ruminal fluid pH was determined on fresh samples. Ruminal fluid samples were strained through four layers of cheesecloth. Freshly prepared 25% (wt vol^{-1}) *m*-phosphoric acid (2 mL) was added to 8 mL of strained ruminal fluid. Samples were then centrifuged (17,000×g for 10 min) and supernatant fluid stored at -20°C for analysis

Table 1: Ingredient and chemical composition of basal diet (DM basis)

Item	(%)
Ingredient composition, % (DM basis)Sudan grass, hay	87.60
Yellow grease	3.00
Molasses, cane	8.00
Urea	0.40
Ammonium sulfate	0.20
Chromic oxide[a]	0.30
Trace mineral salt[b]	0.50
Nutrient composition (DM) basis)[c]NE, Mcal kg^{-1}	
Maintenance	1.37
Gain	0.79
Crude protein, %	12.47
DIP, %	51.05
Calcium, %	0.55
Phosphorus, %	0.31[a]

Chromic oxide added as a digesta marker. [b]Trace mineral salt contained: $CuSO_4$, .068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%; ZnO, 1.24%; $MnSO_4$ 1.07; KI, .52%; and NaCl, 92.96%. [c]Based on tabular values for individual feed ingredients (NRC, 1984) with the exception of supplemental fat, which was assigned NE_m and NE_g values of 6.03 and 4.76, respectively (NRC, 1996). Ruminal DIP values were based on NRC (1996), Level 1

of VFA concentrations (gas chromatography). Upon completion of the experiment, ruminal fluid was obtained from all steers and composited for isolation of ruminal bacteria via differential centrifugation (Bergen *et al*., 1968). Microbial isolates were prepared for purine and N analysis by oven drying at 55°C and then grinding with mortar and pestle. On the last day of each collection period 4 h after feeding, total rumen evacuation was accomplished on each steer using a wet dry^{-1} vacuum pump (Shop-Vac, Williamsport VA). A 200-g aliquot was obtained for DM and NDF analysis. Duodenal and fecal samples from each steer and within each period were composited for analysis. Feed, duodenal and fecal samples were first oven-dried at 55°C and ground in a laboratory mill (Micro-Mill, Bell-Arts Products, Pequannock, NJ). Samples were then oven-dried at 105°C until no further weight loss and stored in sealed glass jars. Samples were subjected to all or part of the following analysis: Ash, ammonia N, Kjeldahl N (AOAC, 1975); chromic oxide (Hill and Anderson, 1958); GE (adiabatic bomb calorimeter); NDF (Chai and Udén, 1998); ADF (Goering and Van Soest, 1970) and purines (Zinn and Owens, 1986). Microbial Organic Matter (MOM) and N (MN) leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter fermented in the rumen was considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and MOM reaching the duodenum. Feed N escape to the small intestine was considered equal to total N leaving the abomasum minus ammonia-N and MN and thus, includes any endogenous additions. For estimation of ruminal, postruminal and total tract digestion of the N and OM, calculations were adjusted for respective casein contributions. The model of Zinn and Salinas (1999), was used to estimate expected intake. The K_p for ruminal NDF was estimated as follows: $K_p = ((NDFI * (1-RDNDF))/(S * (RNDF/100)))/24$, where: NDFI = NDF intake (g d^{-1}), RDNDF = ruminal NDF digestibility (%), S = ruminal solids (g) and RNDF = ruminal NDF (as % ruminal solids). The Kd for ruminal NDF was determined according to the relationship $RDNDF = K_d (K_d + K_p)$.The experiment was analyzed as a 4×4 Latin square (Hicks, 1973). The treatment effects were tested for the following contrasts: 1) rumen infusion vs abomasum (via rumen), abomasum, and duodenum; 2) abomasum (via rumen) vs abomasum and 3) abomasum (via rumen) and abomasum vs duodenum.

Table 2: Effect of sodium caseinate infunsion site[a] on ruminal, postruminal and total tract digestion characteristcs in steers calves fed with forage based diet

Item	Rmn	AbR	Abm	Ddn	SEM	C1[b]	C2	C3
Body Weight, kg	258	258	258	258	-	-	-	-
Intake, g d^{-1}								
DM	6191	5679	5889	6204	210	.34	.86	.10
OM	5529	5071	5259	5540	188	.34	.86	.10
NDF	3141	2881	2987	3147	107	.34	.86	.10
ADF	2047	1877	1947	2051	70	.34	.86	.10
N	95.2	87.3	90.6	95.4	3.3	.34	.86	.10
Leaving abomasum, g d^{-1} abomasum								OM
	3258	3089	3104	3242	188	.99	.99	.20
NDF	1211	1046	1109	1204	87	.50	.99	.30
ADF	1019	886	890	995	49	.10	.99	.10
N	116	95	91	112	9	.13	.99	.10
Ammonia N	8.00	8.65	6.04	6.18	7.00	.20	<.01	.20
Non-ammonia N	108	87	85	106	9	.17	.99	.10
Microbial N	55.6	46.1	44.7	54.0	3.0	.07	.99	
.06Nonammonia-nonmicrobial N							Total	52.3
	81.3	81.5	93.0	6.4	<.01	.99	.18	
Feed N	52.3	40.5	40.7	52.2	6.5	.36	.99	.18
Rumen digestion,%								
DM	34.5	30.5	31.4	33.6	2.4	.99	.99	.99
OM	50.5	46.9	48.8	47.8	1.7	.20	.68	.99
NDF	61.4	63.4	62.8	62.1	2.0	.99	.99	.99
ADF	50.0	52.4	54.2	51.5	1.0	<.01	.20	.10
Feed N	44.5	51.7	55.1	45.6	6.5	.51	.99	.37
Microbial efficiency[c]	20.6	20.3	17.7	20.4	1.2	.60	.18	
.50Postruminal digestion, % flow to duodenum							OM	29.1
	37.1	35.6	38.0	2.2	.02	.99	.99	
N	62.5	69.5	68.9	71.6	1.4	<.01	.99	.18
Total tract digestion %								
of intakeOM	57.5	57.9	58.3	58.7	1.2	.99	.99	.99
NDF	54.3	55.8	55.0	56.2	1.1	.36	.99	.99
ADF	50.5	50.9	50.9	51.4	1.6	.99	.99	.99
N	68.1	68.1	68.6	68.1	.8	.99	.99	.99
Digestible Energy, %	56.2	56.9	57.0	57.4	1.3	.99	.99	.99

[a]Rmn = Rumen infusion, AbR = abomasum infusion throughout rumen, Abm = abomasum infusion, Ddn =Duodenum. [b]C1= Rmn vs AbR, Abm and Ddn; C2 = AbR vs Abm; C3 = AbR and Abm vs Ddn. [c]Grams of microbial N kg^{-1} of organic matter fermented

RESULTS AND DISCUSSION

Treatment effects on DMI and characteristics of ruminal digestion are shown in Table 2. Casein infusion did not enhance ($p>0.20$) DMI. Although, consistent with previous studies (Titgemeyer and Merchen, 1990; Alvarez and Zinn, 1998), infusion of casein into the abomasum via the reticulo-omasal orifice tended ($p = 0.17$) to depress DMI. Apparently, positioning the cannula in the abomasum via the reticulo-omasal orifice obstructs or otherwise interferes with passage of digesta to the abomasum. Direct abomasal casein infusion increased DMI of sheep fed a semipurified diet (Papas *et al*., 1974). However, for the most part, infusion of casein directly into the abomasum via an abomasal cannula has not enhanced DMI (Holstein cows fed corn silage, (Broderick *et al*., 1970) Holstein cows fed alfalfa hay, (Clark *et al*., 1977) steer fed low protein grass hay diet, (Johnson *et al*., 1981) steers fed a high concentrate diet, (Johnson *et al*., 1982). In contrast, infusion of casein into the proximal duodenum at levels similar to that used in the present study (0.65 g N kg^{-1} $BW^{.75}$) has increased DMI (Egan and Moir, 1965; Egan, 1970; Garza *et al*., 1991a).

Inconsistencies in the effects of duodenal casein infusion on DMI may be attributable to differences in basal levels of DMI. It is expected (NRC, 1984; Minson, 1990) that DMI by cattle with ad libitum access to forage-based diets will be within the range of 98 to 113 g kg^{-1} $BW^{.75}$. Dry matter intakes during ruminal casein infusion of steers in the present study averaged 97 g kg^{-1} $BW^{.75}$ (Table 2). Thus, notwithstanding the intensive experimental conditions and close confinement of the cattle, the level of DMI attained in our study approximated the expected maximal. In contrast, in studies where duodenal casein infusion has enhanced DMI over that of ruminal casein infusion, the basal level of DMI was considerably less than anticipated (40.4 g DM $BW^{-1}{}_{kg}{}^{.75}$), (Egan, 1965a) 33.4 g DM $BW^{-1}{}_{kg}{}^{.75}$, (Egan and Moir, 1965).

There were no treatment effects ($p>0.20$) on flow of OM, NDF and ADF to the small intestine. Flow of non-ammonia N to the small intestine when casein was infused ruminally averaged 108 g d^{-1}. Given that 80% of the non-ammonia N leaving the abomasum is % amino N and that 80% of this is truly digestible in the small intestine (Zinn and Owens, 1982), the estimated metabolizable protein supply was 432 g d^{-1}. Based on NRC (1996) the metabolizable protein requirements of a 269 kg steer calves consuming 5.9 kg d^{-1} of the basal diet is 412 g d^{-1}. Thus, the basal diet exceed the metabolizable protein requirements of the steers by 5%. Flow of non-ammonia N to the small intestine was greater ($p<0.01$) when casein was infused postruminally then when it was infused into the rumen. This was expected due to the high ruminal degradability of casein (Zinn *et al*., 1981; Köster *et al*., 1996; Dhiman and Satter, 1997).

Flow of MN to the small intestine was greater ($p<0.10$) when casein was infused ruminally then when it was infused postruminally. Although ruminal casein infusion increased DIP from 151 to 270 g kg^{-1} total tract digestible OM (DOM), the basal level of DIP (151 g kg^{-1} DOM) was markedly in excess (151%) of that shown to be optimal for microbial growth (Zinn and Shen, 1998). The increase in MN was apparently due to a tendency (6%, $p = 0.20$) for greater ruminal OM digestion when casein was infused ruminally then when it was infused postruminally. Indeed, there were no treatment effects ($p>0.10$) on ruminal microbial efficiency (g MN kg^{-1} OM fermented). The difference in ruminal OM digestion can be attributed to the high digestibility of the infused casein, per se. In previous studies (Egan and Moir, 1965; Garza *et al*., 1991b, Taniguchi *et al*., 1995; Klevesahl *et al*., 2003), casein infusion has not affected ruminal digestibility of basal dietary OM.

Ruminal degradability of feed N was not affected ($p>0.20$) by treatments. Observed DIP of the basal diet averaged 49.2%, in close agreement with the expected (51.0%; NRC, 1996; Level 1). Adjusting the N flow to the small intestine when casein was infused ruminally for N flow to the small intestine when casein was infused postruminally, the estimated ruminal degradability of casein-N was 86%. In several of the previous studies evaluating casein digestion (McDonald and Hall, 1957; Hume, 1974; Axford *et al*., 1975) ruminal degradation of infused casein was also less then 100%.

There were no treatment effects ($p>020$) on ruminal NDF digestion. However, ruminal ADF digestion was greater (5%, $p<005$) when casein was infused postruminally then when it was infused ruminally. Hudson *et al*. (1970) also observed an increase in cellulose digestion when soy protein was infused into abomasum. This effect may have been due, in part, to the effects of postruminal protein infusion on abomasal acid secretion. Bruchem and Klooster (1980) noted that postruminally infused protein increases abomasal acid secretion and Deswysen and Ellis (1988) observed that solubilization of hemicellulose in the abomasum increased proportionally with abomasal hydrochloric acid secretion. As expected, postruminal casein infusion increased postruminal digestion of the OM (21%, $p<005$) and N (12%, $p<0.01$). Similar to Swanson *et al*. (2004), there were no treatment effects ($p>0.20$) on total tract digestion of OM, N, ADF, NDF and GE.

Table 3: Effect of sodium caseinate infusion site[a] on abomasal chyme composition flowing to small intestine and digestive kinetics in steer calves fed with a forage-based diet

Item	Rmn	AbR	Abm	Ddn	SEM	C1[b]	C2	C3
Flowing from abomasums								
Chyme, Kg d^{-1}	78.2	77.4	68.6	83.7	5.2	0.99	0.28	0.13
NAN[c], % DMI	1.69	2.19	2.07	2.40	10.66	<0.01	0.62	0.09
N, as % in mL	0.06	0.09	0.103	0.123	0.01	<0.01	0.44	0.04
Ash, as % in mL	0.694	0.676	0.693	0.708	0.035	0.99	0.99	0.99
Osmotic pressure, mOsm	258	270	266	261	9	0.74	0.99	0.99
Flowing from abomasum,								
g L^{-1} OM	43.8	41.4	47.0	43.1	1.5	0.99	<0.01	0.99
NDF	16.0	13.9	16.6	14.9	1.0	0.67	0.10	0.99
ADF	13.8	11.9	13.5	12.7	0.6	0.13	0.10	0.99
N	1.56	1.83	2.02	1.95	0.90	<0.01	0.17	0.99
NAN	1.45	1.72	1.93	1.88	0.10	<0.01	0.16	0.99
MN	0.76	0.61	0.66	0.67	0.03	<0.01	0.21	0.47
Feed N	0.69	1.11	1.26	1.21	0.09	<0.01	0.30	0.99
Ruminal fluid Volume, Kg	35.2	28.7	32.6	33.4	4.0	0.68	0.90	0.99
DM, %	14.1	14.9	14.9	14.2	0.5	0.42	0.99	0.33
Solid, Kg	4.92	4.28	4.81	4.70	0.50	0.99	0.82	0.99
Liquid, Kg	30.3	24.4	27.8	28.7	3.6	0.63	0.93	0.99
Rate of flow, L h^{-1}	2.71	2.09	2.52	2.31	0.38	0.49	0.67	0.99
Turnover time, times d^{-1}	2.29	2.12	2.03	2.49	0.25	0.99	0.99	0.21
NDF dependent Model K_p	2.003.93	1.88	1.85	1.99	0.20	0.99	0.99	0.99
K_d	3.25	3.28	3.18	3.23	0.32	0.99	0.99	0.99
Maximum intake, g d^{-1}	7.757	7.431	7.331	7.669	683	0.99	0.99	0.99
Maximum intake, O/E[d]	0.95	0.79	0.91	0.88	0.10	0.76	0.59	0.99
NDF fill limit, g $kg^{-1.75}$	43.8	37.1	42.5	41.0	4.7	0.95	0.65	0.99

[a]Rmn = Rumen infusion, AbR = abomasum infusion throughout rumen, Abm = abomasum infusion, Ddn =Duodenum, [b]C1= Rmn vs AbR, Abm and Ddn; C2 = AbR vs Abm; C3 = AbR and Abm vs Ddn. [c]Nonammonia N [d]Observed/Expected ratio.

Characteristics of chyme entering the small intestine, nutrients outflowing from abomasum and ruminal kinetics are shown in the Table 3. Contrary to the hypothesis of this study, casein infusion did not influence (p>0.20) flow of chyme to the small intestine. We had expected that postruminal protein infusion might increase the abomasal turnover of chyme, allowing for greater passage rate of chyme from the rumen and thus, increase feed intake. Previously, Zinn *et al*. (1981) observed that the proportion of N in the chyme leaving the abomasum remained remarkably constant (2.6 g L^{-1}; CV = 9.6%) over a wide range of dietary N sources and intakes. In their study the flow of chyme (L d^{-1}) fluctuated closely with N flow to the small intestine. Accordingly, they proposed the existence of an enterogastric regulation mechanism, possibly keyed to % amino N sensors in the proximal duodenum that regulated outflow of protein from the abomasum. Because the total flow of chyme increased proportionately with the total flow of N, it seemed that this might form the basis for earlier findings (Egan and Moir, 1965; Egan, 1965a,b; Garza *et al*., 1991; Chung and Chamberlain, 1992), wherein postruminal casein infusion had enhanced DMI (hence, the hypothesis that the presence of increased proportions of protein in the abomasum will increase the flow of chyme from the abomasum, which may, in turn, increase the rate of passage of chyme from the rumen, allowing for greater intake). However, we did not observe an effect (p>0.20) of casein infusion on rate of passage of chyme from the rumen and ruminal turnover. As was observed by Liebholz and Hartmann (1970) and Gregory *et al*. (1985) the flow of chyme to the small intestine was primarily a function of DMI (chyme, L = 5.3 + 12.1DMI; R^2 = 0.50).

In contrast with Zinn *et al*. (1981) postruminal casein infusion increased (75%, p<.01) the soluble N content of duodenal chyme (Table 3). However, the increase in soluble N concentration did not affect (p>0.20) the tonicity of the chyme, averaging 264 mOsm (Table 3). Soluble ash concentration of the chyme (ash content of chyme following centrifugation at 20,000×g for 10 min) was also not affected (p>0.20) by treatments. Furthermore, there was very little (R^2 = 0.05) relationship between tonicity of chyme and passage rate of chyme from the abomasum to the small intestine. Carter and Grovum (1990) proposed that tonicity of chyme leaving the abomasum might be an important regulator of DMI. However, in other studies (Cottrell and Iggo, 1984; Gregory and Miller, 1989), increasing the tonicity of duodenal chyme to as high as 1,500 mOsm (using NaCl infusions) has not affected ruminal, abomasal or small intestinal motility.

Consistent with (Egan, 1970), casein infusion did not influence (p>0.20) the DM percentage of ruminal contents (Table 3). Ruminal DM content (17.5 g DM kg^{-1} $BW^{.75}$) was less (50%) then that reported by Olson *et al*. (1999), even though DMI in their study (94 " 2 g kg^{-1} $BW^{.75}$) was similar to that of the present study (97 g kg^{-1} $BW^{.75}$).

As with Olson *et al*. (1999), casein did not influence (p>0.20) total ruminal solids (77 g kg^{-1} $BW^{.75}$) or liquid

Table 4: Effect of sodium caseinate infusion site[a] on ruminal pH and volatile fatty acids profiles and ruminal volume in steers fed with a forage based diet

Item	Rmn	AbR	Abm	Ddn	SEM	C1[b]	C2	C3
pH	6.6	6.6	6.6	6.4	.08	.99	.99	.13
Acetate	73.7	73.6	73	71.8	.5	.14	.50	.05
Propionate	18.3	18.8	19.1	20	.35	.06	.99	.06
Butyrate	7.9	7.6	8.0	8.0	.25	.99	.44	.26
Acetate: Propionate	4.03	3.92	3.85	3.6	.09	.06	.99	.05

[a]Rmn = Rumen infusion, AbR = abomasum infusion throughout rumen, Abm = abomasum infusion, Ddn =Duodenum, [b]C1= Rmn vs AbR, Abm and Ddn; C2 = AbR vs Abm; C3 = AbR, Abm vs Ddn.

(471 g kg^{-1} $BW^{.75}$). Garza *et al.* (1992) observed that casein infusion into the duodenum increased the volume of liquid in the rumen in steers fed alfalfa hay.

There were no treatment effects ($p>0.20$) on indigestible NDF intake, averaging 26 g kg^{-1} $BW^{.75}$. Lippke (1986) observed that when dietary indigestible NDF was greater than 15%, maximum INDF intake ranged between 13 and 20 g kg^{-1} $BW^{.75}$.

Casein infusion did not affect ($p>0.20$) ruminal NDF fill, averaging 41.1 g kg^{-1} $BW^{.75}$ (Table 3). This value, observed in steer calves, is considerably less then the 75 g NDF fill kg^{-1} $BW^{.75}$ proposed by Mertens and Ely (1979) for Holstein cows. Zinn and Salinas (1999) developed a model for predicting maximal DMI in growing cattle under conditions of intensive feeding management [DMI_{max} = ((.098 * IW_{kg}) + 26.24) / ((.01*RNDF * (1 - (.01 * PRDNDF))) / ((.77 - (.00386*eNDF)) *(-.037 + (.042*RNDF) - (.00031*$RNDF^2$)))); where: RNDF = dietary NDF, % (DM basis), PRNDF = % ruminal NDF digestion, eNDF = effective NDF, % NDF]. In this model NDF fill is described as a function of the Initial Weight (IW) of cattle when placed under intensive management conditions [NDF fill, g kg^{-1} $BW^{.75}$ = (.098 * IW_{kg}) + 26.24]. Accordingly, the expected NDF fill and maximal DMI for calves in this trial are 47g kg^{-1} $BW^{.75}$ and 113 g kg^{-1} $BW^{.75}$, respectively, in good agreement with observed (Table 3). There were no treatment effects ($p>0.20$) K_p and K_d of ruminal NDF. The K_p of ruminal NDF, averaging 1.93%/h, is lower (26%) than expected based on NRC (1996) level 2 model (2.6%/h).

There were no treatment effects ($p>0.20$) on ruminal pH (Table 4). When using similar ruminal infusion rates, Köster *et al.* (1996) and Olson *et al.* (1999) observed that casein infusion decreased (4%) ruminal pH. Mathis *et al.* (1999) also found a small decline in ruminal pH when supplementing SBM to low-quality forage diets. However, Doyle *et al.* (1988) and Taniguchi *et al.* (1995) observed decreased ruminal pH when casein was infused into the abomasum as compared with the rumen infusion. The ruminal acetate: Propionate molar ratio was greater ($p<0.10$) when casein was infused in the rumen then when it was infused postruminally. The ruminal acetate:propionate molar ratio was also greater ($p<0.05$) when casein was infused into the abomasum then when it was infused into the proximal duodenum. Why the ruminal acetate: Propionate ratio decreased as the site of casein infusion moved progressively further from the rumen is not certain.

CONCLUSION

Protein composition of abomasal chyme does not play an important role in regulating intake of cattle fed forage-based diets. Independently of N status, increasing protein supply to the small intestine will not facilitate an increase in dry matter intake. Instead, limits to maximal dry matter intake in cattle fed forage-based diets appears to be largely a function of ruminal neutral detergent fiber fill capacity and rates of passage and digestion of neutral detergent fiber.

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